

**Neural and psychological mediators of trait impulsivity:  
cortico-striatal circuitry and catecholaminergic  
neuromodulation**



**Chiara Toschi**

Department of Psychology University of Cambridge

This dissertation is submitted for the degree of  
Doctor of Philosophy

Darwin College

April 2021



# Declaration

The work described in this dissertation was carried out between October 2016 and March 2021 at the Department of Psychology, University of Cambridge under the supervision of Professor Jeffrey W. Dalley.

This dissertation has resulted from my own work and all collaborations are specified in the text. This dissertation is not substantially the same as any that I have submitted or is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University of similar institution, except as specified in the text. I have made every attempt to reference properly for any idea or finding that is not my own. The length of this dissertation does not exceed the word limit of the School of Biology degree committee i.e., 60,000 words.

Chiara Toschi, April 2021

# Acknowledgements

First of all, I would like to thank my supervisor, Jeff Dalley for supporting me throughout these years and giving me the possibility to explore my ideas freely, even if that meant that sometimes I made some mistakes! Thank you also for the prompt feedback on my work and for having an ‘open door’ policy which meant that I could always come and discuss scientific ideas with you, it’s been a pleasure to learn from such interactions. Thank you also to my advisor, Trevor Robbins, for reminding me of the importance of remembering papers ;) and for being a fountain of knowledge at easy reach, I learned a lot from our discussions and your way of thinking about behaviour and the brain. Thank you also to post-docs in the lab who had to put up with my moments of PhD-induced GAD! Thank you Johan for your wisdom, funny and relaxed attitude and for making sure I always did the right calculations! Thank you Karly for the very interesting discussions about behaviour and for showing me what it means to be passionate about a subject whilst still having a life outside the lab ☺ Thank you Mona for all the in-depth existential discussions that we had and for reminding me that personal development is the priority no matter where one is in his/her career. Thank you Mick for being so patient with me and with my mistakes, for being a loyal friend and someone I could always rely on, I admire your sense of responsibility and generosity! Thank you Colin for always being up for a laugh and for helping me in the dark moments of my experimental journey. Thank you Julietta for listening to my depressive rants about lab-life, for helping me whenever you saw I was struggling and for having such a cooperative and generous attitude despite us following a similar academic track. Thank you Parisa for helping me with experiments and telling me fascinating tales about Iran during boring immunostaining practices! I hope we can meet some day in our respective countries! Thank you to all the other people in the lab: Peter, Laura, Ethan, Jolyon, Dhaval, Livia, Katherine for creating a fun and relaxed working environment, I’m sad to leave y’all!

Thank you to my college family, the Darmates! who made life outside the lab a very appealing and joyful alternative, I cherish all the moments we spent together from our baby steps into this Cambridge experience to our mature walks as final year PhD students (aka senile members of the college). Thank you Claudia, John and Clark for the interesting discussions on neuroscience and

for all of our non-neurosciency crazy fun moments. Thank you to the fantastic Ms!! Miriam B., Marzia, Mara, Miriam L., thank you making people think that in Italy everyone's name starts with 'M' eheh, thank you for all the Italian aperitivi and for being by my side in the ups and the downs, thanks for all the chats on life and science and may the journey continue together wherever we end up going! Thank you to my flatmates for these 4 years together and for all the crazy stuff we went through during this pandemic, it would have been a very grim experience had you not been sharing it with me (I say this even in light of Thiago's neurotic bouts, so it means a lot to me). Thank you Sachina for listening and for baking endlessly, famine was not our problem during these harsh times; Thank you Thiago for all the preaching (mostly interesting, new stuff) and for showing me that mindfulness has a true practical component that needs to be practiced! Thank you Nadi for all the interesting talks on the middle east and apologies for my ignorant comments sometimes, but don't take it personally ;) Thank you Chiaretta for being STRONG throughout and for lowering the level of anxiety in the house; Thank you Sandrina for your fiducia attiva and your optimism, I will learn from that ☺ Thank you Alessio for being such a calming presence and for all the tasty apple pies! Thank you Ludo for your bubbly presence ehe. Thanks to my friends in Italy, my life companions, thank you for being there in sickness and in health and for showing me depth of relationships I am grateful I have in my life ☺ Thank you to my lovely family, for supporting me and being there no matter what, mamma, papa', Cate sapete che siete la mia cuccia calda dove a volte ho bisogno di rifugiarmi quando mi sento un po' persa, sono contenta che nonostante gli alti e bassi sappiamo sempre divertirci ed essere uniti!. Finally thank you to my goofball, Paolino, who stuck with me despite the tortuous path, you know how important you are to me and how amazing the last ride has been with you by my side ☺

# Abstract

Impulsivity is a multidimensional trait in humans and other mammalian species. It is widely regarded as the tendency to act rapidly, without appropriate foresight and underlies many psychiatric disorders, including attention-deficit/hyperactivity disorder (ADHD), mood disorders such as depression and mania, and substance use disorder (SUD). Research has shown that impulsivity is a non-unitary construct, characterised by distinct behavioural manifestations and, accordingly, distinct neural substrates.

This thesis focuses on elucidating the psychological and neural mechanisms of waiting impulsivity in experimental rats trained on the 5-choice serial reaction time task (5CSRTT). The psychological processes involved in this behaviour are yet to be fully understood. However, aspects of sustained attention, urgency, motivation, and delay-aversion are all likely to play a role in shaping this behaviour. Although the neural substrates and neurotransmitter systems underlying waiting impulsivity have been extensively investigated, there are still many unanswered questions. Some of the questions that this work aims to address are: 1) can we refine our understanding of the psychological mechanism that influence premature responses, specifically the extent to which these are driven by motivated behaviour or, instead, negative urgency? 2) Does waiting impulsivity confer advantages in specific experimental contexts and what are the neurotransmitter mechanisms regulating this? 3) Can we refine our understanding of the precise neural circuits involved in the execution of a premature response? 4) How does impulsivity and attention on the 5CSRTT compare with performance on other tasks of sustained attention? Are there any shared neural processes?

To investigate these questions, I used a range of experimental approaches spanning behavioural testing to systemic and local (intra-cerebral) pharmacology, brain lesions and chemogenetics. In chapter 2, I show that premature responses are influenced by reinforcement rate and motivation, and that negative urgency does not seem to play an important role in the genesis of these responses. In chapter 3, I found that high levels of trait impulsivity confer an advantage in contexts that require rapid focusing and action. I also showed that this advantage might be conferred putatively by elevated striatal dopamine (DA) release in the striatum. In chapter 4, I

show that inhibition of the mesolimbic DA system reduces premature responses but was unable to pinpoint the midbrain-striatal loop responsible for this effect. Finally, in chapter 5, two tasks were compared that assess sustained visual attention. PFC lesions effected using the excitotoxin quinolinic acid profoundly affected performance on the 5CSRTT but had negligible effects on a signal detection attentional task.

Taken together, these findings suggest that impulsive (premature) responding in the 5CSRTT is linked with the motivation to perform the task but does appear to depend on negative urgency. Trait impulsivity confers an advantage in specific contexts and depends on striatal DA function. Finally, sustained attention on the 5CSRTT is distinct from other forms of sustained attention both at the psychological and neural levels. Some of these findings are consistent with studies on impulsive individuals, thus highlighting the potential for translational research between rodents and humans. Ultimately, this work expands our understanding of the psychological and neural circuit mechanisms underlying waiting impulsivity.

# Publications arising from this Ph.D

- **Toschi, C.**, El-Sayed Hervig, M., Moazen, P., Parker M., Dalley, J. W., Gether, U., Robbins, T. W. Adaptive aspects of impulsivity and interactions with effects of catecholaminergic agents in the 5-choice serial reaction time task: implications for ADHD. *Psychopharmacology. under revision (Chapter 4)*
- **Toschi, C.**, El-Sayed Hervig, Burghi, T., Sell, T., Gether, U., Robbins, T. W., Dalley, J. W. Does negative urgency play a role in premature responses on the 5CSRRT?. *In preparation (Chapter 3)*
- **Toschi, C.**, El-Sayed Hervig, Robbins, T. W., Dalley, J. W. Sustained attention and impulsivity: comparing 5CSRRT and SDT at the behavioural and neuropsychological level. *In preparation (Chapter 6)*

# Table of Contents

<i>Declaration</i> .....	3
<i>Acknowledgements</i> .....	4
<i>Abstract</i> .....	6
<i>Publications arising from this Ph.D</i> .....	8
<i>Table of Contents</i> .....	9
<i>List of Abbreviations</i> .....	16
List of Abbreviations and Acronyms.....	16
<b>Chapter 1 Introduction</b> .....	<b>20</b>
<b>1.1 Defining impulsivity</b> .....	<b>20</b>
<b>1.2 Clinical significance of impulsivity</b> .....	<b>21</b>
<b>1.3 Assessment of impulsivity</b> .....	<b>23</b>
1.3.1 Humans .....	23
1.3.2 Experimental approaches in rodents .....	31
<b>1.4 Psychological mechanisms of impulsivity: focus on response inhibition</b> .....	<b>35</b>
1.4.1 Sustained attention .....	35
1.4.2 Incentive motivation .....	38
1.4.3 Urgency.....	39
1.4.4. Sensitivity to negative outcomes.....	41
<b>1.5 Neural substrates of impulsivity: waiting impulsivity</b> .....	<b>44</b>
1.5.1. Basal Ganglia.....	45
1.5.2 The role of the BG in movement initiation .....	49
1.5.3 The role of the BG in waiting impulsivity .....	55
<b>1.6 Thesis overview</b> .....	<b>61</b>
1.6.1 Summary.....	61
1.6.2 Aims.....	61

<b>Chapter 2 General methods and materials.....</b>	<b>64</b>
<b>2.1 Subjects .....</b>	<b>64</b>
<b>2.2 5CSRTT apparatus .....</b>	<b>65</b>
<b>2.3 Five-choice serial reaction time task .....</b>	<b>66</b>
2.3.1 Training.....	66
2.3.2 vITI challenge .....	66
<b>2.4 Stereotaxic surgery .....</b>	<b>67</b>
<b>2.5 Perfusion and brain extraction .....</b>	<b>67</b>
<b>2.6 Statistical methods .....</b>	<b>67</b>
<b>Chapter 3 .....</b>	<b>69</b>
<b>3.1 Introduction.....</b>	<b>69</b>
<b>3.2 Methods .....</b>	<b>78</b>
<b>3.2.1 Subjects .....</b>	<b>78</b>
<b>3.2.2 5CSRTT .....</b>	<b>79</b>
3.2.2.1 Training.....	79
3.2.2.2 vITI challenge .....	79
<b>3.2.3 Behavioural manipulations on 5CSRTT .....</b>	<b>79</b>
3.2.3.1 Experiment 1 .....	79
3.2.4 Analysis .....	81
<b>3.3 Results.....</b>	<b>85</b>
<b>3.3.1 Experiment 1 .....</b>	<b>85</b>
3.3.1.1 Effects of partial reinforcement on 5CSRTT performance .....	85
3.3.1.2 Effects of partial reinforcement on premature responses .....	88
3.3.1.3 Consequences of a rewarded or non-rewarded trial on premature responses.....	91
<b>3.3.2 Experiment 2 - Effects of reinforcement omission on behaviour: increasing the ITI .....</b>	<b>94</b>
3.3.2.1 Results (Cohort 1).....	94
3.3.2.2 Consequences of a rewarded or non-rewarded trial on premature responses.....	97

<b>3.3.3 Experiment 3 and 4 - Effects of reinforcement omission and timeout reduction on premature responses.....</b>	<b>98</b>
3.3.4.1 Results.....	99
3.3.4.2 Comparison with previous manipulations with 5 s ITI.....	102
<b>3.3.5 Experiment 5 - Effects of reinforcement omission and reward magnitude on premature responses.....</b>	<b>103</b>
3.3.5.1 Results.....	104
3.3.5.2 Consequences of rewarded or non-rewarded trials on premature responses.....	106
<b>3.4 Discussion.....</b>	<b>109</b>
<b><i>Chapter 4 - Adaptive aspects of impulsivity and interactions with effects of catecholaminergic agents.....</i></b>	<b>118</b>
<b>4.1 Introduction.....</b>	<b>118</b>
<b>4.2 Methods and materials.....</b>	<b>122</b>
<b>4.2.1 Subjects .....</b>	<b>122</b>
<b>4.2.2 Five-choice serial reaction time task: training .....</b>	<b>122</b>
<b>4.2.3 Experiment 1 – Effects of impulsivity trait on behavioural performance at variable ITI .</b>	<b>122</b>
4.2.3.1 Variable ITI challenge .....	122
4.2.3.2 Short vITI challenge: rapid stimulus presentation .....	122
4.2.3.3 Data analysis.....	123
4.2.3.4 Progressive Ratio .....	123
<b>4.2.4 Experiment 2: Effects of methylphenidate, atomoxetine, amphetamine, atipamezole and phenylephrine on vITI performance .....</b>	<b>125</b>
4.2.4.1 Variable ITI challenge .....	125
4.2.4.2 Systemic drug administration.....	125
4.2.4.3 Data analysis.....	125
<b>4.3 Results.....</b>	<b>126</b>
<b>4.3.1 Experiment 1 .....</b>	<b>126</b>
4.3.1.1 Effects of trait-like impulsivity trait on behavioural performance assessed during a vITI challenge ..	126
4.3.1.2 Short vITI challenge .....	130
4.3.1.3 Progressive Ratio .....	131

<b>4.3.2 Experiment 2</b> .....	<b>134</b>
4.3.2.1 Effects of methylphenidate, atomoxetine, amphetamine, atipamezole and phenylephrine on vITI performance .....	134
4.3.2.2 Effects of methylphenidate and atomoxetine .....	134
4.3.2.3 Effects of amphetamine, atipamezole and phenylephrine .....	137
<b>4.4 Discussion</b> .....	<b>139</b>
<b>Chapter 5 - Role of mesolimbic dopamine circuits in impulsivity</b> .....	<b>147</b>
<b>5.1 Introduction</b> .....	<b>147</b>
<b>5.2 Experiment 1 - VTA cannulation</b> .....	<b>150</b>
<b>5.2.1 Materials and methods</b> .....	<b>150</b>
5.2.1.1 Animals.....	150
5.2.1.2 Five-choice serial reaction time task: training and screening for impulsivity .....	150
5.2.1.3 Intracranial surgery .....	150
5.2.1.4 Drugs.....	151
5.2.1.5 Intracranial microinfusions .....	151
5.2.1.6 Cannulae tip placement.....	152
5.2.1.7 Data Analysis .....	152
<b>5.2.2 Results VTA</b> .....	<b>152</b>
5.2.2.1 Histology.....	152
5.2.2.2 Effects of microinfusions of quinpirole into VTA during performance on 5CSRTT.....	153
<b>5.3 Experiment 2 - circuit-specific manipulations</b> .....	<b>155</b>
<b>5.3.1 Pilot experiment</b> .....	<b>155</b>
<b>5.3.2 Chemogenetics in the context of 5CSRTT</b> .....	<b>156</b>
5.3.2.1 Methods and Materials.....	157
5.3.2.2 Results.....	162
<b>5.3.2.3 Discussion</b> .....	<b>170</b>
<b>Chapter 6 – A comparative study between 5CSRTT and SDT</b> .....	<b>176</b>
<b>6.1 Introduction</b> .....	<b>176</b>
<b>6.2 Methods and materials</b> .....	<b>180</b>

6.2.1 Subjects.....	180
6.2.2 5CSRTT training and testing .....	180
6.2.3 SDT training and testing .....	181
6.2.4 Considerations about comparing baseline sessions on the SDT and 5CSRTT .....	182
6.2.5 Surgeries .....	182
6.2.6 Performance on the SDT and 5CSRTT tasks following PFC lesions .....	183
6.2.7 Histology.....	183
6.2.8 Immunohistochemistry .....	184
6.2.9 Analyses.....	184
<b>6.3 Results.....</b>	<b>185</b>
<b>6.3.1 Baseline .....</b>	<b>185</b>
6.3.1.1 SDT.....	187
6.3.1.2 5CSRTT.....	187
6.3.1.3 In summary .....	188
<b>6.3.2 Variable SD challenge .....</b>	<b>190</b>
<b>6.3.3 Effects of mPFC lesions on SDT and 5CSRTT performance.....</b>	<b>194</b>
<b>6.3.3.1 Histology.....</b>	<b>194</b>
<b>6.3.3.2 Baseline .....</b>	<b>195</b>
6.3.3.2.1 Accuracy .....	195
6.3.3.2.2 Omissions.....	196
6.3.3.2.3 Anticipatory responses.....	197
6.3.3.2.4 Response latencies .....	197
<b>6.3.3.3 Variable SD challenge .....</b>	<b>199</b>
6.3.3.3.1 Accuracy .....	200
6.3.3.3.2 Omissions.....	201
6.3.3.3.3 Anticipatory responses.....	201
6.3.3.3.4 Response Latencies.....	203
6.3.3.3.5 In summary .....	203
<b>6.4 Discussion.....</b>	<b>205</b>
<b>6.4.1 A comparative analysis of the SDT and 5CSRTT .....</b>	<b>205</b>
<b>6.4.2 Behavioural effects of mPFC lesions .....</b>	<b>209</b>

<b>Chapter 7</b> .....	<b>213</b>
<b>7.1 General discussion</b> .....	<b>213</b>
<b>7.2 Translational implications and further considerations</b> .....	<b>225</b>
<b>7.3 Limitations and alternative approaches</b> .....	<b>227</b>
<b>7.4 Future directions</b> .....	<b>229</b>
<b>Bibliography</b> .....	<b>232</b>
<b>Appendix A - Chapter 3</b> .....	<b>297</b>
<b>A1.1 Analyses</b> .....	<b>297</b>
<b>A1.2 Results</b> .....	<b>298</b>
A1.2.1 Experiment 1.....	298
<b>A1.2.2 Experiment 2</b> .....	<b>302</b>
A1.2.2.1 Consequences of a rewarded or non-rewarded trial.....	302
<b>A1.2.3 Experiment 3</b> .....	<b>305</b>
<b>A1.2.4 Experiment 4</b> .....	<b>309</b>
A1.2.4.1 Consequences of a rewarded or non-rewarded trial.....	309
<b>A1.2.5 Experiment 5</b> .....	<b>311</b>
A1.2.5.1 – Effects of rr and reward magnitude on indexes of motivation.....	311
A1.2.5.2 Consequences of a rewarded or non-rewarded trial.....	313
<b>Appendix B - Chapter 4</b> .....	<b>320</b>
B1.1 Data Analysis.....	320
B1.2 Progressive ratio.....	320
<b>B2.1 Results</b> .....	<b>321</b>
B2.1.1 Experiment 1.....	321
B2.1.2 Experiment 2.....	325
B2.1.2.7 Effects of amphetamine, atipamezole and phenylephrine.....	334
<b>Appendix C - Chapter 5</b> .....	<b>335</b>
<b>C1 Experiment 1</b> .....	<b>335</b>
C1.1 Data Analysis.....	335

C1.2 Results .....	336
<b>C2 Experiment 2 - circuit-specific interventions .....</b>	<b>336</b>
C2.1 Pilot experiment .....	336
C2.2 Chemogenetics intervention in the 5CSRTT .....	346
<b><i>Appendix D - Chapter 6</i> .....</b>	<b>354</b>
<b>D1 Methods and materials.....</b>	<b>354</b>
D1.1 Immunohistochemistry.....	354
D1.2 Data Analysis .....	355
D1.3 Results.....	359

# List of Abbreviations

## List of Abbreviations and Acronyms

4-CSRTT = The 4-Choice Serial Reaction Time Task

5-CSRTT = The 5-Choice Serial Reaction Time Task

5C-CPT = 5-choice continuous performance task

5-HT = 5-hydroxytryptamine

AC = adenylyl cyclase

ACC = anterior cingulate cortex

ADHD = Attention-Deficit/Hyperactivity Disorder

AIC = Akaike information criterion

AMPH = d-amphetamine

ASPD = Antisocial Personality Disorders

ATI = atipemazole

ATO = atomoxetine

AUD = alcohol use disorders

BART = The Balloon Analogue Risk Task

BD = Binge drinkers

BG = Basal ganglia

BIS = Barratt Impulsivity Scale

CNO = clozapine N-oxide

CPT = The Continuous Performance Task

CR = conditioned response

CS = conditioned stimulus

DA = dopamine

DAT = dopamine transporter

DBS = deep brain stimulation

DI = Dysfunctional impulsivity

DREADDs = Exclusively Activated by Designer Drugs

DS = dorsal striatum

DMS = dorso-medial striatum  
DLS = dorso-lateral striatum  
DRL = differential reinforcement of low-rate schedules  
EPI = Eysenck Personality Inventory  
EIQ = Eysenck Impulsiveness Questionnaire  
EEfRT = The Effort Expenditure for Rewards Task  
FI = Functional impulsivity  
FFM = five-factor model of personality  
FMRI = functional magnetic resonance imaging  
FP = frontal panel  
GABA =  $\gamma$ -amino-butyric acid  
GAD = GABA decarboxylase  
GIRKs = G-protein sensitive inwardly rectifying potassium channels  
GPe = external segment of the globus pallidus  
GPi = internal segment of the globus pallidus  
GT = goal-trackers  
HI = high impulsive  
IGT = The Iowa Gambling Task  
ITI = intertrial interval  
IST = The Information Sampling Task  
LMEM = Linear Mixed-Effects Model  
LI = low impulsive  
MFFT = The Matching Familiar Figures Test  
MID = mid impulsive  
MPH = methylphenidate  
MSNs = medium-sized spiny projection neurons  
mPFC = medial prefrontal cortex  
NA = noradrenaline  
NAcb = nucleus accumbens  
NR = correct non-rewarded trials  
OFC = orbitofrontal cortex

PavCA = Pavlovian conditioned approach task  
PET = positron emission tomography  
PFC = prefrontal cortex  
PHEN = phenylephrine  
PI = Peak-Interval procedure  
PKA = protein kinase A  
PR = progressive ratio  
PUM = positive urgency measure  
R = correct rewarded trials  
RDT = the Risky Decision Making Task  
ROE = reinforcement-omission effect  
RPE = reward prediction errors  
RP = rear panel  
rr = reinforcement rate  
RT = reaction times  
SAT = sustained attention task  
SD = stimulus duration  
SDT = signal detection task  
SNc = substantia nigra  
pre-SMA = pre-supplementary motor area  
SSRT = Stop-Signal Reaction Time Task  
ST = sign-tracking  
STN = subthalamic nucleus  
SUD = Substance use Disorder  
SURPS = Substance use risk profile scale  
TD = tryptophan depletion procedure  
TH = tyrosine hydroxylase  
US = unconditioned stimulus  
vSD = variable stimulus duration  
vITI = variable ITI  
VMAT2 = vesicular monoamine transporter 2

VS = ventral striatum

VTA = ventral tegmental area

# Chapter 1 Introduction

## 1.1 Defining impulsivity

Impulsivity is a multidimensional trait in humans and other mammalian species. It is widely regarded as the tendency to act rapidly, without appropriate foresight and underlies many psychiatric disorders, including Attention-Deficit/Hyperactivity Disorder (ADHD), mood disorders such as depression and mania (Moeller et al., 2001; Swann et al., 2008), schizophrenia (Ouzir, 2013), and substance use disorder (SUD, Dalley and Robbins, 2017; Jentsch and Taylor, 1999). Research has shown that impulsivity is a non-unitary construct, characterised by distinct behavioural manifestations and, accordingly, distinct neural substrates (Dalley & Robbins, 2017; Winstanley et al., 2006). Some have even suggested that impulsivity fails to meet the requirements of a psychological construct, and that instead more specific terms should be used to describe the higher order domains of ‘impulsivity’ (Stricklan & Johnson, 2021).

Impulsivity has long been considered a personality trait (Hamilton, et al., 2015b; Odum, 2011). A trait is regarded as a lasting, consistent individual feature and in humans it is usually assessed *via* self-report questionnaires (Odum, 2011; Roberts & Jackson, 2008; Vassileva & Conrod, 2019). In the context of impulsivity, these have proven to be particularly useful in detecting endophenotypes for substance abuse (Audrain-McGovern et al., 2009; Ersche et al., 2010; Long et al., 2018) thus strengthening the validity and clinical relevance of studying impulsivity as a trait. Advances in molecular biology have grounded the study of personality traits in genetics, supporting the idea that traits are pre-existing, biologically defined dispositions (Roberts & Jackson, 2008). This is relevant for the study of impulsivity, as various sub-components of impulsivity have strong genetic underpinnings (Anokhin et al., 2011; Eisenberg et al., 2007; Gray et al., 2018).

There has also been research on the nature of the state variables that influence impulsivity (Cyders & Coskunpinar, 2011). These alter behaviour over a short time frame and are

determined by the ‘environmental context and the current state of the individual’ (Vassileva & Conrod, 2019). State dimensions are typically measured by performance on neurocognitive tasks (Dalley & Robbins, 2017; Dixon et al., 2006; Vassileva & Conrod, 2019), and in humans have the advantage, compared to trait measurements, of being independent of self-report biases (Cyders & Coskunpinar, 2011). Different taxonomies have been suggested to categorize the different subtypes of impulsive behaviour (Bari & Robbins, 2013; Dalley & Robbins, 2017), with most behaviours falling under the domains of either ‘response inhibition’ (or motor impulsivity) or ‘deferred gratification’ (or choice impulsivity). The former refers to impulsivity as the inability to delay or stop a motoric response, even if this results in a maladaptive choice or less rewarding outcome. The latter, on the other hand, refers to the preference for small, immediate rewards over rewards that are larger but more distant in time (Hamilton, et al., 2015b; Odum, 2011).

## **1.2 Clinical significance of impulsivity**

The study of impulsivity has received much attention over the last thirty years due to the involvement of this dimensional construct in numerous psychiatric conditions, including Attention-Deficit/Hyperactivity Disorder (ADHD), pathological gambling, substance use disorders (SUD), Antisocial Personality Disorders (ASPD), suicidal and aggressive behaviours. In the context of SUDs, for example, impulsivity has been found to be consistently associated with the development of alcohol and drug abuse (Dalley & Ersche, 2019; Vassileva & Conrod, 2019), both as a consequence and as a determinant of the disorder (Audrain-McGovern et al., 2009; de Wit, 2009; Kreek et al., 2005; Moffitt et al., 2011). Importantly, rather than simply instigating drug use, impulsivity is thought to determine the development of drug addiction, presumably due to failures in inhibitory control (Dalley & Ersche, 2019; Ersche et al., 2013; Vassileva & Conrod, 2019). For this reason, recent theories of addiction conceptualise impaired impulse control as the core mechanism underlying drug seeking and relapse (Lyvers, 2000; Vassileva & Conrod, 2019). There have been attempts at addressing impulsivity with the aim to treat the symptoms of addiction (Odum, 2011). This is a particularly important clinical objective in light of evidence showing that high levels of impulsivity can predict relapse (Evren et al.,

2012) and treatment drop out (Gerard Moeller et al., 2001; Loree et al., 2015). However, to date, this has been successful only in the context of nicotine addiction (Sheffer et al., 2018).

Linked to failures of impulse control is also another important disorder which originates in childhood and tends to persist throughout adult life, that is ADHD (Vassileva & Conrod, 2019). ADHD is characterised by poor sustained attention, impulsiveness, and hyperactivity (American Psychiatric Association, 2013), has been primarily associated with deficits of motor impulsivity (Barkley, 1997; Vassileva & Conrod, 2019). Specifically, to unify different theories of ADHD, Barkley (1997) proposed a new model suggesting that the core mechanism underlying most impairments in ADHD is poor response inhibition, defined as: a) a failure to inhibit an initial prepotent response which is immediately associated with reinforcement; b) a failure to halt an ongoing stopping response; c) an inability to protect one's self-directed actions from interference by external competing events. Barkley suggested that deficits in executive functions typical of ADHD, including those in working memory and in self-regulation of motivation/arousal, were secondary to this primary deficit in response inhibition. This fits with research showing that individuals with ADHD display poor inhibitory control as measured, for example, with the stop task (Chamberlain et al., 2011; Lijffijt et al., 2005; Lipszyc & Schachar, 2010). However, as pointed out by Ma and colleagues (2016), deficits in response inhibition only account for roughly half of cases of ADHD (van Mourik et al., 2005; Willcutt et al., 2005) and indeed other theories have been put forward to understand the underlying aetiological factors of this disorder. Thus, three separate theories have been proposed, all pointing to abnormalities in reinforcement sensitivity and in the functioning of the neurotransmitter dopamine (DA) (for a review see Plichta and Scheres, 2014).

## 1.3 Assessment of impulsivity

In light of how widely impulsivity is implicated in psychiatric disorders, it is important to study the behavioural and neural dimensions of this construct. Research has explored these both in humans and in animals, with work in the latter being useful in precisely defining the neural circuits and neurochemistry underlying impulsivity. Any psychological phenomenon, however, is limited in the way it is studied by the means through which it is validated (Smith, 2005). Thus, it is important to describe how impulsivity has been assessed both in humans and in animals.

### 1.3.1 Humans

#### 1.3.1.1 Self-Report Measures

The study of impulsivity is grounded in the idea that impulsivity has a trait dimension, and as such it is regarded as a stable and lasting personality characteristic that can be assessed *via* self-report questionnaires. This notion is supported by empirical evidence showing that impulsivity has a strong genetic component (variance due to genes: 0.50), as well as unique environmental influences (Bezdjian et al., 2011). A number of different questionnaires and scales have been developed over the years to capture different sub-domains of the multi-faceted construct that impulsivity (Eben et al., 2020; Hamilton, 2015a; Hamilton, 2015b; Sharma et al., 2014; Stricklan & Johnson, 2021; Vassileva & Conrod, 2019). For an overview of this see **Table 1.1**.

Reference	Scale	About
Eysenck and Eysenck (1968)	Eysenck Personality Inventory (EPI) and Eysenck Impulsiveness Questionnaire (EIQ)	Combines the dimension of impulsivity with that of sociability to construct the Extraversion scale of the Eysenck Personality Inventory. Later revisions of this shifted many impulsivity items to the Psychoticism scale, leaving Extraversion to be composed primarily of sociability and, to some extent, venturesomeness (Eysenck et al., 1985).
Barratt (1959)	Barratt Impulsivity Scale (BIS)	Aimed to study impulsivity within a more multi-dimensional framework. The BIS is now one of the most widely administered self-report measures for the assessment of impulsivity and divides this construct into three sub-categories or second-order factors: motor impulsiveness (acting without thinking), non-planning impulsiveness (lack of forethought), and attentional impulsiveness (inability to focus attention, Patton et al., 1995; Stanford et al., 2009)
Dickman (1990)	Functional impulsivity (FI) and Dysfunctional impulsivity (DI).	Assesses the adaptive aspects of impulsivity. Differentiates the dimension of FI from that of DI. The latter refers to the typical conceptualisation of impulsivity as rash behaviour in situations where this is nonoptimal, while the former is defined as ‘the tendency to engage in rapid, error-prone information processing when such a strategy is (..) optimal’, (Dickman, 1990).
Whiteside and Lynam (2001)	UPPS Impulsive Behaviour Scale	Examines the relation between seventeen impulsivity personality scales and the five-factor model of personality (FFM; McCrae and Costa, 1990) through factor analysis. The UPPS Impulsive Behaviour Scale, identifies 4 main components of impulsivity: (1) Urgency, which is the propensity to engage in rash or maladaptive actions due to intense negative affect; (2) Lack of Premeditation, which refers to the inability ‘to think and reflect on the consequences of an act before engaging in that act’ (p.686, Whiteside and Lynam, 2001); (3) Lack of Perseverance, which refers to the inability to

		resist distracting stimuli and stay focused on a task and (4) Sensation seeking, which is defined as the propensity to pursue new, exciting experiences.
Cyders and Smith (2007)	Positive Urgency Measure (PUM)	Assesses the propensity to act rashly in response to positive affective states.
Woicik and colleagues (2009)	Substance Use Risk Profile Scale (SURPS)	Based on a model of personality risk for substance abuse in which four personality dimensions (hopelessness, anxiety sensitivity, impulsivity, and sensation seeking) are thought to differentially relate to specific patterns of SUD

**Table 1.1 A summary of the different impulsivity scales used to assess impulsivity in humans.**

Self-report measures of impulsivity have been shown to be useful from a clinical point of view, especially in the context of SUD (Castellanos-Ryan et al., 2011, 2013; Moffitt et al., 2011; Vassileva & Conrod, 2019). For example, disinhibited personality, as measured with the EPI, was shown to be an important predictor of self-report and laboratory-based drinking behaviour (Conrod et al., 2000; Conrod et al., 1997), and was associated with higher rates of ASPD and cocaine dependence (Conrod et al., 2000). More recently, high impulsivity as assessed with the SURPS (Woicik, et al., 2009), reliably predicted substance use, hyperactivity, and conduct problems in a large sample of adolescents (Castellanos-Ryan et al., 2011, 2013).

Thus, measures of impulsivity through personality questionnaires have been shown to have clinical validity and when assessed early in childhood to predict later psychopathology (Vassileva & Conrod, 2019). However, the use of self-report measures to assess impulsiveness has received some criticism (Stricklan & Johnson, 2021; Vassileva & Conrod, 2019; Woicik et al., 2009). Firstly, some of the early personality scales failed to conceptualise impulsivity as a multi-dimensional construct and thus to model different sub-domains or to frame impulsivity as part of a broader inventory of personality dimensions (Stricklan & Johnson, 2021; Woicik et al., 2009). Some of the instruments intended to overcome this problem, such as the NEO-Five Factor Inventory (NEO-FFI; Costa and McCrae, 1992), failed to include personality factors that have been shown to be relevant predictors of clinical conditions such as SUDs (Woicik et al., 2009). This led Woicik and colleagues (2009) to construct a more comprehensive instrument, the SURPS, that could assess distinct and independent personality factors at once, and importantly, that had direct clinical applicability.

Finally, other short-comings of personality questionnaires are: i) the biases inherent to self-report assessments and the lack of objectivity (Bari & Robbins, 2013; Cyders & Coskunpinar, 2011); ii) the fact that they often rely on a single assessment and fail to take into account within-person variability (Vassileva & Conrod, 2019); and iii) the intractability of these measures in pre-clinical research, thus limiting translational research (Bari & Robbins, 2013).

### **1.3.1.2 Behavioural Measures**

Somewhat independently from the study of self-report measures (Stricklan & Johnson, 2021), impulsivity and its sub-components have also been studied in its ‘state’ dimension. This refers to performance-based behavioural manifestations that have a more transient nature and are more susceptible to environmental context (Dalley & Robbins, 2017; Vassileva & Conrod, 2019). A broad distinction that has often been implemented in the field is that between ‘motor impulsivity’ (or impulsive action) and ‘choice impulsivity’ (or impulsive choice). While the former refers to deficits in motor inhibition and the inability to withhold a prepotent dominant response, the latter refers to deficits in reward-based responding and the propensity to choose a small, immediate rewards over larger but delayed rewards (Bari & Robbins, 2013; Eben et al., 2020; van Gaalen et al., 2006). For an overview of the different tasks used to measure impulsivity in humans see **Table 1.2** (choice impulsivity) and **Table 1.3** (motor impulsivity).

Impulsivity type	Impulsivity sub-type	Description	Examples of tasks
Choice impulsivity	Temporal discounting	Preference for small, readily available rewards over larger but delayed rewards. An individual choosing mostly the small, immediate option is said to have steeper (or greater) discounting of the large reward and thus to be more impulsive (Hamilton, et al., 2015b).	<a href="#">Two Choice Impulsivity</a> (Dougherty et al., 2003)
			<a href="#">The Single Key impulsivity paradigm</a> (Dougherty et al., 1999)
	Effort discounting	Preference for low expenditure of effort associated with a small reward over choices that require greater exertion of effort but are associated with greater rewards (Treadway et al., 2009)	<a href="#">The Effort Expenditure for Rewards Task</a> (EEfRT; Treadway et al., 2009)
	Risky decision-making	Propensity to choose large but unlikely rewards over small but certain outcomes and taps onto the ‘risk-based aspects of impulsive decision making’ (Dalley & Robbins, 2017)	<a href="#">The Iowa Gambling Task</a> (IGT, Bechara et al., 1994)
			<a href="#">The Cambridge Gamble Task</a> (Lawrence et al., 2008)
			<a href="#">The Balloon Analogue Risk Task (BART)</a> (Lejuez et al., 2003)
	Reflection impulsivity	‘tendency to make rapid decisions without adequate accumulation and consideration of the available evidence’ (Dalley & Robbins, 2017).	<a href="#">The Matching Familiar Figures Test</a> (MFFT; Kagan, 1966)
			<a href="#">The Information Sampling Task</a> (IST Clark et al., 2006).

**Table 1.2 Examples of tasks used to assess choice impulsivity in humans**

Impulsivity type	Impulsivity sub-type	Description	Examples of tasks
Motor impulsivity	Action cancellation (stopping)	Participants are required to respond as quickly as possible to a specific stimulus, but then halt that response -after it has been initiated- when another cue, the stop signal, is presented.	The Stop-Signal Task (SST, Logan and Cowan, 1984)
	Action restraint/withholding (no go)	Participants are required to respond to a predetermined stimulus (Go) on most trials, but then withhold that response when another stimulus (No Go) is presented.	The Go/No Go Task (Marczinski & Fillmore, 2003)
			The Continuous Performance Task (CPT, Kirmizi-Aslan et al., 2006)
	Action postponing (waiting)	Participants are required to refrain from responding until a defined amount of time has elapsed	The 4-Choice Serial Reaction Time Task (4-CSRTT, Worbe et al., 2014)
The Traffic Light Task (Adam et al., 2012)			

**Table 1.3 Examples of tasks used to assess motor impulsivity in humans**

To clarify whether the different behavioural tasks used to study impulsivity measure similar or overlapping psychological processes, Reynolds and colleagues (2006) applied a principal-components analysis on four behavioural tasks used to measure behavioural inhibition (Stop Task, Go/No-Go Task), reward delay (Delay-discounting Task) and risk taking (BART). The principal component analysis revealed two overarching factors: one relating to ‘impulsive disinhibition, with scores on the Stop Task and the Go/No-Go Task loading significantly on this factor; the second factor, relating to ‘impulsive decision-making’ with scores on the Delay-Discounting task and the BART loading significantly on this factor. This suggests that perhaps motor impulsivity and choice impulsivity do in fact represent two distinct psychological domains. This was confirmed in a more recent study (Broos et al., 2012) also looking at the relationship between impulsive action and impulsive choice through principal component analysis.

To assess psychological constructs, behavioural tasks have been criticised for suffering from the ‘impurity problem’ (Bari & Robbins, 2013; Burgess, 1997), meaning that successful performance on these tasks often requires the recruitment of executive functions other than the ones that the task is set out to test. In the case of behavioural measures assessing impulsivity Bari and Robbins (2013) point out that these, for example, also recruit elements of compulsivity and that the two constructs are sometimes difficult to dissociate (Fineberg et al., 2010; Robbins et al., 2012). Nonetheless performance-based tests have some advantages compared to self-report measures (as mentioned above), such as for example: they constitute more objective evaluations made by the experimenter based on objective data and they can be adapted to be tested in different species, thus allowing for translational research (Bari & Robbins, 2013).

There is evidence that performance-based measures of impulsivity, similarly to self-report measures, can be clinically relevant tools when studying the causes and consequences of psychopathology (Audrain-McGovern et al., 2009; Castellanos-Ryan et al., 2011; Morin et al., 2019; Vassileva & Conrod, 2019; Whelan et al., 2012). For example, one large longitudinal study (N= 3,659, Morin et al., 2019) looking at adolescents’ substance use and cognitive development over a period of 4 years, showed that deficits in inhibitory control (and working memory), as tested with the passive avoidance learning paradigm (Castellanos-Ryan et al., 2011)

which resembles the Go/No Go task, predicted cannabis and alcohol misuse. In addition, cannabis use during the duration of the study was associated with further impairments in inhibitory control. Thus, similarly to personality questionnaires, impulsivity as assessed through behavioural measures can be a useful tool in determining the likelihood of developing SUDs and in estimating the effects of substance misuse on cognition and behaviour.

The degree to which self-report and behavioural measures tap into similar underlying constructs has long been the subject of debate (Bari & Robbins, 2013; Cyders & Coskunpinar, 2011; Stricklan & Johnson, 2021). A meta-analysis of 27 published research studies exploring this debate (Cyders & Coskunpinar, 2011) showed that the relationship between these measures is significant but in practice very small ( $r=0.097$ ). As Stricklan and Johnson (2021) point out, a small relationship between these two different methods, despite both being useful at predicting clinically relevant behaviour, is not unique to the construct of impulsivity and indeed has also been observed in the context of, for example, intelligence (Joseph & Newman, 2010) and empathy (Murphy & Lilienfeld, 2019). Dang and colleagues (2020) suggested reasons as to why there might be such a dissociation, including: low test-retest reliability of performance-based measures (also mentioned by Bari and Robbins, 2013) and, simply, that personality questionnaires and behavioural tasks assess different aspects of impulsivity.

### **1.3.2 Experimental approaches in rodents**

The introduction of laboratory-based tasks to assess impulsivity paved the way for preclinical research in non-human species to investigate behavioural and neural mechanisms of impulsivity at a level that cannot be reached in humans. Thus, many of the tasks described above, used to measure both motor impulsivity and choice impulsivity in humans, have been successfully translated to rodents.

With regards to choice impulsivity, tasks measuring both temporal discounting (Cardinal, 2006; Reynolds & Berridge, 2002) and probabilistic discounting (Pais-Vieira et al., 2007; Rivalan et al., 2009; van den Bos et al., 2006; Winstanley, 2011; Zalocusky et al., 2016; Zeeb et al., 2009) have been developed from existing human tasks. With regards to the former, while there are

many variants, the general procedure is very similar to the task developed for humans and involves animals being presented with the option of having a small, immediate reward or a larger but delayed reward. Impulsive animals are those who exhibit steeper reward discounting, meaning that as the reward is delayed the value attributed to this diminishes more rapidly than for non-impulsive animals (Kirby et al., 1999). With regards to probabilistic discounting, numerous tasks have been developed resembling the IGT (Pais-Vieira et al., 2007; Rivalan et al., 2009; van den Bos et al., 2006; Winstanley, 2011; Zeeb et al., 2009) and for this reason have been referred to as rodent-IGT (r-IGT). In the paradigm designed by Zeeb and colleagues (2009), rats can nose poke in any of 4 different holes following an interval of 5 seconds. Each hole is associated with a specific reward magnitude and a probability of reward delivery that is inversely related to the reward magnitude, such that higher gains are associated with a higher probability of punishment/loss. Impulsive rats are those who choose ‘riskier’ options, thus aiming for a larger but less probable reward. A simpler version of probabilistic discounting, involving just two levers and small/certain reward versus large/uncertain reward, has also been implemented (Zalocusky et al., 2016). Finally, the Risky Decision Making Task (RDT) is similar to this simpler version, however the uncertainty associated with the large reward is that it is paired with the risk of a mild foot shock (Simon et al., 2009).

With regards to motor impulsivity, tasks have also been developed to assess action cancellation (stopping), action restraint/withholding (no go), and action postponing (waiting) in rodents. Stopping behaviour is widely assessed with the stop-signal reaction time task (SSRTT, Eagle and Robbins, 2003) which requires rodents to abort an action that has already been initiated, following the presentation of an auditory or visual ‘stop signal’ stimulus. Action restraint has been measured with a variety of operant paradigms, such as for example a Go/No-Go conditional visual discrimination task (Harrison et al., 1999). Here, animals are instructed to earn a reward by either poking into a central hole or withholding a response, depending on two different visual cues (or discriminanda) positioned to the sides of the central hole. A fast visual discriminanda indicates ‘go trials’ and is correctly performed by poking into the central hole, while a slow visual discriminanda indicates ‘no-go trials’ and is correctly performed by withholding a response. Impulsivity is measured as errors of commission, that is the number of times the animal is unable to withhold a response during no-go trials and pokes into the central hole.

Finally, action postponing or waiting impulsivity in rodents has been measured with a variety of tasks, including differential reinforcement of low-rate schedules (DRL, Richards et al., 1993) and the 5-choice serial reaction time task (5CSRTT, Robbins, 2002). In the latter, animals are trained to detect a visual cue (i.e., a brief appearance of light) in one of five different apertures inside the operant chamber. A correct, and thus rewarded, response is one where the animal accurately identifies the port where the visual cue was presented and ‘pokes’ into the port within the time constraints imposed by the task. A response occurring before the appearance of the visual cue, that is during the intertrial interval (ITI) between the start of the trial and the onset of the target stimulus, is considered ‘premature’ or impulsive and is punished by the absence of a reward.

There is some evidence of a correlation in performance between tasks assessing motor impulsivity and those assessing choice impulsivity (Barrus et al., 2015; Robinson et al., 2009). Robinson and colleagues (2009), for example, showed that rats that made many premature responses on the 5CSRTT were also more likely to choose the small, immediate reward in a delay discounting task. Performance on these tasks however was not related to impulsive responding on the SSRT, in line with previous findings (van den Bergh et al., 2006). A relationship between waiting impulsivity and delay-discounting, however, was not always detected (Broos et al., 2012; Winstanley et al., 2005 here they used a ‘one-hole’ version of the 5CSRTT), and indeed neural and pharmacological manipulations tested on both tasks have often yielded different findings (Dalley and Robbins, 2017 and see below). This is in line with research in humans (Reynolds et al., 2006) and has strengthened the notion that impulsivity is a multifaceted construct, with independent sub-components and, accordingly, distinct underlying neural processes (Dalley & Robbins, 2017). A recent meta-analysis of 211 manipulation-naive male animals, however, found a correlation between motor impulsivity as measured with the 5CSRTT and risky decision making as assessed with the r-IGT (Barrus et al., 2015).

Despite choice and motor impulsivity being largely independent, research in humans and animals has shown that these two sub-components of impulsivity are implicated both as causes (Belin et al., 2008; Dalley, et al., 2007a; Diergaarde et al., 2008; Marusich & Bardo, 2009; Oberlin & Grahame, 2009; Poulos et al., 1995; Radwanska & Kaczmarek, 2012; Sanchez-Roige et al., 2014) and consequences (Dalley, et al., 2007b; Kayir et al., 2014; Peña Oliver et al., 2009;

Walker et al., 2011) of substance abuse. Waiting impulsivity has been shown to predict cocaine self-administration (Belin et al., 2008; Dalley, et al., 2007a), nicotine self-administration (Diergaarde et al., 2008) and sucrose consumption (Diergaarde et al., 2009). In a similar fashion, steeper delay discounting (high choice impulsivity) was shown to confer vulnerability to stimulants (Anker et al., 2009; Marusich & Bardo, 2009; Perry et al., 2008), to predict consumption of nicotine despite increments in the effort required to obtain it (Diergaarde et al., 2012) and to seek nicotine consumption during abstinence (Diergaarde et al., 2008). Both waiting impulsivity (Dalley, et al., 2007b) and delay discounting (Kayir et al., 2014) were shown to worsen following exposure to drugs of abuse.

Evidence pointing to both motor and choice impulsivity predicting alcohol consumption is more mixed. While some found that both waiting (Loos et al., 2013; Radwanska & Kaczmarek, 2012; Sanchez-Roige et al., 2014) and delay-discounting (Oberlin & Grahame, 2009; Poulos et al., 1995) predicted alcohol intake, others did not observe these associations (Diergaarde et al., 2012; Pattij et al., 2020; Peña-Oliver et al., 2015; Stein et al., 2015). However, it was shown that impulsivity can worsen following alcohol consumption (Pattij et al., 2020; Peña Oliver et al., 2009; Walker et al., 2011). Finally, neither waiting impulsivity (McNamara et al., 2010) nor delay-discounting (Schippers et al., 2012) were found to confer vulnerability to heroin taking. The association between behavioural impulsivity and psychostimulant addiction and the lack of such association with opiate addiction (and to some extent with alcohol addiction) may be due to the fundamental differences underlying the neurobiology of these types of addiction (Badiani et al., 2011; Pattij et al., 2020).

Behavioural measures to assess impulsivity have been successfully translated into operant tasks suitable for preclinical research, allowing for cross-species investigation of the neuropsychological basis of impulsivity. Importantly, not only do animals manifest impulsive responding in such tasks (similarly to humans), but crucially this behaviour is implicated as a determinant and consequence of SUDs. This mirrors research in humans and confirms the importance of preclinical research in the quest to understand the psychological and neural mechanisms of psychopathology.

## **1.4 Psychological mechanisms of impulsivity: focus on response inhibition**

The act of responding impulsively involves a strong desire, urge or habit to make an action at a time that is not deemed ‘appropriate’, coupled with the inability to successfully inhibit this act (Bari & Robbins, 2013). This implies that there are several psychological processes that can play a role in premature responding including: urgency, sensitivity to reward and to negative outcomes, sustained attention and response inhibition (Voon, 2014).

### **1.4.1 Sustained attention**

The manner in which impulsivity has been investigated at the behavioural level is strongly connected to elements of sustained attention. This is because the behavioural paradigms that have been used to measure premature responses were originally developed to measure vigilance and sustained attention (Robbins, 2002). This is the case for CPT in humans (Rosvold et al., 1965), which was widely used to investigate attention and distractibility in children affected by ADHD (Huang-Pollock et al., 2012). This is also the case for the 5CSRTT in rodents (Robbins, 2002) and the 4-CSRTT in humans (Voon, 2014), where subjects must pay close attention to stimuli being presented unpredictably in any of the possible locations. Sustained attention is particularly recruited when the task involves elements of unpredictability (Robbins, 2002) and requires constant monitoring to either discriminate between noise and target stimuli (Huang-Pollock et al., 2012) or detect when (temporal unpredictability) and in which location (spatial unpredictability) a target stimulus will appear (Robbins, 2002). Specifically, in both the 5CSRTT and the 4-CSRTT the difficulty of the task can be varied in different ways including: i) by reducing the stimulus duration (SD) of the target (5CSRTT: Baunez and Robbins, 1997; Blondeau and Dellu-Hagedorn, 2007; Caballero-Puntiverio et al., 2017; Callahan et al., 2019; Grottick and Higgins, 2002; Milstein et al., 2007; Robinson, 2012; 4CSRTT: Voon et al., 2014), by reducing the brightness of the target stimulus (5CSRTT: Carli et al., 1983; Sirviö et al., 1993), or the frequency (5CSRTT: Blondeau and Dellu-Hagedorn, 2007; Callahan et al., 2019; Carli et al., 1983; Milstein et al., 2007; Navarra et al., 2008; Paterson et al., 2011; Sirviö et al., 1993;

4CSRTT: Voon et al., 2016); by introducing distractors (5CSRTT: Carli et al., 1983; Robinson, 2012; 4CSRTT: Voon et al., 2014); by lengthening the session (5CSRTT: Blondeau and Dellu-Hagedorn, 2007); and by manipulating the number of locations in which the target visual stimulus can be presented (5CSRTT: Dalley et al., 2002; Murphy et al., 2008).

These manipulations generally lead to decrements in sustained attention, but these are not always coupled with changes in the propensity to make premature responses. Decrements in attention are indexed by percentage of omissions and accuracy, with the latter being sometimes operationalised as percentage of correct responses, (that is, presumably, number of correct responses over total number of trials multiplied by 100, Navarra et al., 2008, on long ITI) and other times as  $\text{correct}/(\text{correct}+\text{incorrect})$  (Dalley, et al., 2007a; Voon et al., 2014; Worbe et al., 2014). Thus, in healthy subjects, measures of accuracy were not related to the number of premature responses in the 4-CSRTT (Voon et al., 2014), however in healthy participants with reduced serotonin (5-hydroxytryptamine, 5-HT) induced by the dietary tryptophan depletion procedure (TD), premature responses correlated positively with accuracy measures and with response to reward feedback (Worbe et al., 2014). In addition to this, premature responding correlated negatively with age, in line with previous findings showing that impulsivity declines with increasing age (L. Steinberg et al., 2008).

In animals, there is a large body of evidence exploring the relationship between premature responses and measures of sustained attention. Thus, a strong noise distractor (85 dB) was found to increase the propensity to make a premature response, without affecting accuracy, if presented shortly before the presentation of the target stimulus (Carli et al., 1983), however when this was presented randomly during the ITI and was of a lesser intensity, it was not found to alter premature responses (Robinson, 2012). On the contrary, when the ITI was lengthened it was shown to significantly increase the number of premature responses (Blondeau & Dellu-Hagedorn, 2007; Caprioli et al., 2013; Dalley, et al., 2007a; Navarra et al., 2008; Paterson et al., 2011; Robinson, 2012). In fact, lengthening the ITI is a known strategy to increase the occurrence of premature responses (Bari et al., 2008a). This manipulation was not reported to affect omission responses (Dalley, et al., 2007a; Paterson et al., 2011; Robinson, 2012), and did not lead to decrements in accuracy (Blondeau & Dellu-Hagedorn, 2007; Dalley, et al., 2007a;

Paterson et al., 2011), except in some cases (Caprioli et al., 2013; Navarra et al., 2008 on long ITI). Reduction in brightness had no effect on premature responses, however it did reduce accuracy and increase omissions (Carli et al., 1983). Evidence for an effect of reduction of SD on premature responses is less clear. Some studies reported a slight increase in premature responses with shorter SDs, however it was not clear whether this difference was significant (Grottick & Higgins, 2002; Robinson, 2012). Blondeau and Dellu-Hagedorn (2007) reported a negative correlation between premature responses and omissions, during standard training conditions. When testing a manipulation of reduced SD, the authors reported a significant increase in premature responses with decreasing SD and, mirroring this, a decrease in premature responses with increasing SD. This manipulation did not affect omissions but it did decrease the percentage of correct responses. In line with this, Baunez and Robbins (1997) also reported a decrease in premature responses with increasing stimulus duration, with no change in accuracy but with a concomitant decrease in omission responses. Thus, evidence suggests that a longer (thus easier to discriminate) SD is associated with a reduction in premature responses, and *vice versa*. However, these SD-dependent changes in the propensity to make a premature response are not always coupled with changes in selective attention. Finally, when tested over long sessions of 250 trials, towards the end of the session, animals were slower at making a correct response, made fewer correct response, made more omissions but also fewer premature responses (Grottick & Higgins, 2002). Thus, in this case, decrements in accuracy were due to fatigue and this, instead of increasing premature responses, had the opposite effect.

Thus, the relationship between decrements in attention and premature responses is not straightforward, and these two measures are distinct and vary independently from each other (Christakou et al., 2004). This conclusion is corroborated by neural and pharmacological evidence showing effects on premature responses that are independent of changes in selective attention (Cole & Robbins, 1989; Harrison et al., 1997; Muir et al., 1996), and *vice versa* (Chudasama et al., 2003; Granon et al., 2000). This suggests that the propensity to make a premature response, in tasks of waiting impulsivity, cannot be accounted for solely by impairments in attention and distractibility. What appears to be a robust finding, instead, is that increasing the waiting time (the ITI), dramatically increases the number of premature responses. Finally, evidence that fatigue decreases the propensity to make premature responses and that

these in some cases correlate negatively with omission responses, suggest that motivation and sensitivity to reward might also play a role in waiting impulsivity (Voon et al., 2014).

## 1.4.2 Incentive motivation

An impulsive act is said to occur when dysfunctional inhibitory processes are unable to control ‘a strong desire, urge or habit’ (Robbins, 2002). Within that definition, there is the idea that impulsivity could be influenced by the craving for something rewarding. Within this ‘hedonic’ framework, Hofmann and colleagues (2009) defined an impulse as a “primitive hedonic reaction to a tempting stimulus that is immediate in a temporal and a spatial sense”. Hofmann and co-authors further suggested that specific experiences that are associated with rewarding outcomes get reactivated, in specific contexts, by perceptual input and internal states (e.g., hunger or thirst) to confer a sense of ‘preparedness’, or motor readiness, that allows the individual to act swiftly in response to one’s needs and past learning experiences. Premature responses seem to fit, at least in part, this description in that they are rash responses and usually occur in animals that are food restricted and are trained to work for sugar in an operant task. When animals are allowed to eat *ad libitum* sometime before being tested on the 5CSRTT, fewer premature responses are elicited (Bizarro & Stolerman, 2003; Carli & Samanin, 1992; Grottick & Higgins, 2000; Nemeth et al., 2010; Semenova & Markou, 2007, but see also: Baunez and Robbins, 1997; Grottick and Higgins, 2002). In humans, premature responses have not been found to correlate with the amount of money won, or motivation for monetary feedback (Voon, 2014). However, both in humans and in animals, impulsive action has been associated with risky decision-making (Barrus et al., 2015; Gabriel et al., 2019; Ioannidis et al., 2019) and substance-abuse (Belin et al., 2008; Dalley, et al., 2007a; Diergaarde et al., 2008, 2009; Voon, 2014). These are both regarded as indices of greater sensitivity to reward-predicting cues (Diergaarde et al., 2009; Gabriel et al., 2019). Further, premature responses have been associated with increased responsivity for high-incentive foods such as sucrose (Diergaarde et al., 2009), increased magnitude of a food reward (King et al., 2016) and sign-tracking (ST) behaviour (King et al., 2016; Lovic et al., 2011). This is the propensity to approach a food-associated cue in a Pavlovian conditioned approach task (PavCA) and is seen as indicative of greater sensitivity to the incentive motivational properties of reward-related cues (Robinson & Flagel, 2009). Thus, given this evidence it is possible that

motor impulsivity is associated with enhanced value attribution to reinforcers and is influenced by changes in motivation. Recent neuro-imaging data in humans performing the 4-CSRTT supports this hypothesis (Mechelmans et al., 2017).

### 1.4.3 Urgency

Personality theories of impulsivity postulated ‘urgency’ to be one of the factors driving impulsive behaviour (Cyders et al., 2007; Whiteside & Lynam, 2001). Specifically, negative urgency was first conceptualised by Whiteside and Lynam (2001) who defined it as the ‘tendency to commit rash or regrettable actions as a result of intense negative affect’ (Whiteside & Lynam, 2001). The authors developed the UPPS scale to measure whether this emotional state, among other factors, could explain impulsive behaviour. Building on this, (Cyders & Smith, 2007, 2008) later redefined the construct and more clearly differentiated between the two sub-components of *positive* and *negative* urgency (Eben et al., 2020), with the former referring to the “tendency to engage in rash action in response to extreme positive affect”. In humans, negative urgency has been linked to unfavourable behavioural dispositions (for a review see: Berg et al., 2015; Smith and Cyders, 2016) including aggression (Carlson et al., 2013); problematic alcohol and other substance abuse use (Latzman et al., 2013; Magid & Colder, 2007); suicidality (Nock & Prinstein, 2004; Yen et al., 2009), and disordered eating (Rosval et al., 2006; Stojek et al., 2014).

From behavioural and cognitive perspectives, Frijda (2010) conceptualised negative urgency as ‘appraisal’ (Frijda, 2010) elicited by negative emotions that readily translate into action to modify the relationship between the agent and the object in the environment that generated that negative emotion. Along the same lines, Carver (2006) suggested that negative emotions such as frustration and anger provide rapid feedback on how distant an agent is from their goal and serve a motivational role to adjust the rate of progress to achieving this. Thus, negative urgency has been framed by these accounts as a negatively valenced sense of arousal that invigorates behaviour and keeps an individual engaged with a specific task (Eben et al., 2020). This framework has received support from empirical evidence. For example, it has been shown that gamblers are faster at initiating the next gamble after a loss than after a win (Dixon et al., 2013;

Forder & Dyson, 2016; Shao et al., 2013; Verbruggen et al., 2017). Along the same lines, a study in healthy controls showed that when participants were prevented from obtaining a reward, they reported greater levels of frustration and they exhibited greater response vigour when key-pressing a button box to perform the task (Yu et al., 2014). In at least two studies (Verbruggen et al., 2017; Yu et al., 2014), frustration-dependent invigoration of behaviour scaled positively with the size of the omitted reward. Finally, in an attempt to reconcile work on self-report questionnaires with behavioural measures of negative urgency, Gipson and colleagues (2012) tested whether scoring high on the negative urgency scale of the UPPS would predict performance in a task where rewards were occasionally omitted. The authors found that scoring highly on negative urgency was linked to more operant responses in trials where the reward was omitted unexpectedly. Thus, there is evidence that negative outcomes in the context of potential rewards can invigorate behaviour and lead to impulsive actions (Eben et al., 2020).

In animals, invigoration of behaviour in a partial reinforcement schedule was first studied by Amsel and Roussel (1952; for an extensive review of this topic, see Papini & Dudley, 1997). The authors showed that, in an apparatus where two separate goal boxes are connected by two runways, rats tended to run faster to collect reinforcement in the second goal box if reinforcement in the first goal box was omitted unpredictably. This led to the hypothesis that negative affect or frustration, derived from an omitted reinforcement, could invigorate behaviour on a second instrumental response. This first set of results generated much interest and led to the conceptualisation of what is now known as the reinforcement-omission effect (ROE, Kello, 1972; Stout et al., 2003). Similarly, to its early formulation by Amsel and Roussel (1952), ROE refers to “a learning phenomenon defined as greater response strength (e.g., higher rate, shorter latency, etc.) immediately following non-rewarded trials than immediately following rewarded trials in a partial reinforcement situation” (p.438, Stout et al., 2003). ROEs have been observed in different experimental settings, for example as greater response rates on a lever both in Pavlovian (Dudley and Papini, 1995) and instrumental (Gipson et al., 2012; Judice-Daher et al., 2011) training schedules. Frustration theory makes a series of predictions as to what modulates this negatively valenced frustration that results from a violation of reward expectation (Amsel, 1958; Amsel, 1962; Anselme & Robinson, 2019). For example, the intensity of frustration, and thus its capability to energise behaviour, is directly related to the magnitude of the expected, but

omitted, reward (Peters & McHose, 1974). There is some evidence that this is the case (Flaherty, 1999; Hughes et al., 1974; Judice-Daher et al., 2011; Peckham & Amsel, 1967; Wilton et al., 1969) however not all studies have been able to replicate this phenomenon (Jensen & Fallon, 1973; McHose & Gavelek, 1969; Shettleworth & Nevin, 1965) and among those that did not, Jensen and Fallon (1973) suggested that whenever a reward magnitude-dependent ROE was observed, it was due to substantial discrepancies between the small and large rewards utilised.

Alternative theories have been put forward to explain the apparent facilitatory effect that omitted reinforcement has on behaviour. Seward and colleagues (1957), for example, suggested that ROE may be in part driven by suppression of behaviour after reinforcement, rather than solely by response invigoration after lack of reinforcement. This was supported by evidence (Jensen and Fallon, 1973; McHose and Gavelek, 1969) showing that invigoration of behaviour following reward omission did not scale with reward magnitude, while post-consummatory inhibition of behaviour following reward collection did scale positively with reward magnitude. Post-reinforcement pausing has been observed in a variety of contexts, as reduced lever presses but also as slowing of reaction times following receipt of reward, both in animals (Peters et al., 2010) and in humans (Dixon et al., 2013; Raio et al., 2020). To reconcile these two interpretations, a series of experiments by Stout and colleagues (2003) showed that both phenomena, that is response suppression after a reward and response facilitation after a non-reward, play a role during schedules of partial reinforcement and the extent to which one component prevails over the other depends on the choice of training parameters, such as the length of the inter-trial interval.

#### **1.4.4. Sensitivity to negative outcomes**

Learning from punishment or the negative consequences of one's action is critical for updating one's response strategy to events in the environment and achieve effective inhibitory control (Roos et al., 2015). Failure to learn from negative outcomes can result in the perseveration of maladaptive behaviour (Nilsson et al., 2015). For this reason, diminished sensitivity to negative outcomes could be linked to impulsive responding and to the propensity to develop SUDs

(Izquierdo & Jentsch, 2012; Voon, 2014). However, this learning mechanism has not been extensively explored in the context of waiting impulsivity.

In humans, there is some evidence that increasing the punishment for premature responses leads to a reduction of these responses (Bizarro et al., 2004; Voon, 2014). For example, as it currently stands, the 4-CSRTT has a time-out penalty for premature responses. However, when an explicit monetary loss (instead of a time-out) was piloted during task development, this resulted in a dramatic reduction in premature responses (Voon, 2014). Thus, healthy volunteers performing the 4-CSRTT were able to regulate their impulsive responses if these were paired with substantial negative consequences. In the 5-CSRTT, Stolerman and colleagues (Bizarro et al., 2004; Hahn et al., 2002; Mirza & Stolerman, 1998) removed the time-out punishment after premature responses. They did not compare this manipulation with a paradigm that included the time-out punishment, thus it is difficult to draw definitive conclusions on the effects of time-out on premature responses. Nonetheless, when focusing on data from, for example, Mirza and Stolerman (1998) who tested rats on a 5 s ITI without a time-out punishment, the premature responses reported are well within the range (if not less) of premature responses that are usually observed during standard training and testing procedures of the 5-CSRTT (Fernando et al., 2012). This would suggest that premature responses, in the context of the 5-CSRTT, are not dramatically affected by the presence of the time-out punishment. Another open question is whether high impulsive (HI) rats on the 5-CSRTT exhibit such elevated impulsive responding due to a failure to learn from the time-out punishment that occurs when they incur in a premature response. Another task measuring waiting impulsivity in animals is the DRL (Richards et al., 1993). This task requires animals to withhold a lever press for a predetermined amount of time and then respond to obtain a reward. Lever presses occurring before the predetermined amount of time has elapsed leads to the omission of reinforcement and resets the trial. A task equivalent to this that does not punish premature responses is the Peak-Interval procedure (PI, Buhusi and Meck, 2002). This consists of the presentation of trials, under a fixed-interval schedule of reinforcement, which are sometimes paired with a reward and sometimes are not. A fixed-interval schedule of reinforcement refers to a reinforcement schedule where a lever press is rewarded only if this occurs after a specified amount of time has elapsed. Thus, the PI task has been used extensively to study how rats process temporal information, because during trials that

are not reinforced rats will display a ‘peak’ in lever pressing around the time that they expect a reward to be delivered. What is interesting is that rats start to lever press as early as 30s before the delivery of the reinforcement in anticipation of that event. This shows that when premature responses are not punished, rats may engage in such responses as a consequence of the motivating and energising effect that anticipation of reward has on behaviour (Robinson et al., 2014). Thus, it could be expected that removal of the time-out punishment leads to ‘uncontrolled’ anticipatory responses in the form of premature responses, similarly to what is observed during the PI task. However, it has been shown that the form of a conditioned stimulus can influence the degree to which it acquires incentive motivational properties (Meyer et al., 2014). Thus, it is possible that anticipatory responding manifests differently depending on whether a lever or nosepoke response is required.

Aside from waiting impulsivity, an association between impulsivity and the ability to learn from negative and positive feedback has been explored in the healthy population (Cáceres & Martín, 2017; Franken et al., 2008; Romer et al., 2009) and in ADHD subjects (Frank et al., 2007). In both cases, elevated impulsivity was associated with overall impairments in learning about reward contingencies and in exploiting these to maximise performance. In some cases, healthy participants with elevated (reflection) impulsivity (Cáceres & Martín, 2017) were more impaired at learning from negative feedback than from positive feedback, however this was not always the case (Franken et al., 2008). Franken and colleagues (2008) for example studied whether high impulsive subjects on the IQP (Eysenck et al., 1985), exhibited impairments when tested on a probabilistic reversal-learning task. Reversal learning tasks require participants to flexibly adapt to the changing reinforcement contingencies of different stimuli, for example two decks of cards. Trial-by-trial responses to wins and losses are sometimes measured by computing win-stay and lose-shift probabilities (Alsiö et al., 2019). Franken and colleagues (2008) found that high-impulsive subjects had a higher proportion of stay responses after large loss choices, and a lower proportion of stay responses after large gain choices. This suggests that the impairments in reward processing observed in impulsive individuals are not necessarily due to problems with learning from negative feedback, but perhaps more with the ability to adequately respond to reinforcement contingencies and adjust such responding through executive control (Romer et al., 2009). Romer and colleagues (2009), for example, found in a sample of 387 adolescents that

those who scored highly on the Impulsiveness Scale (Eysenck et al., 1985) not only showed impairments in reversal learning as observed before (Franken et al., 2008) but also in working memory capacity. This refers to the ability to store information in mind temporarily to guide decision-making and behaviour (Kimberg & Farah, 1993). The association between working memory capacity and impulsive behaviour has been observed before, both in relation to risky decision making, such as the IGT (Bechara et al., 1998, 2001; Fellows & Farah, 2005) and in the clinical setting (Klingberg et al., 2005). For example, Klingberg and colleagues (2005) found that improvements in working memory capacity in ADHD children, through training, resulted in a reduction in symptoms of inattention and hyperactivity/impulsivity.

## **1.5 Neural substrates of impulsivity: waiting impulsivity**

Key to the understanding of the various components of impulsivity and to design therapies that can treat the maladaptive aspects of this, is the characterisation of the neural processes underlying this construct. Research in humans and in animals have combined methods that assess impulsivity at the behavioural level (self-report and especially performance-based) with tools in neuroscience to characterise the neural substrates of the different sub-components of impulsivity. The following paragraphs discuss the neural circuits, neurochemistry and neuropharmacology of waiting impulsivity and where relevant how these overlap with those underlying other forms of impulsivity. Generally speaking, waiting impulsivity features an impulsive act, be this a strong desire, urge or habit, that is not being appropriately regulated by an inhibitory process (Bari and Robbins, 2013). Research on the neural substrates of waiting impulsivity has thus attempted to explore the neural mechanisms regulating both the urgency to act and inhibitory control. Across species, the most consistent finding has been that of abnormal processes in networks involving the prefrontal cortex (PFC) and the basal ganglia (BG), with alterations in the signalling of three important neurotransmitter systems such as DA, noradrenaline (NA) and 5-HT (for a review on this see Barrus and Winstanley, 2017; Basar et al., 2010; Dalley et al., 2011; Dalley and Ersche, 2019; Dalley and Robbins, 2017).

## 1.5.1. Basal Ganglia

The BG is a major neural system involved in action initiation, both self-paced and reward-driven (Klaus et al., 2019). Waiting impulsivity is operationalised by premature responses, which are defined as uncued, quick actions occurring earlier than optimal, usually in instrumental paradigms (Dalley & Robbins, 2017). For this reason, it is important to describe the anatomical and cytoarchitectural organization of the BG and describe how this neural structure has been characterised functionally.

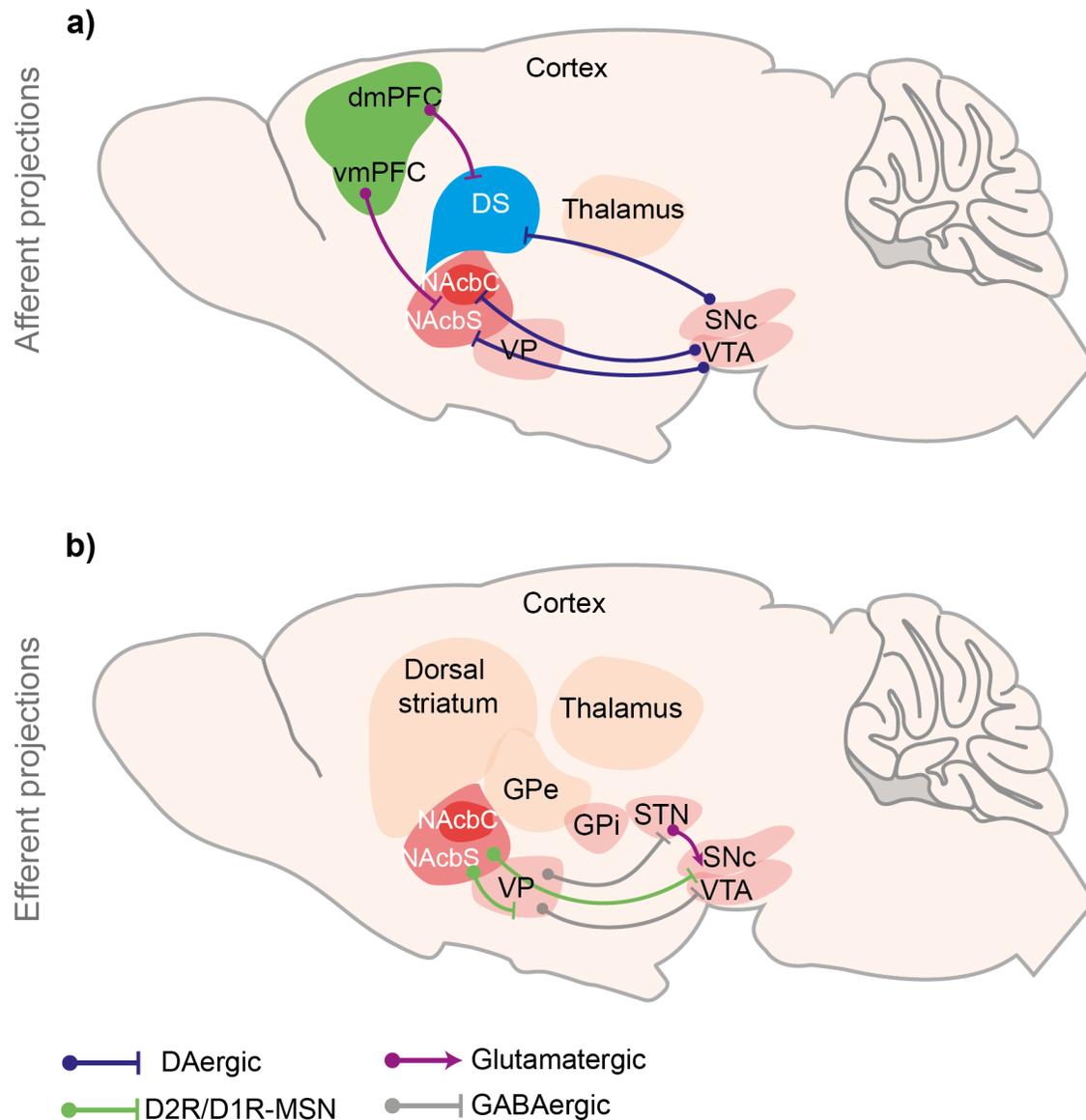
### 1.5.1.1. Anatomical organization

The BG is a complex network of dynamically interacting brain areas and the function of this has been heavily associated with the execution and suppression of motor commands (Frank, 2006; Klaus et al., 2019). The striatum is the largest basal ganglia input nucleus, it is primarily composed of striatal medium-sized spiny projection neurons (MSNs). These release the neurotransmitter  $\gamma$ -amino-butyric acid (GABA), and receive glutamatergic projections from the cerebral cortex, thalamus, and limbic regions, including the hippocampus and amygdala (Valjent & Gangarossa, 2020). The pattern of cortical inputs into the striatum helps establish the correspondence between striatal regions in humans and rodents (Chuhma et al., 2017). Thus, the primary motor and premotor cortices project onto the sensorimotor striatum, which in rodents corresponds to the lateral portion of the dorsal striatum (Smith et al., 2004). Association areas of the cortex such as the dorsolateral prefrontal cortex project onto the associative striatum, which in rodents corresponds to the medial portion of the dorsal striatum (DMS, Berendse et al., 1992; Parent and Hazrati, 1995). Finally, the anterior cingulate cortex (ACC) and the orbitofrontal cortex (OFC) project onto the ventral striatum (VS), which in addition to these also receives inputs from limbic structures such as the amygdala and hippocampus (Smith et al., 2004).

In rodents the VS comprises primarily the nucleus accumbens (NAcb). The NAcb is itself a heterogeneous structure composed of shell and core sub-regions (Everitt et al., 1999; Floresco, 2015; Zahm, 1999). These areas differ both with regards to their efferent and afferent projections and together with their intrinsic dopaminergic innervation form distinct midbrain-striatal loops

(Corbit et al., 2001; Dalley & Robbins, 2017; Deutch & Cameron, 1992; Haber et al., 2000). More precisely, DA neurons from the ventral midbrain map onto the striatum topographically: the ventral tegmental area (VTA) sends DA projections primarily to the NAcB, while more lateral DA neurons in the SNc project mainly to the dorsal striatum (Chuhma et al., 2017). The density of DA input into the VS also follows this pattern, with the dorsal striatum and the lateral shell receiving a higher concentrations of DA inputs than the core and the medial shell (as identified by mRNA expression of dopaminergic marker genes tyrosine hydroxylase (TH), vesicular monoamine transporter 2 (VMAT2) and dopamine transporter (DAT), Lammel et al., 2008). For a graphical representation of the afferent and efferent projections to the VS see Figure 1.1.

With regards to cortical inputs, the NAcB core receives afferents primarily from dorsal regions of the medial prefrontal cortex (mPFC), including the dorsal prelimbic and anterior cingulate areas, as well as from the parahippocampal cortex (Basar et al., 2010; Berendse et al., 1992). The shell, on the contrary, receives cortical inputs from the more ventral regions of the PFC, including the infralimbic and ventral prelimbic areas (Berendse et al., 1992b; Brog et al., 1993; Heidbreder & Groenewegen, 2003). Efferent projections from the NAcB also differ between the shell and core regions. Thus, the former target primarily the medial ventral pallidum, VTA, and hypothalamus; neurons originating in the NAcB core, instead, more densely innervate the lateral ventral pallidum, the subthalamic nucleus (STN), and the medial substantia nigra pars reticulata (Groenewegen et al., 1999; Heimer et al., 1991; Tripathi et al., 2010). Finally, the NAcB shell and core regions differ substantially with regards to their neurotransmitters and receptors distribution patterns. For example, the core shows strong immuno-reactivity for the calcium binding protein calbindin-D28K (Basar et al., 2010a; Jongen-Rêlo et al., 1994; Zahm & Brog, 1992). The NAcB shell, instead, shares a variety of histochemical features and connections with the bed nucleus of the stria terminalis and for this reason it was suggested to be a rostral extension of the extended amygdala (Alheid & Heimer, 1988; Everitt et al., 1999; Lennart Heimer & van Hoesen, 2006).



**Figure 1.1 Afferent and efferent projections to the ventral striatum (VS).** (a) The VS receives projections primarily from the ventral portion of the medial prefrontal cortex (vmPFC), while the dorsal striatum receives projections from the dorsal portion of the medial prefrontal cortex (dmPFC). Dopaminergic projections from the substantia nigra (SNc) target primarily the dorsal striatum (DS), while dopaminergic projections from the ventral tegmental area (VTA) target the nucleus accumbens (NAcb). Projections from the VTA follow a medio-lateral gradient, with medial VTA projecting to the NAcb medial shell (NAcbS) and the more lateral portion of the VTA projecting to the NAcb core sub-region (NAcbC). (b) As shown by recent evidence (Kupchik and Kalivas, 2016) the distinction between direct- and indirect pathway in the VS depends more on the efferent target of the neuron, rather than on its molecular make-up. Thus, both D1R and D2R neurons project to the ventral mesencephalon (VTA and SNc, canonically the direct pathway) *and* to the ventral pallidum (VP, canonically the indirect pathway). Adapted from Soares-Cunha et al., 2016.

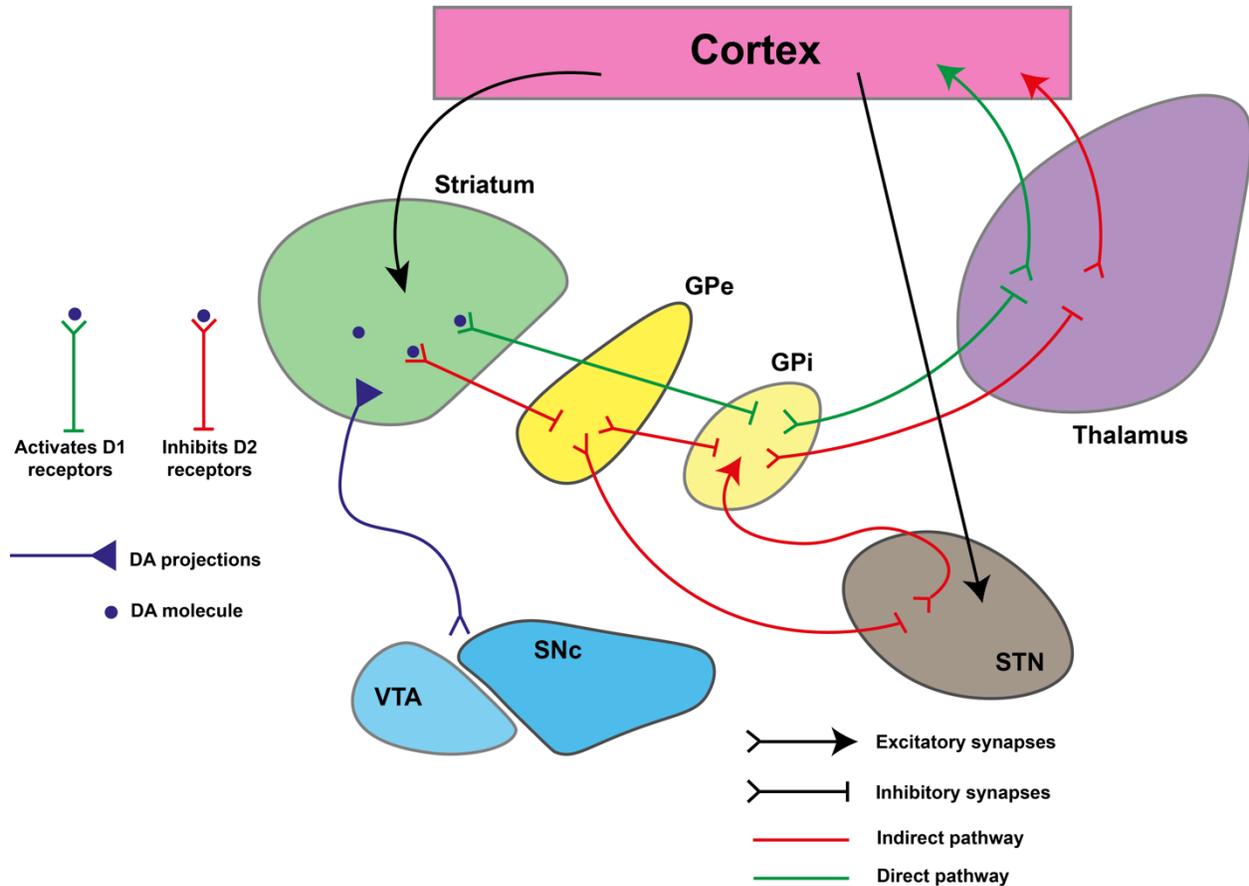
### 1.5.1.2 Cytoarchitectural organization

The principal neurons of the striatum are MSNs, which comprise more than 95% of the total neuronal population. These contain the neurotransmitter GABA and are further divided into two subgroups, those containing the neuropeptides substance P and dynorphin, and those containing enkephalin (Baser et al., 2010). Other cells in the striatum comprise cholinergic and GABAergic interneurons (Meredith, 1999).

MSNs constitute the two major projection pathways that are thought to depart from the striatum: the direct pathway (“Go”) or striatonigral pathway directly innervates and inhibits the internal segment of the globus pallidus (GPi), which in turn disinhibits the thalamus and facilitates the execution of a motor response considered in cortex (Frank, 2006; Gerfen & Wilson, 1996). The indirect pathway (“No Go”) or striatopallidal pathway directly projects to and inhibits the external segment of the globus pallidus (GPe), releasing the tonic inhibition of GPe onto GPi, thus suppressing motor activity (Aubert et al., 2000; Frank, 2006). GPe neurons also project to the STN, which in turn innervates the GPe and both the GPi and the substantia nigra (SNc). The STN receives direct excitatory projections from the cortex, and for this reason it is said to be part of the “hyperdirect” pathway, through which the cortex communicates the BG outputs, bypassing the striatum (Gerfen & Surmeier, 2011; Nambu et al., 2000). For a graphical representation of the direct and indirect pathways see Figure 1.2.

In the classical model, the direct pathway and indirect pathway are thought to differ with regards to DA receptor segregation. Thus, MSNs comprising the direct pathway are thought to express D1-type dopamine receptors (D1-MSNs), while MSNs comprising the indirect pathway are thought to co-express D2-like DA and adenosine A2a receptors (D2-MSNs, Gerfen et al., 1990). D1 receptors are Gs-coupled receptors, their activation stimulates cyclic AMP production, which in turn activates protein kinase A (PKA) and results in an increase in neuronal firing. D2 receptors, instead, are Gi-coupled receptors and their activation inhibits adenylyl cyclase (AC) activity and leads to a reduction in neuronal firing (Hasbi et al., 2011; Vallone et al., 2000). Recent studies have questioned the complete segregation of the direct/indirect pathways to D1- and D2-MSNs (Kupchik et al., 2015). Thus, while the correspondence between DA receptor

distribution and afferent inputs seems to hold for the most part in the dorsal striatum (Gerfer & Surmier, 2011), in the VS D1- and D2-MSNs (dis)inhibit thalamic activity more depending on their projection patterns than on their genetic characteristics (Kupchik et al., 2015).



**Figure 1.2 Graphical representation of the direct and indirect pathways of the Basal Ganglia.** The direct pathway (“Go”) or striatonigral pathway directly innervates and inhibits the internal segment of the globus pallidus (GPi), which disinhibits the thalamus and facilitates the execution of a motor response considered in cortex. The indirect pathway (“No Go”) or striatopallidal pathway directly projects to and inhibits the external segment of the globus pallidus (GPe), releasing the tonic inhibition of GPe onto GPi, thus suppressing motor activity. GPe neurons also project to the sub-thalamic nucleus (STN), which in turn innervates the GPi. The STN receives direct excitatory projections from the cortex, and for this reason it is said to be part of the “hyperdirect” pathway, through which the cortex modulates the output of the basal ganglia, bypassing the striatum. SNc = substantia nigra; VTA = ventral tegmental area; DA = dopamine. BioRender (2022).

## 1.5.2 The role of the BG in movement initiation

The function of the BG has been researched extensively over the past years (for a review see Klaus, et al., 2019). This complex neural structure, *via* inhibition of thalamocortical and cortical centres, has been shown to play a key role in regulating the initiation and termination of specific

movements (Hikosaka et al., 2000). Dopaminergic inputs in this region are essential to the process of action initiation (da Silva et al., 2018; Hamid et al., 2016). Early studies on patients with Parkinson's disease, which is characterised by a degeneration of DA neurons in the SNc (Carlsson et al., 1958), had provided an intuition for this as these patients had difficulties self-initiating movements and performing vigorous actions. Since then, DA projections onto the BG have been studied both in the context of self-paced spontaneous movements and in the context of reward-based tasks, where actions are generated (either cued or uncued) with the purpose to secure a reward (Klaus et al., 2019). The BG pathways involved in these two different types of behaviours have been suggested, broadly, to differ (Klaus et al., 2019). The circuit involved, predominantly, in self-paced movement has been suggested to be the nigro-striatal pathway, concerning DA input originating in the SNc and projecting onto the dorsal striatum. Instead, the circuit involved, predominantly, in reward-driven or cued movement is the midbrain-accumbens pathway, implicating DA fibres from the VTA to the NAc.

### **1.5.2.1 Self-paced movement: the nigro-striatal pathway**

MSNs in the dorsal striatum serve a particularly important role in movement execution as they integrate information from the cortex (Shima & Tanji, 2006) and from other afferent inputs carrying sensory and contextual information (Klaus, et al., 2019). In line with this, activity in the MSNs of the dorsal striatum has been observed to occur both during self-paced action initiation and termination (Cui et al., 2013; Jin & Costa, 2010). It has also been shown to be independent of the reinforcement associated with the action and to be movement specific (e.g., being different for left and right lever presses, Cui et al., 2013; Jin et al., 2014; Jin and Costa, 2010).

Classical models of the function of the BG circuit pose that, as a result of their different receptor and connectivity profiles, the direct and indirect pathway behave in an antagonistic manner to allow action initiation (DeLong, 1990; Graybiel, 2000). Specifically, the direct pathway was thought to be active during movement execution, upon DA release, serving its pro-kinetic function through the recruitment of D1-MSNs. The indirect pathway, instead, was thought to exhibit less firing activity during movement, due to inhibition of D2-MSNs, and to be active during immobility (Alexander & Crutcher, 1990). Recently this view has been challenged and D1-MSNs and D2-MSNs have both been observed to be recruited during movement initiation

(Cui et al. 2013). Differences between these two populations of neurons, instead, seem to emerge during the execution of a motor sequence, rather than at the start of action sequence (Barbera et al., 2016; Jin et al., 2014). In light of this recent evidence, an alternative framework to the classical one has been suggested. This postulates that coactivation of both pathways is necessary for successful action execution, with the direct pathway facilitating the release of the desired movement, and the indirect pathway suppressing competing actions (Klaus et al., 2019; Yttri & Dudman, 2016).

Within this framework, DA release from the SNc onto MSNs in the dorsal striatum is thought to play a key role in modulating the probability of action initiation and action vigour (da Silva et al., 2018; Dodson et al., 2016; Howe & Dombeck, 2016). For example, da Silva and colleagues (2018) showed, in mice, that a large portion of DA neurons in the SNc become active prior to self-paced movement initiation (~300 ms before movement). They also showed that activity in these neurons represents information about action vigour, with cells firing more strongly before higher vigour movements. Finally, the authors also showed that silencing these cells using optogenetics prior to action initiation, but not during ongoing movement, significantly impaired movement initiation in mice. This shows that dopaminergic projections, from the SNc to the dorsal striatum, play a fundamental role in modulating the probability and latency of sequence initiation. A working model of the neural bases of action initiation proposes that cortical projections onto the striatum determine action specificity, by representing different motor plans, while DA inputs from the SNc regulate the likelihood that (and the vigour with which) such motor plans are executed (Klaus et al., 2019).

### **1.5.2.2 Reward-driven movement: the midbrain-accumbens pathway**

The NAcb has long been thought to be one of the key structures within the brain's reward circuitry, with a plethora of studies showing its role in directing attention and guiding behaviour toward appetitive stimuli (for a review see Floresco, 2015; Mogenson et al., 1980). Being located at the interface between cortico-limbic projections and motor efferents (Corbit et al., 2001), it serves the function of converting motivationally-significant information into behavioural output (Ito & Hayden, 2011).

One of the key modulators of the NAc, is mesolimbic DA originating from cell bodies of the VTA (Ikemoto, 2007). DA neurons in this region have long been studied in the context of reward processing and associative learning. Specifically, mesoaccumbens DA is characterised by two types of cell firing: *phasic* firing which consists of brief (<1s) bursts of activity by DA cells (Schultz et al., 1997) and *tonic* firing which changes on slower timescales (minutes) (Floresco, 2015). Seminal work by Schultz and colleagues (1993) in the early '90s showed that DA cells would fire phasically upon the presentation of unexpected stimuli associated with future reward, however, would cease to be active once these stimuli had become expected. This property of DA phasic activity was later defined as the encoding of reward prediction errors (RPEs, Montague, 1997). Thus, RPEs encode the discrepancy between expected and actual rewards and generate estimates of future rewards (or values) to guide decision-making and maximise reward (Berke, 2018). This reward-dependent learning was captured already, at a theoretical level, by the model of Rescorla and Wagner (1972) however the discovery of RPEs paved the way for a multitude of studies on the role of DA signalling in associative learning. For example, it was shown that the magnitude of these DA phasic signals varies with the size and probability of the expected reward (Day, 2011; Fiorillo et al., 2003; Sugam et al., 2012). Recent advances in optogenetics confirmed the dopaminergic nature of RPEs (Cohen et al., 2012; Eshel et al., 2016) and established a causal role for these signals in cue-reward learning (Steinberg et al., 2013).

On slower timescales, mesolimbic DA signalling is also implicated in approach behaviour towards reward-related stimuli (Botvinick & Braver, 2015; Wyvell & Berridge, 2001). For example, DA transmission in the NAc was shown to be essential during tasks of effort/cost decision-making, where the animal needed to overcome an obstacle (e.g., a barrier in a maze) to obtain a reward (Salamone et al., 1994; Yohn et al., 2016). The idea that tonic DA activity is involved in motivated behaviour was developed computationally by Niv and colleagues (2005, 2007). Specifically, the authors suggested that the expectation of future reward determined the rapidity/vigour with which the operant action was performed (by acting as an opportunity cost) and that this computation could be reported by DA fluctuations in the striatum. Thus, higher levels of DA release would increase expectation of future reward and determine faster and more vigorous responding. This idea was later validated empirically by evidence showing that DA

release in the NAcB is concomitant with reward-driven approach behaviour (Howe et al., 2013; Phillips et al., 2003; Syed et al., 2016; Wassum et al., 2012); is contingent on action initiation and not just the expectation of reward (Syed et al., 2016); scales flexibly with both the distance and size of the rewards (Howe et al., 2013); and correlates positively with reward density as well as latency to initiate behaviour in anticipation of future reward (Hamid et al., 2016; Mohebi et al., 2019; Saunders et al., 2018a; Wassum et al., 2012). Importantly, DA transients were not simply shown to report how engaged an animal was in a task. Instead, manipulations that augmented DA release in the striatum were found to increase the probability that an animal would engage in learned actions (Phillips et al. 2003, Hamid et al. 2016), thus confirming a causal role of (non-burst) DA signalling in motivated behaviour.

The difference between the function of fast (burst) and slower (non-burst) DA signalling in modulating behaviour led to the idea that the role of DA in behaviour was indeed dependent upon the time scales of its signalling modes (Schultz, 2007). Thus, bursting activity was thought to be involved in associative learning and serve the function of updating the values of past actions. Slower, non-burst activity, instead, was thought to use prediction of these values to invigorate behaviour (Berke, 2018). This idea however has been recently challenged. Mohebi and colleagues (2019), for example, tested mice on an operant ‘bandit’ task, where animals had to withhold their response in a central port and then approach either a left port or a right port depending on what cue was being presented. Importantly, they varied the reward rate of the task making it probable that reinforcement was being delivered upon entry into the correct port. DA release as detected with fibre photometry showed rapidly evolving ramps of DA that scaled in magnitude with probability and proximity of the reward (replicating previous findings with voltammetry, Hamid et al., 2016; Howe et al., 2013; Wassum et al., 2012). These ramps became larger and more frequent as the reward became more probable. Importantly, the authors observed that changes in DA signalling, related to the expectation of future reward (or state values), were uncoupled from activity of DA cells, as shown by electrical recordings of VTA DA neurons. This suggests that DA dynamics may be controlled by mechanisms other than DA cells firing, such as for example local dynamics at the level of the axon terminal (Floresco et al., 1998; Jones et al., 2010; Threlfell et al., 2012), and that these alternative ways of regulating DA release may serve different functions. This would explain how DA signalling can operate on different time-

scales and with different effects on behaviour, such as those involving learning and motivation (Klaus et al., 2019; Mohebi et al., 2019).

#### **1.5.2.2.1 The midbrain-accumbens pathway: different functions of the NAcb core and the NAcb shell**

Over the years, mounting evidence has revealed a role of the NAcb core in instigating approach behaviour to incentive stimuli (di Ciano et al., 2001; Parkinson et al., 2000; Saunders & Robinson, 2012). For example, Saunders and colleagues (2018) found that optogenetic stimulation of DA fibres in the NAcb core, but not shell, during cue presentation resulted in approach behaviour to the cue and in lever pressing to receive laser stimulation, a sign of acquired primary reinforcement. They also showed that previous stimulation of these fibres concomitant with cue presentation, resulted in the cue acquiring conditioned or ‘secondary’ reinforcement, with rats becoming willing to work (press a lever) to receive the presentation of a conditioned stimulus (CS) in the absence of laser activation.

This is in line with findings from instrumental paradigms showing that inactivation of the NAcb core, but not the shell, slowed approach to the lever and reduced lever presses, in animals trained to press a lever upon presentation of an auditory cue (CS+) to obtain a reward (Ambroggi et al., 2011). Interestingly, inactivation of both regions but especially of the shell, dramatically increased (spurious) lever presses during presentation of an auditory stimulus not predictive of reward (CS-). Pharmacological findings were confirmed by electrophysiological recordings showing more frequent cue-related responses in cells of the NAcb core compared to the NAc shell, during CS+ presentation (Ambroggi et al., 2011). Thus, evidence points to the NAcb core as playing a fundamental role in incentive salience and approach behaviour towards reward-predicting cues (Floresco et al., 2008; Nicola, 2010).

The NAcb shell, instead, seems to be preferentially involved in suppressing behavioural responses that may interfere with goal seeking (Everitt et al., 1999; Floresco, 2015; Zahm, 1999). For example, Ambroggi and colleagues (2011) observed a greater proportion of neurons in the NAcb shell that were excited by the CS- (auditory stimulus not predictive of reward). Given that

activation of the shell increased responding to a lever press during presentation of the CS-, it is possible that the NAc shell plays a role in suppressing responding to nonrewarded stimuli. Specifically, the NAc shell has been suggested to ‘actively suppress extinguished and non-reinforced instrumental behaviour’ (Piantadosi, 2018). More recently, Piantadosi and colleagues (2017, 2018) showed that lesions of the NAc shell but not the core disrupted performance in a passive avoidance task, where rats need to withhold or decrease pressing a lever to avoid a foot-shock. Thus, the NAc shell seems to play a role in suppressing ‘behaviour that may be directed toward irrelevant, non-rewarded, or less preferable outcomes, thus keeping the reward seeker on task and ensuring rewards may be obtained more efficiently’ (Floresco, 2015).

### **1.5.3 The role of the BG in waiting impulsivity**

Given its role in modulating action initiation and reward processing, the BG has been extensively studied in the context of waiting impulsivity (a form of motor impulsivity), both in humans and in animals.

#### **1.5.3.1 Humans**

The neural correlates underlying waiting impulsivity in humans have only been recently investigated with the development of the 4-CSRTT. Specifically, Morris and colleagues (2016) tested healthy volunteers both on the 4-CSRTT and the SSRT with a novel multi-echo resting-state functional magnetic resonance imaging (fMRI) sequence. The authors found that greater premature responding in the 4-CSRTT was negatively correlated with connectivity between subthalamic nucleus (STN) and right VS and between STN and subgenual cingulate cortex. This network was not the same responsible for action cancellation in SSRT, as performance on this task was associated with lower connectivity between hyperdirect projections of the right pre-supplementary motor area (pre-SMA) and left STN along with dorsal caudate and STN connectivity. Interestingly, the authors also tested binge drinkers (BD) and abstinent subjects with alcohol use disorders (AUDs). In this study BD were found to have greater premature responding, while AUD had been shown to exhibit this behaviour in previous studies (Voon et

al., 2014), in line with work in rodents on the effects of alcohol on waiting impulsivity (Peña Oliver et al., 2009; Sanchez-Roige et al., 2014; Walker et al., 2011). Compared to healthy volunteers, both AUD and BD had reduced STN connectivity with subgenual cingulate cortex and the inferior parietal cortex. Given that both patient groups were found to have increased premature responding, these findings confirm the importance of the network involving the STN and subgenual cingulate in waiting impulsivity, with this potentially being an endophenotypic marker of alcohol misuse (Morris et al., 2016).

These findings are in line with previous work in humans (Ballanger et al., 2009; Frank et al., 2007; Wylie et al., 2010) implicating the STN in impulsivity (however see also Lhommée et al., 2012). Specifically, deep brain stimulation (DBS) of the STN in patients affected by Parkinson's disease was found to: decrease reaction times (RTs) and increase choice impulsivity during high-conflict win/win decisions (Frank et al., 2007); decrease RTs and impair response inhibition in a Go/No Go task (Ballanger et al., 2009); increase fast premature response errors during high-conflict trials during performance of the Simon task (Wylie et al., 2010). In healthy volunteers STN activation correlated with faster stopping abilities on the SSTTR (Aron & Poldrack, 2006; Li et al., 2008). As described above, the STN is part of the indirect pathway of the BG, communicating with the GPe and the GPi to facilitate action initiation (Frank, 2006). The STN is also part of the 'hyperdirect' pathway, meaning that it receives direct projection from the cortex, without these being relayed by the striatum (Nambu et al., 2000). According to contemporary models of action selection, activity of the STN has the function to suppress behaviour (*via* indirect inhibition of the thalamus) that would otherwise compete with the desired action plan actuated by the direct pathway of the BG (Kropotov & Etlinger, 1999; Redgrave et al., 1999; Wylie et al., 2010). Abnormal functioning of this network can thus result in impulsive action, as observed empirically (Frank et al., 2007; Ballanger et al., 2009; Wylie et al., 2010).

### **1.5.3.2 Preclinical studies**

In line with data on humans, preclinical research has also implicated the STN in impulse control (Aleksandrova et al., 2013; Baunez et al., 1995, 2001; Baunez & Robbins, 1997; Eagle et al., 2008). For example, excitotoxic lesions of the STN increased motor impulsivity both in the

5CSRTT (Aleksandrova et al., 2013; Baunez et al., 1995; Baunez and Robbins, 1997; Winstanley et al., 2005) and led to impairments in stopping in the SSRT (Eagle et al., 2008). In the specific case of the 5CSRTT, it was shown that such an increase in premature responses was also achieved through disconnection of the PFC-STN pathway (Chudasama et al., 2003), suggesting that impulsive-action control may be regulated in part through cortico-STN circuitry (Eagle & Baunez, 2010).

Most work on waiting impulsivity in the 5CSRTT has explored the contribution of the VS towards the performance of premature responses (for a review see Eagle and Baunez, 2010; Dalley and Robbins, 2017; Bari and Robbins, 2013; Barrus and Winstanley, 2017; Basar et al., 2010). However, some work has been conducted also on the dorsal striatum, for example lesions of the dorso-medial striatum (DMS), but not of the dorso-lateral striatum (DLS), were found to increase premature and perseverative responses on the 5CSRTT (together with an increase in omissions and a decrease in accuracy, Rogers et al., 2001). These deficits were also observed by Christakou and colleagues (2001) following disconnection of the mPFC from the DMS. This work provided strong evidence for a role of the mPFC-DMS pathway in regulating visual attention function and inhibitory control. These early results have recently been corroborated by Terra and colleagues (2020) who recorded activity in mPFC neurons projecting onto the DMS and found that reduced and advanced onset of change in activity in these neurons is associated with premature responses in the 5CSRTT. Confirming a causal role of this pathway in inhibiting impulsive responding, the authors also showed that chemogenetic inhibition of mPFC-DMS neurons increases premature responses, albeit only when the ITI is sufficiently long to induce a high number of premature responses.

Aside from the 5CSRTT, recent work studying waiting impulsivity on a different task confirmed a role of the DMS in sustaining response inhibition and refined, at a cellular level, the micro-circuits implicated in this response (Cruz et al., 2020). Specifically, Cruz and colleagues (2020) tested mice on a two-alternative interval categorization task wherein subjects were required to hold their snout into a central port until an auditory cue was presented after a certain delay. The delay was chosen from a set of 6 intervals, symmetric about 1.5s, and ranging from 0.6s to 2.4s. Mice were then required to categorize the intervals and respond either left or right depending on

whether the interval was shorter than 1.5s ('short choice') or longer than 1.5s ('long choice'). Cruz and colleagues recorded with fibre photometry whether direct striatonigral medium spiny neurons (D1-MSNs) and indirect striatopallidal medium spiny neurons (D2-MSNs) in the dorsal striatum were differentially involved in this task. Interestingly, they found that activity of D2-MSNs, but not of D1-MSNs, was increased during interval presentation, when mice were required to wait and suppress movement. Confirming a functional role of these cells, optogenetic inhibition of D2-MSNs, but not of D1-MSNs, led to gross impairments in the ability to suppress movement during interval presentation. All these findings point at the DMS, and in particular at mPFC projections on this region and MSNs of the indirect pathway, as playing an important role in controlling movement suppression during a delay.

The role of the NAcB has also been extensively studied in the context of waiting impulsivity. For example, Christakou and co-workers (2004) showed that combined unilateral lesions of the mPFC and NAcB core significantly increased premature responses, in particular following non-rewarded trials. Work further to this provided evidence of a functional opponency between NAcB core and shell in regulating waiting impulsivity. For example, in the DRL task, Pothuisen and colleagues (2005) found that lesions of the NAcB core, but not the NAcB shell, increased premature responses, albeit only during sessions where rats had to wait for an extended period (12s) before being allowed to work for food. In the 'forced choice' (FC) task, which is a simpler version of the 5CSRTT (only one hole is available) and resembles the DRL task, Murphy and colleagues (2008) reported no effects of lesions to the NAcB core or shell (perhaps because the ITI was not long enough to reveal any deficits in NAcB functioning). However, when animals were administered d-amphetamine, which increases extracellular levels of DA in the VS (Upadhyaya et al., 2013), the authors did see a differential effect on behaviour depending on the location of the lesion. Specifically, core lesions potentiated and shell lesions attenuated the increase in anticipatory responses that is generally induced by the drug (Robbins & Cole, 1987) and that was observed in sham animals.

Building on this work, Feja and colleagues (2014) investigated whether the NAcB shell and core are differentially involved in impulsive responding on the 5CSRTT. Using a reversible lesion approach, the authors found that intra-shell infusions of muscimol increased both premature

responses and responding during the time-out phase. Inactivation of the core, instead, caused general impairments in task performance, such as a profound increase in omissions and very poor accuracy, and did not lead to any premature responses. This is in line with aforementioned studies on NAc core and shell pointing to a role of the former in incentive salience and CR, and of the latter in suppressing spurious behaviour not associated with reward (Floresco, 2015). Finally, the functional opponency between NAc core and shell was supported by an MRI study (Caprioli et al., 2014) showing that *only the core* subregion of high impulsive (HI) rats was characterised by decreased grey-matter density, reductions of dendritic spine and microtubule markers and decreased expression of GABA decarboxylase (GAD). Reduction of GAD could indicate impairments in GABA-ergic function in the MSNs of this region. Importantly, reducing the expression of GAD in the NAc core of low-impulsive (LI) rats, through the infusion of a messenger RNA interference, was sufficient to elevate spontaneous premature responses in this group of animals. These findings suggest that structural abnormalities and GABA functioning in the NAc core might play a role in the aetiology of premature responses.

### 1.5.3.2.1 Neurochemical substrates: dopamine

Research has been conducted to investigate the neurotransmitter processes contributing to premature responding (for a review see Dalley and Robbins, 2017). Early evidence pointed to a role of the dopaminergic mesolimbic pathway in the manifestation of this behaviour. For example, it was shown that systemic administration of the indirect DA agonist, d-amphetamine, increased premature responding; an effect that was blocked by selective depletion of DA within the NAcB (Cole and Robbins, 1989) and intra-accumbens infusions of a D2 receptor antagonist (Pattij et al., 2007). In addition to this, rats characterised by a trait-like form of impulsivity (HI rats) show a reduced density of the DAT and DA D2/3 receptors in the VS (Dalley et al., 2007a) and specifically in the shell sub-region (Jupp et al., 2013). Brain slice studies *in-vitro* have found that DA release is increased in the NAcB shell but decreased in the NAcB core of HI rats compared with low-impulsive (LI) rats (Diergaarde et al., 2008). On the basis of all these findings, at least two hypotheses can be advanced to describe the aetiology of premature responding. The first is that impulsive responding arises from increased dopaminergic release from VTA terminals into the NAcB shell, perhaps due to decreased functioning of DAT and a decrease in D2/3 autoreceptors controlling DA release in this region (Dalley and Robbins, 2017). Human findings support this hypothesis by showing that highly impulsive individuals are characterised by a reduction in midbrain D2/3 autoreceptors and by an increase in amphetamine-induced DA release in the striatum (Buckholtz et al., 2010). Additional evidence, in humans, of a negative correlation between impulsivity and D2/3 availability in VS has been reported in a recent positron emission tomography study (PET, Lee et al., 2009). However, PET is unable to resolve pre- *versus* postsynaptic populations of D2/3 receptors. Indeed, even classical pharmacological approaches with selective D2/3 receptor antagonists are unable to resolve the precise contribution of D2/3 receptors to impulsivity (e.g., Besson et al., 2010; Pattij et al., 2007). Thus, it remains possible (second hypothesis) that post-synaptic D2/3 receptors in the VS modulate impulsive responding in the 5CSRTT. This second theory may extend to postsynaptic D1 receptors since microinfusions of the D1 receptor antagonist in the NAcB shell reduced impulsivity in the 5CSRTT (Pattij et al., 2007). Indeed, impulsivity could result from an imbalance of DA-binding at the striatal level, with increased midbrain DA release predominantly targeting D1 receptors in VS due to a decreased availability of D2/3 receptors.

## **1.6 Thesis overview**

### **1.6.1 Summary**

Impulsivity is a multifaceted construct composed of different sub-domains. Waiting impulsivity, is one of such domains and is defined as the maladaptive inability to withhold a motoric response for a specific amount of time. The psychological processes involved in this behaviour are yet to be fully understood, however aspects of sustained attention, urgency, motivation, and delay-aversion are all likely to play a role in shaping this behaviour. The neural structure of the BG, together with its afferent neurotransmitter systems, has been extensively studied in the context of waiting impulsivity. While there is a broad understanding of the neural circuits and neurochemistry involved, there are still many unanswered questions. The many questions yet to be addressed reflect the complexity of this behaviour both at the psychological and neural level. Thus some of the questions that this work aims to address are: 1) whether we can refine our understanding of the psychological mechanism that influence premature responses, specifically the extent to which these are driven by motivated behaviour or, instead, negative urgency; 2) whether waiting impulsivity confers some advantages in specific experimental contexts and the neurotransmitter mechanisms regulating this; 3) whether we can refine our understanding of the precise neural circuits involved in the execution of a premature response; 4) how impulsivity and attention on the 5CSRTT compare with performance on other tasks of sustained attention and whether there are any shared neural processes.

### **1.6.2 Aims**

In this thesis, waiting impulsivity was assessed in experimental rats trained on the 5CSRTT. To address some of the point outlined above, I used a range of experimental approaches, including behavioural manipulations to parameters of the 5CSRTT to study the context within which premature responses occur; pharmacological manipulations to explore the neurochemistry regulating premature responses; neural manipulations to dissect the contribution of specific circuits and brain regions in waiting impulsivity. A graphical representation of the core aims of this thesis is summarised in Figure 1.3.

Chapter 2 describes the general methods, including a description of the 5CSRTT which is the experimental task utilised in all the results chapters.

Chapter 3 aims to understand the psychological processes regulating premature responses. In this chapter, the reinforcement rate and other parameters of the 5CSRTT were systemically adjusted to determine whether premature responses are invigorated by reward-based motivation or instead by frustration.

Chapter 4 aims to elucidate whether waiting impulsivity, as operationalised by premature responses on the 5CSRTT, can confer some advantages in specific contexts, as is the case for impulsive individuals and ADHD patients. This chapter also aims to describe the neurotransmitter mechanisms that enable behaviour to adapt to different environmental constraints. Here the frequency of stimulus presentation on the 5CSRTT was modified to have both fast-paced and slow-paced trials. I then administered different pharmacological agents that modulate DA and NA levels in the brain to explore the contribution of these systems to the behaviour observed.

Chapter 5 focuses on a more standard paradigm of waiting impulsivity and aims to determine the precise neural circuits in the midbrain-accumbens pathway that regulate the execution of premature responses.

Chapter 6 evaluates performance on the 5CSRTT, both in the domain of impulsivity and in that of sustained attention, in comparison to another task of sustained attention to understand whether these two tasks recruit similar psychological processes. This chapter also explores whether lesions of the mPFC affect behaviour on the two tasks similarly. This component of the thesis is intended to expand our understanding of the role of mPFC in sustained attention and impulsivity, as measured with two different behavioural paradigms.

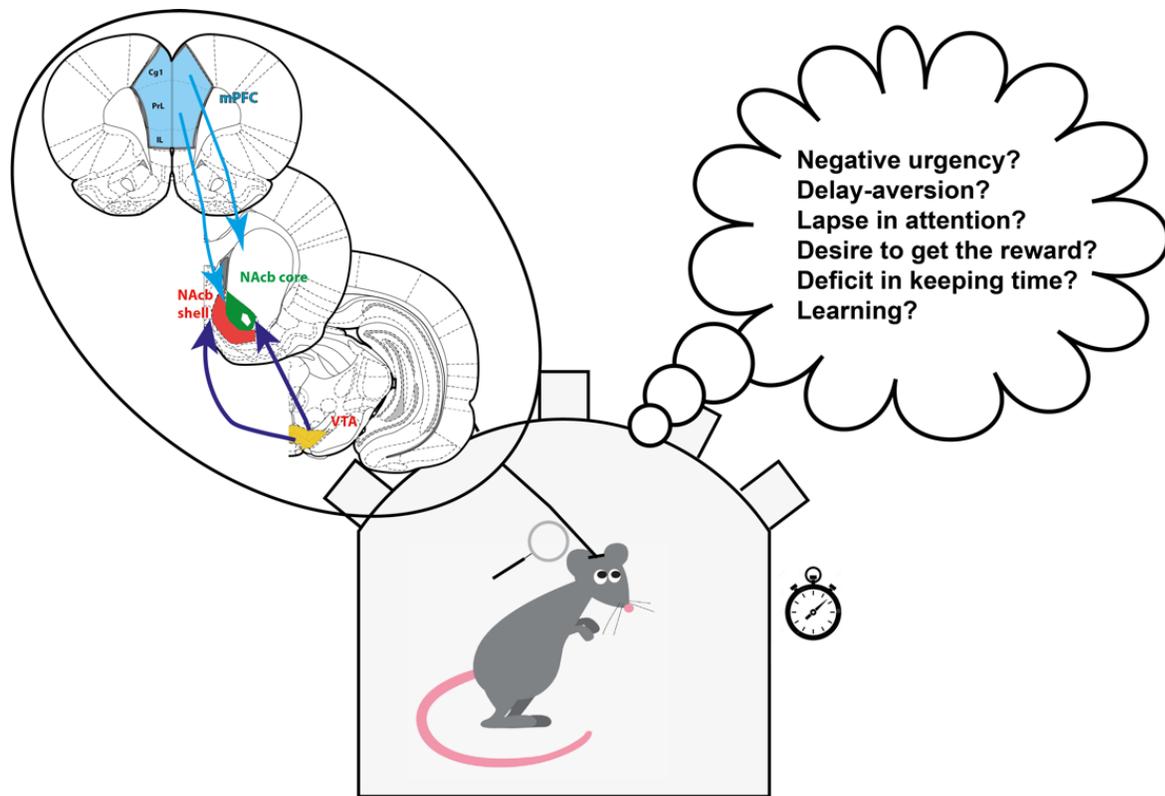


Figure 1.3 Graphical representation of the key scientific questions addressed in this thesis.

# Chapter 2 General methods and materials

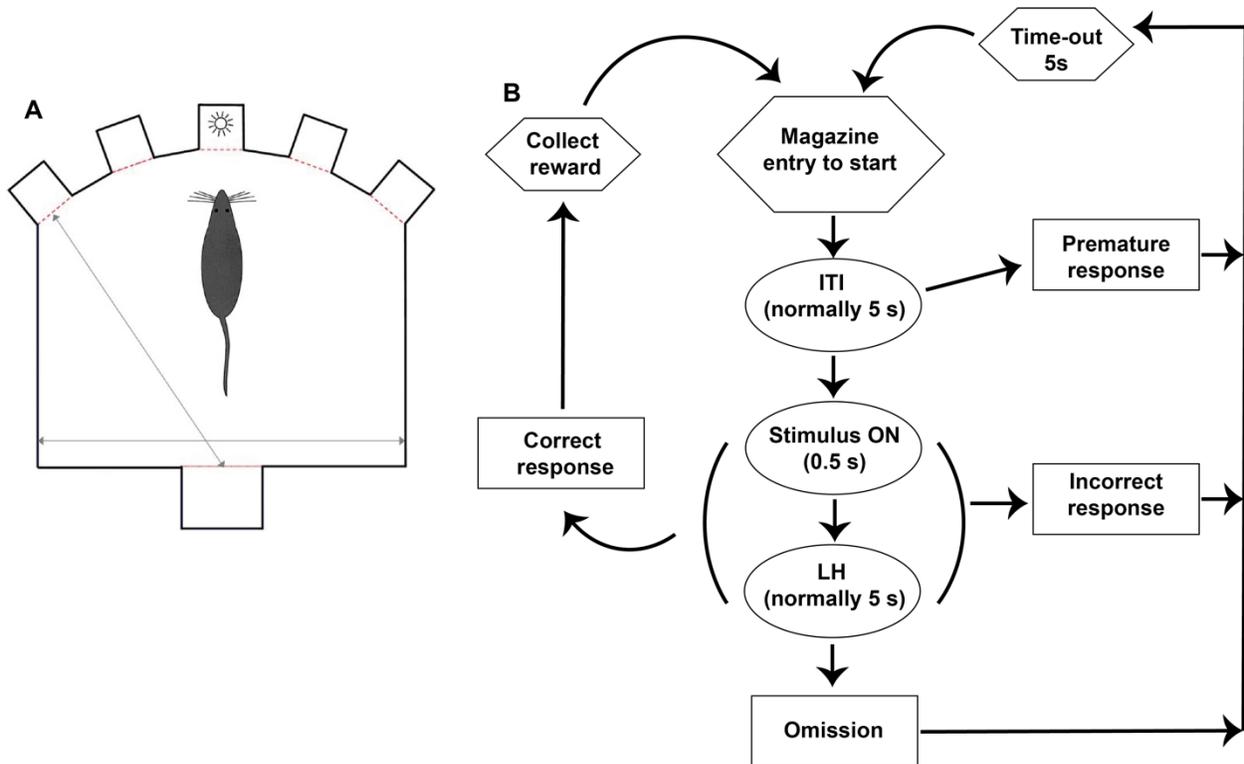
This chapter summarises the methodology common to most experimental chapters. Further specifications to the methods given in this chapter and all other methods specific to individual experiments are described in the relevant chapter.

## 2.1 Subjects

Subjects were male Lister Hooded rats (Charles River, Margate, UK) weighing 280–300 g at the beginning of the experiments. Animals were acclimatised to the animal facility under a 12 h:12 h light cycle (lights off at 7 AM) for a minimum of 7 days before any procedure began. When rats reached a body weight of approximately 300 g, they were food-restricted to maintain approximately 90% of their free-feeding weight trajectory (19 g of Purina rodent chow per animal and day; adjusted for reward pellet consumption during testing). Water was available *ad libitum* and food was given at the end of each day's testing. All procedures conformed to the UK (1986) Animal (Scientific Procedures) Act (Project licence 70/7548 and PA9FBFA9F: Neurobehavioural mechanisms of mental health, held by Dr. A. L. Milton) and were approved by the local Ethics Committee at Cambridge University.

## 2.2 5CSRTT apparatus

Twelve five-hole operant chambers (Med Associates, Georgia, VT) controlled by two computers and Whisker Control software (Cardinal & Aitken, 2010) were used. Each chamber was enclosed in a ventilated sound-attenuating box, fitted with five apertures in a curved wall and a food magazine on the opposite wall of the box that delivered rodent sugar pellets (TestDiet®, Purina, UK). A yellow light-emitting diode stimulus was placed at the rear of each aperture. The food magazine and entire chamber was illuminated by light emitting diodes. Infrared beams detected responses in the magazine and apertures.



**Figure 2.1 (A) Schematic diagram of the 5CSRTT (B) Possible trial sequences of the 5CSRTT.** (B) A trial is initiated by the rat entering the food magazine. A brief light stimulus is then presented in one of five possible apertures after a 5-s inter-trial interval (ITI) has elapsed. Rats are required to scan the five apertures for the appearance of the light stimulus and to respond in the ‘correct’ aperture with a nose-poke response to earn a single food pellet. If the rat responds before the stimulus (‘premature response’) or in an adjacent incorrect aperture (‘incorrect response’), a 5-s time-out (TO) period is introduced where the house light is extinguished, and no food reward is provided. A failure to respond within the limited hold (LH) period results in an ‘omission’ and a subsequent 5-s TO period. After collecting the reward or—on punished trials—at the end of the TO period, a head entry in the food magazine starts a new trial. Adapted from Bari et al., 2008

## 2.3 Five-choice serial reaction time task

### 2.3.1 Training

All rats were trained in the 5CSRTT as described previously (Bari et al., 2008). **Figure 2.1a** outlines a schematic diagram of the task. Animals were trained to detect a brief visual cue appearing in one of five apertures of the operant chambers. Each trial is initiated when the rat pokes into the food magazine and the visual cue is presented after an ITI of 5 s. A response was deemed ‘correct’ if the animal poked into the hole where the light was presented within 5 s of target presentation. A nose-poke response occurring before the appearance of the visual cue was considered ‘premature’, while a response occurring in any of the apertures where the light was not presented was considered ‘incorrect’. A failure to respond within 5 s of target presentation was recorded as an ‘omission’ of response. Only correct responses were rewarded with a food pellet (Noyes dustless pellets, Research Diets, UK), while incorrect, premature and omission responses were punished with a time-out period of 5 s. During a time-out, the animal was required to wait for the beginning of the next trial to engage again with the task. Nose-pokes in any of the holes made after a correct or incorrect response, but prior to reward collection, were deemed ‘perseverative’ but were not signalled by punishment. Each session lasted a maximum of 100 trials or 30 min, whichever limit was reached first. During the training session, stimulus duration was set at 30 s and was gradually decreased over sessions until animals reached stable baseline performance (accuracy, >80% correct choice and <20% errors of omission). **Figure 2.1b** shows the possible trial sequences of the task.

### 2.3.2 vITI challenge

The vITI session consisted of a pseudo-random presentation of trials with 3 s, 5 s, 7 s and 9 s ITI (mean of 6 s). Each ITI was presented at least 50 times and the session ended when animals had completed 200 trials or after 2 h (whichever event occurred first). Animals could not predict which ITI was going to be presented on each trial. Time-out (0.5 s) and stimulus duration (specified in each chapter for each cohort of rats) were kept constant at the same level as their baseline training. To identify which animals exhibited extreme impulsivity phenotypes rats underwent an impulsivity screening procedure. This procedure consisted in testing rats on the vITI challenge for multiple days (ranging between 2 and 3, see each chapter for details on this),

with at least 1 day of baseline in between. Premature responses across the different days of vITI challenge were averaged and the upper (i.e., the highest-impulsive rats) and lower quartiles (i.e., the lowest-impulsive rats) were selected. Animals falling between these two extremes were classified as mid-impulsive (MID) rats.

## **2.4 Stereotaxic surgery**

Surgical procedures were performed following standard stereotaxic techniques for cannula implantation (Chapters 4), expression of viral vectors (Chapter 4) and neurotoxic lesions (Chapter 5). For all these surgeries, rats were anaesthetised using isoflurane in 5% oxygen and secured in a stereotaxic frame fitted with atraumatic ear bars. Anaesthesia was generally maintained at 2.5-3% isoflurane. Baytril (1 mg/kg; 100 mg/ml; Bayer, Germany) and Metacam (1 mg/kg; 5 mg/ml; Boehringer Ingelheim, Germany) diluted in distilled water 1:1 were injected subcutaneously prior to surgery. Following surgery animals were monitored for 5 days and administered Metacam orally (analgesic) for the first 3 days.

## **2.5 Perfusion and brain extraction**

Rats were anaesthetised with an overdose of sodium pentobarbital and transcardially-perfused with saline followed by 10% buffered formalin. The brain was removed and stored for at least 48 h in a 30% sucrose solution. The brain was sectioned using a Leica CR cryostat (chamber temperature:  $-19^{\circ}\text{C}$ ; sample temperature:  $-18^{\circ}\text{C}$ ) and coronal sections (60  $\mu\text{m}$ ) were collected across the whole brain or for specific regions of interest depending on the experiment. More details on this in each individual chapter.

## **2.6 Statistical methods**

Statistical tests were performed with RStudio, version 1.2.1335 (RStudio, Inc). Data were subjected to Linear Mixed-Effects Model (LMEM) analysis with the lmer package in R. In all experiments, percentages or probabilities were arcsine square root transformed, integer numbers (e.g., number of reinforcers earned) were square root transformed. Latencies were log-transformed. Transformations were applied to avoid incurring issues of non-normal data

distributions. Wherever accuracy is reported this was always calculated as following: number of correct responses/(correct + incorrect responses).

To validate whether the data transformations improved model fit I compared the Akaike information criterion (AIC) values of the models with transformed and non-transformed data. The model with transformed data yielded the lowest AIC values for all variables. The appendix associated with each chapter describes the fixed factors of each LMEM model used to analyse data for that chapter. All models with a within-subject factor had the factor 'subject' modelled as a random slope to account for individual differences between rats across testing sessions. When significant three-way interactions were found, further analysis was performed by conducting separate multilevel models on a specific variable of interest. For all analyses, significance was considered at  $\alpha = 0.05$ . When significant interactions were found, further analysis was performed by conducting post-hoc Tukey's corrected pairwise comparisons. For drug manipulations post-hoc testing was used to determine differences with vehicle treatment only.

# Chapter 3

## 3.1 Introduction

Negative urgency, in humans, is regarded as one of the dimensions of impulsivity (Whiteside and Lynam, 2001; Cyders et al., 2007) and is conceptualized as a negatively valenced sense of arousal that invigorates behaviour, leading to impulsive responding (Eben et al., 2020). This framework has received support from empirical evidence. For example, it has been shown that gamblers are faster at initiating the next gamble after a loss than after a win (Dixon et al., 2013; Forder & Dyson, 2016; Shao et al., 2013; Verbruggen et al., 2017). In healthy controls, Gipson and colleagues (2012) found that scoring highly on negative urgency was linked to more operant responses in trials where the reward was omitted unexpectedly, suggesting that negative outcomes in the context of potential rewards can invigorate behaviour and lead to impulsive actions (Eben et al., 2020). Interestingly, in at least two studies (Verbruggen et al., 2017; Yu et al., 2014) frustration-dependent invigoration of behaviour scaled positively with the size of the omitted reward.

In animals, Amsel and Roussel (1952) were the first to study negative urgency and to show that omission of reinforcement can have invigorating effects on behaviour. Specifically, the authors showed that rats tended to run faster to collect reinforcement in a second goal box if reinforcement in the first goal box was omitted unpredictably. Since then, ROEs have been explored in different contexts, for example as greater response rates on a lever both in Pavlovian (Dudley and Papini, 1995) and instrumental (Judice-Daher et al., 2011; Gipson et al., 2012) training schedules. To the best of my knowledge, however, negative urgency has not received much attention as an aetiological factor of premature responses. Few studies looked at the distribution of premature responses after rewarded or non-rewarded trials (Christakou et al., 2004; Donnelly et al., 2014). For example, Donnelly and colleagues (2014) found that premature responses occurred more frequently after non-rewarded trials, while Christakou and colleagues (2004) observed the opposite, that premature responses occurred more frequently after correct rewarded (R) trials. None of these studies, however, introduced specific manipulations to study

the effects of negative urgency on premature responses. Thus, this chapter explored the extent to which, negative urgency, induced by omitting a reward following a correct trial in schedules of partial reinforcement, plays a role in the occurrence of premature responses on the 5CSRTT. In Experiment 1, two separate cohorts of rats trained on the 5CSRTT and screened for impulsivity, were exposed to standard versions of the 5CSRTT but with reinforcement schedules of 0.2, 0.5, 0.8 and 1 reinforcement rates (rr), to explore whether increasing or decreasing the ROE events had a linear impact on premature responses. This experiment was done to test one of the tenets of the frustration hypothesis, whereby frustration should ‘increase in strength as a function of nonrewarded trials’ (Amsel, 1992). In addition, screening for impulsivity was carried out to test the extent to which rats with varying levels of trait impulsivity were susceptible to frustration-induced premature responding. To explore the extent to which the frustration hypothesis can explain the occurrence of premature responses on the 5CSRTT, data was analysed both at the *macro* and *micro* level. The *macro*-level compares overall performance of rats across sessions to detect the effects of ROEs on behaviour on the 5CSRTT at a more global scale. The *micro*-level dissects performance on the 5CSRTT on a trial-by-trial basis to explore whether rats are more likely to make a premature response after a correct non rewarded (NR) trial, compared to a correct rewarded (R) trial.

A partial reinforcement schedule of rr 0.5 has an equal distribution of NR and R trials, thus allowing to evaluate more precisely, both at the *macro*- and *micro*-level, the impact of NR and R on performance. For this reason, Experiment 2 to 5 focused on comparing performance on rr 0.5 with that on a continuous reinforcement schedule on several additional manipulations. In detail, Experiment 2 tested the frustration hypothesis in a setting in which premature responses occur more frequently such as during a 7 s ITI session. Experiment 3 tested the consequences of reducing the punishment for premature responses on a standard session of the 5CSRTT, both on a continuous and partial reinforcement schedule. Experiment 4 was identical to Experiment 3 except that it was tested during a long ITI session (7 s), to explore the additive effects of manipulating rr, time-out and ITI. Finally Experiment 5 tested another tenet of the frustration hypothesis, and specifically whether the intensity of frustration, and thus its capability to energise behaviour, is directly related to the magnitude of the expected, but omitted, reward (Peters & McHose, 1974). In Experiment 5 performance of rats was tested and compared on two

separate rr 0.5 sessions, one delivering 1 pellet and the other delivering 3 pellets as the appetitive reward. Experiment 5 also informed on whether an unequal number of premature responses after NR compared to R was due to premature responses being influenced by frustration-dependent invigoration of behaviour (following the omission of a reward), or rather by suppression of behaviour after reinforcement (Seward et al., 1957). For a more detailed discussion of this see Chapter 1, section 1.4.3.

Alternative theories have been put forward to explain the facilitatory effect that omitted reinforcement has on behaviour. For example, building on a different set of experiments and literature, Anselme (2015; Robinson and Anselme, 2019) disputes the idea that invigoration of behaviour, under partial reinforcement, is (solely) due to frustration. In his so-called ‘incentive hope’ hypothesis, it is suggested that during partial reinforcement, the stimulus associated with reward (CS) acquires a greater conditioned response (CR) or ‘wanting’ because, upon presentation, it elicits ‘hope’ that it will be paired with a reward. In psychological terms, this ‘hope’ translates into incentive motivation and behaviourally into stronger approach behaviour (Anselme, 2015; Anselme and Robinson, 2019). Anselme and colleagues (2013; Robinson et al., 2014) tested this idea on sign-tracking behaviour, which is considered to be a behavioural manifestation of incentive motivation (or ‘wanting’). In this procedure, rats are trained to perform an autoshaping task whereby a lever, the CS, is paired with the delivery of a sugar pellet (unconditioned stimulus, US). For some rats, the sign-trackers (STs), the lever CS does not just acquire predictive properties but also motivational significance, and STs develop a strong attraction towards the CS, approaching it, sniffing it and nibbling on it. Goal-trackers (GTs), on the other hand, preferentially approach the food magazine. Of interest for the present discussion are two studies by Anselme and co-workers (2013; Robinson et al., 2014) where the authors tested how sign-tracking behaviour, an index of incentive motivation, changed in the context of reward uncertainty. Interestingly, when rats could no longer predict whether and how much reinforcement was going to be delivered, they approached the CS lever to a much greater extent than they would in conditions of reward certainty. In a separate experiment, the authors also showed that, under a partial reinforcement schedule, animals approached the lever-CS both after rewarded and non-rewarded trials, and in a way that was stable throughout the session (Anselme and Robinson, 2019). These findings argued against the frustration hypothesis as an explanation

for increased sign-tracking behaviour under uncertainty and led the authors to conclude that reward uncertainty triggers increased incentive motivation (Anselme & Robinson, 2019).

The authors suggested that the neurobiological mechanism whereby uncertainty can drive incentive motivation is via sensitisation of the mesolimbic DA pathway (Robinson, et al., 2014). The link between increased sign-tracking in uncertain contexts and elevated DA transmission is supported by different lines of evidence. Firstly, STs are characterised by greater DA release in the NAc core in response to the lever-CS -compared to GTs - and pharmacological blockade of DA transmission during CS-US acquisition prevents the emergence of sign-tracking behaviour (Flagel et al., 2011). Secondly, there is some evidence of a link between reward uncertainty and increased DA transmission (Fiorillo et al., 2003; Hart et al., 2015; Linnet et al., 2012). For example, Fiorillo and colleagues (2003) showed in monkeys that population activity of DA neurons in VTA have their highest sustained activation under conditions of maximal uncertainty, such as the 50% partial reinforcement schedule. This in turn could suggest greater DA release in the accumbens under conditions of uncertainty. Thus, the incentive hope hypothesis also predicts an increase in premature responses during partial reinforcement compared to continuous reinforcement. This would be driven by an uncertainty-induced invigoration of behaviour and a stronger incentive motivation (or ‘wanting’) towards cues associated with rewards. The micro-level analyses should inform whether an increase in premature responses during partial reinforcement is driven by frustration as opposed to incentive hope. While the former theory predicts that premature responses will occur mostly after NR, the incentive hope hypothesis expects premature responses to occur equally after all trial types as they are driven by a ‘hope’ to be rewarded in the upcoming trial.

Finally, it is also possible that premature responses will occur with greater frequency during continuous reinforcement as opposed to partial reinforcement. I will call this the sensitivity to reward hypothesis. Sensitivity to reward is defined as ‘sensitivity to positive incentives that increases the propensity for approach behavior’ (p.346, Colder et al., 2013). The relationship between impulsivity and sensitivity to reward has long been addressed both in personality theory (Cyders & Smith, 2007; Gray, 1987) and psychiatric research (e.g. Luman et al., 2005; Uebel et al., 2010). Gray (1987) was one of the first to study this construct in the context of personality

theory. He postulated that motivated behaviour is regulated by two major systems of emotion: the behavioural approach system (BAS) and the behavioural inhibition system (BIS) (Corr, 2002). The former is thought to control an individual's response to appetitive stimuli and to underlie the personality factor of impulsivity. Building in part on this work, Cyders and colleagues (2007) explored the extent to which positive, rewarding contexts can foster impulsive decision-making. The authors developed the construct of positive urgency and showed that the tendency to engage in impulsive, risky actions in response to positive affect could identify individuals at risk of problem gambling and alcoholism. In healthy controls, impulsivity has also been associated with SR (Cools et al., 2005; Corr et al., 1997; Wallace & Newman, 1990). For example, Cools and colleagues (2005) showed that individuals who score highest on 'Non-planning Impulsiveness' of the Barratt Impulsivity Scale (BIS) scale, responded more rapidly relative to low-impulsive subjects when the opportunity to gain rewards was highest.

Evidence of an association between impulsivity and sensitivity to reward is also present in the animal literature. For example, HI rats, as assessed with the 5CSRRT, are generally faster at making operant responses, and this can be interpreted as indicating enhanced value attribution to reinforcers or greater subjective utility of food reward (see Chapter 4; Niv et al., 2005). In addition, HI rats on the 5CSRRT have been shown to be more susceptible to the reinforcing properties of natural reinforcers, such as cocaine (Belin et al., 2008; Dalley, et al., 2007a), nicotine (Diergaarde et al., 2008) and sucrose (Diergaarde et al., 2009). When exposed to these substances, HI rats show elevated operant responding and persistent drug seeking despite negative consequences, thus exhibiting stronger addiction-like behaviour (Voon, 2014). Finally, impulsive action is greater when reward magnitude increases (King et al., 2016) and has been associated with ST (King et al., 2016; Lovic et al., 2011). This is the propensity to approach a food-associated cue in a Pavlovian conditioned approach (PavCA) task and is seen as indicative of greater sensitivity to the incentive motivational properties of reward-related (Robinson & Flagel, 2009).

The association, at the behavioural level, between sensitivity to reward and impulsive action is validated by neurobiological evidence showing a common neural substrate between impulsivity and reward processing. Thus, it has been shown in humans that high impulsive subjects have

greater activation in the ventral striatum, a key hub of the neural reward circuit (Haber & Knutson, 2010), in response to positive stimuli, including during reward anticipation (for a review see Kennis et al., 2013). In animals, research has confirmed a role of the ventral striatum in the representation of reward and motivation (Berke, 2018). More specifically, it has been shown that the release of DA in the ventral striatum is concomitant to reward-driven approach behaviour (Wassum et al., 2012; Phillips et al., 2003; Howe et al., 2013; Syed et al., 2016) and - of particular interest for the present study- correlates strongly with expected future reward (Berke, 2018; Mohebi et al., 2019; Hamid et al., 2016). Berke and his team (2018; Mohebi et al., 2019; Hamid et al., 2016), for example, showed that as an animal experiences frequent rewards, in an operant decision-making task, their expectation of future rewards increases and results, on average, in greater DA release. The authors also showed that the change in reinforcement rate (rr) scales inversely with latency to make an operant response, thus as the reinforcement became more frequent, the animals became faster at performing the task. Latency to make an operant response has been interpreted as reflecting the motivational value of an action (Niv et al., 2007), and DA release in the accumbens has been suggested to play a fundamental role in encoding this signal (Niv et al., 2007; Klaus et al., 2019). In line with evidence suggesting a link between SR and rapid responding, HI rats in the 5CSRTT have been postulated to be characterised by a hyper-dopaminergic state (Dalley and Robbins, 2017). On the basis of behavioural and neural evidence suggesting a link between sensitivity to reward and impulsive action, the sensitivity to reward hypothesis would predict that premature responses occur with greater frequency during a continuous reinforcement schedule, which is known to boost motivation and DA release in the NAcB (Mohebi et al., 2019).

### Scientific predictions

Different theoretical frameworks support different predictions in relation to the effects of partial reinforcement on premature responses. Firstly, if increases in motivation drive impulsive action due to increased phasic DA release with frequent or continuous reward delivery, it is predicted that premature responses will be greater during the 100% rr and diminish accordingly with lower rrs in Experiments 1-5. With specific references to Experiment 5, this framework also predicts that premature responses on 5CSRTT will be greater when the reward magnitude is increased from 1 pellet to 3 pellets, as was the case in a previous study (King et al., 2016). It is an open

question whether HI rats will adapt to lower rrs differently from non-impulsive rats. It is possible that, if HI rats show greater sensitivity to reward, their performance on lower rrs will not change as steeply as that of non-impulsive rats. Equally, it could be the case that differences between impulsivity groups are only evident in highly rewarding contexts, as was the case in humans (Cools et al., 2005).

According to the frustration hypothesis, it is predicted that a partial reinforcement schedule will increase premature responses due to a frustration-dependent invigoration of behaviour, in Experiments 1-5. Since frustration is dependent on a violation of expected reward, it is also predicted that in Experiment 5 increasing the magnitude of the reward (from 1 to 3 pellets) will increase the frustration effect still further. Importantly, manipulating the reward magnitude should inform whether any observed increase in the likelihood to make a premature response is due to frustration or to a post-consummatory inhibitory effect. Thus, the former would predict a *higher* likelihood of making a premature response after a NR trial in the 3-pellets as opposed to the 1-pellet condition. The latter, on the contrary, would predict a *lower* likelihood to make a premature response after a NR trial in the 3-pellets as opposed to the 1-pellet condition. If HI rats are more sensitive to negative urgency, it is predicted that they will make more premature responses than the non-impulsive animals.

Probability to make a premature response after different trial types, that is the *micro*-level analysis, will be explored with a first-order Markov chain model. This tests the prediction that actions in the current state  $t$  (in this case a response type in a trial) can determine responding in the future state  $t+1$  (Davison, 2003). For more details on this see the Methods section of this chapter (see methods). Because the omission of an expected reward generally causes an immediate frustration-dependent behavioural response, it is reasonable to explore whether ROEs during NR trials have an impact not just on premature responses in subsequent trials but also on the perseverative responses in the frontal panel (FP), which occur immediately after reward omission. Given that frustration should have an immediate effect on behavioural activation, two additional measures will be explored to test whether 1) premature responses in trial  $t$  are driven by frustration following a NR trial ( $t-1$ ) and whether 2) response types other than premature responses in trial  $t$  following NR trials ( $t-1$ ) are driven by frustration. To address both points, the

time elapsed between making a specific response type, including premature responses, in trial  $t$  and making a NR trial in  $t-1$  will be calculated. If this time window is shorter than, for example the time window between the same response types and correct rewarded trials, then this could potentially indicate a frustration effect as this would energise behaviour and thus drive responses earlier in time.

Finally, according to the incentive hope hypothesis, a partial reinforcement schedule should also drive an invigoration of behaviour and thus a higher number of premature responses, in Experiments 1-5. However, differently from the frustration hypothesis, the incentive hope hypothesis would not predict a trial-by-trial difference in the likelihood of making a premature response and thus the outcome of the previous trial should not affect the likelihood of making a premature response in all Experiments and particularly in Experiment 5. The incentive hope hypothesis postulates that invigoration of behaviour in paradigms of reward uncertainty is driven by an increase in DA release (Anselme and Roberts, 2019). Since HI rats are thought to be characterised by greater levels of DA in the shell subregion of the ventral striatum (Dalley and Robbins, 2017), it is reasonable to hypothesise that they might be more sensitive to uncertainty-driven increases in DA efflux and in turn engage in more premature responses. This is supported by studies in the human population suggesting that greater DA release in the striatum and paradigms of reward uncertainty are two of the main factors that drive pathological gambling, a form of risky, impulsive behaviour (Anselme, 2018). For a summary of the different predictions put forward by the accounts described above see **Table 3.1**.

	<b>Macro-level</b>	<b>Micro-level</b>
<b>Frustration hypothesis</b>	Premature responses should increase in sessions with lower probability of reinforcement	Premature responses should occur with higher probability after frustrating events and especially after NR responses
<b>Sensitivity to reward hypothesis</b>	Premature responses should decrease in sessions with lower probability of reinforcement	Premature responses should occur with higher probability after R trials

<b>Incentive hope hypothesis</b>	Premature responses should be highest with maximal uncertainty so when $rr = 0.5$	Premature responses should occur with equal probabilities after each trial type, that is trial type of the previous trial should not determine the likelihood of a premature response in the following trial
----------------------------------	---	--

**Table 3.1 Table summarising the different predictions of the various accounts presented to explain premature responses.** Macro-level refers to predictions formulated considering the experimental session as a whole, while micro-level refers to predictions at the trial-by-trial level, within each session.

## 3.2 Methods

### 3.2.1 Subjects

Animals were kept under the conditions specified in Chapter 2 (see section 2.1). A total of 60 male Lister-Hooded rats (Charles River, UK) were used for this study. Rats were housed in groups of four. Two cohorts of rats were used for this study, the first consisted of 24 animals and the second consisted of 36 animals. Not all cohorts were used for all the behavioural manipulations reported in this experiment. Specifically, Experiment 1 was run both on cohort 1 and 2; Experiment 2-4 were run on cohort 1 only; Experiment 5 was run on cohort 2 only. For a better understanding of the experimental timeline for both cohorts of animals, with regards to experiments concerning this chapter, see **Figure 3.1**. For a broader perspective on how experiments described in this chapter fit with experiments described in other chapters on the same cohort of rats see **Table 2.1** in Chapter 2.

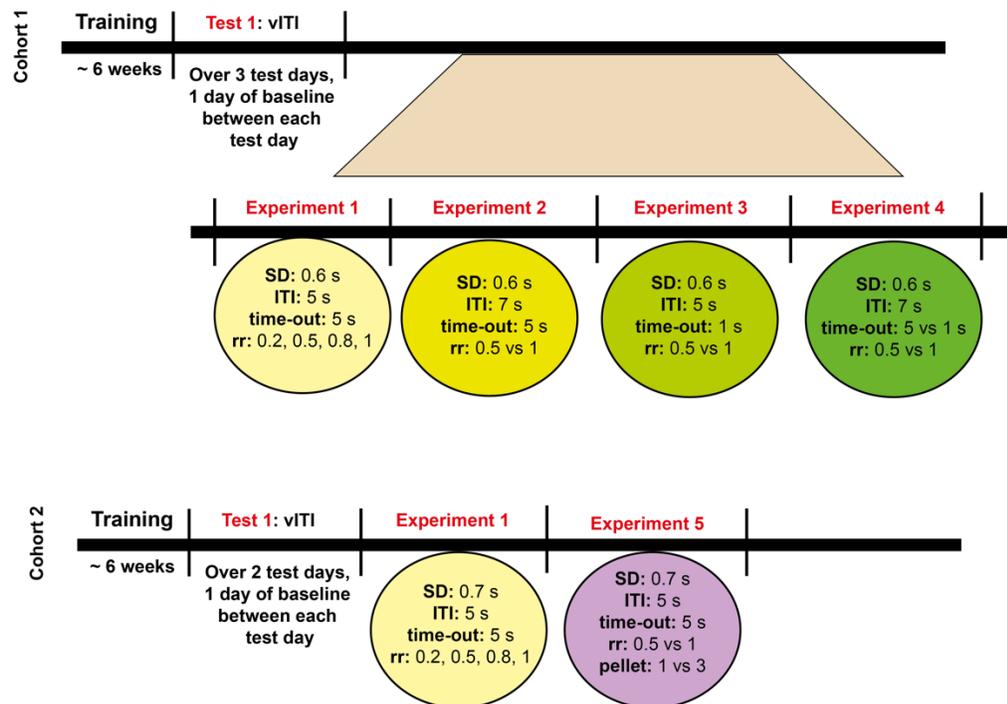


Figure 3.1 Timeline of experiments described in this chapter, for cohort 1 and cohort 2

## **3.2.2 5CSRTT**

### **3.2.2.1 Training**

Rats were trained on the 5CSRTT as described in Chapter 2 (section 2.3). Rats in cohort 1 (N=24) were trained to reach a stable baseline performance on the 5CSRTT with a final stimulus duration of 0.6 s and an ITI of 5 s. Rats in cohort 2 (N=36) were trained to reach a stable baseline performance on the 5CSRTT with a final stimulus duration of 0.7 s and an ITI of 5 s. Each session lasted a maximum of 100 trials or 30 min, whichever limit was reached first.

### **3.2.2.2 vITI challenge**

Rats in Cohort 1 and 2 were screened for impulsivity using a variable ITI (vITI) procedure as described in Chapter 2 (section 2.3.2). For cohort 1 premature responses were averaged across 3 days of vITI challenge, whereas for cohort 2 premature responses were averaged across 2 days of vITI challenge.

## **3.2.3 Behavioural manipulations on 5CSRTT**

### **3.2.3.1 Experiment 1**

Rats were challenged on a standard version of the 5CSRTT (5s ITI; 5s time-out) with varying degrees of partial reinforcement. Specifically, rats were tested on rr 0.2, 0.5, 0.8 and 1 (continuous reinforcement) and these different rr sessions were administered in a Latin square (LSQ) design. Between each test day, rats were tested on a baseline day of standard 5CSRTT with rr 1. Reinforcement of correct trials was pseudo-randomised such that every 20 trials rats were exposed to all the planned rewarded and non-rewarded contingencies according to the probability of each specific session ( $p(R)=0.2, 0.5, 0.8$  or 1), as determined by the Latin square design. Reinforcement probability only changed between sessions and not within a session.

### **3.2.3.2 Experiment 2**

Rats were challenged on a ‘long’ version of the 5CSRTT (7s ITI; 5s time-out) both on partial reinforcement rr 0.5 and on continuous reinforcement rr 1. These sessions were administered in a cross-over design with 1 day of baseline testing in between.

### **3.2.3.3 Experiment 3**

Rats were challenged on a standard version of the 5CSRTT with a shorter time-out (5s ITI; 1s time-out) both on partial reinforcement rr 0.5 and on continuous reinforcement rr 1. These sessions were administered in a cross-over design with 1 day of baseline testing in between.

### **3.2.3.4 Experiment 4**

Rats were challenged on a long version of the 5CSRTT both with a short and long time-out (7s ITI; 1s time-out vs 7s ITI; 5s time-out) both on partial reinforcement rr 0.5 and on continuous reinforcement rr 1. Specifically, rats were first tested on continuous reinforcement (rr 1: 7s ITI; 1s time-out vs 7s ITI; 5s time-out) in a cross-over design with 2 days of baseline in between. A week after this, rats were tested under the same ITI and time-out parameters as the first cross-over, but on partial reinforcement (rr 0.5: 7s ITI; 1s time-out vs 7s ITI; 5s time-out) in a cross-over design with 2 days of baseline in between. These sessions were spaced out in such a way to avoid training effects on the 7s ITI sessions and thus to ensure a high level of premature responses.

### **3.2.3.5 Experiment 5**

Rats were challenged on a standard version of the 5CSRTT (5s ITI; 5s time-out) on partial reinforcement rr 0.5 but with two different reward magnitudes: 1 pellet and 3 pellets. Rats were first tested on rr 0.5 and 1 pellet and, after a day of baseline, were tested on rr 0.5 with 3 pellets. This order was chosen to avoid having some animals whose first experience of partial reinforcement was with an increased reward. The order was also chosen to ensure that all animals experienced both reward magnitudes at the same time and thus attributed to these the same values, thus avoiding having half of the cohort encountering the smaller reward after the bigger reward and perhaps attributing an even lower value to this because of the order of experiences.

### 3.2.4 Analysis

Analyses were performed as described in Chapter 2 (section 2.6). The main variables of interest across experiments are reported in **Table 3.2**.

Variable	Description
Latency to make a correct response (ms)	Time between the end of the ITI and the moment the rat pokes into a ‘correct’ hole.
Premature responses	Number of responses occurring before a cue has appeared in the 5-choice visual array.
Latency to re-start a trial after a time-out	Calculated from the time the time-out ended to the time the animal poked into the food magazine to start a new trial.
Proportion of perseverative responses either in the frontal panel (FP) and in the rear panel (RP)	Calculated by dividing number of perseverative responses (both FP and RP) specific to each response type by number of responses of each response type (e.g. ratio of FP during premature responses= FP during prematures/number of prematures)

**Table 3.2** Main dependent variables analysed in this study.

For details on the fixed factors included in all LMEM models see Appendix A (section A1.1).

#### 3.2.4.1 Consequences of R and NR trials - a Markov chain model

To test whether premature responses occur mostly after NR trials, which are designed to occur during a partial reinforcement schedule and are hypothesised to lead to frustration, a first-order Markov chain model was fit to the data. This describes whether there are any dependencies between transitions from one state, in this case a trial ( $t$ ), to the next state, in this case the next trial ( $t+1$ ). In the present case the states that are being considered are 5, that is all the possible trial types: R, NR, premature, incorrect, and omissions. A large sample size increases the statistical robustness and evidence when comparing the model to an independence model using a likelihood-ratio test (see below), thus wherever there were not group differences in the number of premature responses, trials from all animals were pooled together to build one transition probability matrix. Only in Experiment 2 and 5 a specific transition probability matrix was built for HI rats only, as these made significantly more premature responses than the other two groups of LI and MID rats. In general, for each manipulation, regardless of whether it was specific to an

impulsivity group or not, the 5x5 transition matrix that was created was irreducible, meaning that each state can be accessed by any other, and was assumed to be homogeneous, meaning that time-dependent changes in the transition probabilities between states are ignored (Davison, 2003). For each matrix a diagnostic test was applied to verify whether the transition probability matrix considered classifies as a first-order Markov chain or whether instead it is better described by a zero<sup>th</sup>-order Markov chain, or independence model, where no dependencies are assumed between states. Independence was assessed through the application of the likelihood ratio statistic  $W$  for each transition matrix (as explained by Davison, Chapter 6, 2003). The likelihood ratio statistic  $W$  approximately equals Pearson's statistic  $P = \sum(O - E)^2/E$  and indeed follows approximately the same probability distribution as the Chi-squared distribution with  $(S - 1)^2$  degrees of freedom, where  $S$  denotes the number of states in the matrix. Thus, in the present case, under the independence model, the  $W$  for each matrix would follow a distribution with 16 degrees of freedom. To represent graphically the contribution of each transition probability to the overall value of the  $W$  statistic and thus to show which transition probabilities deviated the most from the independence model, the quantity  $Y=(O-E)/E^{1/2}$  was calculated for each transition probability (cell) of the matrix and plotted in probability plots for the normal distribution. In these, a straight line in the middle of the graph -the linear function- corresponds to the expected normal distribution. Under the independence model,  $Y$  follows standard normal distributions such that a close alignment with the linear function indicates a good fit of the independence model to the observed data. However, a situation in which  $Y$  values deviate from the linear function, and thus from the normal distribution, indicates violation of the independence model and informs of the specific transition probabilities that are more strongly driving this deviation.

Once the  $W$  statistic was assessed for the whole transition matrix and the transition probabilities deviating the most from the independence model were graphically represented in normal probability plots, further tests were applied to test whether specific transition probabilities of interest deviated from the independence model. **Figure 3.3** shows the transition matrix for performance on rr 0.5 in Experiment 1 (cohort 1) and what that transition matrix would look like under the independence model. For the present analysis, those of interest were frequencies of one-step transitions that lead to a premature response, frequencies of one-step transitions having NR trials as the starting states and those having R trials as the starting states. For a graphical

representation of the possible starting states leading to a premature response see **Figure 3.4**. To do this I used standard asymptotic theory for multinomial or normal distributions  $X^2$  (as explained by Anderson and Goodman, 1957). The choice of degrees of freedom (df) for each chi-square test differed depending on the frequencies of one-step transitions considered. This is because ‘in statistical terms the degrees of freedom relate to the number of observations that are free to vary’ (p.38, Field et al., 2012) and those that are free to vary, in a Markov chain model, depend on which transition probabilities are being considered. For example, the row-wise transition probabilities of the Markov chain matrix must add up to 1 thus only S-1 values are free to vary in these specific transitions. For this reason, goodness-of-fit chi-square tests considering, for example, R or NR responses as the starting states (1st and 2nd rows respectively of the transition probability matrix, **Figure 3.3**), will have S-1 df and in this case 4 df. On the contrary, when considering transition probabilities leading to a specific end state, thus for example those leading to a premature response (3rd column of the probability matrix in **Figure 3.3**), these vary independently without an overall constraint that they sum to one. For this reason, goodness-of-fit chi-square tests considering one-step transitions leading to premature responses will have S df and in this case 5 df.

**First-order Markov Chain**

	R	NR	Prem	Omiss	Inc
R	331	335	42	54	137
NR	315	289	102	67	128
Prem	75	74	33	11	32
Omiss	63	64	12	42	21
Inc	118	142	31	24	59

**Independence model**

	R	NR	Prem	Omiss	Inc
R	312	312	76	68	130
NR	312	313	76	69	131
Prem	78	78	19	17	33
Omiss	70	70	17	15	29
Inc	130	130	32	28	54

**Figure 3.3 Fit of independence model to performance of the 5CSRTT: observed (left) and fitted (right) frequencies of one-step transitions.** The pink column represents starting states, whereas the blue row represents end states. The observed data (left) is the raw data obtained from performance on the 5CSRTT, whereas the fitted data (right) is the frequencies of one-step transitions that we would observe if there were no dependencies between trials.

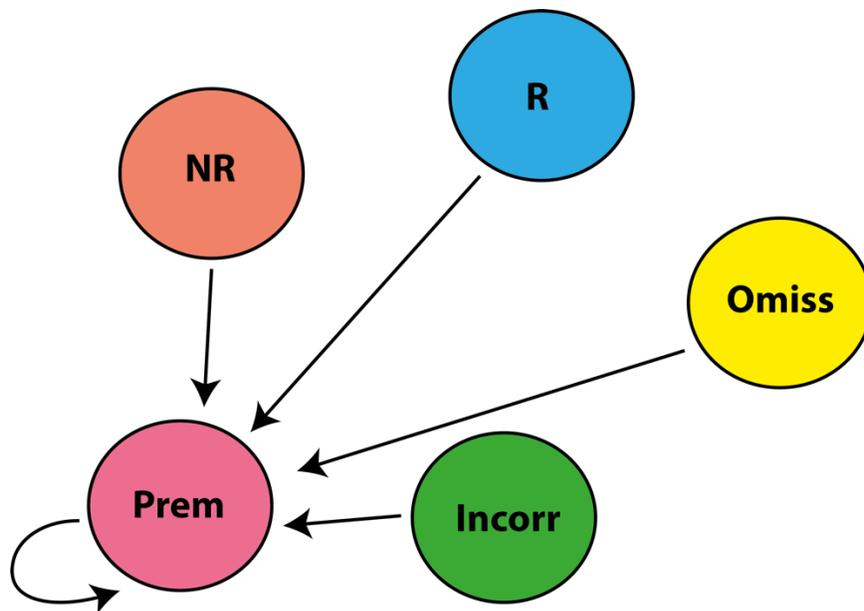


Figure 3.4 Graphic representation of the possible starting states (or trials) leading to a premature response.

To reject the independence model, which states no dependency in transition probabilities between states, both the  $W$  and  $X^2$  should be greater than the  $\alpha$  significant point of the  $X^2$  distribution with the degrees of freedom considered in each specific test. We set  $\alpha=0.05$  as is common in behavioural neuroscience applications. Thus, in the context of the  $W$  statistic, the independence model is rejected when  $W$  is greater than the critical value 26.30 (based on the  $X^2$  distribution with 16 df). With regards to the goodness-of-fit chi-square tests applied to transition probabilities leading to premature responses, the independence model is rejected when  $X^2$  is greater than the critical value of 11.07 (based on the  $X^2$  distribution with 5 df). On the contrary, for transition probabilities having R or NR trials as the starting states the independence model is rejected when  $X^2$  is greater than the critical value of 9.49 (based on the  $X^2$  distribution with 4 df).

Only transition probabilities between trials in the rr 0.5 condition were analysed, as this condition guarantees an even distribution of R and NR trials and thus controls for exposure to frustrative and non-frustrative events.

## 3.3 Results

### 3.3.1 Experiment 1

Data were analysed with the intent to address the scientific predictions set out in the Introduction. Thus, to test whether premature responses are modulated by level of motivation (sensitivity to reward hypothesis), it was first necessary to evaluate the extent to which partial reinforcement affected motivation to engage with the task. This was done by looking at different measures that are generally regarded as indices of motivation: latency to make a correct response, number of correct responses, number of omissions, latency to start a new trial after a period of time-out and collection latency. The latter, in the context of partial reinforcement, identifies the collection of a food pellet during R trials but during NR trials simply indicates the time it took the rat to poke into the food magazine to initiate a new trial. Latency to make a correct response was further analysed comparing R vs NR trials to verify whether the rat could predict if the upcoming trial was rewarded or not.

Experiment 1 was conducted on two separate cohorts of animals to test for replicability of findings, thus data for both cohorts is reported below separately.

#### 3.3.1.1 Effects of partial reinforcement on 5CSRTT performance

##### 3.3.1.1.1 Cohort 1

**Table 3.2** shows the effects of rr on the number of correct and omission responses, latency to make a correct response and latency to re-start a trial, across rrs. Briefly, rats made fewer correct and omission responses in rr 0.2 compared to all the other reinforcement rates. In addition, animals were slower to make a correct response in rr 0.2 and rr 0.5 compared to rr 1 and were slower to re-start a trial during rr 0.2 compared to all other rrs. For details on the statistical results see **Table 3.3**.

<b>Cohort 1</b>	rr 0.2	rr 0.5	rr 0.8	rr 1
Correct responses	<b>58.57 (2.90)*</b>	68.14 (1.75)	73.90 (1.56)	74.52 (1.51)
Omission responses	<b>23.42 (3.02)*</b>	11.81 (1.61)	6.71 (0.77)	7.24 (0.92)
Latency correct responses (ms)	<b>824.23 (23.65)*</b>	<b>803.47 (38.70)*</b>	760.47 (31.34)	705.90 (33.56)
Latency to restart a trial following time-out (ms)	<b>7916.17 (753.92)*\$^</b>	5688.88 (694.316)	5357.08 (767.05)	5328.27 (678.76)

**Table 3.3 Cohort 1. Effects of rr on number of correct and omission responses, latency to make a correct response and to re-start a trial following a time-out.** Mean and standard error (SE) in brackets. For correct responses, there was a main effect of reinforcement rate [ $F(3,54)=15.33$ ,  $p<0.001$ ], with rats making significantly **fewer** correct responses in rr 0.2 compared to all the other reinforcement rates (rrs,  $p<0.01$  for all comparisons). For omission responses there was a main effect of rr  $F(3,54)=25.62$ ,  $p<0.001$ , with rats making more omissions in rr 0.2 compared to all the other rrs ( $p<0.001$  for all comparisons). Reinforcement rate also influenced latency to make a correct response [ $F(3,54)=6.95$ ,  $p<0.001$ ]. Specifically, animals were slower in rr 0.2 and rr 0.5 compared to rr 1 ( $p<0.01$ ). There was a main effect of rr on latency to start a trial after a time-out,  $F(3,53.34) = 5.46$ ,  $p=0.002$ . Animals were slower during rr 0.2 compared to all other rrs ( $p<0.05$  for all comparisons). \*statistical significant difference with rr 1 <sup>S</sup> statistical significant difference with rr 0.8 <sup>^</sup> statistical significant difference with rr 0.5.

Importantly, latency to make a correct response was analysed separately for R responses and NR responses to test whether rats could predict which correct response was going to be rewarded. There was no effect of trial outcome on latency to make a correct response [ $F(1,90)=0.13$ ,  $p=0.715$ ]. For collection latency, there was an interaction between trial outcome and rr, [ $F(2,90)=5.50$ ,  $p=0.005$ ]. Specifically, there was an effect of rr on latency to poke into the food magazine solely after a NR trial, with animals being faster during rr 0.8 compared to the rr 0.2 and rr 0.5 ( $p<0.05$ , for all comparisons). Animals were also faster to poke into the food magazine after rr 0.5 compared to rr 0.2 ( $p=0.03$ ).

### Summary

As expected, and in line with previous research (Mohebi et al., 2019), partial reinforcement had an effect of motivation to engage with the task, with rats being slower to make a correct response with decreasing rrs. Other measures that changed with decreasing rrs were (1) lower number of correct responses (2) higher number of omissions and (3) slower latencies to initiate a new trial both after NR responses and after a time-out. Importantly, when making a correct response, rats could not predict whether this was going to be rewarded, as shown by identical latencies for R and NR trials

### 3.3.1.1.2 Cohort 2

Similar to cohort 1, rr had an effect on number of correct and omission responses for cohort 2. As shown in **Table 3.4**, rats made the least number of correct responses and the highest number of omissions in rr 0.2 compared to the other rrs. In addition, rats were faster at making a correct response in rr 1 compared to rr 0.5 and rr 0.2, while they were slower at re-starting a trial in rr 0.2 compared to the other rrs. For details on the statistical results see **Table 3.4**. Similar to cohort 1, latency to make a correct response was analysed as a function of trial outcome (R vs NR responses) to check whether animals could predict whether the upcoming trial would be rewarded or not when making a correct response. There was no effect of outcome on latency to make a correct response [ $F(1,165)=0.85$ ,  $p=0.358$ ]. There was an interaction for collection latency between trial outcome and rr, [ $F(2,165)=10.94$ ,  $p<0.001$ ]. In details, rr did not affect collection latency when a correct response was rewarded, but it did affect latency to poke into the food magazine when a correct response was not rewarded. Specifically, animals were slower in rr 0.2 compared to rr 0.8 ( $p<0.001$ ) and rr 0.5 ( $p<0.001$ ). Animals were also slower in rr 0.5 compared to rr 0.8 ( $p=0.008$ ).

<b>Cohort 2</b>	rr 0.2	rr 0.5	rr 0.8	rr 1
Correct responses	<b>68.27 (2.33)*<sup>§</sup></b>	74.03 (1.25)	76.72 (1.44)	76.75 (1.33)
Omission responses	<b>16.17 (1.82)*<sup>§</sup><sup>^</sup></b>	<b>9.36 (1.16)*</b>	6.36 (0.98)	4.81 (0.71)
Latency correct responses (ms)	<b>769.97 (25.90)*<sup>§</sup></b>	<b>723.89 (23.36)*</b>	667.01 (19.28)	649.94 (24.34)
Latency to restart a trial following time-out (ms)	<b>6165.45 (636.97)*<sup>§</sup><sup>^</sup></b>	4497.46 (441.64)	3874.65 (344.96)	3833.48 (311.274)

**Table 3.4 Cohort 2. Effects of rr on number of correct and omission responses, latency to make a correct response and to re-start a trial following a time-out.** Mean and standard error (SE) in brackets. Reinforcement rate had an effect on number of correct responses for cohort 2 [F(3,99)=5.58, p=0.001]. Animals made less correct responses in rr 0.2 compared to rr 1 (p=0.004), rr 0.8 (p=0.003) and rr 0.5 (trend level of significance, p=0.057). Similarly to cohort 1, reinforcement rate affected number of omissions during a session, [F(2,99)=20.29, p<0.001]. Specifically, animals made more omission responses in rr 0.2 compared to rr 1, rr 0.8 and rr 0.5 (p<0.001 for all comparisons). Animals also made more omissions during rr 0.5 compared to rr 1 (p=0.024). Latency to make a correct response was affected by rr, [see Table 3.2, F(3,99)=11.06, p<0.001]. Animals were faster at making a correct response in rr 1 compared to rr 0.5 (p=0.023) and rr 0.2 (p<0.001). Animals were also faster at making a correct response in rr 0.8 compared to rr 0.2 (p<0.001). \*statistical significant difference with rr 1 p<0.05 <sup>§</sup>statistical significant difference with rr 0.8 p<0.05. <sup>^</sup>statistical significant difference with rr 0.5 p<0.05.

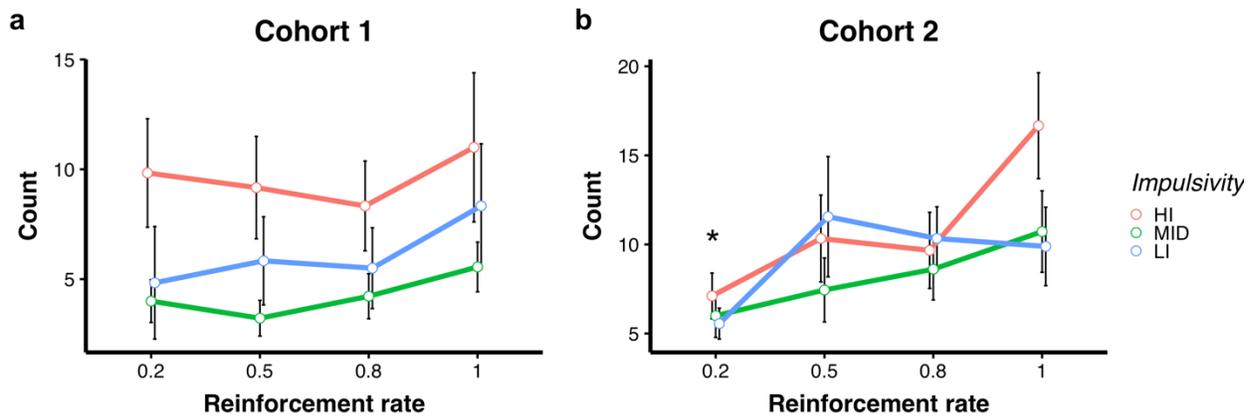
### Summary

Similar to cohort 1, also for cohort 2, partial reinforcement affected the motivation to engage with the task, with animals being slower and less motivated with decreasing rr. This was reflected by (1) slower latencies to make a correct response (2) a decreased number of correct responses and (3) a greater number of omissions, with decreasing rr. In addition, when rr was low, animals were slower to initiate a trial after a correct response and after a time-out. As for cohort 1, when making a correct response, rats could not predict whether this was going to be rewarded, as shown by identical latencies for R and NR trials.

### **3.3.1.2 Effects of partial reinforcement on premature responses**

After having established that partial reinforcement modulates motivation to engage with the task, it was important to test whether changes in motivation affected the occurrence of premature responses. This was aimed at testing the validity of the sensitivity to reward hypothesis.

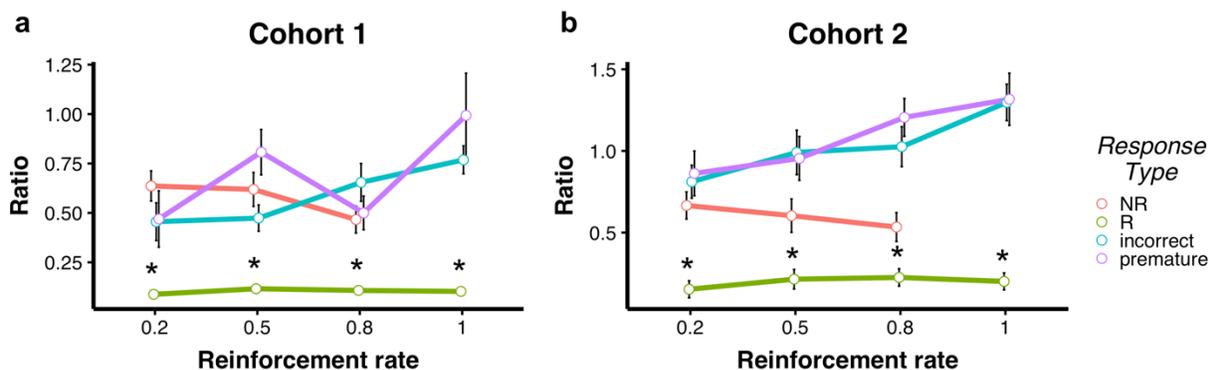
### 3.3.1.2.1 Cohort 1



**Figure 3.5** Premature responses during different reinforcement rates (a) Cohort 1. (b) Cohort 2 \*statistically significant difference with rr 1,  $p < 0.05$

**Figure 3.5a** shows the effects of rr on premature responses. Animals tended to make more premature responses during rr 1 compared to the other reinforcement rates, however this difference was not significant [main effect of rr:  $F(2,18)=3.25$ ,  $p=0.062$ ]. However, there was a negative correlation between number of prematures and latency to make a correct response, thus for rr 0.2:  $r=-0.50$ ,  $p=0.022$ ; rr 0.5:  $r=-0.49$ ,  $p=0.024$ ; rr 0.8:  $r=-0.46$ ,  $p=0.036$ ; rr 1:  $r=-0.45$ ,  $p=0.041$ .

Other types of responses that could be affected by the omission of an expected reward were explored. Specifically, the proportion of perseverative responses in the FP were analysed to investigate whether rats manifested frustration in these specific response types, which occur at the time of the omission of the reward and could thus be a direct measure of frustration.



**Figure 3.6** Ratio of perseverative responses in the FP following either a R, a NR, a premature or an incorrect response. **(a)** Cohort 1: there was an interaction between rr and trial type [ $F(6,193)=2.28, p=0.038$ ]. In details, during a premature response, rats made a higher proportion of FP perseverative responses during rr 0.5 compared to rr 0.2 ( $p=0.007$ ). In addition, in all rrs animals made a lower proportion of FP preservative responses during R trials as opposed to all other trial types considered ( $p<0.01$  for all comparisons). The only other difference in proportion of FP perseverative responses between trial types was during rr 0.2, where animals made slightly more FP preservatives during NR trials compared to premature responses ( $p=0.054$ , trend level). **(b)** Cohort 2: there was an interaction between rr and trial type [ $F(6,358)=2.56, p=0.019$ ]. During all rrs animals made less FP perseveratives during correct rewarded trials compared to all other trial types ( $p<0.01$  for all comparisons). During both rr 0.5 and rr 0.8, animals made more FP perseveratives during incorrect and premature trials compared to correct non rewarded trials ( $p<0.01$  for all comparisons), while there were no differences between incorrect and premature trials. In addition, rats made proportionally more FP during premature responses in rr 0.8 than in rr 0.2 ( $p=0.001$ ).

**Figure 3.6a** shows how response type and rr affected the ratio of perseverative responses in the FP. To test whether premature responses occurring after a NR trial happened earlier in time compared to premature responses occurring after a R trial, as this could be a sign of frustration-dependent invigoration of behaviour, the time window between these responses was explored. There was no significant effect of response type on time elapsed between a correct response in trial  $t$  and a premature response in trial  $t+1$ , during any rr [ $F(1,47)=2.51, p=0.120$ , data not shown]. Finally, the extent to which animals tend to respond faster or slower following a R or NR trial was explored. This was again done to verify whether NR trials in trial  $t$  generate frustration which drives early responding in trial  $t+1$ . There was only an effect of rr [ $F(2,90)=10.27, p<0.001$ ], with response latency being slower in rr 0.2 compared to rr 0.8 ( $p<0.001$ ) and in rr 0.5 compared to rr 0.8 ( $p=0.044$ ).

### 3.3.1.2.2 Cohort 2

**Figure 3.5b** shows the effects of rr on premature responses. There was a significant effect of rr on premature responses for cohort 2, [ $F(3,99)=3.83, p=0.012$ ], with animals making fewer

premature responses during rr 0.2 compared to rr 1 ( $p=0.012$ ). In all rrs, except rr 0.2, there was a negative correlation between the number of premature responses and the latency to make a correct response, thus for rr 0.5:  $r=-0.54$ ,  $p<0.001$ ; rr 0.8:  $r=-0.39$ ,  $p=0.018$ ; rr 1:  $r=-.52$ ,  $p=0.001$ . **Figure 3.6b** shows how response type and rr affected the ratio of perseverative responses in the FP. It was next evaluated whether the time elapsed between making a correct response in trial t and making a premature response in trial t+1 differed depending on whether the correct response was rewarded or not. There was a main effect of trial outcome [ $F(1,121)=9.62$ ,  $p=0.002$ ], specifically, the time window between a correct response in trial t and a premature response in trial t+1 was greater when t was a NR trial ( $p=0.003$ ). Finally when looking at whether, in general, the presence or omission of an expected reward in trial t-1 affects latency of response in trial t, there was a main effect of rr on latency to make a response in trial t [ $F(2,165)=19.63$ ,  $p<0.001$ ], but no effect of trial outcome in t-1. Responses were slower in rr 0.2 compared to rr 0.5 ( $p=0.002$ ) and rr 0.8 ( $p<0.001$ ), and slower in rr 0.5 compared with rr 0.8 ( $p=0.020$ ).

### Summary

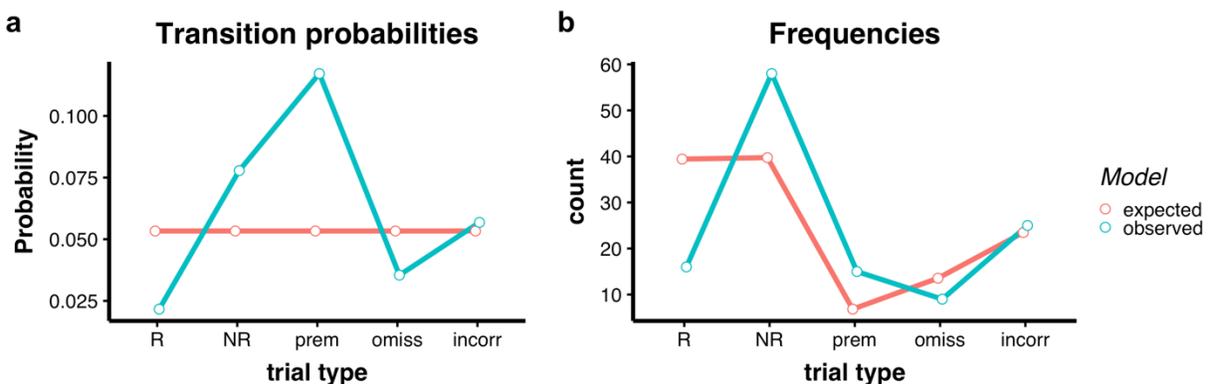
For both cohorts of animals, rr affected premature responses as these decreased with decreasing rr, however this difference reached significance only in the second cohort of animals. When the omission of an expected reward occurred animals made more FP perseverative responses than when the expected reward was delivered. In both cohorts there was no difference in latencies to make a response following R versus NR trials. Finally, in both cohorts the time window between making a premature response in trial t and making a correct response in trial t-1 was longer when t-1 was a NR trial. This difference was significantly different in cohort 2 but not in cohort 1.

## **3.3.1.3 Consequences of a rewarded or non-rewarded trial on premature responses**

### **3.3.1.3.1 Cohort 1**

Transition probabilities between trial types were analysed to test whether animals were more likely to make a premature response after a frustrative event, such as a NR trial. First it was verified whether a Markov chain model that was built taking all trials from all animals into consideration violated the independence model. A W statistic of 73.61 indicated violation of the

independence model with a significance level of  $p < 0.001$ , meaning that the probability to transition to a state  $t+1$  does depend on the current state  $t$ . The contribution of each cell to this overall result was then assessed to further explore which specific transition probability is particularly deviating from the independence model. To do this, for each cell of the matrix, the deviance from the independence model was calculated as  $Y = (O - E)/E^{1/2}$  and plotted in normal probability plots as shown in **Figure 3.1A** (and as explained by Davison, 2008). The points deviating the most from the linear function are those deviating the most from the independence model. To more precisely quantify the extent to which transition probabilities leading to a premature response deviate from the independence model, a chi-square test was run on the frequencies of one-step transitions leading to a premature response. This showed that these are significantly different from the distribution that would be expected if there were not dependencies between trials,  $X^2=33.71$ ,  $p < 0.001$  (under the chi-squared distribution with five degrees of freedom). **Figure 3.7a** and **b** show how the transition probabilities and frequencies of one-step transitions, respectively, leading to a premature response, deviated from the independence model. The biggest deviations from the independence model were a lower-than-expected probability to transition to a premature response from a R trial ( $Y=-3.72$ ), and a higher-than-expected probability to transition to a premature response from a premature response ( $Y=3.13$ ).

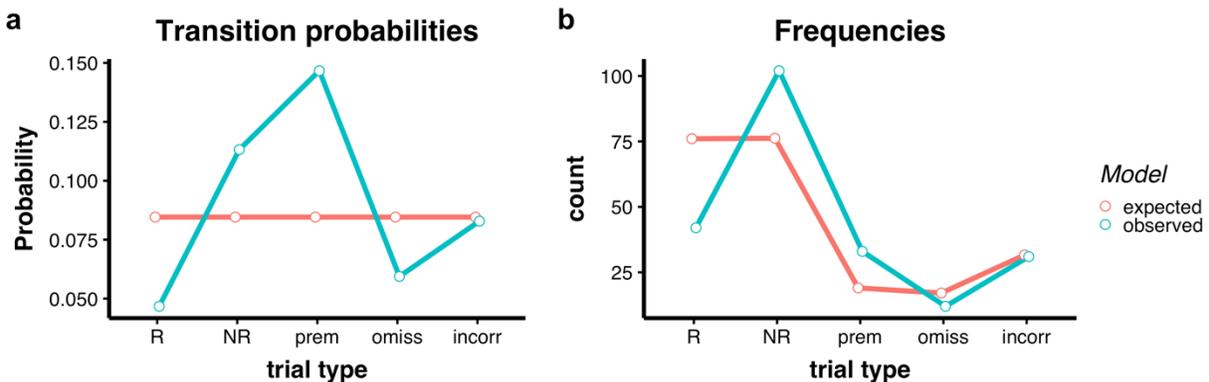


**Figure 3.7 Experiment 1. Cohort 1. (a) Transition probabilities and (b) frequencies of one-step transitions leading to a premature response (end state).** x axis shows starting states. Red = independence model; Blue = observed data.

The same tests were run considering frequencies of one-step transitions starting from R and NR trials. For more details on this see Appendix A (section A1.2.1.1.1).

### 3.3.1.3.2 Cohort 2

Similar to Batch 1, the Markov chain model was fit to the performance data of Batch 2 and was tested for a violation of the independence model. The independence model was violated as shown by a W statistic of 85.84 with significance level  $p < 0.001$ . The specific contribution of each cell was then explored and **Figure 3.4A** shows which observations considerably deviated from the independence model taking all transition states into account. A chi-square test on the frequencies of one-step transitions leading to a premature response showed that these are significantly different from the distribution that would be expected if there were not dependencies between trials,  $X^2 = 35.75$   $p < 0.001$ . **Figure 3.8a** and **b** show how the transition probabilities and frequencies of one-step transitions, respectively, leading to a premature response deviated from the independence model. The biggest deviations from the independence model were a lower-than-expected probability to transition to a premature response from a R trial ( $Y = -3.90$ ) and a higher-than-expected probability to transition to a premature response from a premature response ( $Y = 3.20$ ).



**Figure 3.8** Experiment 1. Cohort 2. (a) Transition probabilities and (b) frequencies of one-step transitions leading to a premature response (end state). x axis shows starting states. Red = independence model; Blue = observed data.

The same tests were run considering frequencies of one-step transitions starting from R and NR trials. For more details see Appendix A (section A1.2.1.1.2).

#### Summary

A Markov chain model applied to the data showed that there are dependencies between trial types, thus a response made in trial  $t+1$  depends on the current state in trial  $t$ . In both batches, rats had the highest probability to transition to a premature response from another premature

response. They were also more likely than chance levels to make a premature response after a NR trial and they were less likely than chance level to make a premature response after a R trial.

### **3.3.2 Experiment 2 - Effects of reinforcement omission on behaviour: increasing the ITI**

In Experiment 1 partial reinforcement rate did not seem to increase the number of premature responses and, if anything, these seem to decrease with decreasing rr. This argues against the frustration hypothesis; however animals are very well trained to respond to a 5 s ITI schedule, thus it could also be the case that any effect of ROE on premature responses does not emerge due to the scarcity of premature responses in this condition. For this reason, the difference between continuous and partial reinforcement (only rr 0.5 in this case) was tested on a longer 7 s ITI, which should induce a greater number of premature responses and thus potentially unmask any effects of ROE on omissions.

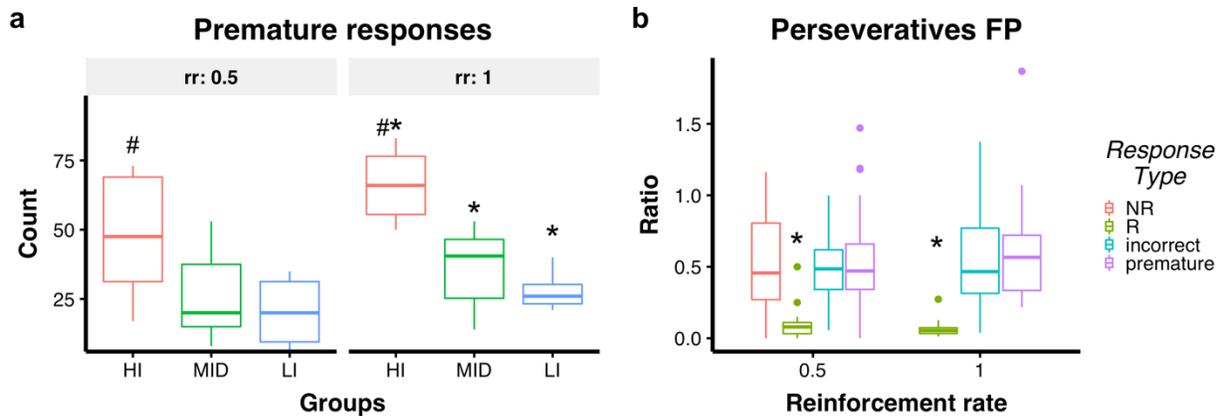
#### **3.3.2.1 Results (Cohort 1)**

Firstly, indices of motivation were analysed to explore how differences in rr affected performance. Rats were faster at making a correct response in rr 1 compared to rr 0.5 and were faster at re-starting a trial following a time-out punishment in rr 1 compared to rr 0.5. A summary of these results is shown in **Table 3.5**.

	Latency to make a correct response (ms)		Latency to restart a trial following time-out (ms)	
	rr 1	rr 0.5	rr 1	rr 0.5
HI	<b>544.46 (19.18)*</b>	581.65 (30.58)	<b>3632.58 (381.52)*</b>	5732.17 (913.26)
MID	<b>661.14 (32.31)*</b>	693.47 (57.87)	<b>3875.25 (606.52)*</b>	4482.98 (676.76)
LI	<b>619.75 (22)*</b>	738.47 (28.46)	<b>4912.15 (1064.87)*</b>	5904.73 (915.23)

**Table 3.5 Effects of rr on latency to make a correct response and latency to re-start a trial after a time-out punishment. Latency to make a correct response changed across rrs and impulsivity groups.** Mean and standard error (SE) in brackets. There was a main effect of rr [ $F(1,19)=5.36, p=0.032$ ] and a trend for a main effect of impulsivity [ $F(2,19)=3.42, p=0.054$ ]. Specifically, animals were faster at making a correct response in rr 1 compared to rr 0.5 ( $p=0.032$ ), with HI rats being non-significantly faster than MID ( $p=0.066$ ) and LI ( $p=0.097$ ). When focusing on rr 0.5 only, latency to poke into the food magazine following a correct response was found to depend on trial outcome [ $F(1,19)=102.22, p<0.001$ ]. Rats were faster at poking into the food magazine following a correct response when such response was rewarded, compared to when it was not ( $p<0.001$ ). Latencies to re-start a trial following a time-out depended on rr [ $F(1,19)=11.98, p=0.002$ ], with animals being faster at re-starting a trial during rr 1 as opposed to rr 0.5 ( $p=0.003$ ). \*statistically significant difference with rr 0.5,  $p<0.05$

The number of premature responses were then compared across rrs and impulsivity groups. As shown in **Figure 3.9a** there was a main effect of rr [ $F(1,19)=10.37, p=0.005$ ] and a main effect of impulsivity groups [ $F(2,19)=9.38, p=0.001$ ]. Specifically, animals made more premature responses during rr 1 as opposed to rr 0.5 ( $p=0.005$ ). Across conditions, HI rats made more premature responses than MID ( $p=0.002$ ) and LI ( $p=0.008$ ). In rr 1 only there was a significant negative correlation between number of prematures and latency to make a correct response, rr 1:  $r=-.51, p=0.016$ . FP perseverative responses were analysed to test for effects of frustration on these responses, results are summarised in **Figure 3.9b**.



**Figure 3.9 Effects of rr, during 7 s ITI challenge, on a) premature responses and b) ratio of perseverative responses in the FP.** For proportion of FP perseverative responses there was a main effect of response type [ $F(3, 57)=22.16, p<0.001$ ]. Specifically, animals made proportionally fewer FP perseverative responses during R trials compared to all other trial types ( $p<0.001$  all comparisons). a) #significant difference with MID and LI impulsivity groups ( $p<0.05$ ); \*significant difference with rr 0.5 ( $p<0.05$ ); b) \* significant difference with NR, incorrect and premature response types ( $p<0.05$ )

The latency to make a premature in trial  $t$  was also analysed to look for frustration effects. For this analysis, the time-window considered was that within which the rat makes a correct response in trial  $t$  and following this makes a premature response in trial  $t+1$ . More time passed between these two responses when the animal made a NR trial as opposed to a R trial [ $F(1,19)=16.20, p<0.001$ ]. Finally, when comparing the latency of responses following either a R trial during rr 0.5, a NR trial during rr 0.5 and a correct trial during rr 1, there were no main effect of session.

### Summary

Partial reinforcement led to a decrease in incentive motivation, with animals being slower at making correct responses and starting new trials during rr 0.5 as opposed to rr 1. Extending the ITI increased premature responses, however similarly to what was observed during the 5 s ITI sessions, more premature responses occurred during rr 1 as opposed to rr 0.5. Also, in line with results from the 5 s ITI sessions, animals made fewer FP perseverative responses during R trials compared to the other response types. Finally, the time that passed between the rat making a correct response in trial  $t$  and making a premature response in trial  $t+1$  was longer for premature responses occurring following a NR response. This is probably due to the fact that during NR trials rats are making more FP perseverative responses, compared to R trials.

### 3.3.2.2 Consequences of a rewarded or non-rewarded trial on premature responses

The transition probabilities between trial types were modelled with a Markov chain, to test whether animals were more likely to make a premature response after a frustrative event, such as a NR trial. Because HI rats made significantly more premature responses than the other groups, two separate Markov chain models were fitted, one for HI rats and one for LI and MID rats combined. Both Markov chains models violated the independence models: HI rats had a W statistic of 54.62  $p < 0.001$ , while the combined group had a W of 152.51,  $p < 0.001$ .

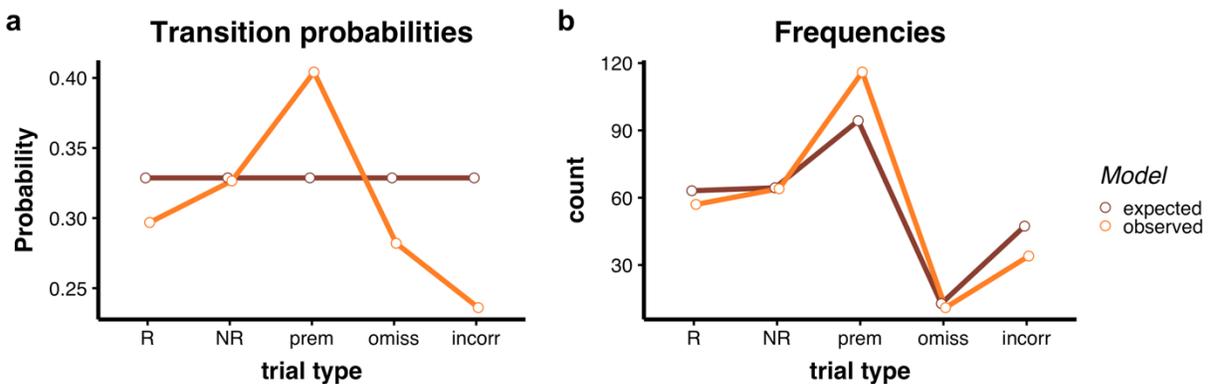


Figure 3.10 Experiment 2. Cohort 1. HI rats. (a) Transition probabilities and (b) frequencies of one-step transitions leading to a premature response (end state). x axis shows starting states. Brown = independence model; Orange = observed data.

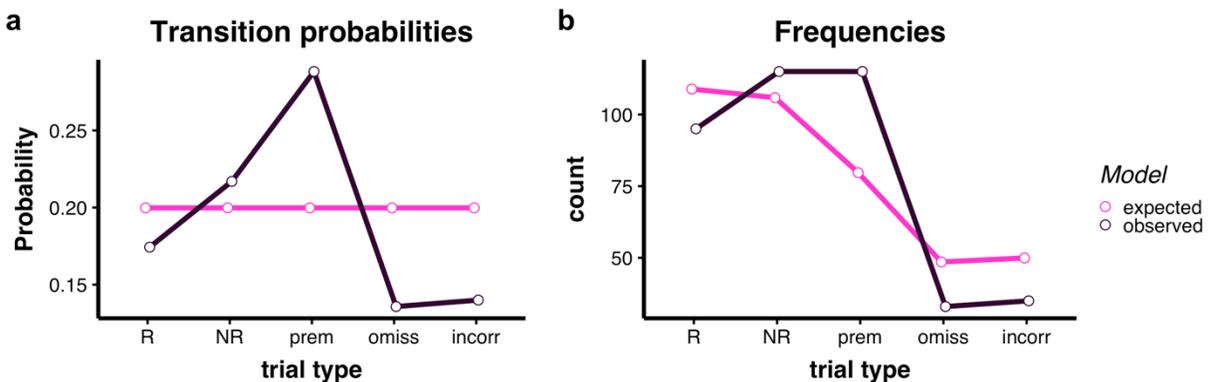


Figure 3.11 Experiment 2. Cohort 1. MID and LI rats. (a) Transition probabilities and (b) frequencies of one-step transitions leading to a premature response (end state). x axis shows starting states. Pink = independence model; Purple = observed data.

**Figure 3.7A** shows which specific transition probabilities deviated strongly from the independence model, both for HI and the combined group. A chi-square test on the frequencies of one-step transitions leading to a premature response showed that, for HI rats, these were not significantly different from an expected distribution under the independence model  $X^2=9.58$ ,  $p>0.05$  (see **Figure 3.10a and b**), however for the combined group they were,  $X^2=27.72$ ,  $p<0.001$  (see **Figure 3.11a and b**). For the combined group, the biggest discrepancy was that of a higher probability of a premature response following a premature response ( $Y=3.95$ ). The same tests were run considering frequencies of one-step transitions starting from R and NR trials. For more details see Appendix A (section A1.2.2.2).

#### In summary

There are dependencies between trials in a partial reinforcement session with 7s ITI. When focusing on transition probabilities to make a premature response, the greatest deviation from the independence model, across groups, is a higher likelihood to make a premature response after a premature response. However, this was significant only for the combined group of MID and LI rats.

### **3.3.3 Experiment 3 and 4 - Effects of reinforcement omission and timeout reduction on premature responses**

In light of evidence showing that a continuous, as opposed to a partial reinforcement rate increases premature responses, I next tested whether increasing the pace of the task (and in this way the reinforcement rate) by reducing the time-out had an effect on the number of premature responses. Reducing the timeout also decreases the punishment for premature responses, thus another important point of these Experiments was to verify the extent to which reducing the punishment for premature responses increases the likelihood of performing this response type. Finally, it was also interesting to explore whether some of the transition probabilities observed in the rr 0.5 condition were influenced by the 5 s timeout. For these reasons, a group of rats (cohort 1) were tested with a reduced time-out both on rr 1 and on rr 0.5. Experiment 3 tested this manipulation during a standard 5 s ITI paradigm and is described in Appendix A (section

A.1.2.3). Experiment 4 (described below) tested this manipulation during a 7 s ITI paradigm, which is known to exacerbate the occurrence of premature responses.

### 3.3.4.1 Results

**Table 3.6** and **3.7** summarise results for latency to make a correct response and latency to re-start a trial after a time-out, respectively. Briefly, rats were faster at making a correct response and at re-starting a trial during rr 1 compared to rr 0.5 only when the time-out was set to 5 s.

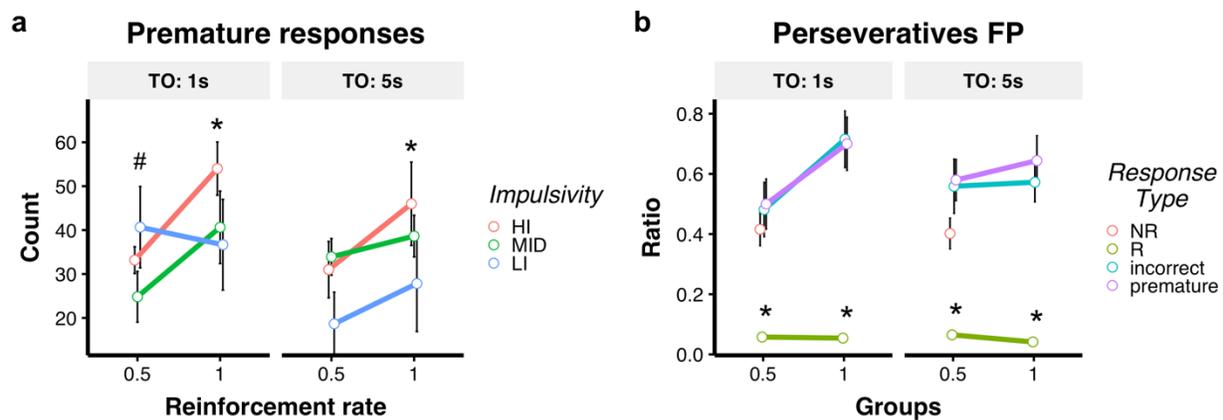
Latency to make a correct response				
	rr 1		rr 0.5	
	timeout 5 s	timeout 1 s	timeout 5 s	timeout 1 s
HI	588.43 (39.25)*	579.84 (25.60)	745.73 (62.73)*	625.641 (31.0)
MID	595.37 (20.12)* <sup>§</sup>	676.07 (41.06)	617.18 (36.79)* <sup>§</sup>	705.913 (51.07)
LI	650.22 (24.06)* <sup>§</sup>	619.604 (42.59)	860.31 (91.905)* <sup>§</sup>	632.20 (42.12)

**Table 3.6 Effects of rr and timeout on latency to make a correct response.** Mean and standard error (SE) in brackets. There was an interaction between rr and time-out [ $F(1,60)=4.95$ ,  $p=0.030$ ] and between time-out and impulsivity [ $F(2,60)=11.23$ ,  $p<0.001$ ]. In details, when time-out was 5 s rats were significantly faster at making correct responses during rr 1 compared to rr 0.5 ( $p<0.001$ ). The only difference between impulsivity groups that was observed was during 5 s timeout with MID rats being faster than LI rats ( $p=0.033$ ), this difference disappeared in the 1 s time-out because LI rats got significantly faster with this time-out compared to the 5 s time-out ( $p=0.002$ ). \*statistically significant difference in time-out 5 s between rr 0.5 and rr 1.

<sup>§</sup>statistically significant difference in time-out 5 s between MID and LI rats.

Latency to re-start a trial after a punishment time-out				
	rr 1		rr 0.5	
	timeout 5 s	timeout 1 s	timeout 5 s	timeout 1 s
HI	3307.84 (788.582)*	4327.87 (623.41)	5349.39 (1555.66)*	3401.49 (441.42)
MID	3426.49 (544.68)*	4629.51 (448.24)	6029.43 (1884.79)*	6557.83 (1386.78)
LI	6286.08 (977.66)*	8610.22 (3371.57)	21155.63 (15223.82)*	5612.97 (1274.66)

**Table 3.7 Effects of rr and timeout on latency to re-start a trial after a timeout punishment.** There was an interaction between rr and time-out [ $F(1,60)=5.04$ ,  $p=0.0284$ ]. In details, when time-out was 5 s rats were significantly faster at re-starting a trial during rr 1 compared to rr 0.5 ( $p=0.012$ ). \*statistical significance difference in time-out 5 s between rr1 and rr0.5. §statistical significance difference in timeout 5 s between MID and LI rats.



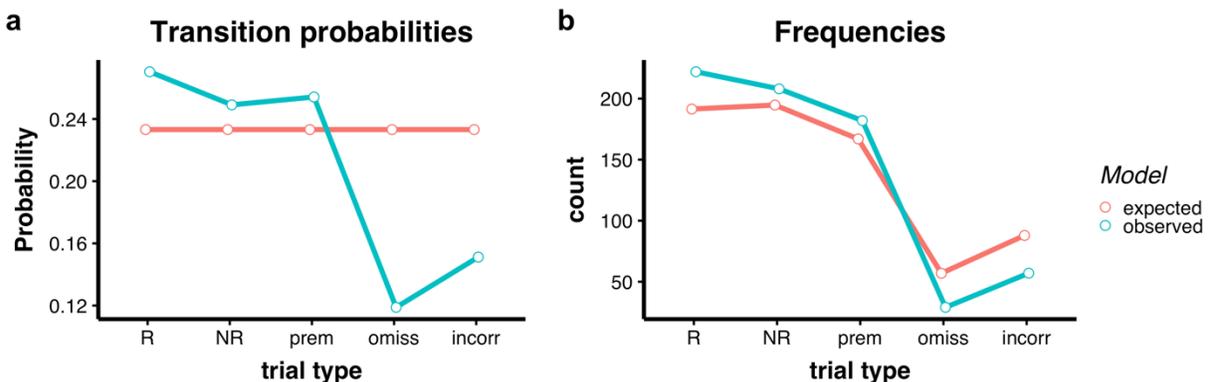
**Figure 3.12 Effects of rr and time-out on (a) premature responses and (b) ratio perseverative responses in the FP.** (a) \*statistical significant difference with rr 0.5,  $p<0.05$ . (b) For the ratio of perseverative responses in the FP there was a main effect of response type [see Figure 3.14b,  $F(3,276)=129.82$ ,  $p<0.001$ ], with rats making proportionally fewer responses during R trials compared to all other trial types ( $p<0.001$ ). TO = time-out. \*significant difference of perseverative responses in R vs those in all other response types  $p<0.05$ .

As shown in **Figure 3.12a**, for premature responses there was a main effect of rr [ $F(1,60)=6.52$ ,  $p=0.013$ ] and an interaction between time-out and impulsivity [ $F(2,60)=3.74$ ,  $p=0.029$ ].

Specifically, animals made more premature responses during rr 1 as opposed to rr 0.5 ( $p=0.013$ ) and only LI made more premature responses when the time-out was 1 s as opposed to when it was 5 s ( $p=0.018$ ). In addition to this, HI and MID rats made more premature responses than LI

in the 5 s time-out session, however this only showed a trend level of significance ( $p=0.074$  and  $p=0.052$ , respectively). There was a strong negative correlation between making a correct response and premature responses in all sessions except during rr 1 with 1 s time-out, thus: for rr 0.5 and 1 s time-out  $r=-0.69$ ,  $p<0.001$ ; for rr 0.5 and 5 s time-out  $r=-0.52$   $p=0.010$ ; for rr 1 and 5 s time-out  $r=-0.47$   $p=0.025$ . **Figure 3.12b** summarises results for ratio of FP perseveratives. Focusing just on the rr 0.5, there was a main effect of response outcome for time elapsed between making a correct response in trial  $t$  and making a premature response in trial  $t+1$  [ $F(1,58)=9.74$ ,  $p=0.002$ ]. Specifically, significantly more time elapsed between premature responses and NR trials, when compared to R trials ( $p=0.003$ ).

A Markov chain model was fit to the data from the rr 0.5 session with 1s time-out. Because there were not any differences in premature responses across impulsivity groups, a transition probability matrix was created pooling all animals together. The transition probability matrix violated the independence model with a  $W$  of 118.50,  $p<0.001$ . **Figure 3.17A** shows the observations that deviated the most from the independence model. A chi-square test on the frequencies of one-step transitions leading to a premature response showed that these deviated significantly from the independence model,  $X^2=31.68$ ,  $p<0.025$ .

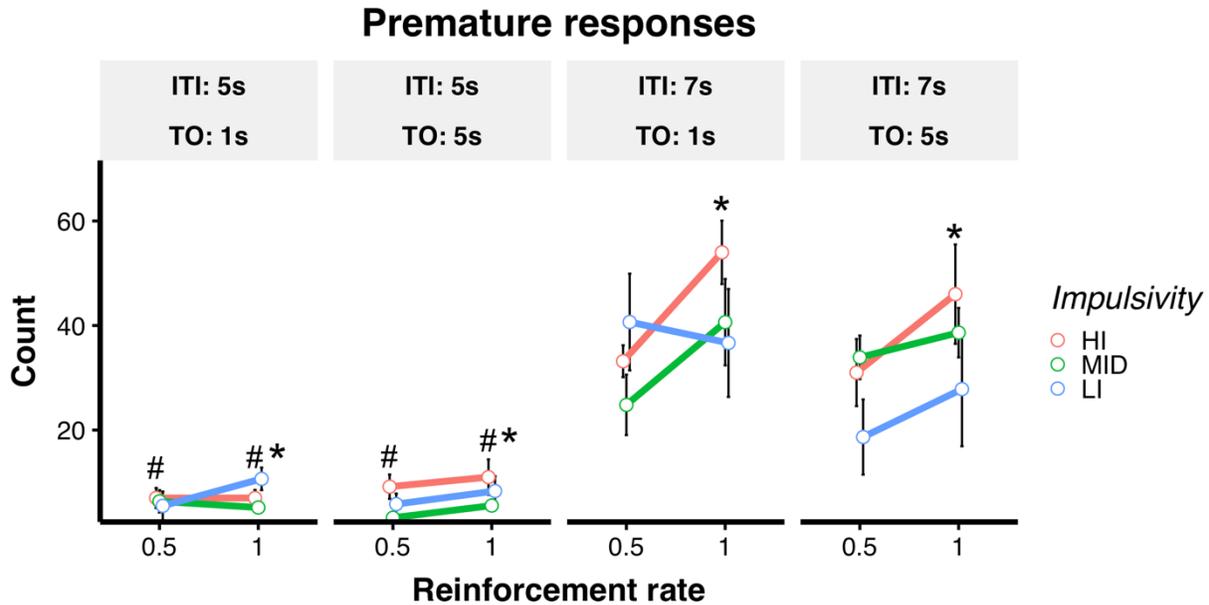


**Figure 3.13** Experiment 4. Cohort 1. (a) Transition probabilities and (b) frequencies of one-step transitions leading to a premature response (end state). x axis shows starting states. Red = independence model; Blue = observed data.

**Figure 3.13a** and **b** show how the transition probabilities and frequencies of one-step transitions, respectively, leading to a premature response deviated from the independence model. The biggest deviations from the independence model were a lower-than-expected probability to make a premature after an omission response ( $Y=-3.70$ ) and after an incorrect response ( $Y=-3.30$ ). The

same tests were run considering frequencies of one-step transitions starting from R and NR trials. For more details on this see Appendix A (section A1.2.4.1).

### 3.3.4.2 Comparison with previous manipulations with 5 s ITI



**Figure 3.14** Effects of rr, time-out and ITI on premature responses across experiments. TO = time-out. #significantly different from ITI 7s. \*significantly different from rr 0.5.

Data from all manipulations was then merged to create a model that included two different ITIs: 5 s and 7 s; two different time-outs: 5 s and 1 s; and two different rrs: 1 and 0.5. The factors that affected premature responses the most were then explored. As shown in **Figure 3.14**, the model revealed a main effect of rr [ $F(1,136)=10.21$ ,  $p=0.002$ ], a main effect of ITI [ $F(1,136)=264$ ,  $p<0.001$ ], and an interaction between time-out, ITI and impulsivity [ $F(2,136)=3.51$ ,  $p=0.033$ ]. *Post-hoc* contrasts showed that rats made more premature responses during rr 1 compared to rr 0.5, across all manipulations ( $p=0.002$ ). In addition, all impulsivity groups made more premature responses during the 7 s ITI sessions compared to the 5 s ITI sessions ( $p<0.01$  for all comparisons). Differences between impulsivity groups were only seen in the 7 s ITI, 5s time-out session with HI rats making more premature responses than LI and MID rats ( $p<0.05$ ). Finally,

differences in timeout only affected LI rats in a 7 s ITI session, where more premature responses occurred during a 1s timeout session compared to a 5 s timeout session.

#### In summary

A reduced timeout in a 7 s ITI session did seem to affect motivation to perform the task, at least in the partial reinforcement sessions, with rats being faster under rr 0.5 and 1 s time-out than under rr 0.5 and 5 s time-out. With regards to premature responses, rats made more premature responses during rr 1 and 7 s ITI sessions. Changing the timeout did not significantly impact premature responses, except for LI rats, who tended to make more premature responses when the time-out was 1 s as opposed to 5 s. A 1 s timeout also affected the transition probabilities leading to a premature response, when compared to a rr 0.5, 5 s time-out session. Specifically, while in the rr 0.5, 5 s timeout session rats were more likely to make a premature response after a premature response, however during a rr 0.5, 1 s timeout session rats were as likely to make a premature response after a NR response as they were after a premature response, in line with data from the 5 s ITI, 1 s time-out sessions.

### **3.3.5 Experiment 5 - Effects of reinforcement omission and reward magnitude on premature responses**

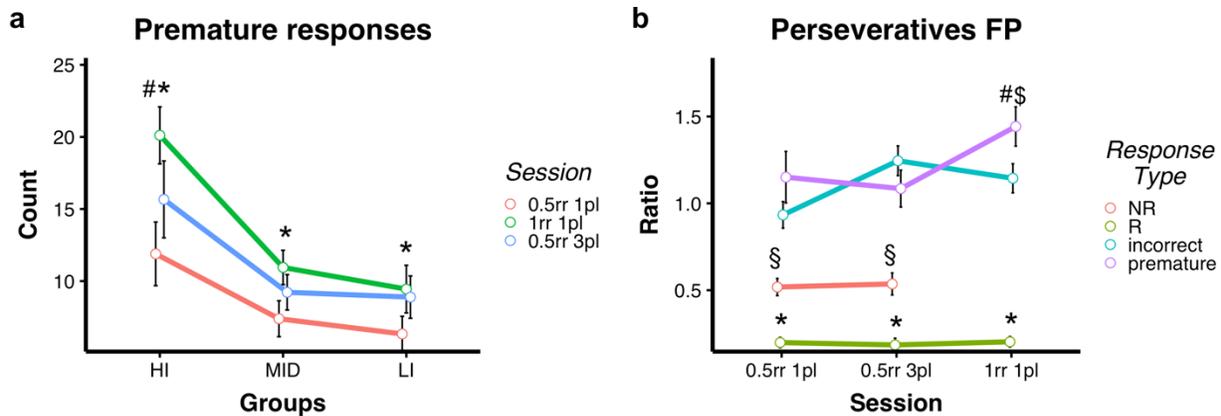
Finally, the effect of increasing the reward magnitude in a partial reinforcement setting on premature responses was investigated. This experiment tested the frustration hypothesis, the sensitivity to reward hypothesis, and the incentive hope hypothesis. To test this, performance in a partial reinforcement setting with a reward of the size of 3 pellets was compared with performance on rr 0.5 and rr 1 with a reward of the size of 1 pellet.

### 3.3.5.1 Results

	rr 0.5, 1 pellet	rr 0.5, 3 pellets	rr 1, 1 pellet
HI	645.86 (48.92) <sup>§</sup>	746.761 (61.32)* <sup>§</sup>	612.22 (37.07) <sup>§</sup>
MID	772.86 (35.96)	822.785 (32.28)*	675.44 (23.63)
LI	795.70 (48.07)	830.13 (49.13)*	778.53 (24.44)

**Table 3.8 Effects of rr and pellet size on latency to make a correct response.** Mean (in ms) and standard error (SE) in brackets. There was a main effect of session [ $F(2,66)=11.66$ ,  $p<0.001$ ] and of impulsivity groups [ $F(2,33)=3.44$ ,  $p=0.044$ ]. Specifically, animals were slower in the 3-pellets condition compared to the 1-pellet condition with rr 0.5 ( $p=0.024$ ) and the 1-pellet condition with rr 1 ( $p<0.001$ ). HI rats were faster across conditions compared to LI rats ( $p=0.040$ ). \*significantly different from the other conditions  $p<0.05$ . <sup>§</sup>significantly different from LI rats  $p<0.05$ .

**Table 3.8** shows that rats were slower in the 3-pellets condition compared with the 1-pellet condition with rr 0.5 and the 1-pellet condition with rr 1. In addition, HI rats were faster across conditions compared to LI rats. When looking at latency to re-start a trial following a timeout, rats were on average faster in the 3-pellets condition compared to the 1-pellet condition, but this did not reach significance. Further analyses were carried out to investigate the slowing of responses in the 3-pellets condition. These are described in detail in Appendix A (section A1.2.5.1). Briefly, rats made more perseverative responses in the rear panel (RP) following a R trial in the 3-pellets condition compared to 1-pellet condition (rr 0.5), leaving the food magazine later in the next trial. This likely caused a slowing of responses and an increase in omissions in the 3-pellets condition compared with 1-pellet condition (rr 0.5).



**Figure 3.15** Effects of rr and reward size on a) premature responses and b) proportion of FP perseverative responses. For ratio of FP perseverative responses there was a significant interaction between these session and response type [ $F(5,329)=3.41$ ,  $p=0.005$ ]. Post-hoc comparisons showed that, across pellet conditions, rats made proportionally fewer FP perseverative responses during R trials compared to all the other response types ( $p<0.001$ ). In the rr 0.5 sessions rats also made fewer FP perseverative responses during NR trials compared to premature and incorrect trials ( $p<0.001$  for both comparisons) while there was no difference in FP perseverative responses between incorrect and premature responses. Finally, the only differences between sessions was an increase of FP perseverative responses during incorrect trials in the rr 0.5, 3 pellet condition compared to the rr 0.5, 1 pellet condition ( $p=0.003$ ); and an increase of FP perseverative responses during premature trials in the rr 1 condition compared to both the rr 0.5 conditions ( $p<0.005$ );

Premature responses made during continuous reinforcement (rr 1, 1 pellet) were compared against those made during the two partial reinforcement sessions (rr 0.5, 1 and 3 pellets). As **Figure 3.15a** shows, there was both an effect of session [ $F(2,66)=8.73$ ,  $p<0.001$ ] and of impulsivity groups [ $F(2,33)=9.27$ ,  $p<0.001$ ]. Specifically, rats made fewer premature responses during the rr 0.5 with 1 pellet compared to the rr 1 ( $p<0.001$ ) and rr 0.5 with 3 pellets ( $p=0.042$ ). In addition, HI rats made more premature responses than MID and LI ( $p<0.01$  for both). It was then evaluated whether latency to make a correct response correlated negatively with number of premature responses, across pellets. Considering data showing that animals slow down after R trials in the 3-pellets condition (as described in Appendix A section A1.2.5.1), the latency to make a correct response after a R trial was analysed separately from latency to make a correct response after a NR trial. Thus, when considering the former, there was a significant negative correlation between premature responses and latency to make a correct response for the 1-pellet condition only,  $r=-0.50$ ,  $p=0.002$ . However, when looking at latency to make a correct response after a NR trial there was a correlation between this measure and premature responses in both pellet conditions: 1 pellet  $r=-0.43$ ,  $p=0.008$ ; 3 pellets:  $r=-0.49$ ,  $p=0.003$ . **Figure 3.15b** summarises results on the proportion of FP perseverative responses as a function of sessions (rr 0.5, 1 pellet vs rr 1, 1 pellet vs rr 0.5, 3 pellets) and response types.

### **3.3.5.2 Consequences of rewarded or non-rewarded trials on premature responses**

A Markov chain model was fitted to the data on the rr 0.5 session both with 1 and 3 pellets. Because there were differences in premature responses between HI rats and the two other groups, for each pellet condition two separate transition probability matrices were created, one for HI rats and one pooling together MID and LI rats (combined group).

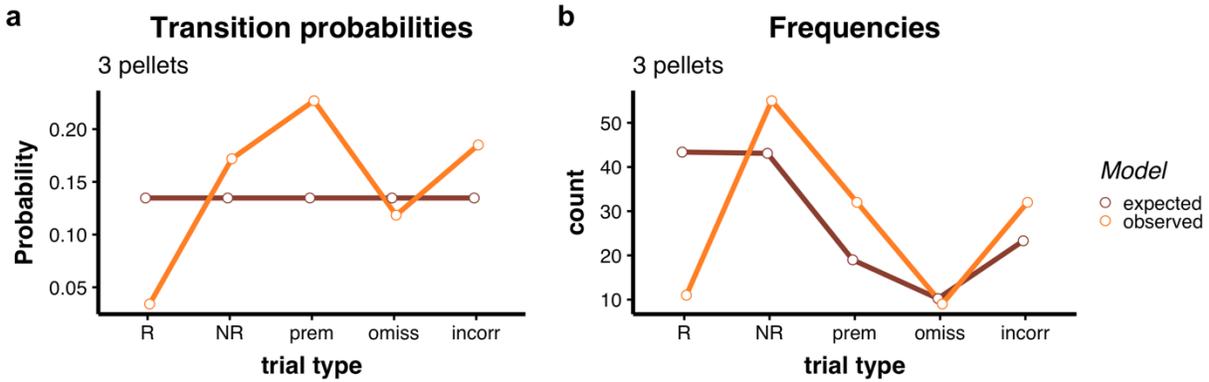
#### **3.3.5.2.1 One pellet condition (Experiment 5a)**

Because a Markov chain model has already been fitted on performance of this cohort on a rr 0.5, 1 pellet condition (see Experiment 1, section 3.3.1.3), analyses of this condition are reported in Appendix A (section A1.2.5.2.1).

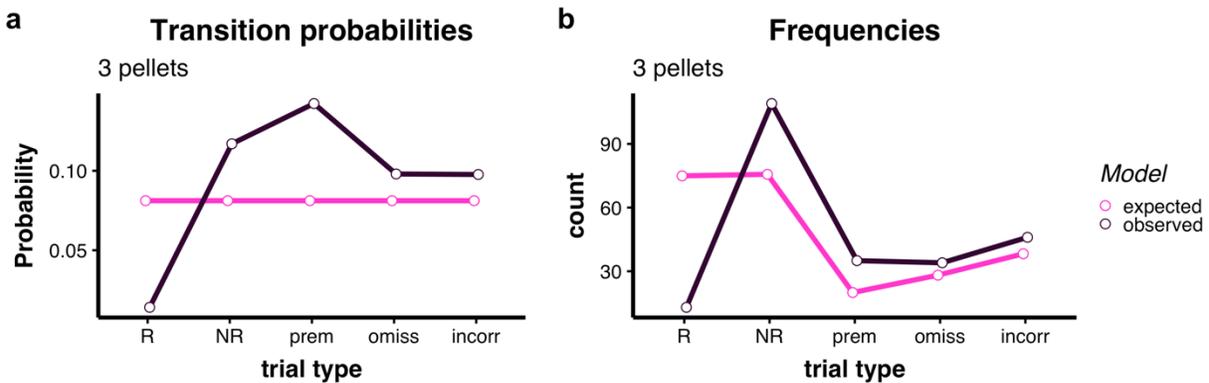
#### **3.3.5.2.2 Three pellets condition (Experiment 5b)**

For both HI rats and the combined group, the transition probability matrices violated the independence model: HI rats exhibited a W statistic of 95.76 ( $p < 0.001$ ) while the combined group had a W statistic of 262.41 ( $p < 0.001$ ). **Figure 3.20A** shows which transition probabilities deviated the most from the independence model. A chi-square test on the frequencies of one-step transitions leading to a premature response showed that, both for HI rats and for the combined

group, there were significant differences from the independence model,  $X^2=39.75$  and  $X^2=79.96$ , respectively ( $p<0.001$  in both cases).



**Figure 3.16** HI Experiment 5b. Cohort 2. HI rats. (a) Transition probabilities and (b) frequencies of one-step transitions leading to a premature response (end state). x axis shows starting states. Dark brown = independence model; Orange = observed data.



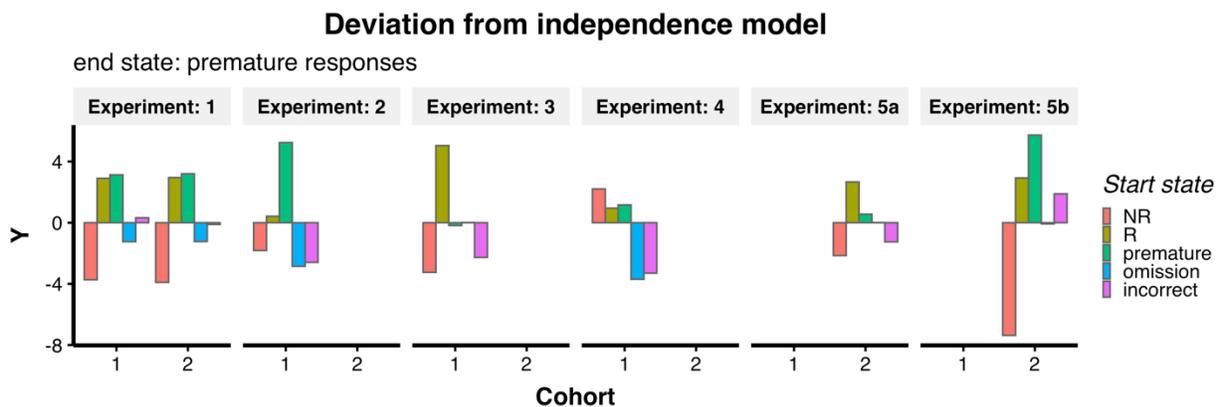
**Figure 3.17** Experiment 5b. Cohort 2. MID and LI rats. (a) Transition probabilities and (b) frequencies of one-step transitions leading to a premature response (end state). x axis shows starting states. Light pink = independence model; Dark purple = observed data.

For HI rats, **Figure 3.16a** and **b** show how the transition probabilities and frequencies of one-step transitions, respectively, leading to a premature response deviated from the independence model. For the combined group, **Figure 3.17a** and **b** show how transition probabilities and frequencies of one-step transitions, respectively, leading to a premature response deviated from the independence model. For HI rats, the biggest deviation from the independence model was a lower likelihood of making a premature response after a R trial ( $Y=-4.92$ ); similarly, for the combined group the biggest deviation from the independence model was a very low likelihood of making a premature response after a R trial ( $Y=-7.16$ ). The same tests were run considering

frequencies of one-step transitions starting from R and NR trials. For more details see Appendix A (section A1.2.5.2.2).

In summary

Somewhat surprisingly the partial reinforcement 3-pellet condition presented the slowest latencies to respond to correct trials. This was probably due to animals being slowed down by the collection and consumption of a 3-pellet reward, since the slowest response latencies occurred following R trials. In line with this, following these trials, animals made more RP perseverative responses and left the RP later in time within the next trial, thus having less time to orient towards the FP for the upcoming new trial. With regards to premature responses, the effect of rr on these trial types was confirmed, with animals making the most premature responses during continuous reinforcement. Interestingly, during partial reinforcement, animals made more premature responses when the reward magnitude was greater, thus in the 3-pellet condition. The effect that a greater reward pellet had on performance was also evident from the Markov chain transition probability matrices, where the strongest deviations from the independence model occurred following a R trial.



**Figure 3.18** Summary of the deviation from the independence model of transition probabilities leading to premature responses, across all experiments.

For an overview of the extent to which transition probabilities leading to a premature response deviated from the independence model in all Experiments, see **Figure 3.18**.

## 3.4 Discussion

These findings show that rr has an impact on premature responses and, importantly, analyses both at the *macro*- and *micro*-level support the sensitivity to reward hypothesis as a valid framework for understanding the occurrence of premature responses.

Across all manipulations, a continuous reinforcement rate (rr 1) was shown to speed latency to make a correct response when compared to partial reinforcement paradigms (rr 0.2; rr 0.5; rr 0.8). This finding is consistent with previous research and has been interpreted as indicating increased motivation to engage with the operant task (Mohebi et al., 2019; Hamid et al., 2016; Niv et al., 2007). Indeed, response vigour has been postulated to be controlled by the opportunity cost of not acting, whereby shorter latencies are worth the energetic cost if they allow the individual to gain a higher amount of reward per unit of time (Niv et al., 2007). Concomitant with a shortening of latencies, during continuous reinforcement, there was also an increase in the number of premature responses (*macro*-level analysis). Importantly, across almost all manipulations latency to make a correct response correlated negatively with the number of premature responses. Thus, under the assumption that latency to make a correct response reflects increased motivation to engage with the operant task, these findings show that premature responses are influenced by increases in motivation. In line with this, increasing the reward magnitude from 1 pellet to 3 pellets also elevated the number of premature responses.

The present results thus support the sensitivity to reward hypothesis and are against both the frustration and incentive hope hypothesis which predict, albeit for different reasons, an increase in premature responses during partial reinforcement. Specifically, the incentive hope hypothesis (Anselme, 2015; Anselme and Robinson, 2019) postulates that during partial reinforcement, uncertain rewards acquire greater incentive value than certain rewards because each successful outcome is never fully predicted and thus it is valued to a greater extent than during continuous reinforcement. ‘Incentive hope’ refers to the increase in value attributed to rewarded outcomes in the context of non-rewarded events. Further, Anselme (2015) explains that, at the behavioural level, incentive hope is expressed as increased salience attributed to the CS and thus stronger

approach behaviour, or conditioned response (CR). This theory posits to explain why certain activities that involve uncertain rewards such as gambling become attractive (Anselme & Robinson, 2013; Costikyan, 2013), and continue to be pursued despite a history of unsuccessful outcomes (Breen & Zuckerman, 1999). With relevance to the animal literature, this theory developed in light of evidence that animals trained on a PavCA task display stronger CR (i.e., lever presses) during partial reinforcement as opposed to continuous reinforcement. Specifically, reward uncertainty was shown to increase the attractiveness of the CS, which in turn led to shorter latencies to approach the CS-lever and increased ST behaviour.

Sign-trackers have been shown to display increased action impulsivity (Lovic et al., 2011; King et al., 2016) and both ST and motor impulsivity have been associated with elevated DA release in the NAcB (Dalley & Robbins, 2017; Flagel et al., 2011) and the development of SUDs (Dalley, et al., 2007a; Saunders & Robinson, 2010). Premature responses can be thought of rapid responses directed at, or better anticipating, the CS, thus there were reasons to believe that reward uncertainty could increase the display of these responses under the incentive hope hypothesis, however this was not the case. One reason why the incentive hope hypothesis might not be appropriate for the present context is that this theory was developed within the settings of a Pavlovian learning task, where the display of excessive or minimal CR does not have direct consequences on reward outcome. The 5CSRTT, however, is an operant task in which specific kinds of responses, for example premature responses, are punished. Thus, it is possible that in the context of an operant task, where actions matter when it comes to securing a reward, different psychological mechanisms are at play in shaping action selection and operant responding. To this end, it would be interesting to run the same manipulations that we did but removing the punishment from premature responses altogether. Experiment 3 and 4 came close to answering this question, as the time-out punishment here was reduced from 5 s to 1 s. However, this only led to an increase in premature responses in LI rats in Experiment 4, that is during the 7 s ITI session. There were no other changes in the other impulsivity groups in Experiment 4 nor, across groups, in Experiment 3, where the shortened time-out was paired with a 5 s ITI. Even when there was an increase in premature responses in LI rats during rr 0.5 with 1s time-out, these were not statistically greater than those recorded during rr 1 with 1 s time-out thus making it unlikely that incentive hope was driving such increase in premature responses. The theory indeed poses

that CR should be greater than that observed in contexts with reward certainty (Anselme, 2015). On the contrary, the increment in premature responses in LI rats during rr 0.5 with 1 s time-out was concomitant with a decrease in latencies to make a correct response. Thus, instead, it is possible that reducing the time-out increased the pace of the task (and thus the rr) which in turn rescued, in LI rats, some of the slowing effects induced by partial reinforcement, driving more premature responses. This interpretation would be further evidence in support of the sensitivity to reward hypothesis.

With regards to the frustration hypothesis, this posits that the omission of an expected reward generates frustration, which is experienced as a negative emotion and, behaviourally, has the capacity to invigorate the dominant response (Amsel, 1992; Papini, 2003, 2006). Some of the reported aftereffects of frustration are shorter latencies on subsequent operant responses (Amsel and Russel, 1952) or increased lever presses (Judice-Daher et al., 2012; Stout et al., 2003). In the present context, this theory would predict that when the animal is not rewarded on some of the correct trials, during partial reinforcement, the frustration-induced invigoration of behaviour could facilitate approach to the FP prematurely and nose-poke, as this is the dominant response in the 5CSRTT. The idea that the frustration hypothesis could explain, in part, the occurrence of premature responses was supported by evidence that, during continuous reinforcement, animals tend to make premature responses after non-rewarded trials (Donnelly et al., 2014). To test for this more precisely, we introduced correct non-rewarded (NR) responses as these are trials that violate the expectation of a reward and thus should be the most frustrating events within a session. As mentioned however, *macro*-level analyses of performance did not reveal an increase in premature responses during sessions with partial reinforcement. This was the case also for sessions with a greater proportion of NR trials, that is rr 0.2, which were conducted to test one of the tenets of the frustration hypothesis, advanced by Amsel (1992), whereby frustration should ‘increase in strength as a function of nonrewarded trials’ (p. 43).

While *macro*-level analyses of performance did not reveal an effect of frustration on premature responses, it is still possible that the occurrence of premature responses in trial  $t$  is influenced by the frustration of a non-reward in trial  $t-1$ , be this a NR or a time-out punishment following the occurrence of incorrect, premature or omissions responses. For this reason, evaluation of

performance on a trial-by-trial basis, i.e., at the *micro*-level, was implemented on rr 0.5 sessions, which have an equal distribution of R and NR trials and thus allow a more controlled assessment of the influence of frustration on premature responses. In the *micro*-level analysis two main things were evaluated: the likelihood of a premature response to occur after a specific trial type (R, NR, premature, omission or incorrect response) and the delay between making a correct response in trial  $t$  and making a premature response in trial  $t+1$ . This temporal gap between responses was not analysed for premature, omission and incorrect responses as this would be confounded by the presence of a 5 s timeout punishment.

To test for dependencies between trial types, and for example to test whether premature responses are more likely to follow specific responses, a first-order Markov chain model was fit to all the possible transitions between trials and across animals. A first-order Markov chain is a probability model for certain time series in which the probability to transition to a future state depends solely on the current state (Anderson and Goodman, 1957). This model was adopted to test whether engaging in a specific response in trial  $t+1$  depends on trial  $t$ . The model creates a matrix of transition probabilities between all possible trial types, that is: premature, R, NR, omission and incorrect. Once a matrix of transition probabilities was obtained, a diagnostic test was run to verify whether the probabilities to transition from one trial type to another were statistically different from an ‘independence’ model, that is a model where trial types follow each other without there being any relationship between these transitions (i.e., by chance). In Experiment 1 and 2, animals were more likely to transition to a premature after having done a NR trial and, with a slightly higher probability, after having done a premature response. In addition to this, rats were much less likely than ‘chance’ to make a premature response after a R trial. This could potentially support the frustration hypothesis if premature responses occurring in the preceding trial were considered frustrating events (see below for a discussion on this). However, analysis of the time elapsed between making a correct response (NR or R) in trial  $t$  and a premature response in trial  $t+1$ , showed that in almost all Experiments less time elapsed between making an R response in trial  $t$  and a premature response in trial  $t+1$ . This does not support the frustration hypothesis as this would predict that a premature response, by being the expression of frustration, occurs immediately after the loss of the reward. The only instances in which the temporal gap between premature responses and R trials was not smaller than that

between premature responses and NR trials, was in Experiment 3 and 5 where time elapsed between correct responses and premature responses did not differ on the basis of outcome of the correct response. One reason why more time elapsed between making a NR response in trial  $t$  and making a premature response in  $t+1$ , was because upon realising that a correct response was not rewarded, rats engaged in many FP perseverative responses. These responses were thus analysed to evaluate whether these could be signs of frustration. The most consistent finding was that rats tended to make proportionally fewer FP perseveratives during a R trial compared to all other trial types. This was presumably the case because as rats detected that a reward had been delivered, during R trials, they would rush to the food magazine to collect it and thus not spend time making perseverative responses in the FP. This could indicate that FP perseverative responses occur as a result of frustration-dependent invigoration of behaviour.

As mentioned, rats were more likely to make a premature response after another premature response. The few instances in which this was not the case were: in Experiment 5a (which has the same settings as Experiment 2 but without being part of a larger Latin square design of other rrs) and in Experiment 3 and 4, where the time-out was reduced to 1 s. This points to an interesting effect of the time-out, whereby in its longer, 5 s version it seems to increase the propensity to make premature responses in succession. This could be because a wider temporal gap between trials disorients the rat and interferes with the timing of the task, thus perhaps driving a premature response in the trial following this gap. Another reason why longer time-outs could be driving premature responses in succession of one another could be due to the fact that a lengthy time-out by definition increases the waiting period, thus perhaps augmenting urgency and thus driving a greater propensity to make a premature response in the following trial. Both interpretations would be in line with evidence that trials ending in premature responses have a faster start of the waiting period (Donnelly et al., 2015). However, if a 5 s timeout was indeed driving premature responses for the reasons described above, one would expect premature responses to occur following all trial types that are punished by a timeout, including incorrect and omission responses (not just premature responses). However, in sessions with 5 s timeout, premature responses were less likely than 'chance' to occur after incorrect or omission responses. Thus, there may be other reasons why rats tended to make a premature response following another premature response. One way to reconcile these findings with the frustration

hypothesis would be to equate premature responses to NR trials and thus to postulate that rats do not predict to be punished for making a premature response (thus being surprised and frustrated when this happens). Punishing premature responses would violate the expectation of a reward if rats either 1) do not realize that they are responding prematurely or 2) do realise that they are responding earlier in time but are slower at learning that such a response is punished with a time-out. The former case begs the question of why rats would respond 'randomly' in any hole (i.e., uncued), however rats do that in the case of incorrect responses, presumably because they do not know or remember which hole of the five apertures illuminated and make a guess as to where the cue might have appeared. Incorrect (and omission) responses have much slower latencies compared to correct responses, suggesting that rats have low confidence when they make such choices. This is not the case for premature responses, which are very rapid responses, casting doubts on the validity of interpretation 1). Interpretation 2) is also possible however because rats are tested after extensive training and over many sessions it is unlikely that at the time that these experiments were conducted rats were not familiar with the contingencies of the task.

To carryout a final test on whether premature responses occur as a result of frustration, Experiment 5 attempted to manipulating and specifically to amplify frustration by increasing the reward magnitude. Thus rats were challenged with a session in which the appetitive reward was increased from 1 to 3 pellets, in a rr 0.5 partial reinforcement schedule. A greater number of premature responses during this session, however, could also be due to increases in rr (in favour of the sensitivity to reward hypothesis), thus for the frustration hypothesis to be supported, any greater propensity to engage in premature responses during the 3-pellet condition should not be uniformly distributed across the session, but instead, should follow correct non rewarded trials. Comparing the micro-level analysis of the rr 0.5, 3 pellets session to that of the rr 0.5, 1 pellet session would also inform whether premature responses, at the *micro*-level, are more strongly influenced by frustration of the omitted reward as opposed to by the post-reinforcement pause. In detail, a decrease in the probability of a premature to follow an R trial, in the 3-pellet vs the 1 pellet condition, would indicate that premature responses are sensitive to inhibition of behaviour following reinforcement. On the contrary, an increase in the probability of a premature to follow a NR trial, in the 3-pellet vs the 1 pellet condition, would indicate that premature responses are more sensitive to frustration induced by the omission of reward.

At the *macro*-level, rats made significantly more premature responses in the 3 pellet, rr 0.5 condition compared to the 1 pellet, rr 0.5 condition. This is in line with previous research (King et al., 2016) and supports the sensitivity to reward hypothesis that a larger reward exerts a stronger ‘attraction’ to the reward-predicting cue, boosting approach behaviour and thus triggering an elevation in premature responses. At the *micro*-level, when looking at whether these premature responses were more likely to follow NR trials results were not as clear. Specifically, while the probability to make a premature response after a NR trial was greater than that expected under the independence model (i.e., greater than chance, as shown by Y in **Figure 3.18**), deviation from the independence model was actually much greater for premature responses occurring after R trials, of which there were almost none. This is in line with some critics of the frustration hypothesis, who argue that ROEs occur not (just) because frustration leads to invigoration of behaviour, but actually because the receipt of reward induces a post-consummatory inhibition of responding (Seward et al., 1957). This theory was supported by evidence (Jensen & Fallon, 1973; McHose & Gavelek, 1969) showing that slowing of behaviour following reward consumption scaled positively with reward magnitude while frustration-dependent activation of behaviour did not. This reflects well the present findings whereby the Y associated with transition probabilities to make a premature response following a NR in the 3 pellet, rr 0.5 condition is almost identical to that observed during the 1 pellet, rr 0.5 session. On the contrary, Y associated with transition probabilities to make a premature response following a R in the 3 pellet, rr 0.5 condition was dramatically greater than that observed during the 1 pellet, rr 0.5 session. In addition, there was a much higher likelihood to make an omission response following R trials in the 3 pellets condition vs the 1 pellet condition. A lower likelihood to make a premature response and a higher likelihood to make an omission response after a R trial during the 3 pellets condition, could be because, under this manipulation, the rat needs to collect 3 pellets as opposed to 1, thus inevitably spending more time poking into the food magazine and running the risk of not orienting towards the FP in time for the CS presentation of the new trial. This scenario was indeed confirmed by nosepoke data showing a substantial increase in RP perseverative responses and a slower exit from the food magazine (on average ~1.87s into the ITI of the new trial) in the 3-pellet condition compared to the 1 pellet condition (on average ~0.61s into the ITI of the new trial). In light of this it is difficult to interpret the nature of the slowing of behaviour observed following a R trial in the 3-pellet condition. To overcome this

ambiguity this manipulation should be tested again either using a liquid reward with different levels of sucrose concentration to vary the reward magnitude (to avoid having an increase in volume with increasing reward magnitude) or training animals with a longer ITI to allow rats to orient back to the FP in time for the presentation of the new CS. Regardless of this issue, in most manipulations except in Experiment 3 and Experiment 5a, the Y (deviations from the independence model) for premature responses happening after R trials were actually greater than the Y for prematures happening after NR trials. In all these manipulations the reward magnitude is just 1 pellet thus this discrepancy in favour of Y for R trials cannot be explained by excessive time spent in the RP eating. Instead, these findings support the idea put forward by Seward and colleagues (1957), that consummation of reinforcement leads to a temporary suppression of behaviour and in the present case by a reduction in premature responses following rewarded trials.

Differences in premature responses across impulsivity groups were only apparent when waiting behaviour was challenged, i.e., when the ITI was lengthened, in Experiment 2 and 4. This is not surprising considering that HI and LI rats were selected based on premature responses made during long ITI trials (7 s and 9 s) of two sessions of a vITI paradigm. This was done, in accordance with previous research (Caprioli et al., 2013; Dalley, et al., 2007a), because long ITIs are known to challenge waiting impulsivity and thus reveal a vulnerability for an inability to withhold a response (Bari, et al., 2008). Nonetheless, the lack of an interaction between impulsivity phenotype and *rr* on all indices of performance on the 5CSRTT suggests that all impulsivity groups, including HI rats, were equally sensitive to decrements in motivation and the effect that this had on premature responses.

Taken together, these experiments do not provide convincing evidence in support of the frustration hypothesis or of the incentive hope hypothesis as valid frameworks to explain the occurrence of premature responses. On the contrary, Experiments in this chapter suggest that premature responses are influenced by manipulations that affect motivation to perform a task, in favour of the sensitivity to reward hypothesis. These findings add to a body of literature on the association between trait impulsivity and indexes of enhanced subjective value of reinforcement,

observed both in animals (Belin et al., 2008; Dalley et al., 2007a; Diergaarde et al., 2008; 2009) and in humans (Cools et al., 2005; Ioannidis et al., 2019; Mechelmans et al., 2017) .

# **Chapter 4 - Adaptive aspects of impulsivity and interactions with effects of catecholaminergic agents**

## **4.1 Introduction**

Impulsivity is a multifactorial construct (Dalley et al., 2011; Whiteside & Lynam, 2001), more generally understood as the tendency to act prematurely without foresight. It is often regarded as a maladaptive trait and indeed it has been associated with various psychiatric conditions, including substance abuse (Kollins et al., 2005; Kreek et al., 2005) and ADHD (Solanto, 2002). However impulsivity need not be an exclusively dysfunctional trait, and there has been research (Dickman, 1985; Dickman, 1990; Gomez et al., 2004; Smillie & Jackson, 2006) on whether and in which contexts impulsivity can be advantageous. Dickman (1990, 2000), for example, developed the concept of functional impulsivity (FI), that is ‘the tendency to engage in rapid, error-prone information processing (i.e., to act with relatively little forethought) when such a strategy is (..) optimal’ (Dickman, 1990). In a series of experiments, he showed that when the experimental task is very simple, the rapid responding typical of high impulsive individuals does not lead to a higher rate of errors (Dickman, 1985). Similarly, when there is little time available to decide, high impulsive individuals respond with greater accuracy than low impulsive individuals (Dickman & Meyer, 1988).

In line with this early evidence, it was recently shown that trait impulsivity boosts performance in highly rewarding settings (Cools et al., 2005). Similar conclusions on the advantages of impulsivity can be drawn from other contexts, including entrepreneurship (Lerner et al., 2019; Verheul et al., 2015), and creative literature (Lawrence et al., 2008; White & Shah, 2011), and are consistent with the recognised role of context in the expression of ADHD (Barkley, 2002;

Williams & Dayan, 2005). Thus, environments encompassing novel, interesting, and fast-paced activities improve ADHD symptoms among young adults (Lasky et al., 2016).

However, despite there being growing evidence in the human literature that impulsivity can confer some adaptive advantages, animal research on this topic is lacking. Thus, in this chapter I explored whether high levels of impulsivity, as assessed with the 5CSRTT (Robbins, 2002), can be advantageous under certain experimental conditions. To test this, animals were presented with pseudo-randomly interleaved inter trial intervals (ITI) of varying durations from fast intervals, of 3 and 5 s in duration, to slow intervals, of 7 and 9 s in duration. It was predicted that high impulsive animals (HI) would perform better when the task requires them to respond quickly while low-impulsive animals (LI) would be impaired. On the contrary, LI animals were expected to have superior performance when the task requires animals to wait for an extended period of time for the stimulus to appear. To further test how performance on fast trials is affected by context (i.e., the presentation of both slow and fast trials) and the extent to which HI and LI adapt to high-event rate trials, animals were also tested on a session comprising only short trials, that is pseudo-randomly interleaved 3 s and 2 s ITI trials. A variable ITI (vITI) paradigm was chosen because it offers a range of ITIs and can thus allow different behavioural tendencies to emerge. In addition, because the presentation of each ITI is unpredictable, this type of manipulation increases attentional load, while controlling for habituation or timing strategies that the animals might be adopting as the session progresses (Bizarro et al., 2004; Cope et al., 2016). Other studies have looked at performance under a variable ITI in the 5CSRTT (Bizarro et al., 2004; Callahan et al., 2019; Carli et al., 1983; Navarra et al., 2008; Paterson et al., 2011; Robinson, 2012; Sirviö et al., 1993), but none have explored whether HI and LI animals perform differently during this experimental manipulation and whether a specific impulsivity phenotype confers a selective advantage in performance. Blondeau and Dellsu-Hagedorn (2007) tested whether animals segregated based on impulsivity *as well as* accuracy show a selective advantage in long (8 s) ITI as opposed to short (2 s) ITI trials. The authors, however, presented these trials in isolation as separate challenges and did not test how stable performance of different impulsivity phenotypes is over time. On the contrary, differences in performance were explored as a function of impulsivity on multiple sessions and in two separate batches of animals, thus strengthening the validity of our results.

Fast latencies in free-operant tasks are considered an index of motivation as they scale inversely with reward density (Hamid et al., 2016; Mohebi et al., 2019; Niv et al., 2007; Walton & Bouret, 2019), and have been shown to correlate with DA release in the VS (Wassum et al., 2012; Hamid et al., 2016; Mohebi et al., 2019). This region is key in regulating action initiation (Klaus et al., 2019) and effort-based decision making (Ikemoto & Panksepp, 1999; Salamone et al., 2012). From a causal point of view, optogenetic activation of DA neurons in the VTA, which have dense projections into the VS, reduces latencies and promotes movement initiation in rodents (Hamid et al., 2016; da Silva et al., 2018). In line with this, pharmacologically induced high levels of DA in the NAcB have been shown to bias animals into expending more effort to obtain food rewards (Yohn et al., 2016). HI rats have been postulated to have increased DA release in the VS (Dalley and Robbins, 2017), thus I wanted to test whether any observed fast latencies and higher performance in short ITI trials by HI rats were related to other indexes of motivation, such as effort expenditure. To achieve this I tested animals on an effort-based task with two schedules of reinforcement. Specifically, animals were tested both on a progressive ratio (PR) schedule that adds one lever pressing requirement to each new trial and on a PR schedule that follows an exponential progression, similar to that designed by Roberts and Richardson (1992).

Additionally, pharmacological agents that are widely used to treat ADHD – d-amphetamine (AMPH), methylphenidate (MPH) and atomoxetine (ATO) – were administered to investigate how different medications affect the performance of animals segregated on the basis of their impulsivity phenotype. Importantly, drugs with different though overlapping effects on catecholamine transmission were chosen to more precisely dissect the contribution of distinct neurotransmitter systems in the vITI-5CSRTT paradigm. In details, AMPH increases the extracellular levels of catecholamines both by inhibiting their reuptake and by entering the pre-synaptic terminal and pumping neurotransmitters into the synapse by a process called retro-transport (Heal, et al., 2013). MPH, instead, increases extracellular levels of DA and noradrenaline (NA) solely by blocking their re-uptake (Solanto, 1998), while ATO primarily inhibits the uptake of NA and has relatively low affinity for the DA transporter (Swanson, et al., 2006). In line with this difference, MPH has been shown to increase levels of DA (and NA) both in the striatum and in PFC, while ATO increases extracellular levels of DA only in PFC where

NA transporters have been suggested to carry out the removal of DA due to scarcity of DA transporters (Koda et al., 2010; Bymaster, et al., 2002).

Based on evidence showing that AMPH and MPH impair 'waiting' impulsivity (Navarra et al., 2008; Pattij et al., 2007) and decrease response latency (Bizarro et al., 2004), we predicted that administration of these drugs would lead to an improvement of performance in short ITI trials especially. On the contrary, since ATO reduces impulsivity (Blondeau & Dellu-Hagedorn, 2007) and slows responding in some contexts (Callahan et al., 2019) we predicted that ATO would mostly enhance performance on long ITI trials. To better dissect the role that NA plays in modulating performance of HI and LI rats, we also assessed the effects of systemic administration of atipamezole (ATI), an alpha-2a antagonist, and phenylephrine (PHEN), an alpha1 agonist. To the best of my knowledge ATI and PHEN have not been tested on animals segregated based on impulsivity, thus it is unknown how these drugs would interact with this phenotype. In addition, PHEN has never been tested on a vITI paradigm such as the one used in this experiment. On the basis of evidence showing that ATI increases behavioural activation (Ma et al., 2005; Sirviö et al., 1994), while PHEN has the opposite effect (Pattij et al., 2012), we predicted that the former would improve performance during short ITI trials, while the latter would impair performance.

## **4.2 Methods and materials**

### **4.2.1 Subjects**

Sixty outbred male Lister Hooded rats (Charles River, Margate, UK) weighing 280–300 g at the beginning of the experiments were used for this study. Animals were kept under the conditions specified in Chapter 2 (see section 2.1).

### **4.2.2 Five-choice serial reaction time task: training**

See Chapter 2 (section 2.3) for details on 5CSRTT training. In Experiment 1, thirty-six animals were trained to reach a stable baseline performance on the 5CSRTT with a final stimulus duration of 0.7 s and an ITI of 5 s. In Experiment 2, twenty-four animals were trained to reach a stable baseline performance on the 5CSRTT with a final stimulus duration of 0.6 s and an ITI of 5 s. Each session lasted a maximum of 100 trials or 30 min, whichever limit was reached first.

### **4.2.3 Experiment 1 – Effects of impulsivity trait on behavioural performance at variable ITI**

#### **4.2.3.1 Variable ITI challenge**

Thirty-six rats were exposed to two vITI sessions following a procedure described in Chapter 2 (section 2.3.2). Time-out (5 s) and stimulus duration (0.7 s) were kept constant at the same level as that of their baseline training. To identify which animals exhibited extreme impulsivity phenotypes, rats underwent a screening procedure. Specifically, premature responses across the 2 days of vITI challenge were averaged and the upper (N=9) and lower (N=9) quartiles were selected. Animals falling between these two extremes were deemed MID impulsive rats.

#### **4.2.3.2 Short vITI challenge: rapid stimulus presentation**

A day after their last vITI challenge, rats were presented with a short vITI session. This consisted of 100 trials of 3 s ITI and 50 trials of 2 s ITI (mean of 2.6 s). The two different ITI trials were

pseudo-randomly interleaved and the rat could not predict which ITI trial was going to be presented. The session ended when rats had completed 150 trials or after 1 h and 30 min (whichever occurred first). More instances of the 3s ITI were presented compared to the 2s ITI, to avoid making the task too difficult (and risk having floor effects), whilst still exploring whether rats could be challenged with even quicker ITI trials than 3s and whether trait impulsivity differentially affected performance during these two ITIs.

### **4.2.3.3 Data analysis**

The main dependent variables were percentages of premature responses, percentages of omissions, the number of reinforcers earned and response latencies to make correct, incorrect, and premature responses. To assess the temporal profile of responses, we divided the vITI sessions into 5 min bins. Each bin required responses from at least three animals from each impulsivity group to be included in the analyses. The 5 min bins satisfying this criterion were from 5 to 55 min (i.e., 11 bins). Analyses for performance on the 5CSRTT were performed as described in Chapter 2 (section 2.6). For details on the fixed factors included in all LMEM models see Appendix B (section B1.1).

### **4.2.3.4 Progressive Ratio**

#### **4.2.3.4.1 Apparatus and Training**

See Appendix B, section B1.2.1 and B1.2.2.

#### **4.2.3.4.3 Testing**

Rats were first tested on a PR schedule with an *exponential* progression, based on Roberts & Richardson (1992). Under this schedule, the number of target responses required for the delivery of the reward increased in each trial, according to the following formula ( $5 * e^{(0.2*n)} - 5$ ), where n is the trial number. This schedule yielded ratios of ratio requirements of: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145 etc. The test terminated when the rat obtained 100 pellets or when 60 minutes had elapsed since the last reinforcer, whichever occurred first. Following this, on a separate day, rats were tested on a *linear* PR schedule. Under this schedule, the number of target responses required for the delivery of the appetitive reward increased by

one in each trial. This schedule yielded ratios of ratio requirements of: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 etc. The test terminated when the rat obtained 100 pellets or when 60 minutes had elapsed since the last reinforcer, whichever occurred first. The linear PR schedule was tested, as well as the exponential schedule, because it offers less steep effort-requirements and thus may allow for more subtle group-differences to emerge.

#### **4.2.3.4.4 Data Analysis**

The measures of interest were breakpoint (BP), defined as the number of target responses made in the last successfully completed trial for each subject; the total number of lever presses, including those in trials not completed; the post reinforcement pause (PRP), defined as the time interval between collection of the reward in the food magazine and the first lever press on the next trial. Rate of pressing was calculated by counting the number of presses within a trial over the time taken to complete each trial, from the first response (thus, excluding PRP). The first two trials were excluded when calculating this measure because: in the first trial the requirement of one lever press is not sufficient to calculate a rate, and in the second trial the low number of lever presses required would inflate the total response rate. Finally, in the exponential PR the following negative exponential function was fitted to the mean response rates per condition:  $y = a \cdot \exp(x \cdot -b)$ ; while in the linear PR schedule the linear function  $y = a \cdot (x+b)$  was then fitted to the mean response rate per condition. In both cases  $y$  corresponds to the response rate and  $x$  is the trial number. Both equations calculate the predicted peak response rate ( $a$ ) and the decay rate parameter ( $b$ ). The former, ( $a$ ), describes the maximal motoric output of an animal whereas the latter, ( $b$ ), describes the reductions in response rate as the session progresses. Such reductions occur because over the course of the session fatigue increases and perhaps instrumental extinction too (Simpson et al. 2011). However, those rats that are particularly motivated to work for food might display a slower rate of decay because of the excitatory influence of rewards following each trial (Hailwood et al., 2018; Phillips et al., 2017).

## **4.2.4 Experiment 2: Effects of methylphenidate, atomoxetine, amphetamine, atipamezole and phenylephrine on vITI performance**

### **4.2.4.1 Variable ITI challenge**

Twenty-four rats were exposed to three vITI sessions following a procedure described in Chapter 2 (section 2.3.2). Time-out (5 s) and stimulus duration (0.6 s) were kept constant at the same level as that of their baseline training. To identify which animals exhibited extreme impulsivity phenotypes rats underwent a screening procedure. Specifically, premature responses across the 3 days of vITI challenge were averaged and the upper (N=6) and lower (N=6) quartiles were selected. Animals falling between these two extremes were deemed MID impulsive rats.

### **4.2.4.2 Systemic drug administration**

All rats (HI, MID and LI) received control injections of the vehicle 2 days before the start of the experiment. All drugs were administered sub-cutaneously (s.c.) 40 min prior to testing. The drug experiments consisted of two separate randomised within-subject cross-over Latin-square designs, to control for training and crossover effects. These two Latin-square designs were separated by at least three days of washout. In Latin-square 1, vehicle, MPH (1 mg/kg and 3 mg/kg) and ATO (0.3 mg/kg and 1 mg/kg) were administered. In Latin-square 2: vehicle, AMPH (0.2 mg/kg), ATI (0.3 mg/kg) and PHEN (1 mg/kg) were administered. All drugs were dissolved in 0.9% saline (the 'vehicle solution'). Drugs were tested during the vITI challenges only (mean ITI of 6s). Choice of doses, timing and routes was decided based on previous publications with these drugs (Fernando et al., 2012; Sirvio et al., 1994).

### **4.2.4.3 Data analysis**

Analyses for performance on the 5CSRTT is the same as that described for Experiment 1 (section 4.2.3.3) and were performed as described in Chapter 2 (section 2.6). For details on the fixed factors included in all LMEM models see Appendix B (section B1.1).

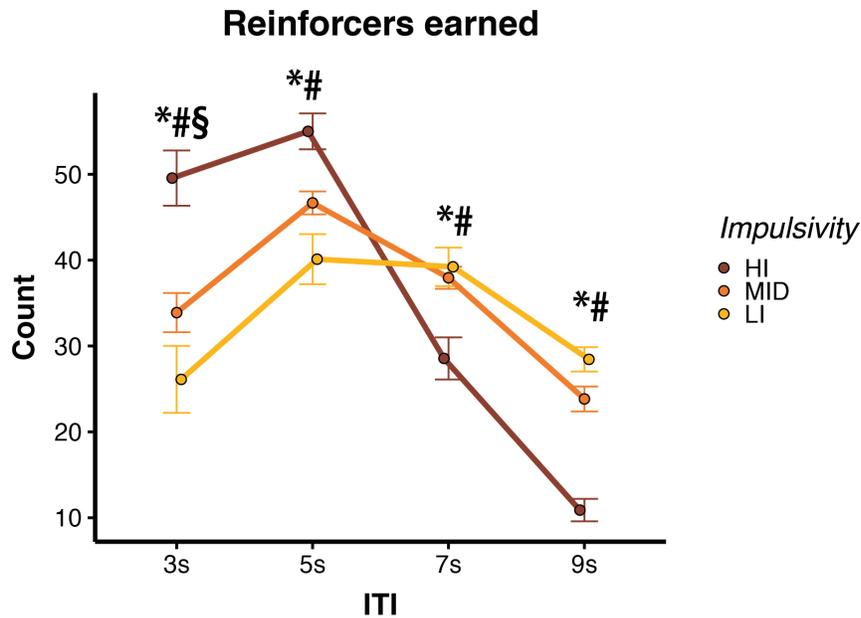
## 4.3 Results

### 4.3.1 Experiment 1

Baseline performance prior to the vITI challenge was analysed and is reported in detail in Appendix B (section B2.1.1.1). Briefly, on baseline, HI rats exhibited elevated premature responses compared with the other two groups.

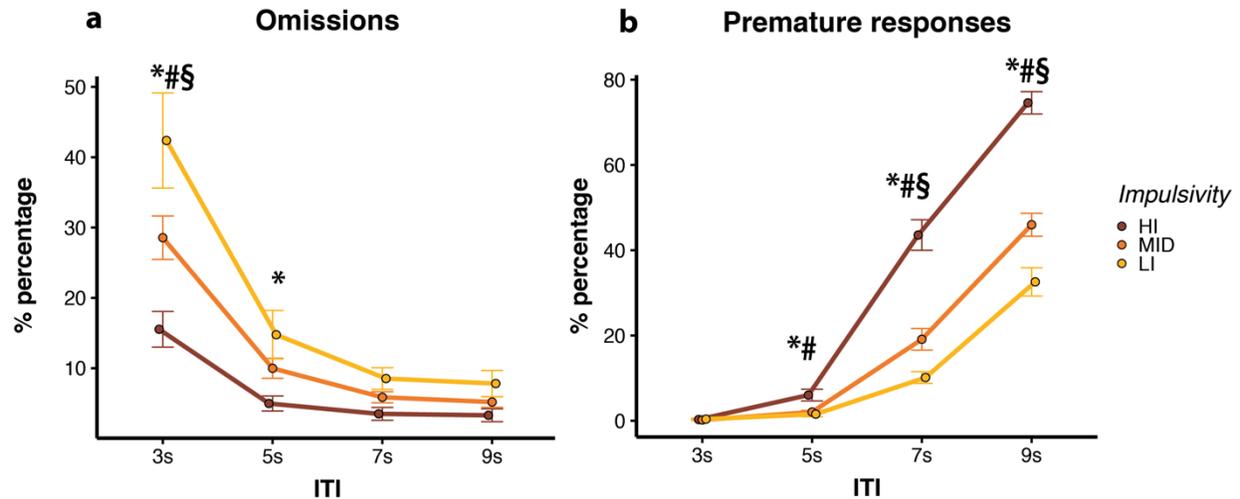
#### 4.3.1.1 Effects of trait-like impulsivity trait on behavioural performance assessed during a vITI challenge

For reinforcers earned, there was a significant Day x ITI x Group interaction [ $F(6,231)=2.89$ ,  $p=0.010$ ]. Since the three-way interaction were significant, separate multilevel models were used to ascertain the Group-dependency of the ITI effects in each Day separately. Impulsivity phenotype determined the efficacy of performance in terms of earned reinforcers at different ITI values for the second day of testing [Group x ITI interaction,  $F(6,99) = 21.91$ ,  $p<0.001$ ]. This is shown in **Figure 4.1** where HI rats obtained more reinforcers than LI rats ( $t=6.30$ ,  $p<0.001$ ) and MID ( $t=4.51$ ,  $p<0.001$ ) on the short 3s ITI trials, with MID rats also earning more reinforcers than LI on the short 3s ITI trials ( $t=2.77$ ,  $p=0.018$ ). HI also earned more reinforcers than LI on the 5 s ITI trials ( $t=2.81$ ,  $p=0.002$ ) but earned fewer reinforcers than LI ( $t=-3.65$ ,  $p=0.012$ ;  $t=-4.69$ ,  $p<0.001$ ) and MID ( $t=-2.87$ ,  $p=0.009$ ;  $t=-2.55$ ,  $p<0.001$ ) on the long 7s and 9s ITI trials, respectively. A similar effect was evident on day 1 of testing as shown by **Figure 4.1B (a)** in Appendix B. In summary, HI rats earned more reinforcers at shorter ITI trials, while LI rats earned more reinforcers at longer ITI trials.



**Figure 4.1 Trait impulsivity modulated reinforcers earned on a vITI paradigm on 5CSRTT.** Group differences for Day 2. \*HI vs LI  $p < 0.05$ ; #HI vs MI  $p < 0.05$ ; §MI vs LI  $p < 0.05$

Both impulsivity phenotype and ITI influenced the incidence of omission responses [Group x ITI,  $F(6,231) = 4.051$ ,  $p < 0.001$ ]. **Figure 4.2a** shows data for the second day of testing. LI rats made proportionally more omission responses than HI rats ( $t = -5.59$ ,  $p < 0.001$ ) and MI ( $t = -4.33$ ,  $p < 0.001$ ) on the short 3 s ITI. LI rats also made proportionally more omission errors than HI rats on the 5 s ITI trials ( $t = -2.67$ ,  $p = 0.025$ ). In summary, LI rats were more prone than HI and MID at making omission errors and these occurred on short ITI trials.



**Figure 4.2 Trait impulsivity modulated (a) % omissions and (b) % premature responses on a vITI paradigm on 5CSRRTT.** Group differences for Day 2. \*HI vs LI  $p < 0.05$ ; #HI vs MI  $p < 0.05$ ; \$MI vs LI  $p < 0.05$

For the proportion of premature responses there was a significant Day x ITI x Group interaction [ $F(6,231)=3.63$ ,  $p=0.002$ ]. Since the three-way interaction was significant, separate multilevel models were used to ascertain the Group-dependency of the ITI effects in each Day separately. Impulsivity phenotype and ITI influenced the frequency of premature responses in the second day of testing [Group x ITI interaction,  $F(6,99) = 17.92$ ,  $p < 0.001$ ]. **Figure 4.2b** shows that HI rats made proportionally more premature response than LI and MID rats during 5 s ITIs ( $t = 3.28$ ,  $p = .004$ ;  $t = 2.92$ ,  $p = 0.012$ , respectively), 7 s ITIs ( $t = 9.16$ ,  $p < 0.001$ ;  $t = 7.41$ ,  $p < 0.001$ , respectively), and 9 s ITIs ( $t = 10.12$ ,  $p < .001$ ;  $t = 8.00$ ,  $p < 0.001$ , respectively). MID rats also made more premature responses than LI rats on the 7 s ITI ( $t = 3.17$ ,  $p = 0.006$ ) and the 9 s ITI ( $t = 3.69$ ,  $p = 0.001$ ). A similar pattern was evident on day 1 as shown by **Figure 4.1B (b)** in the supplementary material. In summary, HI rats and to an extent MI rats made proportionally more premature responses than LI rats and these occurred during the long ITI trials.

I next combined premature, correct and incorrect responses and divided the session into 5 min bins to examine whether HI, MID and LI rats differed in overall rate of responding. There was an effect of impulsivity phenotype on number of active responses per unit of time [ $F(2,33) = 6.32$ ,  $p = 0.005$ ]. Specifically, HI rats were significantly more active than LI rats ( $t = 3.54$ ,  $p = 0.003$ ). For more details on this see **Figure 4.2B** in Appendix B.

I then assessed the relationships between the various behavioural variables. During the first ( $r=-0.46$ ,  $p=0.005$ ) and second ( $r=-0.37$ ,  $p=0.028$ ) day of testing, there was an overall significant negative relationship between making an omission on the 3 s ITI and making a premature response on the 9 s ITI. There was also a strong positive correlation between making a correct response on the 3 s ITI and making a premature response on the 9 s ITI both on day 1 ( $r=0.64$ ,  $p<0.001$ ) and on day 2 ( $r=0.64$ ,  $p<0.001$ ). These correlations are in line with behavioural data analysed by impulsivity phenotype, showing that animals that respond prematurely during long ITI trials are also more likely to respond correctly on short ITI trials. Conversely, animals that do not engage with rapid, short ITI trials, and thus make many omissions on these trials, are more likely to respond correctly when waiting is rewarded. Finally, impulsivity groups and ITI types influenced latency to perform correct and premature responses. For more details see **Table 4.1**.

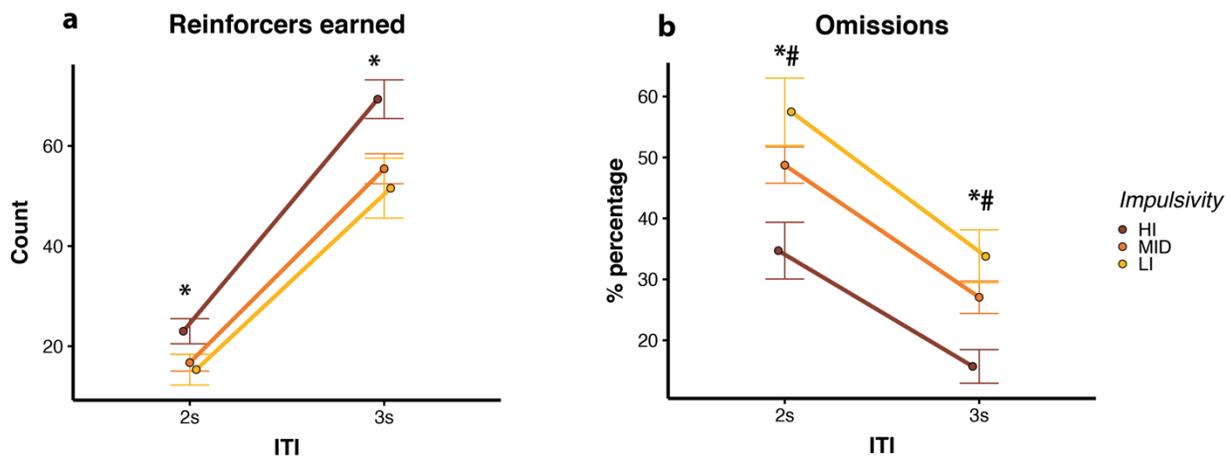
	Correct responses				Incorrect responses				Premature responses	
	3s	5s	7s	9s	3s	5s	7s	9s	7s	9s
HI	937.2 ±82. 4*	643.1 ±22.2 *	596.5 ±19.7 *	660.3 ±53.6 *	2545.9 ±140	1651± 123.6	1014.4 ±106.8	1113.2 ±173.5	5674.8 ± 53°*	6479.4 ± 79.3°*
MID	1101. 7±60 .3	744.4 ±25.5	620.5 ±18.8	680.2 ±27.9	2768.1 ±105.7	1789.6 ±107.9	1445.6 ±110.5	1197.1 ±149.4	5946.5 ±54.2°	6901.1 ±66.6°
LI	1324. 4±94 .5*	901±5 1.1*	758.2 ±25.1 *	709.9 ±27.4 *	2712.2 ±151.5	2176.1 ±117.8	1301.6 ±124.2	1347.6 ±217	5766.8 ±85.3*	7129.9 ±69.6*

**Table 4.1 Experiment 1, vITI challenge. Latencies for correct, incorrect, and premature responses in ms. \*HI vs LI  $p<0.05$ ; °HI vs MI  $p<0.05$ . Mean and standard error (SE) in brackets.**

### 4.3.1.1.1 First encounter with and exposure to the long ITIs

As others have pointed out (Aase & Sagvolden, 2006), subjects with a short delay gradient such as HI rats may rarely experience contingencies where delayed reinforcers are obtained due to their inability to wait. For this reason, it was important to explore whether HI rats encounter long ITIs later in the session compared to the other two groups, across days. Details on the statistical analyses can be found in Appendix B (section B2.1.1.4). Briefly, on Day 1, when the rats first encountered the longer ITIs there were no differences between groups but there was a main effect of ITI, with animals encountering the 9 s ITI later in the session compared to the 7 s ITI. HI rats, however, did make significantly fewer correct responses with the 7 s and 9 s ITIs across days, suggesting that they were not exposed to as many positively reinforced events, with these long ITIs, as the other groups.

### 4.3.1.2 Short vITI challenge

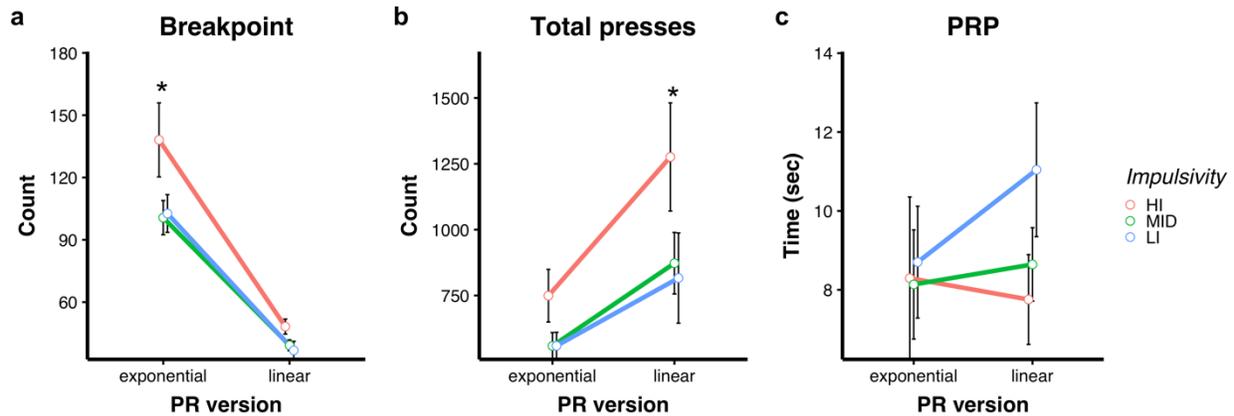


**Figure 4.3 Impulsivity groups and short ITI.** (a) Impulsivity groups differed with regards to reinforcers earned [ $F(2,33) = 3.65$ ,  $p=0.037$ ] with HI rats earning significantly more reinforcers than LI rats ( $t= 2.53$ ,  $p=0.041$ ). (b) Omissions varied as a function of ITI [ $F(1,33) = 209.45$ ,  $p<0.001$ ] and impulsivity group [ $F(2,33) = 7.32$ ,  $p=0.002$ ]. Percentages of omission errors were higher during 2 s ITI trials than 3 s ITI trials ( $t=14.47$ ,  $p<.001$ ) and HI rats made significantly less of these errors than LI ( $t=-3.75$ ,  $p=0.002$ ) and MI ( $t=-2.80$ ,  $p=0.023$ ) rats.

HI rats earned more reinforcers and made fewer omissions on short vITI trials of 2 and 3 s.

**Figure 4.3** summarises these findings. Response latencies varied across ITIs and impulsivity groups and are summarised in **Table B4.1** of Appendix B (section B2.1.1.5).

### 4.3.1.3 Progressive Ratio



**Figure 4.4** Effects of the reinforcement schedule and impulsivity phenotype on performance on PR. (a) The mean breakpoint for both PR versions across impulsivity groups. \*significantly different from the linear model  $p < 0.05$  (b) The total number of presses. \*significantly different from the exponential model  $p < 0.05$  (c) The duration of the post reinforcement pause (PRP).

**Figure 4.4** shows the effects of the reinforcement schedule and impulsivity phenotype on performance on PR. Breakpoint was influenced by manipulation [See **Figure 4.4a**,  $F(1,33) = 589.43$ ,  $p < .001$ ] and at a trend level by impulsivity [ $F(2,33) = 3.00$ ,  $p = 0.063$ ]. *Post-hoc* tests showed that the breakpoint for the exponential manipulation was higher than that for the linear manipulation ( $p < .001$ ), and that HI rats had a higher breakpoint than MID rats, but this was not significant ( $p = 0.076$ ). Total presses during the session were also influenced by manipulation [See **Figure 4.4b**,  $F(1,33) = 43.18$ ,  $p < .001$ ] but not by impulsivity ( $p = 0.0967$ ). *Post-hoc* contrasts showed that in total, animals pressed more in the linear paradigm. PRP did not differ between manipulations ( $p = 0.106$ ) or impulsivity phenotypes ( $p = 0.528$ ) [See **Figure 4.4c**]. Rate of pressing differed between manipulations [ $F(1,33) = 49.34$ ,  $p < .001$ ] but not between impulsivity phenotypes ( $p = 0.400$ ) See **Figure 4.6** and **4.7**. *Post-hoc* contrasts showed that the rate of pressing was higher in the exponential condition. Analysis of the predicted peak response rate (**Figure 4.5a**) and decay rate parameter (**Figure 4.5b**) did not reveal an effect of impulsivity on any of the parameters ( $p > 0.7$  for both comparisons), but it did show an effect of manipulation. The predicted peak response rate was significantly higher when rats were reinforced under the exponential schedule than under the linear schedule [ $F(1,33) = 7.430$ ,  $p = 0.010$ ]. The response rate decay was also significantly greater when rats were tested under the exponential schedule of reinforcement [ $F(1,33) = 71.31$ ,  $p < .001$ ].

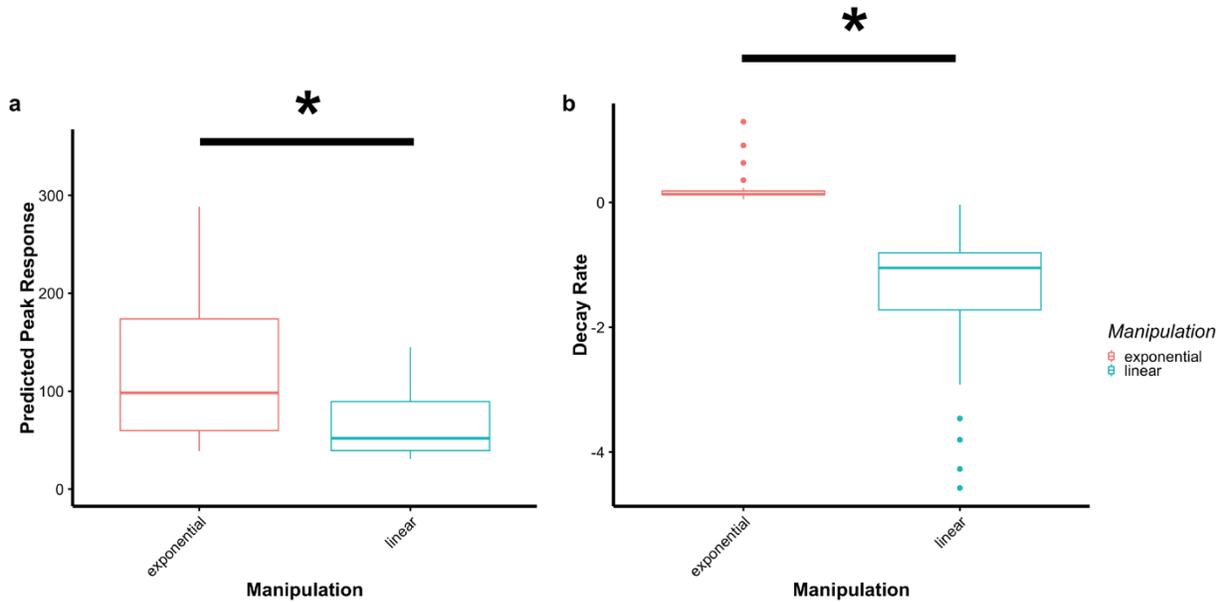


Figure 4.5 Predicted peak response (a) and Decay rate (b) for each PR manipulation. \*significantly different ( $p < 0.05$ ).

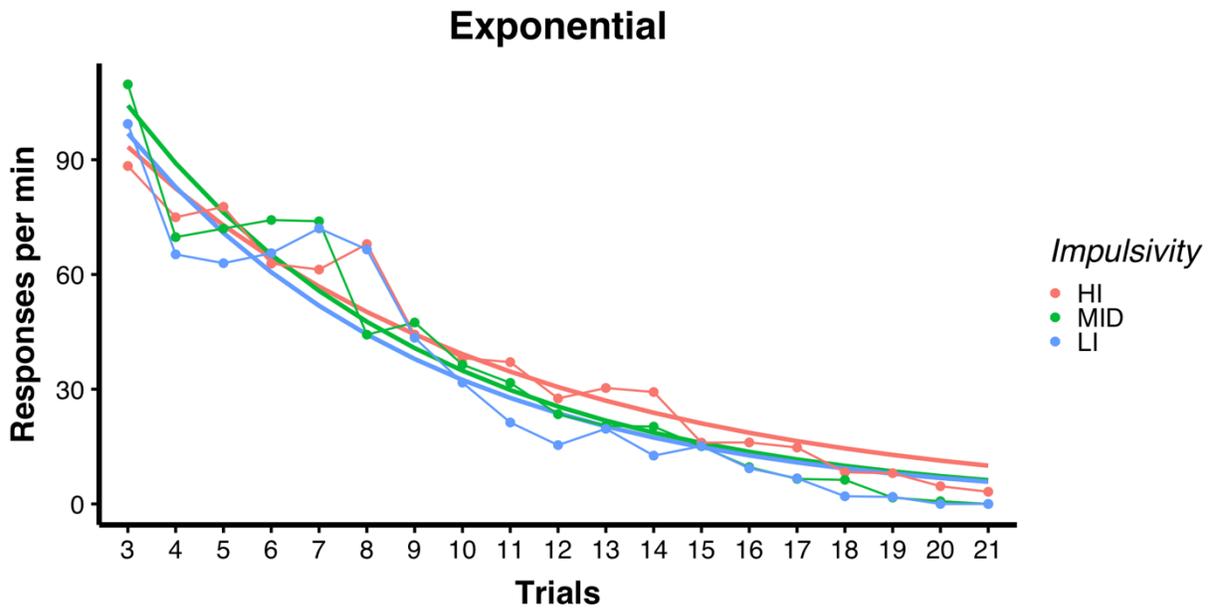
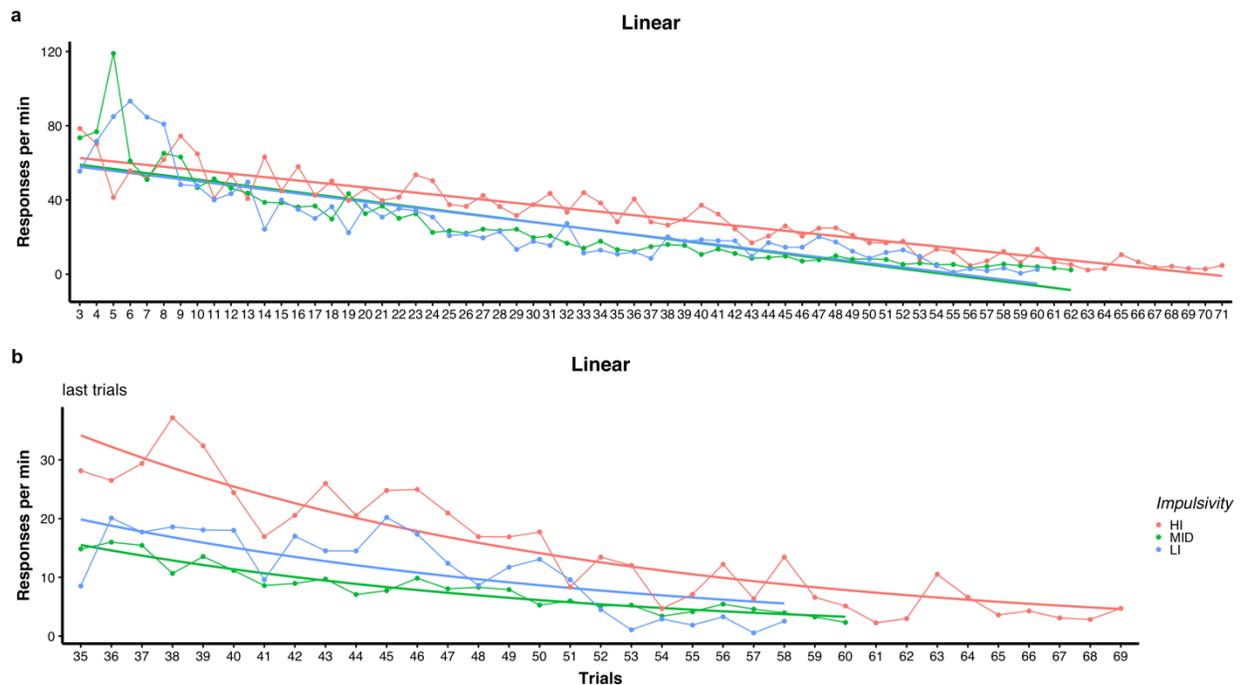


Figure 4.6 Mean response rates across impulsivity groups for each trial, from the third trial onwards for the exponential reinforcement schedule. Trendlines indicate the fitting of the negative exponential function ( $y = -a * \exp(x * b)$ ) to the mean response rates per impulsivity group. In the formula of the negative exponential function  $y$  is the response rate and  $x$  is the trial number.



**Figure 4.7 Mean response rates across impulsivity groups for each trial, from the third trial onwards for the exponential reinforcement schedule. (a) total session (b) last trials of the session. Trendlines indicate the fitting of the linear function ( $y = a + bx$ ) to the mean response rates per impulsivity group. In the formula of the linear function  $y$  is the response rate and  $x$  is the trial number.**

#### 4.3.1.3.1 Relationship with performance on vITI

There was a significant correlation between breakpoint on the exponential schedule of PR and premature responses on the 7s ITI of the vITI manipulation both on Day 1 ( $r=0.50$ ,  $p=0.002$ ) and Day 2 ( $r=0.35$ ,  $p=0.03$ ). There was also a significant correlation between breakpoint on the exponential schedule of PR and premature responses on the 9s ITI of the vITI manipulation on Day 1 ( $r=0.43$ ,  $p=0.010$ ), but not Day 2 ( $r=0.30$ ,  $p=0.074$ ). With regards to the linear schedule of PR, there was a significant correlation between breakpoint on the linear schedule of PR and premature responses on the 7s and 9s ITI of the vITI manipulation on Day 1 ( $r=0.48$ ,  $p=0.002$ ;  $r=0.51$ ,  $p=0.001$ , respectively) but not Day 2 ( $r=0.30$ ,  $p=0.07$ ;  $r=0.25$ ,  $p=0.14$ , respectively).

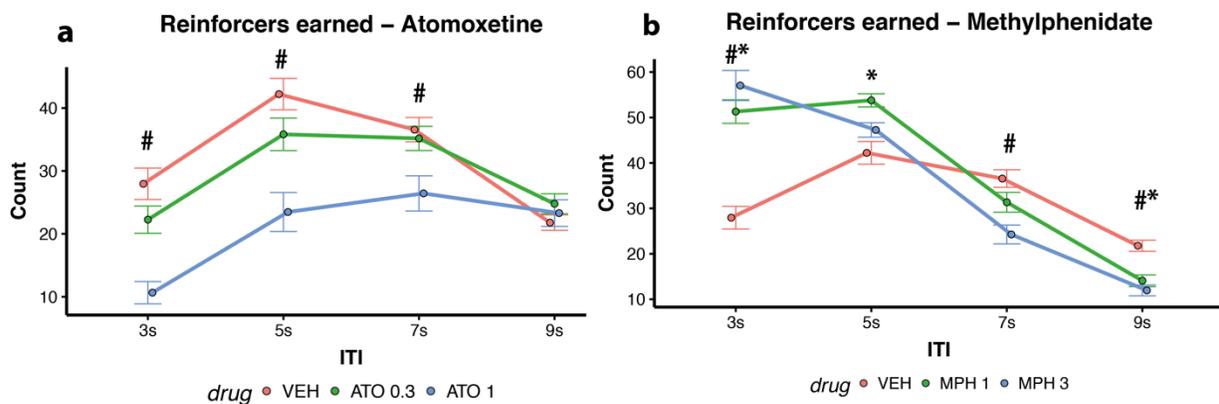
## 4.3.2 Experiment 2

### 4.3.2.1 Effects of methylphenidate, atomoxetine, amphetamine, atipamezole and phenylephrine on vITI performance

#### 4.3.2.1.1 Behavioural performance

Prior to the drug administration studies, rats were trained to a stable baseline level and were tested on three vITI sessions. On baseline, HI rats exhibited elevated premature responses and lower accuracy compared with LI rats (for more details see Appendix B, section B2.1.2.1). Results from the vITI challenges replicated Experiment 1 and are shown in Appendix B (see **Table 4.2B** and **Figures 4.3-4.5B** in section B2.1.2.2). Briefly, HI rats earned more rewards and made fewer omissions when the task required rapid responding in short ITI trials. When the ITI was increased to longer durations, HI rats showed more premature responses than LI rats. LI rats exhibited the opposite behavioural profile with more rewards during long ITI trials and impaired performance during the short ITI trials with increased omissions. Finally, there were no differences between impulsivity groups in the trial number at which animals encounter the 7s and 9s ITI for the first time.

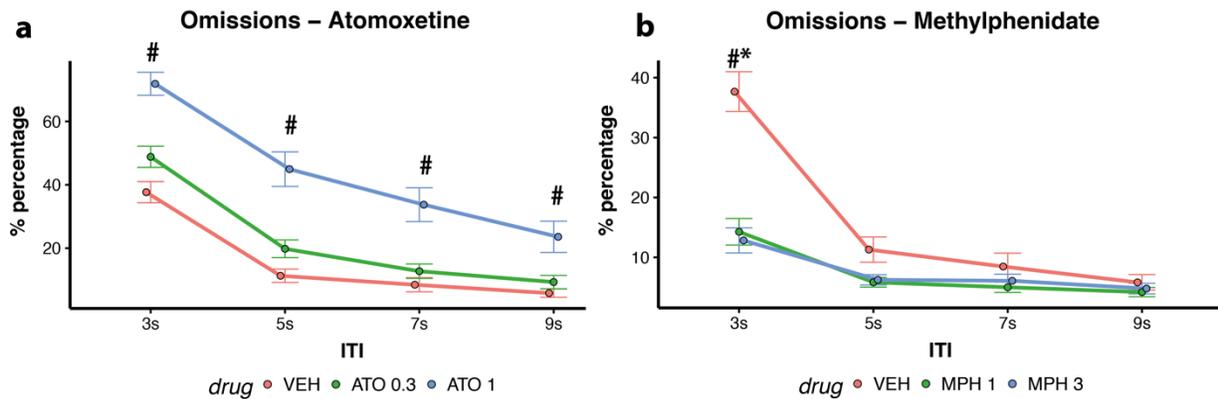
#### 4.3.2.2 Effects of methylphenidate and atomoxetine



**Figure 4.8** Effects of (a) ATO and (b) MPH on the number of reinforcers earned; \*low-dose vs vehicle  $p < 0.05$ ; #high-dose vs vehicle  $p < 0.05$ .

**Figure 4.8a** and **4.8b** show that the effects of ATO and MPH on behaviour depended on the ITI [Drug x ITI interaction,  $F(12,380)=28.52$ ,  $p < 0.001$ ]. During short 3 s and 5 s ITI trials ATO

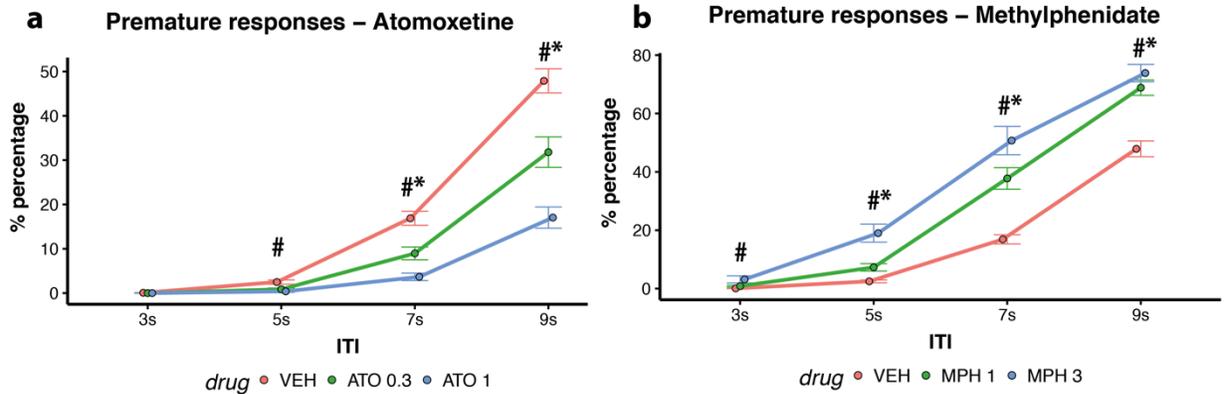
(1mg/kg) significantly decreased the number of reinforcers earned compared with vehicle ( $t=-7.23$ ,  $p<.001$ ;  $t=-6.39$ ,  $p<.001$  respectively for 3 s and 5 s ITI trials). ATO (1 mg/kg) also reduced the number of reinforcers earned during the long 7 s ITIs compared with the vehicle group ( $t=-3.89$ ,  $p<0.001$ ). In contrast, during short 3 s ITI trials, rats earned more reinforcers following the administration of MPH at both low (1mg/kg,  $t=6.91$ ,  $p<0.001$ ) and high (3mg/kg,  $t=7.92$ ,  $p<0.001$ ) doses compared with the vehicle group. The beneficial effect of 1 mg/kg MPH extended to the 5 s ITI compared to vehicle ( $t=3.20$ ,  $p=0.005$ ). However, similar to ATO, during long ITI trials with high-dose MPH (3 mg/kg), performance deteriorated both on the 7 s and 9 s ITI trials ( $t=-4.10$ ,  $p<0.001$ ;  $t=-4.41$ ,  $p<0.001$  respectively). Low-dose MPH (1 mg/kg) impaired performance during the 9 s ITI trials ( $t=-3.49$ ,  $p=0.002$ ).



**Figure 4.9 Effects of (a) ATO and (b) MPH on omissions;** ATO and MPH affected the proportion of omission errors differently depending on the ITI [Drug x ITI,  $F(12,380)=5.68$ ,  $p<0.001$ ]. **(a)** Treatment with ATO (1 mg/kg) increased the percentages of omission responses compared to vehicle on all ITIs (3 s ITI:  $t= 7.45$ ,  $p<0.001$ ; 5 s ITI:  $t= 8.21$ ,  $p<0.001$ ; 7 s ITI:  $t= 6.69$ ,  $p<0.001$ ; 9 s ITI:  $t= 5.23$ ,  $p<0.001$ ). **(b)** Treatment with both high (3 mg/kg) and low-dose (1 mg/kg) MPH reduced the percentage of omission responses on short 3s ITI trials ( $t=-6.47$ ,  $p<0.001$ ;  $t=-6.20$ ,  $p<0.001$  for the high and low doses respectively). \*low-dose vs vehicle  $p<0.05$ ; #high-dose vs vehicle  $p<0.05$ .

ATO and MPH affected the proportion of omission errors differently depending on the ITI.

**Figure 4.9** summarises these findings.



**Figure 410 Effects of (a) ATO and (b) MPH on premature responses.** ATO and MPH affected the proportion of premature responses differently depending on the ITI [Drug x ITI,  $F(12,380)=18.23$ ,  $p<0.001$ ]. **(a)** Administration of ATO both low-dose (0.3 mg/kg) and high-dose (1 mg/kg) decreased the probability of making a premature response on trials with 7s ( $t=-3.37$ ,  $p<0.01$ ;  $t=-6.77$ ,  $p<0.01$  for the low and high doses respectively) and 9 s ITIs ( $t=-3.96$ ,  $p<0.01$ ;  $t=-9.52$ ,  $p<0.01$  for the low and high doses respectively). ATO (1 mg/kg) also decreased the proportion of premature responses on the 5 s ITI trials ( $t=-2.90$ ,  $p=0.015$ ). **(b)** Administration of both low-dose (3 mg/kg) and high-dose (1 mg/kg) MPH increased the proportion of premature responses on the 5 s, 7 s and 9 s ITI trials (low-dose: 5 s ITI  $t=3.26$ ,  $p=0.005$ ; 7 s ITI  $t=7.17$ ,  $p<0.001$ ; 9 s ITI  $t=6.55$ ,  $p<0.001$ ; high-dose: 5 s ITI  $t=8.28$ ,  $p<0.001$ ; 7 s ITI  $t=11.02$ ,  $p<0.001$ ; 9 s ITI  $t=8.28$ ,  $p<0.001$ ). High-dose MPH (3 mg/kg) also increased the proportion of premature responses in the 3 s ITI trials ( $t=3.20$ ,  $p=0.006$ ). \*low-dose vs vehicle  $p<0.05$ ; #high-dose vs vehicle  $p<0.05$

ATO and MPH affected the proportion of premature responses differently depending on the ITI.

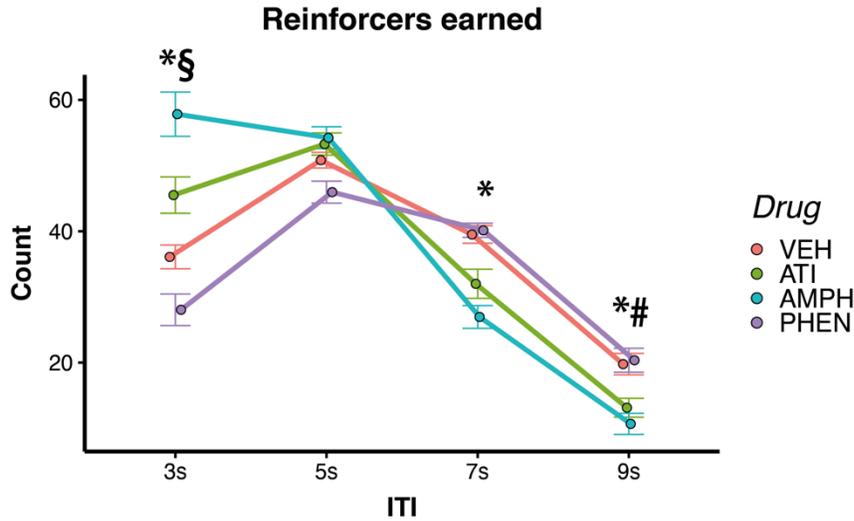
**Figure 4.10** summarises these findings.

While the most obvious effects of ATO and MPH on performance were dependent on ITI, some of these effects also depended on the impulsivity phenotype. For details see Appendix B (section B2.1.2.6.1).

### In summary

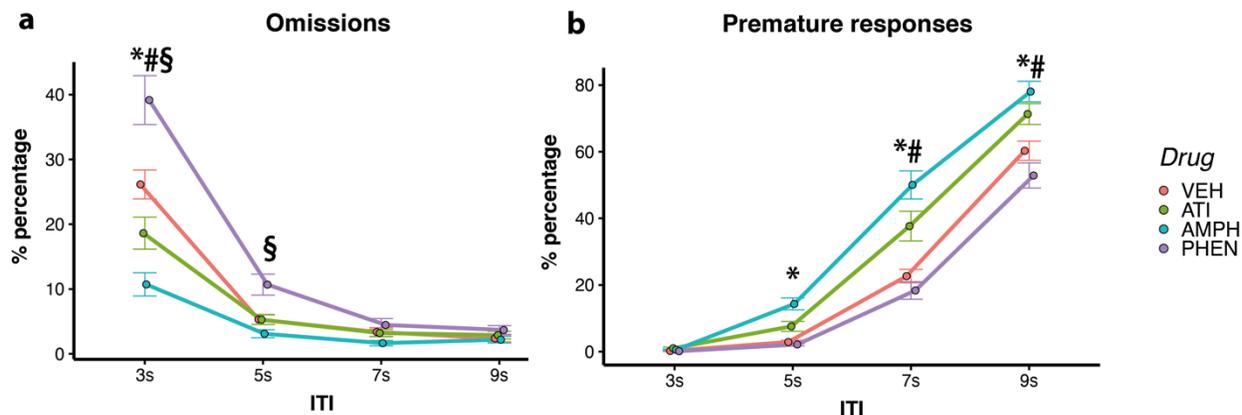
The above findings demonstrate that MPH and ATO have essentially opposite effects on performance. Whereas MPH led to a general activation of behaviour, increasing premature responses, decreasing omissions, and facilitating responding on short ITI trials, the administration of ATO produced a general inhibition of behaviour with reduced premature responses during long ITI trials and increasing omissions, especially during short ITI trials. Finally, the action of ATO was dependent on trait impulsivity and affected MID and LI rats more so than HI rats.

### 4.3.2.3 Effects of amphetamine, atipamezole and phenylephrine



**Figure 4.11** Effects of ATI (0.3mg/kg), AMPH (0.2mg/kg) and PHEN (1mg/kg) on the number of reinforcers earned. \*AMPH vs vehicle  $p < 0.05$ ; #ATI vs vehicle  $p < 0.05$ ; §PHEN vs vehicle  $p < 0.05$

**Figure 4.11** shows that AMPH, PHEN and ATI affected performance on long and short ITI trials differently depending on the ITI [Drug x ITI,  $F(9,285)=17.19$ ,  $p < 0.001$ ]. Specifically, during short 3 s ITI trials, animals earned more pellets after the administration of AMPH compared with vehicle ( $t=5.62$ ,  $p < 0.001$ ), but fewer pellets after the administration of PHEN ( $t=-3.42$ ,  $p=0.002$ ). There was also a trend for animals to earn more pellets on 3 s ITI trials following the administration of ATI compared to vehicle ( $t=2.25$ ,  $p=0.068$ ). During long 7 s and 9 s ITI trials, animals earned significantly fewer pellets following the administration of AMPH compared with vehicle ( $t=-4.28$ ,  $p < 0.001$ ;  $t=-4.69$ ,  $p < 0.001$  respectively). During 7 s ITI trials there was a trend for animals to earn fewer pellets following the administration of ATI compared with vehicle ( $t=-2.27$ ,  $p=0.064$ ). This effect was significant for the 9 s ITI trials ( $t=-3.20$ ,  $p=0.004$ ).



**Figure 4.12 Effects of AMPH, ATI and PHEN on (a) omissions and (b) premature responses.** (a) Omissions were affected differentially by AMPH, ATI and PHEN depending on the ITI [Drug x ITI,  $F(9,285)=7.22$ ,  $p<0.001$ ]. Figure 6b shows that treatment with ATI ( $t=-2.38$ ,  $p=0.05$ ) and AMPH ( $t=-6.12$ ,  $p<0.001$ ) reduced the proportion of omissions on the short 3 s ITI trials, however PHEN increased the proportion of omissions on the 3 s and 5 s ITI trials ( $t= 5.28$ ,  $p<0.001$ ,  $t= 3.58$ ,  $p=0.001$  respectively). (b) Premature responses were affected differently by AMPH, ATI and PHEN depending on the ITI [Drug x ITI,  $F(9,285)=4.40$ ,  $p<0.001$ ]. Administration of AMPH increased the proportion of premature responses in trials with 5 s ( $t=4.84$ ,  $p<0.001$ ), 7 s ( $t=6.73$ ,  $p<0.001$ ), 9 s ( $t=4.60$ ,  $p<0.001$ ) ITIs, whereas ATI only increased premature responses during 7 s ( $t=3.11$ ,  $p=0.006$ ) and 9 s ( $t=2.57$ ,  $p=0.030$ ) ITIs. \*AMPH vs vehicle  $p<0.05$ ; #ATI vs vehicle  $p<0.05$ ; §PHEN vs vehicle  $p<0.05$

Omissions and premature responses were affected differently by AMPH, ATI and PHEN depending on the ITI. **Figure 4.12** summarises these findings. Latencies for correct, incorrect and premature responses following administration of ATI, AMPH and PHEN are shown in Table 4.6B of Appendix B, section B2.1.2.7).

### In summary

The effects of AMPH, ATI and PHEN were mostly dependent on ITI. The administration of AMPH and ATI led to behavioural disinhibition, increasing premature responses, decreasing omissions, and facilitating responding on fast-paced, short ITI trials. Administration of PHEN, instead, led to a general inhibition of behaviour; reducing premature responses and increasing omissions, especially during short ITI trials.

## 4.4 Discussion

The findings reported in this chapter show that high and low levels of impulsivity can be both detrimental and advantageous to task performance, depending on the precise contingencies of the test environment. Specifically, HI rats performed best with short ITIs and fast stimulus presentations while LI rats were superior during the long ITI trials of the vITI-5CSRTT. Moreover, the effects of drugs used to treat ADHD also depended on the context of the test situation as well as baseline levels of impulsivity. Specifically, drugs that increase levels of catecholamines both cortically and subcortically, such as MPH and AMPH, conferred an advantage in the short ITI trials of the 5CSRTT and mimicked the behavioural profile of HI rats. In contrast, the selective NA reuptake blocker ATO (Swanson et al., 2006), which also increases DA release in the PFC but not the striatum (Carboni et al., 2006), decreased impulsivity, slowed response latencies, and improved performance during long ITI trials, mimicking the behaviour of LI rats. Importantly, we observed that the effect of ATO was partly dependent on trait impulsivity and exerted greater effects on behaviour in MI and LI rats.

In two separate experiments, over multiple sessions, HI rats made more correct responses and fewer omissions than LI and MID rats on the short, 3 s and 5 s ITI trials. In addition, HI rats were on average faster at making a correct response, regardless of ITI. This adaptive response was not the result of a strategy chosen based on the available contingencies. Indeed, when animals were challenged with a session that presented short ITIs only, LI rats (and to a lesser extent MID rats) were less able to adapt to the short trials and performed significantly worse than HI rats. On the contrary, when the task required the animals to wait for an extended period before responding, the behavioural phenotype typical of HI emerged, with many more premature responses being made in the long 7 s and 9 s ITIs.

Thus, trait impulsiveness is advantageous under high event rate conditions, unlike low levels of impulsivity. This suggests that there is an adaptive value to the impulsivity phenotype and that the advantage that this trait confers is revealed under conditions where rapid focusing of attention and action are required. Putting behavioural classifications aside, there was a strong

positive correlation between making a correct response on the short, 3 s, ITI trials and making a premature response on the long, 9 s, ITI trials. Thus, animals that made the most correct responses on the short-ITI trials also made the most premature responses in the long-ITI trials. Equally, there was a moderate positive correlation between making an omission on the 3 s ITI trials and making a correct response on the 9 s ITI trials. Therefore, the animals that performed well when the delay to work for food was longer, performed sub-optimally when the delay was short.

These data suggest that animals have an innate preference for responding with a specific pace and that this may be adaptive depending on the nature of the task. A possible reason why HI rats are particularly impaired during the waiting intervals of the vITI paradigm is that they are exposed to longer ITI trials later in the session and with less frequency compared to the other two impulsivity groups. More specifically, if an animal has been trained on a 5 s ITI delay and comes to anticipate this time delay, it will still notice and attend to the 3 s ITI trial as it comes earlier than expected and at a time at which the animal will already be attending to visual stimuli being presented. If, however, the time lag exceeds that which is expected, then an animal might already have a motor plan activated for an operant response and act upon it earlier than appropriate, thus making more premature responses. The more premature responses the animal elicits, the less likely the animal will be exposed to and thus have an opportunity to learn from the new delayed contingencies. In the present experiment, the 7 s ITI contingency was encountered earlier in the session compared to the 9 s ITI contingency (in both experiments, across multiple sessions); however there was no difference between the impulsivity groups with regards to when in the session these two time-contingencies were encountered for the first time. This means that the three groups encounter the new ‘longer’ contingencies around the same time during the session, thus suggesting that HI rats may have detected that they are required to wait for longer to gain a reward during long ITI trials. However, across days, there was a significant difference in frequency of successful trials and thus rewarded events to these ITIs, with HI making the least number of correct responses, across days, on the 7s and 9s ITI trials, compared to LI and MID rats. Thus, from a learning perspective, HI rats may have a weaker representation of the adaptive value of long ITI trials, which probably exacerbates their condition, making them even less able to withhold a response until the required time.

A further notable point is that LI rats tend to ‘give up’ on the fast trials and work mostly for the 7s and 9s ITI trials. Indeed, the variability of responses per unit time was significantly decreased in LI rats compared with HI rats. Short latencies and greater activity-per-unit-of-time have been postulated to require greater energy and thus be more cognitively and motorically more costly (Niv et al., 2005, 2007; Opris et al., 2011; Staddon, 2001). Specifically, it has been shown that animals will respond faster and with greater vigour when the opportunity to gain a reward is high, whereas they will be slower and less active when the expected reward from any given action is low, thus suggesting a cost/benefit decision process (Opris et al., 2011). This idea has been explored computationally by Niv and colleagues (2005; 2007) in two elegant papers where the authors developed a normative account of response vigour, taking both latency and choice of action into account. Specifically, the authors suggest that the expectation of future reward determines the rapidity/vigour with which the operant action is performed by acting as an opportunity cost, that is by determining whether the cost of responding fast and/or frequently is worth the outcome. Under this framework, HI rats would be interpreted as having an enhanced subjective utility of food reward, making them act faster and more vigorously. This would be adaptive when the animal needs to make a rapid operant response to secure a reward; however, it becomes disadvantageous when the animal needs to withhold such responses for periods longer than accustomed. In contrast, LI rats may have a reduced subjective utility of food-reward, and thus would more likely commit to longer trials that do not require rapid responding.

In both rodents and humans, impulsive action has been associated with risky decision-making (Barrus et al., 2015; Gabriel et al., 2019; Ioannidis et al., 2019), substance-abuse (Belin et al., 2008; Dalley et al., 2007a; Diergaarde et al., 2008; 2009; Voon, 2014), and with increased responsiveness for high-incentive foods such as sucrose (Diergaarde et al., 2009). These are all regarded as indices of greater sensitivity to reward-predicting cues (Gabriel et al., 2019; Diergaarde et al., 2009), thus it is possible that motor impulsivity is associated with enhanced value attribution to reinforcers, which is supported by recent evidence in humans (Mechelmans, et al., 2017). Results presented in Chapter 3 are somewhat in agreement with this idea as they show that premature responses are modulated by changes in the reinforcement rate of the task and thus by changes in the motivational value of the environment.

Further to the normative account of response vigour by Niv and colleagues (2005; 2007), the authors speculate that expectation of future reward, which is a key determinant of response vigour, could be signalled by DA transmission in the striatum. Thus, increased DA release might signal the expectation of future reward and determine faster and more vigorous responding. To corroborate this point, recent evidence (Wassum et al., 2012; Hamid et al., 2016; Mohebi et al., 2019, for a review on this see Klaus et al., 2019) has shown that DA release in the striatum (and ventral prefrontal cortex, Mohebi et al., 2019) positively correlates with reward density as well as the latency to initiate behaviour in anticipation of future reward. Importantly, DA transients do not appear to report how engaged an animal is, but instead, sub-second manipulations that augment DA release in the striatum increase the probability that an animal will engage in learned actions (Phillips et al. 2003, Hamid et al. 2016; for a review see Klaus et al., 2019). Indeed, DA release in the striatum has been suggested to play a key role in action initiation, by determining whether action plans that are generated in the cortex and relayed to striatal ensembles reach threshold activity and result in motor output (Klaus et al., 2019).

A surfeit of evidence points to a role of the mesolimbic DA pathway in the manifestation of impulsivity (for a review see Dalley and Robbins, 2017). Thus, high levels of DA release have been linked both to impulsivity and to fast/vigorous actions. Given that effort-based operant responses, including vigour in responding, are regarded as an index of motivation and incentive salience (Salamone & Correa, 2012), I was interested to test whether impulsivity is also associated with higher performance in another task that canonically has been used to test effort-based motivation, specifically the progressive ratio (PR) task. This task taps into cost/benefit decision making processes (Salamone et al., 2009), by requiring animals to perform an instrumental response (in this case lever presses) under increasing work demands (Hailwood et al., 2018). Importantly, performance on this task has also been shown to depend on DA transmission in the striatum (Aberman et al., 1998; Salamone et al., 2005).

A correlation was found between breakpoint -both in the exponential and linear paradigm- and premature responses on the 7 s and 9 s ITIs. However, when inferential statistics were applied to the data to determine group differences on this measure, only a trend level difference between groups was detected. There are multiple reasons for why a strong association between

impulsivity and breakpoint in the PR task was not found. Firstly, the sample may have been too small to observe a significant effect of group, hence why a difference was only detected at a trend level. Secondly, animals were tested only on one effort-based motivation task, however there are many more, for example: operant tasks with choice procedures that offer distinct reinforcers for different effort requirements (Randall et al., 2012; Salamone et al., 1991; Salamone et al., 2007; Salamone & Correa, 2002; Yohn, et al., 2015a); tasks of effort discounting (Bardgett et al., 2009; S. B. Floresco et al., 2008); and T-maze tasks that use a vertical barrier to provide an effort-related challenge (Cousins et al., 1996; Mai et al., 2012; Mott et al., 2009; Salamone et al., 1994). Thus, to have a more complete understanding of the association between impulsivity and motivation rats with varying levels of impulsivity should be tested on different kinds of motivation tasks. Finally, there are reports in the literature of a dissociation between likelihood of making an effort-based choice and speed of responding (Bardgett et al., 2009; Salamone et al., 1994; Yohn, et al., 2015a; Yohn, et al., 2015b). While these reports do not come from time-constrained, operant-conditioning tasks -and so cannot allow a direct comparison with the present experiment- they nonetheless suggest that response latency and effort-related choice have partially overlapping but dissociable neural circuits (Salamone et al., 2007; Yohn, et al., 2015a). Linked to this, HI rats have been postulated to have high extracellular levels of DA in the shell sub-region of the NAcb (Dalley and Robbins, 2017; Jupp et al., 2013), whereas effort-based behaviour has predominantly been associated with high levels of DA in the core sub-region of the NAcb (Salomone et al., 2007). Thus, these points could explain why we did not observe a strong association between motor impulsivity and breakpoint on the PR task.

The findings of this chapter also demonstrate that the effects of drugs used in ADHD are both context and trait dependent. Specifically, drugs that increase the release of DA and NA both cortically and subcortically, such as MPH and AMPH (Bymaster et al., 2002; Kuczenski & Segal, 2001), reduced response latencies and improved performance on fast-paced trials. In long ITI trials, instead, they increased premature responses and impaired performance, mimicking the behaviour of HI rats. Behavioural results obtained with these drugs agree with previous research on impulsive action, whereby increasing extracellular DA levels in the ventral striatum (Economidou et al., 2012), led to enhanced behavioural activation and increased premature

responses (Baarendse & Vanderschuren, 2012; Milstein et al., 2010; Murphy et al., 2008; Navarra et al., 2008; Pattij et al., 2007; Sun et al., 2012). Navarra and colleagues (2008) have also observed an improvement of performance on the 5CSRTT with MPH on short ITI trials. We confirm these findings and extend this effect to low-dose AMPH. These results add to a growing body of literature on the pro-cognitive effects of psychostimulants in animals (Paine et al., 2007; Tomlinson et al., 2014; Turner et al., 2017) and in humans (Pietrzak et al., 2006; Wardle et al., 2011). These results are also in line with computational (Niv et al., 2005; 2007) and empirical evidence (Hamid et al., 2016; Klaus et al., 2019; Mohebi et al., 2019; Wassum et al., 2012) that increased DA transmission in the striatum lowers the threshold for action initiation and invigorates operant responding by a process of activation (Robbins & Everitt, 2007). Importantly, these findings also suggest that the advantage conferred by impulsivity in highly stimulating contexts may be due to increased levels of DA in the striatum. This is in line with evidence suggesting that HI rats exhibit increased synaptic DA levels in the shell sub-region of the ventral striatum, possibly because of reduced expression of the DA transporter and decreased DA D2/D3 receptor availability in this region (Dalley & Robbins, 2017; Jupp et al., 2013).

In contrast to MPH, systemic administration of ATO, which increases extracellular NA and DA levels in the PFC but not the striatum (Carboni et al., 2006; Swanson et al., 2006), improved performance on long ITI trials, increased omissions on short ITI trials and slowed responding in general, mimicking the behaviour of LI rats. These results are in line with previous research on 5CSRTT showing that ATO reduces premature responses in this task (Blondeau & Dellu-Hagedorn, 2007; Economidou et al., 2012; Fernando et al., 2012; Navarra et al., 2008; Robinson et al., 2008). Contrary to previous evidence (Navarra et al., 2008 5 s ITI 1.0 mg/kg; Callahan et al., 2019 2.5 s ITI 3.0 mg/kg), however, we did not observe an increase in the probability to make a correct response during short ITI trials following administration of ATO, but instead, a decrement in performance, consistent with a role of ATO in reducing behavioural activation. Importantly, MID and LI rats were more sensitive to the deactivating effects of ATO than HI rats. Thus, trait-related factors appear to modulate the behavioural effects of ATO. Studies using tasks other than the 5CSRTT have also observed reduced behavioural responding following the administration of ATO, such as an increase in omissions on a cognitive-effort task (Hosking et al., 2015) and a decrease in breakpoint in a PR choice task (Yohn et al., 2016). The specific

mechanisms of how ATO strengthens behavioural inhibition are not well understood. In the context of 5CSRTT, it is possible that ATO reduces premature responses by increasing NA transmission in the NAcb shell (Benn & Robinson, 2017; Economidou et al., 2012). Others have found that administration of ATO reduces DA release in the NAcb core, with a concomitant behavioural effect of decreased willingness to exert effort in a PR task (Yohn et al., 2016). It was speculated from these findings that ATO may be acting *via* alpha-2 adrenergic receptors on VTA DA neurons to reduce NAcb DA release (Guiard et al., 2008; Yohn et al., 2016).

Antagonism of alpha-2a adrenoceptors with ATI yielded results comparable to those of psychostimulants, with a decrease in omissions during short ITI trials and an increase in premature responses during long ITI trials. In contrast, the alpha-1 adrenoceptor agonist PHEN resulted in a behavioural profile like that of ATO, reducing correct responses and increasing the probability of omissions during short ITI trials. These results are consistent with previous research in the 5CSRTT (Koskinen et al., 2003; Pattij et al., 2012; Sirviö et al., 1993) showing that PHEN leads to an overall inhibition of responding while administration of ATI results in an increase in behavioural activation. Given that administration of ATI yielded results markedly different from those of ATO, it is unlikely that ATI acts on pre-synaptic alpha-2a autoreceptors expressed on NA fibres to activate NA transmission (Berridge & Waterhouse, 2003). Instead, ATI may be acting on post-synaptic alpha-2a adrenoceptors subcortically to increase DA release, either *via* its action on alpha-2-autoreceptors on VTA DA cells (Guiard et al., 2008), or by reducing DA release in the striatum (Yavich et al., 2003). Alternatively, ATI may be acting on post-synaptic alpha-2a adrenoceptors receptors located in the PFC. Studies in non-human primates have shown that blockade of these receptors with infusions of yohimbine, an alpha-2a antagonist, in the dorsolateral PFC, increases impulsivity (Ma et al., 2003) and induces locomotor hyperactivity (Ma et al., 2005). Finally, it is possible that the slowing of behavioural activation observed with PHEN could be mediated by a cortical action. This suggestion is based on evidence that activation of alpha-1 adrenoceptors in the PFC impairs working memory performance both in rodents (Arnsten et al., 1999; Birnbaum et al., 2004) and monkeys (Arnsten & Jentsch, 1997; Birnbaum et al., 2004; Mao et al., 1999).

In conclusion, our findings demonstrate that trait impulsivity can be advantageous in certain contexts, specifically when rapid responding and attentional focusing is required. Based on research in humans diagnosed with ADHD, it is apparent that stimulating environments can help remediate decrements in performance. The present findings demonstrate that this is also true for experimental approaches in animals used to assess impulsivity. Importantly, we also explored the role that catecholamines play in the performance of high-event rate tasks and we suggest that drugs that elevate subcortical, as well as cortical DA levels improve performance on fast-paced trials, while drugs that act mainly to block the reuptake of NA, slow responding in such contexts. These results have important implications for our understanding of impulsivity, the context within which it manifests and the pharmacological agents that are used to treat ADHD. In the chapter that follows, the dopaminergic bases of impulsivity is more directed investigated to reveal circuit dynamics that regulate premature responses.

# Chapter 5 - Role of mesolimbic dopamine circuits in impulsivity

## 5.1 Introduction

Substantial research has been conducted to investigate the neural processes contributing to premature responding (for a review see Dalley and Robbins, 2017). Early evidence pointed to a role of the mesolimbic DA pathway in the manifestation of this behaviour. For a review on this see Chapter 1, section 1.5.3.2.1 and Dalley and Robbins (2017). Briefly, there is evidence that trait impulsivity in rodents is associated with reduced density of the DA transporter (DAT) and DA D2/3 receptors in the shell sub-region of the NAcB (Dalley, et al., 2007a; Jupp et al., 2013). Under the assumption that abnormalities in the level of DA D2/3 receptors affect primarily autoreceptors located on DA fibres projecting to the NAcB, these findings led to the hypothesis that impulsive responding arises from increased dopaminergic release from VTA terminals into the NAcB shell ('pre-synaptic hypothesis', Dalley and Robbins, 2017). This hypothesis is supported by findings in rodents that HI rats have reduced mRNA levels of DA D2 receptors in the VTA (Besson et al., 2013) and by findings in humans that highly impulsive individuals are characterised by a reduction in midbrain D2/3 autoreceptors and by an increase in amphetamine-induced DA release in the striatum (Buckholtz et al., 2010). Another possible interpretation of why reduction in D2/3 receptors in the NAcB is associated with trait impulsivity is that this reduction is affecting primarily MSNs in the NAcB ('post-synaptic hypothesis') such that there is an imbalance of DA-binding at the striatal level, with increased midbrain DA release predominantly targeting D1 receptors in NAcB due to a decreased availability of D2/3 receptors. This second hypothesis is supported by evidence that microinfusions of the D1 receptor antagonist in the NAcB shell reduced impulsivity in the 5CSRTT (Pattij et al., 2007). Unfortunately, classical pharmacological approaches with selective D2/3 receptor antagonists are unable to resolve the precise contribution of D2/3 receptors to impulsivity (e.g. Besson et al., 2010; Pattij et al., 2007). Thus, both hypotheses are possible and need not be mutually exclusive.

While plausible, neither of these hypotheses are supported by recent evidence. For example, Boekhoudt and colleagues (2017) reported that chemogenetic activation of tyrosine hydroxylase-positive (TH+) cells in VTA, which are predominantly dopaminergic in phenotype, does not lead to an increase in premature responding but rather impaired attention as shown by a significant increase in omissions in the 5CSRTT. A few points, however, should be considered when interpreting these findings. First, the virus was expressed under the TH promoter, which does not express solely in DA neurons but also in glutamatergic and GABA-ergic neurons (Lammel et al., 2008; Morales & Margolis, 2017). Excitation of non-DA TH+ positive cells could indeed counteract or mask any effect elicited solely by the recruitment of midbrain DA neurons projecting to the ventral striatum. Secondly, even if dopaminergic TH+ cells were predominantly targeted, such a targeting method would still drive excitation both in NAc shell- and core-projecting neurons. Considering evidence showing an ‘opponent’ interaction between these two sub-regions of the NAc, the simultaneous activation of pathways projecting to both regions may again result in a net cancellation and lack of observable behavioural outcome (Dalley and Robbins, 2017).

To further investigate whether trait-like impulsivity is mediated by a hyper-dopaminergic state in the VTA-NAcc shell circuit, two inter-locking experiments were designed. The first experiment aimed at exploring whether pharmacologically reducing the activity of DA cells in the VTA, and thus DA release in the striatum, reduces premature responding. Prior research in the laboratory established that systemic administration of the selective D2/3 receptor agonist quinpirole reduced impulsivity in a dose-dependent manner (Fernando et al., 2012). The neural locus of that effect has not been established, however it is plausible that quinpirole acts presynaptically on VTA DA cells, since infusion of this drug in the NAc core increases impulsive responding (albeit in HI rats only) while infusion in the NAc shell has no effects on premature responses in the 5CSRTT (Moreno et al., 2013). In this experiment quinpirole was infused directly into the VTA and its effects were monitored during task performance. It was predicted that quinpirole would predominately inhibit DA neurons and reduce impulsivity. However, since this approach is unable functionally to dissociate core-projecting from shell-projecting neurons, a second experiment was introduced to chemogenetically inhibit shell-projecting and core-projecting

midbrain cells during performance of the 5CSRTT. Chemogenetics uses Designer Receptors (engineered human muscarinic receptors) that are Exclusively Activated by Designer Drugs (DREADDs) to modulate cell activity (Boender et al., 2014). In this experiment a retrograde Cre CAV2 virus was infused in the shell or core region of rats trained on the 5CSRTT. Concurrently, a second Cre-dependent virus was infused expressing, in a Cre-dependent manner, the hM4(Gi) receptor. Upon delivery of the designer drug clozapine N-oxide (CNO), the hM4(Gi) receptor opens G-protein sensitive inwardly rectifying potassium channels (GIRKs), driving cellular inhibition (Lieb et al., 2019). In this way, neuroanatomical and functional specificity were achieved, the former targeting a projection-specific circuit and the latter silencing VTA-ventral striatal circuits. Prior to the second main experiment, a pilot experiment was also carried out to test the validity of the chemogenetic method. This pilot experiment tested whether inhibiting the VTA-shell pathway *via* the expression of the hM4(Gi) receptor and the delivery of CNO would lead to behavioural changes in locomotion and motivated instrumental behaviour in a progressive ratio test (PR). These behaviours were chosen because DA is involved in the expression of both (Salomone et al., 2007) and because they have been used in previous research to validate the efficacy of DREADDs expressed in the VTA (Boender et al., 2014). Finally, the pilot experiment tested whether clozapine (0.1 mg/kg) could act as a designer drug and activate DREADDs. This was investigated considering recent evidence that CNO reverse-metabolizes to clozapine once administered, thereafter entering the brain and activating transfected DREADDs (Gomez et al., 2017).

## **5.2 Experiment 1 - VTA cannulation**

### **5.2.1 Materials and methods**

#### **5.2.1.1 Animals**

Twelve outbred male Lister Hooded rats (Charles River, Margate, UK) weighing 280–300 g at the beginning of the experiments were used. Animals were kept under the conditions specified in Chapter 2 (see section 2.1).

#### **5.2.1.2 Five-choice serial reaction time task: training and screening for impulsivity**

Twelve rats were trained in the 5-CSRTT as described in Chapter 2 (section 2.3), to reach a stable baseline performance with a final stimulus duration of 0.7 s and an ITI of 5 s. Rats were subsequently exposed to three fixed 7 s ITI sessions, each separated by two days of baseline testing. This was done with the aim to expose animals to the long ITI challenges before the intra-VTA pharmacological manipulations.

#### **5.2.1.3 Intracranial surgery**

Bilateral 22-gauge double guide cannulae (Plastics One, Sevenoaks, UK), extending 4 mm below the plastic pedestal, were implanted bilaterally above the VTA (coordinates in mm relative to Bregma: AP. -5.4; ML. 0.75. DV. -1.6 (below dura); For more details see Chapter 2 (section X). Cannulae were secured to the skull with dental acrylic and stainless-steel screws and occluded by a stylet and a dust cap. After surgery, animals recovered for 7 days in their homecages (single-housed).

### **5.2.1.4 Drugs**

(-)-Quinpirole hydrochloride was purchased from Sigma (St. Louis, MO, USA), dissolved in filtered 0.9% saline, and administered by intracranial infusion (0.25  $\mu$ l per infusion at a rate of 0.125  $\mu$ l/min).

### **5.2.1.5 Intracranial microinfusions**

Drug and vehicle infusions were given 12 min before behavioural testing. Micro-infusions were delivered through a 28-gauge bilateral injector (Plastics One, Roanoke, USA), inserted through the guide cannula and extending 6.5 mm beyond the tip of the guide. Animals were habituated to the infusion procedure over two daily sessions separated by a day of just baseline training on the 5CSRTT (5 s ITI). On the first habituation day, the injector was lowered into the double guide cannula and left in place for 1 min. On the second habituation day, rats received a single vehicle infusion over 2 min (saline, 0.25  $\mu$ l) and were then run on the 5CSRTT as a normal baseline training day (5 s ITI). During the infusion procedure, rats were gently restrained by the experimenter while the obturators were removed from the cannulae and the injectors lowered into the intended brain region. Prior to, and after each infusion, the injector remained in the brain for 1 min. When the injector was removed, the obturator was cleaned with ethanol (2%), rinsed in distilled deionised water, and lowered through the guide cannula. The animal was then placed into the test apparatus.

Following re-establishment of stable performance on the 5CSRTT, intracerebral microinfusions of quinpirole were carried out. Infusions of quinpirole (veh, 0.01, 0.03, 0.3 and 1  $\mu$ g/ $\mu$ l) were delivered according to a randomized Latin-square design and were tested with sessions comprising a long ITI of 7 s. The infusion experiments were run over a 3-day cycle, starting with an initial baseline session (5 s ITI). On day 2, animals received an infusion of drug or vehicle (veh) before testing on a long ITI session (ITI: 7 s; SD: 0.7 s). On day 3, animals were tested again on a baseline session (5 s ITI; SD: 0.7 s).

### 5.2.1.6 Cannulae tip placement

Brains were extracted and sectioned (60  $\mu$ m) as described in Chapter 2 (section 2.5). Every sixth section was mounted on glass slides and stained with Cresyl Violet. The sections were used to verify cannulae tip placement.

### 5.2.1.7 Data Analysis

The main variables of interest across experiments were the number of reinforcers earned; accuracy; number of premature responses; number of omissions; response latency. Analyses were performed as described in Chapter 2 (section 2.6). For details on the fixed factors included in all LMEM models see Appendix C (section C1.1).

## 5.2.2 Results VTA

### 5.2.2.1 Histology

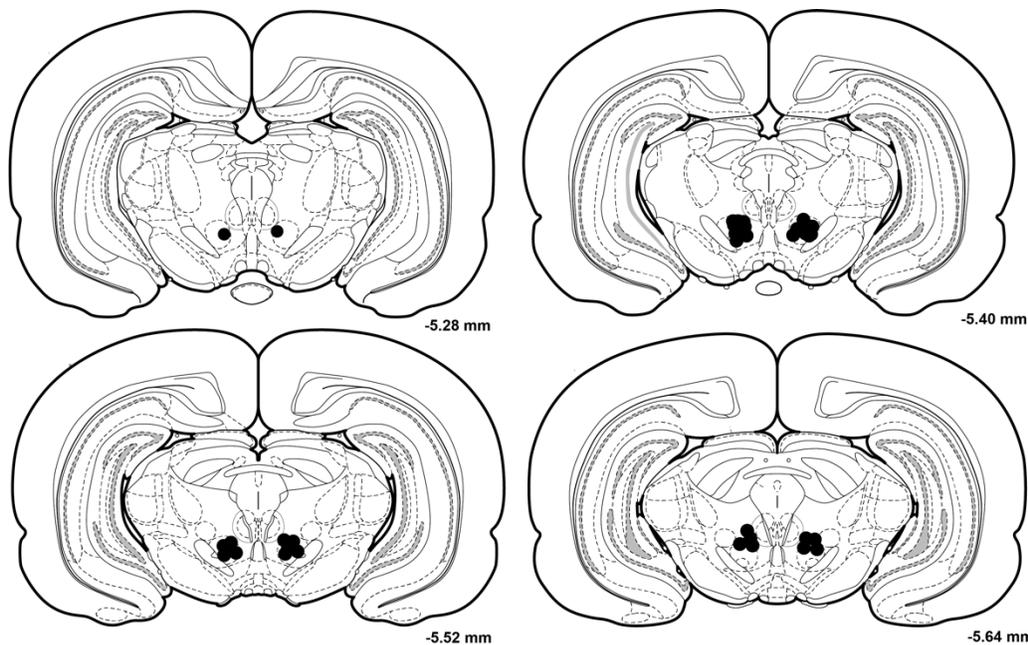


Figure 5.1 Injector tip placements in the VTA. Paxinos and Watson (6th ed.) 2007.

The ventral-most locations of injectors are included in **Figure 5.1**. No rats were excluded from the study. For an exemplar image of a VTA cannulation see Appendix C.

### 5.2.2.2 Effects of microinfusions of quinpirole into VTA during performance on 5CSRTT

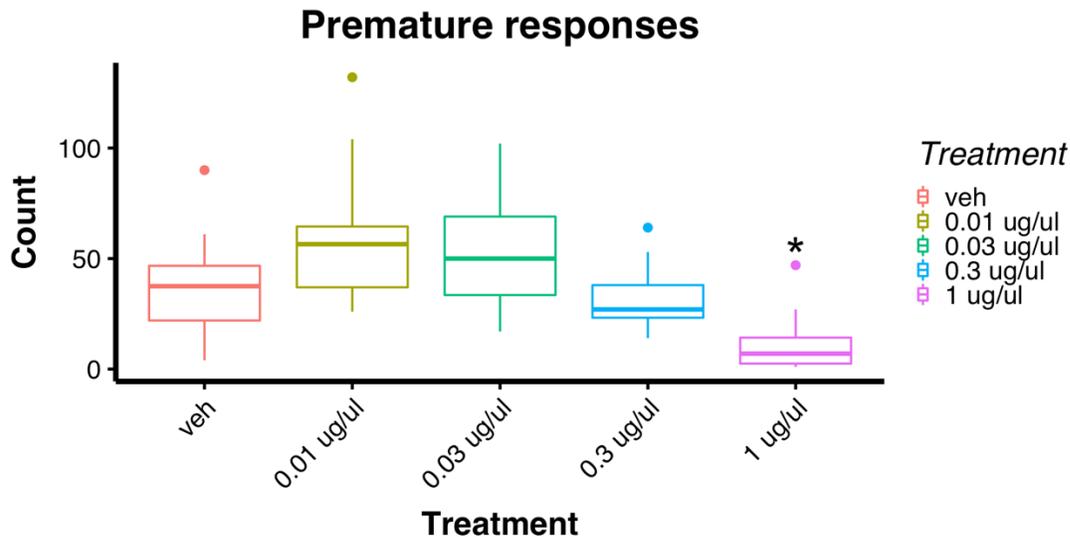


Figure 5.2 Effects of intra-VTA infusions of quinpirole on premature responses in the 5CSRTT. \*significant difference compared with vehicle  $p < 0.05$ .

There were no significant main effects of quinpirole dose on the number reinforcers earned [ $F(4,44)=1.12$ ,  $p=0.359$ ] or accuracy [ $F(4,44)=1.42$ ,  $p=0.243$ ]. As shown in **Figure 5.2**, however, quinpirole did affect the number of premature responses [ $F(4,44)=14.98$ ,  $p < 0.001$ ], with the highest dose significantly decreasing premature responses compared with the vehicle condition and intermediate doses ( $p < 0.05$  for all comparisons). The highest dose also produced more omissions than the vehicle and all other doses; however, this difference did not reach significance ( $p=0.127$ ). **Table 5.1** summarises results for collection and response latencies. Briefly, after administration of the highest dose of quinpirole rats were slower at collecting food and responding to the cue in the FP.

Latencies (ms)	Vehicle	0.01 µg/µl	0.03 µg/µl	0.3 µg/µl	1 µg/µl
Correct latency	675.17 (35.15)	646.10 (31.98)	668.22 (41.20)	682.46 (43.39)	<b>818.05*</b> <b>(55.97)</b>
Incorrect latency	1405.78 (156.59)	1205.24 (120.86)	1226.74 (132.77)	1380.94 (99.84)	<b>1863.14*</b> <b>(188.04)</b>
Collection latency	1509.35 (84.53)	1394.41 (70.88)	1431.24 (93.42)	1513.59 (113.85)	<b>1669.17*</b> <b>(82.93)</b>

**Table 5.1 Response latencies on the 5CSRTT following intra-VTA infusions of quinpirole (0, 0.01, 0.03, 0.3, 1 µg/µl).** Mean (in ms) and standard error (SE) in brackets. Collection latencies were significantly affected by quinpirole [dose:  $F(4,44)=4.81$ ,  $p=0.002$ ] with animals being slower at collecting food after administration of the highest dose 1 µg/µl, compared to 0.01 µg/µl ( $p=0.002$ ) and 0.03 µg/µl ( $p=0.007$ ). Response latencies were influenced by dose [ $F(4,99)=7.58$ ,  $p<0.001$ ] and response type [ $F(1,99)=241.11$ ,  $p<0.001$ ]. Rats were faster at making a correct response compared to an incorrect response regardless of dose ( $p<0.001$ ); however, these responses were significantly slower following administration of the highest dose of quinpirole compared to vehicle and the intermediate doses  $p<0.05$ . \* $p<0.05$  compared to vehicle.

### In summary

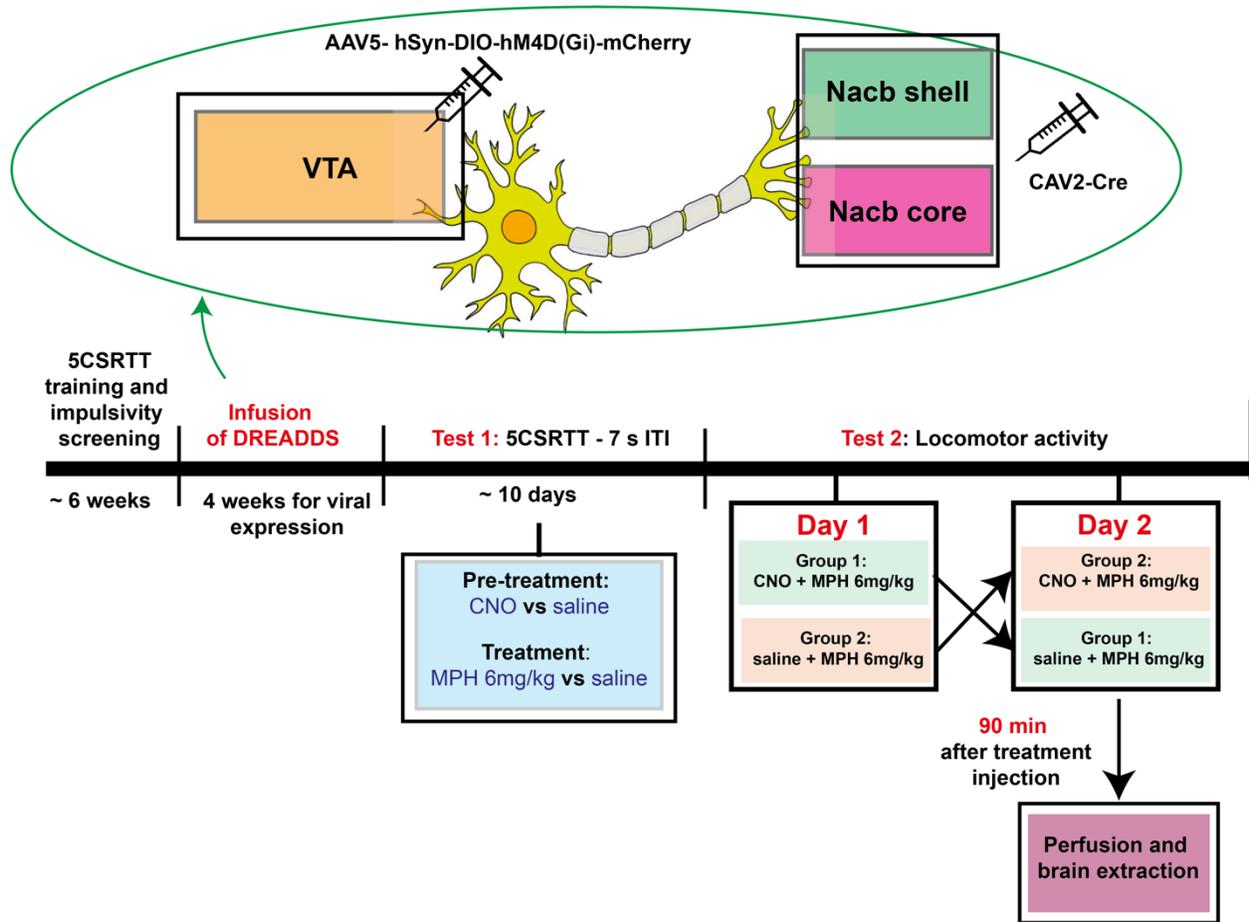
Quinpirole infused in VTA, at the highest dose of 1µg/µl did not affect accuracy or the number of reinforcers earned but decreased premature responses and slowed responding. This in line with data from Fernando and colleagues (2012) who showed that systemic administration of this drug reduced impulsive responding. In addition, the data presented above refine such findings suggesting that the systemic effect of quinpirole that Fernando and co-workers (2012) observed on impulsivity is due to the action of this drug on dopamine cells in the midbrain. Finally, this data also supports the idea that lower levels of impulsivity observed in LI in this and previous chapters may be due to low levels of dopamine release in the ventral striatum.

## **5.3 Experiment 2 - circuit-specific manipulations**

### **5.3.1 Pilot experiment**

This pilot experiment was aimed at exploring whether DREADDs is a reliable technique to silence cells of the VTA-striatal pathway. More specifically, inhibitory DREADDs (hM4D(Gi)) were expressed in cells projecting from VTA to the medial shell 1) to validate with immunohistochemical techniques whether expression of the virus in this pathway is successful and 2) to test whether silencing these cells through delivery of the designer drug clozapine-N-oxide (CNO) significantly affects premature responding and other behavioural variables in the 5CSRTT. For more details see Appendix C (section C2).

### 5.3.2 Chemogenetics in the context of 5CSRTT



**Figure 5.3** Schematic showing the experimental timeline and of the viral manipulation used. CAV2-Cre was injected in the NAcb shell or core sub-regions. This virus is uptaken by VTA terminals in the NAcb and transported retrogradely to the cell body, where it expresses Cre. A second virus was infused into the VTA that encodes for the expression of DREADDs in a Cre-dependent manner. In this way only VTA projections to the NAcb express the DREADDs virus.

Following promising results from the pilot experiment, the experiment described below aimed at dissociating the contribution of the VTA-core vs VTA-shell pathways in the control of premature responses on the 5CSRTT. This was achieved by inhibiting the two pathways, in different animals, with the use of the inhibitory DREADD hM4D(Gi). A timeline of experiments is presented in **Figure 5.3**. Approximately four weeks after viral infusion, rats were tested on a long ITI session of the 5CSRTT following pre-treatment of either CNO (3 mg/kg) or saline and treatment of either MPH (6 mg/kg) or saline 25 min after pre-treatment. To test the efficacy of the chemogenetics intervention, rats were also tested on locomotor activity as done in the pilot

experiment. For this experiment, rats were also pre-treated with either saline or CNO (3 mg/kg) and then, after 25 min, were administered MPH (6 mg/kg). On the second day of this cross-over design, rats were perfused 90 min after the MPH injection, the brains were harvested and stained both for the expression of the virus (in VTA) and of c-fos in the ventral striatum. C-fos is an immediate early gene which is rapidly induced when the neuron is activated (Cohen & Greenberg, 2008; Morgan & Curran, 1991). Its protein product has long been used as a marker of neuronal activation (Cruz et al., 2015). The expression of c-fos was evaluated as a further test of the efficacy of the chemogenetics method. Specifically, MPH is known to amplify DA release by inhibiting its reuptake (Bymaster et al., 2002) and to increase c-fos expression in this region (Brandon & Steiner, 2003). Thus, it was hypothesised that if activation of hM4D(Gi) inhibits dopaminergic fibres projecting from the VTA onto either the NAc shell or core, rats infused with inhibitory DREADDs should show a reduction in c-fos expression in the VS when treated with CNO + MPH. On the contrary, these rats should present strong c-fos expression when treated with saline + MPH. Finally, control rats infused with an empty virus (just the mcherry fluorescent tag) were predicted to show high c-fos expression following MPH delivery, regardless of whether they were pre-treated with saline or CNO.

### **5.3.2.1 Methods and Materials**

#### **5.3.2.1.1 Animals**

Thirty outbred male Lister Hooded rats (Charles River, Margate, UK) weighing 280–300 g at the beginning of the experiments were used. Animals were kept under the conditions specified in Chapter 2 (see section 2.1).

#### **5.3.2.1.2 Five-choice serial reaction time task: training and screening for impulsivity**

Thirty rats were trained in the 5-CSRTT as described in Chapter 2 (section 2.3). Following stable baseline performance on the 5-CSRTT (stage 10, stimulus duration: 0.7, ITI: 5 s), rats were screened for impulsivity. Specifically, rats were challenged with three long ITI sessions (stimulus duration: 0.7, ITI: 7 s) as described previously (Belin et al., 2008; Dalley, et al., 2007a; Economidou et al., 2012). Such sessions were presented at weekly intervals and subjects were

ranked according to their level of impulsivity throughout the 3-week screening procedure. This was done to create groups for the viral manipulation that were balanced for premature responses. One animal was culled prior to the surgery due to spontaneous epileptic seizures.

### 5.3.2.1.3 Intracranial Surgery

All rats were anaesthetised with isoflurane and secured in a stereotaxic frame. To induce DREADD expression selectively in midbrain neurons projecting to medial shell and core we used Cre-dependent DREADD (Addgene, UK) combined with canine-adenovirus2 expressing Cre recombinase (CAV2-Cre, PVM, France). **Table 5.2** shows details of group size and viral manipulations.

Groups	Projection	Virus in VTA (1,00E+09pp/ul)	Coordinates for VTA mm (from bregma)	Coordinates for CAV2- Cre mm (from bregma) (3,33E+09pp/ul)
Group 1 (N=10)	VTA- NAcb medial shell	AAV5- hSyn-DIO- hM4D(Gi)-mCherry	-AP -5.6, -ML at +/-1.82 (angle of 10°), -DV -7.8	-AP +1.45, -ML at +/- 0.75 -3 DV coordinates -5.4; - 5.9; and -6.9 (below dura).
Group 2 (N=10)	VTA- NAcb core	AAV5- hSyn-DIO- hM4D(Gi)-mCherry	-AP -5.6, -ML at +/-2.04 (angle of 10°), -DV -7.5	-AP +1.45, -ML at +/-1.5, -2 DV coordinates -6.1 and -6.6 (below dura).
Group 3 (N=4)	VTA- NAcb medial shell	AAV5- hSyn-DIO- mCherry	-AP -5.6, -ML at +/-1.82 (angle of 10°), -DV -7.8	-AP +1.45, -ML at +/-0.75, -3 DV coordinates: -5.4; -5.9; and -6.9 (below dura).
Group 4 (N=5)	VTA- NAcb core	AAV5- hSyn-DIO- mCherry	-AP -5.6, -ML at +/-2.04 (angle of 10°), -DV -7.5	-AP +1.45, -ML at +/-1.5, -2 DV coordinates -6.1 and -6.6 (below dura).

**Table 5.2 Summary of the virally-mediated interventions.** Coordinates (in mm) are taken from Bregma and the dural surface. Viruses were infused at a rate of 0.1 ul/min. The needle was left in place for 10 min after the last DV infusion, to allow for diffusion of the virus. Light blue shading = groups with DREADDs; purple shading = groups with empty virus.

#### **5.3.2.1.4 Effects of CNO on 5CSR TT performance**

Four weeks after surgery rats were re-baselined on the 5CSR TT. Once a stable baseline level was reached, the animals were tested on a series of pharmacological challenges, in a counter-balanced manner, to investigate the effects of inhibitory DREADDs on premature responses on a long 7 s ITI session in the 5CSR TT. Specifically, rats were pre-treated with an IP injection of either CNO (3 mg/kg, HelloBio, UK) or saline (IP) and then returned to their home cage. After 25 min, animals received injections of either MPH (6 mg/kg, IP) or saline and were tested on the 5CSR TT (stimulus duration: 0.7, ITI: 7s). Animals were then tested on two baseline schedules until performance stabilised before the next treatment. All injections followed a Latin square design.

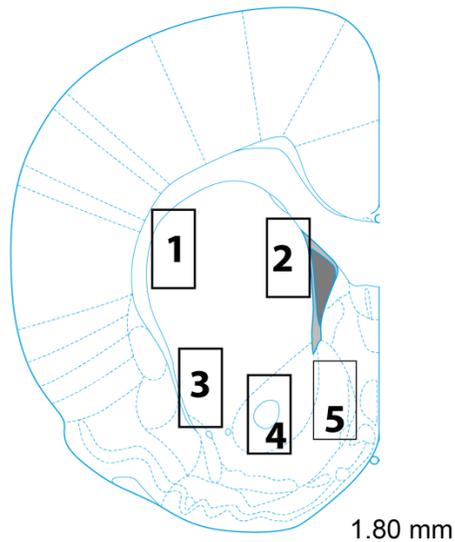
#### **5.3.2.1.5 Effects of CNO on locomotor activity**

Locomotor activity was assessed using 10 activity chambers (MedAssociates; 29.5 x 32.5 x 23.5 cm, USA), equipped with infrared photocell beams and controlled by a PC. Locomotor activity was measured as the number of photocell beam breaks every 5 min for 90 min following the beginning of the session. Rats were pre-treated with an IP injection of either CNO (3 mg/kg, HelloBio, UK) or saline (IP) injections and immediately placed into the activity cages. After 25 min, animals received injections of MPH (6 mg/kg, IP). Animals were assigned to treatment groups in a counterbalanced manner, according to their viral manipulation.

#### **5.3.2.1.6 Immunohistochemistry**

Rats were perfused and brains were extracted as described in Chapter 2 (Section 2.5). For this study, cfos staining was performed on ventral striatum (~1.44 mm anterior to bregma), while TH and mcherry staining was performed on midbrain (~5.4 to ~6.00 mm posterior to bregma). For details on the protocol used see Appendix C (section C2.2.1.1)

### 5.3.2.1.7 Regions of interest



**Figure 5.4 Schematic diagram of a representative coronal brain section selected for immunohistochemical analysis.** Frames delineate the areas analysed within each region of interest: (1) Dorsolateral striatum (DLS); (2) Dorsomedial striatum (DMS); (3) NAcb lateral shell; (4) NAcb core; (5) NAcb medial shell; 1.80 mm from bregma; (Diagrams modified from Paxinos and Watson (6th ed.) 2007.)

Medial NAcb shell and core, lateral shell as well as dorsomedial striatum (DMS) and dorsolateral striatum (DLS) were analysed for c-fos immunoreactivity, within sections 2.16 mm to 1.32 mm anterior from bregma (see **Figure 5.4**). All viral vectors infused in the VTA (both Gi and mcherry-only) were tagged with an mcherry molecule that could be stained for and quantified. VTA was analysed for mcherry-TH colocalisation in sections within -5.50 mm to -6.50 mm posterior from bregma (according to Paxinos and Watson (6th ed.) 2007). The counting frames were placed in similar localizations in each region. For each region, cell counting was carried out using two images: an anterior and a posterior slice of the region of interest.

### 5.3.2.1.8 Semi-quantitative assessment of immunoreactive neurons

Cell counting was carried out manually by a blind observer. The number of positive c-fos cells for each region was averaged across both the anterior and posterior slice and across both hemispheres. To count c-fos in single-stained sections, 10X magnified images of the areas of interest were obtained in a Zeiss Imager.Z1 microscope and c-fos cells were counted with

ImageJ 1.46r software by an automated procedure counting particles of appropriate size with a specific threshold held constant through all the animals in the experiment. The mean density (neurons/mm<sup>2</sup>) was calculated as the number of neurons in one region divided by the area size of that region. For mcherry-TH colocalisation in VTA, 5X magnified images of the area of interest were obtained in a Zeiss Imager.Z1 microscope and the number of mcherry cells which colocalised with TH cells were counted manually with ImageJ 1.46r software. The cells that colocalised with TH staining are presented as a percentage of the total cells that were positive for mcherry.

### **5.3.2.1.9 Data analysis**

Analyses were performed as described in Chapter 2 (section 2.6). For all analyses, the control groups of rats with mcherry expression in the VTA-Core pathway (N=5) and rats with mcherry expression in the VTA-Shell pathway (N=4) were merged into one group to increase statistical power, based on no evidence of a statistical difference between the groups. For details on the fixed factors included in all LMEM models see Appendix C (section C2.2.1).

## 5.3.2.2 Results

### 5.3.2.2.1 Histology

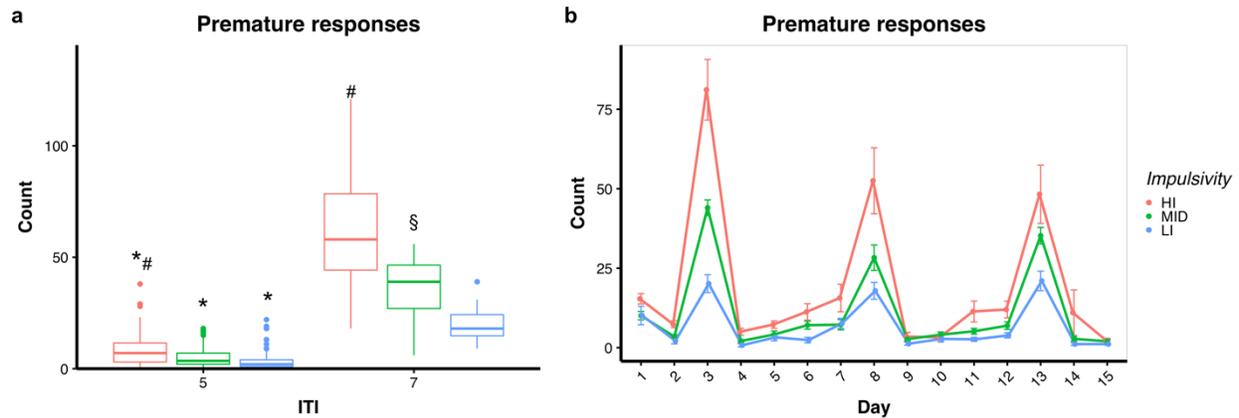
A qualitative analysis of the brain sections revealed that viral expression was present in all animals. The degree of colocalisation between the expression of the virus and TH staining is shown in **Table 5.1**. For an exemplar image of colocalization between TH and mcherry see **Figure 5.5C** in Appendix C.

	mcherry count	% overlap with TH staining
Gi shell	111.33 (13.92)	40.17% (3.99)
Gi core	130.42 (10.28)	44.78% (3.81)
mcherry shell	133 (25.51)	41.99% (8.82)
mcherry core	155.60 (21.54)	46.69% (2.54)

**Table 5.1** Average number of cells positive for mcherry in VTA and % of these cells that co-localised with TH staining. Mean and standard error (SE) in brackets.

### 5.3.2.2.2 Behavioural tests

#### 5.3.2.2.2.1 5CSRTT before DREADDs infusion



**Figure 5.5 Modulation of premature responding by the duration of the ITI.** Rats were assigned to different impulsivity groups according to their performance on three 7 s ITI sessions (separated by 5 days of baseline testing). HI rats made more premature responses both during 5 s ITI and 7 s ITI sessions. \*significantly different from the 7 s ITI session  $p < 0.05$ ; #significantly different from MID rats  $p < 0.05$ .

Model 1 tested differences in behavioural measures of interest between groups later assigned to different viral manipulations, while model 2 explored differences in the same behavioural measures but between impulsivity groups. Prior to the viral infusions, there were no differences in accuracy, premature responses, omission, reinforcers earned and response latency across virus groups ( $p > 0.05$  all comparisons). However, accuracy was influenced by impulsivity and ITI [model 2: ITI x Group,  $F(2,401) = 6.23$ ,  $p = 0.002$ ]. Specifically, HI rats had reduced accuracy compared with LI rats on the long 7s ITI challenge ( $p = 0.034$ ). Reinforcers earned also differed between ITI depending on the impulsivity groups [ITI x Group,  $F(2,402) = 7.26$ ,  $p < 0.001$ ]. In detail, HI rats earned more reinforcers on the 5 s ITI sessions compared to the 7 s ITI sessions ( $p = 0.002$ ), while the opposite was true for LI rats ( $p = 0.029$ ). As shown in **Figure 5.5a and b**, premature responses differed between ITI and impulsivity groups [ITI x Group,  $F(2,402) = 22.18$ ,  $p < 0.001$ ]. Specifically, all impulsivity groups made more premature responses during the 7 s ITI sessions compared to the 5 s ITI sessions ( $p < 0.001$ ). In addition, HI rats made more premature responses than LI rats and MID rats both during the 5 s ITI sessions ( $p < 0.05$ , for both comparisons) and the 7 s ITI sessions ( $p < 0.001$ , for both comparisons). During the 7 s ITI sessions, MID rats also made more premature than LI rats ( $p < 0.001$ ). There were no differences

across impulsivity groups for number of omissions ( $p=0.086$ ), while response latency, both for correct and incorrect responses, differed between impulsivity groups and ITI. For a summary on this see **Table 5.2** and **5.3** (latency for correct and incorrect, respectively).

Correct response latency (ms)	Baseline (ITI 5 s)	Long ITI (ITI 7 s)
HI	687.67 (12.99)	674.199 (26.24)
MID	836.10 (16.63)	<b>730.58 (23.70)*</b>
LI	773.89 (17.03)	<b>698.24 (20.49)*</b>

**Table 5.2 Correct response latencies for each impulsivity group and ITI challenge.** Mean (in ms) and standard error (SE) in brackets. The latency to make a correct response differed between ITI challenges depending on impulsivity sub-group [ITI x Group,  $F(2,401)=3.56$ ,  $p=0.029$ ]. MID rats and LI rats were faster during the 7 s ITI sessions compared with the 5 s ITI sessions ( $p<0.004$  for both comparisons). There were no significant differences between sessions for HI rats ( $p=0.467$ ). \*significantly different from the 5 s ITI session

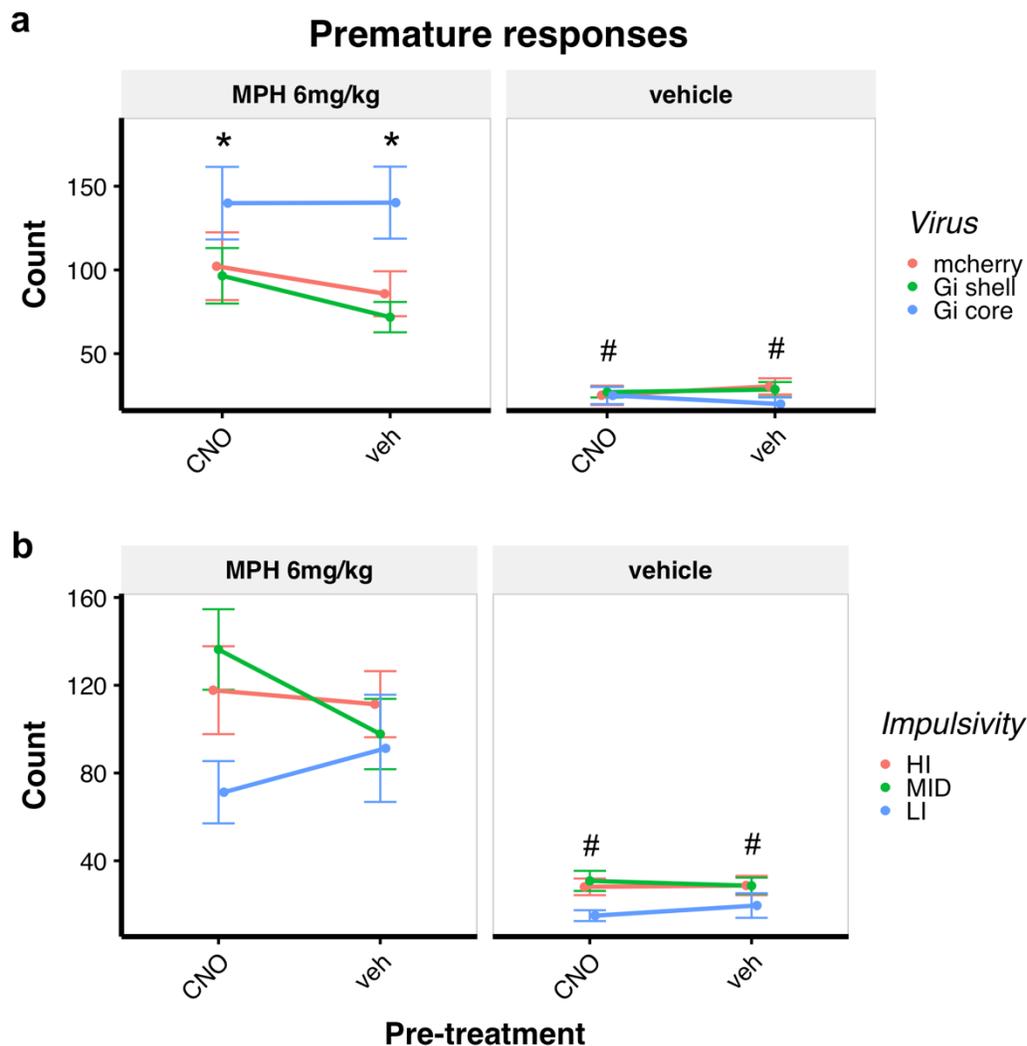
Incorrect response latency (ms)	Baseline (ITI 5 s)	Long ITI (ITI 7 s)
HI	1954.60 (51.61)	<b>1335.12 (73.40)*</b>
MID	2040.06 (40.65)	<b>1227.42 (58.34)*#</b>
LI	2188.65 (58.78)	<b>1580.50 (82.19)*</b>

**Table 5.3 Incorrect response latency for each impulsivity sub-group and ITI challenge.** Mean (in ms) and standard error (SE) in brackets. The latency to make an incorrect response differed between impulsivity groups and ITI [ITI x Group,  $F(2,401)=4.40$ ,  $p=0.012$ ]. All impulsivity groups were faster on the 7 s ITI sessions compared to the 5 s ITI sessions ( $p<0.001$  for all comparisons). In addition, LI rats were slower than MID rats on the 7 s ITI sessions, compared to the 5 s ITI sessions ( $p=0.015$ ). \*significantly different from the 5 s ITI session. #significantly different from LI rats.

### 5.3.2.2.2.2 5CSRRT performance prior to DREADDs activation

Model 1 tested differences in behaviour and across treatments/pre-treatments between groups assigned to different viral manipulations, while model 2 tested differences in behaviour and across treatments/pre-treatments between impulsivity groups. Following infusions, accuracy on the 5CSRRT was affected by treatment only, both in model 1 [ $F(1,78)=58.18$ ,  $p<0.001$ ] and in model 2 [ $F(1,78)=50.32$ ,  $p<0.001$ ]. Specifically, in both models, accuracy was lower when rats were administered MPH compared to saline, regardless of pre-treatment and groups (for all

comparisons). Mirroring these results, reinforcers earned were also fewer following administration of MPH, both in model 1 [ $F(1,78)=85.72, p<0.001$ ] and in model 2 [ $F(1,78)=77.80, p<0.001$ ]. The number of omission responses were greater following treatment with MPH, both in model 1 [ $F(1,78)=102.05, p<0.001$ ] and in model 2 [ $F(1,104)=98.37, p<0.001$ ]. However, in model 2 omission responses also differed across impulsivity groups [ $F(2,104)=10.77, p<0.001$ ], with LI rats making more omissions than HI ( $p=0.001$ ) and MID ( $p<0.001$ ) rats, regardless of treatment or pre-treatment.



**Figure 5.6** Effects of viral manipulation and drug administration on premature responses on the 5CSRRT. (a) Virus groups: all rats made more premature responses when administered MPH (6mg/kg) compared to saline. In addition, Gi core rats made more premature responses than the other groups when administered MPH (6mg/kg). (b) Impulsivity groups: all rats made more premature responses when administered MPH (6mg/kg) compared to saline and there were no group effects. \*significantly different than the other virus groups. #significantly different from treatment with MPH (6mg/kg).

As shown in **Figure 5.6a**, in model 1 the number of premature responses was influenced by test group and treatment [Group x Treatment,  $F(2,78)=7.37$ ,  $p=0.001$ ], with Gi core rats making more premature responses following the administration of MPH compared to Gi shell rats ( $p=0.014$ ). All rats made more premature responses following treatment with MPH, compared to treatment with vehicle ( $p<0.001$ ). This was true also for model 2 [see **Figure 5.6b**, main effect of treatment,  $F(1,78)=162.28$ ,  $p<0.001$ ]. **Table 5.4** and **5.5** show how viral infusion and the administration of drugs affected response latency.

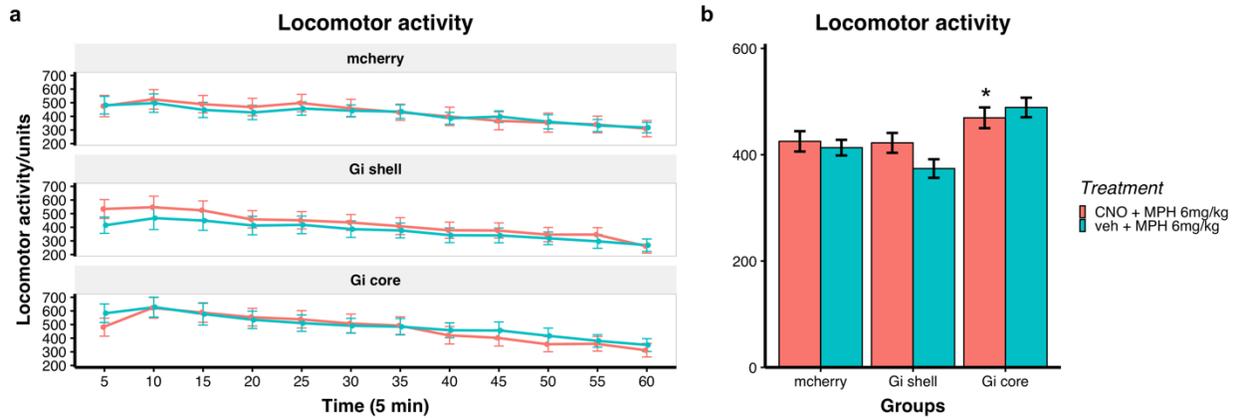
	Latency correct response		Latency incorrect response	
	veh	MPH (6mg/kg)	veh	MPH (6mg/kg)
Gi-core	648.46 (30.47)	<b>772.78 (38.82)*</b>	1515.02 (92.59)	<b>1607.89 (82.37)*</b>
Gi-shell	647.24 (33.56)	<b>723.77 (45.00)*</b>	1401.47 (98.42)	<b>1648.19 (133.71)*</b>
mcherry	668.31 (24.93)	<b>716.48 (32.40)*</b>	1344.41 (88.31)	<b>1732.14 (119.20)*</b>

**Table 5.4 Effects of viral manipulation and MPH on response latencies.** Mean (in ms) and standard error (SE) in brackets. The latency to make a correct response was shorter following vehicle treatment compared with MPH (6 mg/kg) [model 1,  $F(1,78)=10.90$ ,  $p=0.002$ ]. The latency to make an incorrect response was also shorter following treatment with MPH compared to vehicle [model 1,  $F(1,78)=6.51$ ,  $p=0.012$ ]. \*significant difference with vehicle  $p<0.05$

	Latency correct	Latency correct	Latency incorrect	Latency incorrect
	veh	MPH (6mg/kg)	veh	MPH (6mg/kg)
HI	588.80 (24.51)	<b>685.03 (49.33)*</b>	1412.68 (76.34)	<b>1529.40 (98.53)*</b>
MID	663.65 (28.20)	<b>746.24 (33.46)*</b>	1250.53 (63.11)	<b>1612.65 (113.29)*</b>
LI	<b>704.24 (29.17)<sup>§</sup></b>	<b>779.06 (36.17)*<sup>§</sup></b>	<b>1713.28 (120.84)<sup>§</sup></b>	<b>1868.79 (95.09)*<sup>§</sup></b>

**Table 5.5 Effects of viral manipulation and MPH on response latencies.** Mean (in ms) and standard error (SE) in brackets. The latency to make a correct response was shorter following vehicle treatment compared to MPH (6 mg/kg) [model 2,  $F(1,78)=10.50$ ,  $p=0.002$ ]. The latency to make an incorrect response was also shorter following treatment with MPH (6 mg/kg) [model 2,  $F(1,104)=4.97$ ,  $p=0.028$ ]. Here the latency to make an incorrect response was also influenced by impulsivity phenotype [ $F(2,104)=7.20$ ,  $p=0.001$ ], with LI rats being slower than HI rats ( $p=0.039$ ) and MID rats ( $p=0.003$ ), across treatments and pre-treatments. \*significant difference with vehicle <sup>§</sup>significant difference with HI and MID rats.

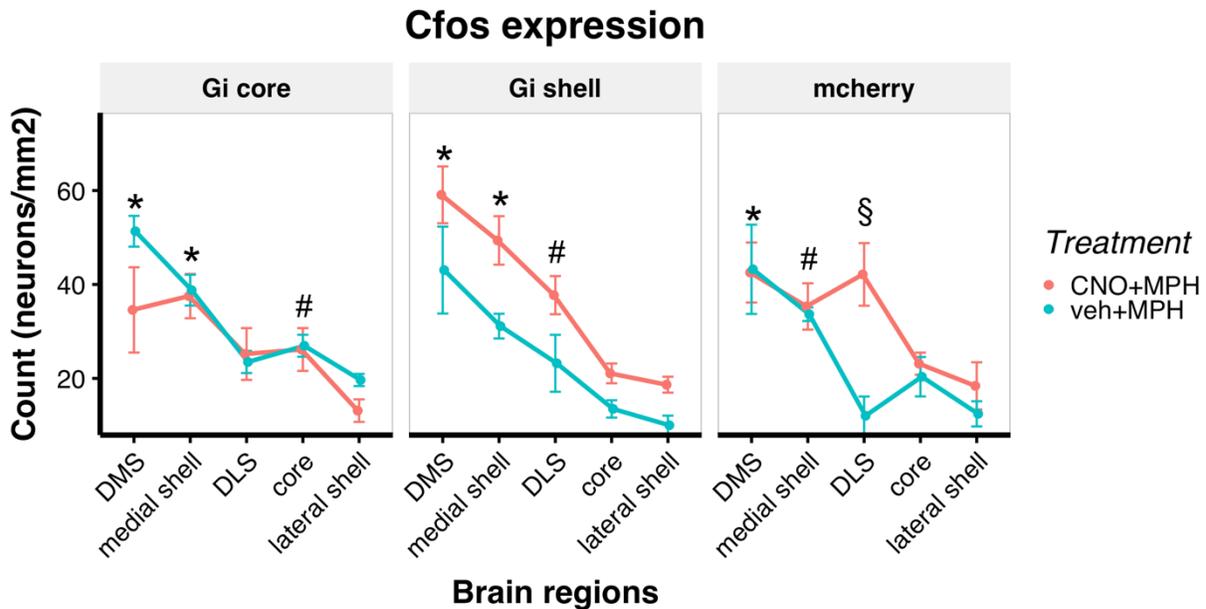
### 5.3.2.2.3 Locomotor activity



**Figure 5.7** Locomotor activity following pre-treatment with either CNO (3 mg/kg) or vehicle and treatment with MPH (6 mg/kg). Gi core rats displayed decreased locomotor activity following the administration of CNO + MPH 6mg/kg compared to the administration of saline + MPH 6mg/kg \* $p < 0.001$ .

**Figure 5.7a** and **b** shows locomotor activity in each group. Locomotor activity was influenced by test group and treatment [ $F(2,904)=6.50$ ,  $p=0.002$ ]. Specifically, there was no difference in locomotor activity for mcherry and Gi shell animals between treatments ( $p > 0.05$ ), however Gi core rats displayed decreased locomotor activity following the administration of CNO + MPH 6 mg/kg compared to the administration of saline + MPH 6 mg/kg ( $p=0.001$ ).

### 5.3.2.2.4 C-fos analyses



**Figure 5.8 C-fos expression in the striatum following pre-treatment with either CNO (3 mg/kg) or saline and treatment with MPH (6 mg/kg) in different virus groups.** C-fos expression followed a medio-lateral gradient, being greater in more medial structures compared to more lateral structures. Gi core: \*significant difference with core, lateral shell and DLS ( $p < 0.05$ ), #significant difference with lateral shell; Gi shell: \*significant difference with core, lateral shell and DLS ( $p < 0.05$ ), #significant difference with core and lateral shell; mcherry: \*significant difference with core, lateral shell and DLS ( $p < 0.05$ ), #significant difference with lateral shell, §significant difference with DLS following treatment with veh+MPH.

A significant Treatment x Group x Area interaction [ $F(8,206)=2.05$ ,  $p=0.042$ ] was further analysed by group separately. **Figure 5.8** summarises the distribution of c-fos expression across different regions for each test group. To see an exemplar image of each brain area and c-fos distribution and staining see **Figures 5.6C-5.10C** in Appendix C. For Gi-core rats c-fos activation varied depending on the area, regardless of treatment [ $F(4,71)=14.47$ ,  $p < 0.001$ ]. In detail, c-fos expression followed a medio-lateral gradient, being greater in more medial structures compared to more lateral structures. Specifically, DMS and medial shell had greater c-fos expression compared to core, lateral shell and DLS ( $p < 0.05$ ). The core had greater c-fos expression than the lateral shell ( $p=0.039$ ), but not of DLS ( $p=0.848$ ), and c-fos expression in DLS did not differ from that in lateral shell ( $p=0.362$ ).

For Gi shell rats c-fos activation also varied depending on the area, regardless of treatment [ $F(4,72)=32.89$ ,  $p < 0.001$ ]. C-fos expression for this group of rats also followed a medio-lateral

gradient, being greater in more medial structures compared to more lateral structures. Specifically, DMS and medial shell had greater c-fos expression compared to core, lateral shell and DLS ( $p < 0.05$ ). DLS had greater c-fos expression than the core and lateral shell ( $p = 0.02$ ), while c-fos expression in core and lateral shell did not differ from one another ( $p = 0.711$ ).

For mcherry rats, c-fos activation varied between different brain areas, depending on treatment [ $F(4,63) = 3.87$ ,  $p = 0.007$ ]. The only difference in c-fos expression between treatments was observed in the DLS, where c-fos expression in animals that received CNO prior to MPH was greater than that in animals that received vehicle prior to MPH. Except for the DLS, animals that received CNO + MPH, c-fos expression for mcherry rats also followed a medio-lateral gradient, being greater in more medial structures compared to more lateral structures. In detail, DMS had greater c-fos expression compared to DLS, core and lateral shell ( $p < 0.02$ ), while medial shell had greater c-fos expression only of lateral shell ( $p < 0.001$ ).

For no groups were there any significant correlations between c-fos count and locomotor activity in the first half hour following MPH treatment.

### **5.3.2.2.3 In summary**

Pre-treatment with CNO did not have any effects on performance of the 5CSRTT, neither in DREADDs rats nor in control rats. However, following viral infusion, Gi-core rats made more premature responses following administration of MPH (6 mg/kg) compared with the vehicle group (irrespective of pretreatment) suggesting that the presence of the virus might have caused molecular changes at the level of DA uptake. Pre-treatment with CNO did reduce locomotor activity in Gi-core rats, when compared to pre-treatment with vehicle. C-fos staining did not reveal an effect of pre-treatment with CNO in DREADDs rats, and in both groups c-fos expression followed a medio-lateral gradient, being greater in more medial structures (DMS and medial shell) compared to more lateral structures (DLS, lateral shell). This same pattern of c-fos expression was evident in control (mcherry-only) rats, except that in this group pre-treatment with CNO led to an increase in c-fos expression in the DLS compared with the vehicle group.

### 5.3.2.3 Discussion

The two main experiments described in this chapter were designed to investigate the functional significance of the mesolimbic DA system in the expression of premature responding in the 5CSRTT, an operational measure of impulsivity. The specific focus of this investigation was to dissociate the contribution of DA inputs to the NAc shell and core, which previously have been hypothesized to interact in a functionally-opponent manner to regulate impulsivity (Dalley and Robbins, 2017 and references therein). Ultimately, this research aimed at elucidating the nature of this interaction and specifically whether functionally-opponent interactions are also present at the level of DA cell bodies located in the VTA. Since previous research has shown that D2 receptors are downregulated in the NAc of HI rats compared with LI rats, the DREADD experiment targeting two different populations of neurons in the VTA was intended to infer whether dysregulated D2 receptor function in HI rats is predominately a presynaptic or postsynaptic phenomenon. The discussion that follows critically evaluates the findings of this study.

#### *Experiment 1: microinfusions of quinpirole into VTA during performance on 5CSRTT*

To further elucidate the role of activity of midbrain dopaminergic neurons on impulsivity, as assessed in the 5CSRTT, Experiment 1 was designed to diminish activity of DA-ergic fibres, and accordingly extracellular DA release into the NAc. To this end, the D2/3 receptor agonist, quinpirole was infused at doses 0, 0.01, 0.03, 0.3 and 1  $\mu\text{g}/\mu\text{l}$  into the VTA of rats tested for premature responses on a long 7s ITI session of the 5CSRTT. Premature responses were significantly reduced when animals were infused with the highest dose of quinpirole (1  $\mu\text{g}/\mu\text{l}$ ), however this effect was not observed when animals were infused with the intermediate doses (0.01, 0.03 and 0.03  $\mu\text{g}/\mu\text{l}$ ). These findings are in line with predictions that diminished firing of VTA DA fibres, *via* activation of D2 somatodendritic autoreceptors, would lead to a decrease in premature responses. As application of quinpirole on midbrain DA cells is known to decrease DA overflow in the NAc (Anzalone et al., 2012; Schmitz et al., 2002), it is suggested that the decrease in premature responses observed with application of quinpirole 1  $\mu\text{g}/\mu\text{l}$  results from diminished DA efflux onto accumbal neurons, thus confirming a role of mesolimbic DA in anticipatory behaviour. These findings also enrich previous evidence in the lab that decreased

premature responding observed following systemic administration of quinpirole (0.01, 0.03 and 0.1 mg/kg, Fernando et al., 2012), may result predominantly from changes in midbrain DA-ergic firing. The highest dose, which affected premature responses, did not have any effects on accuracy and reinforcers earned, suggesting that reduction of DA release in the ventral striatum does not affect attentional performance on the 5CSRTT. However, it did reduce response latency and it slightly increased omission responses (albeit the latter not significantly), supporting a role of DA in action initiation and speed of responding (Klaus et al., 2019; Mohebi et al., 2019).

### *Experiment 2 - Mapping of midbrain projections to NAcc shell and core*

To investigate the function of circuit-specific projections, from midbrain cells to the NAcc shell and core, in the execution of premature responses on the 5CSRTT, two separate experiments were run. An initial pilot experiment tested the efficacy of the combined use of CAV2-DREADDs in 1) transfecting VTA cells in a retrograde manner and 2) silencing activity of the transfected cells in a manner that has consequences on behaviour. The second experiment was conditional upon the success of the first pilot experiment and was aimed at elucidating, with chemogenetics, the function of the VTA-shell and VTA-core pathways in the performance of a long ITI session of the 5CSRTT, which is known to elicit premature responding.

In the first pilot experiment, DREADDs were tested on the VTA-shell pathway only, as a proof-of-concept study. Compared with animals infused with empty virus (mcherry-only rats), rats infused with inhibitory DREADDs hm4D(Gi) exhibited reduced locomotor activity when CNO was administered both on its own and 25 min prior to MPH 6 mg/kg. This is in line with evidence involving the VTA-shell pathway in stimulant-induced increases in locomotor activity (Parkinson et al., 1999; Salamone et al., 2007) and with recent evidence that chemogenetic excitation of the VTA-NAcc pathway increases locomotor activity in rats (Boender et al., 2014). Boender and colleagues (2014) also showed that chemogenetic excitation of the VTA-NAcc pathway (not restricted to either the shell or core sub-regions) also improved PR performance. However, this was not replicated in the present pilot experiment. A reason why this might be the case is because research has shown the NAcc core to be more strongly involved in tasks requiring increasing exertion of effort to gain an appetitive reward, such as the PR task

(Salomone and Correa, 2012). However, in the present study the VTA-shell pathway was singly manipulated, thus potentially explaining why an effect of CNO on PR was not observed in the present study (but was observed in experiments targeting the NAcB more broadly, Boender et al., 2014).

In light of the initial promising results on locomotor activity, a second experiment was conducted to target both VTA-shell and VTA-core pathways and explore their function in the execution of premature responses on the 5CSRTT. This was done to refine, at a circuit-level, the results observed with pharmacological inhibition of VTA in Experiment 1 and, more broadly, to clarify the precise contribution of the different pathways projecting from VTA onto the NAcB. Despite the first pilot experiment yielding promising results, the second main experiment failed to replicate this effect. First, it did not replicate the effect of inhibitory DREADDs in VTA-shell on locomotor activity. A reduction in locomotor activity was, however, observed in rats infused with inhibitory DREADDs in the VTA-core, following administration of CNO. This apparent effect of inhibitory DREADDs on behaviour did not extend to performance on the 5CSRTT. Specifically, both DREADD groups of rats (Gi-shell and Gi-core) and control rats (mcherry-only) were tested on a long ITI session of the 5CSRTT, following pre-treatment of either CNO or saline *and*, after 25 min, treatment of either MPH (6mg/kg) or saline. Results showed that neither group of rats showed differential performance on the 5CSRTT following pre-treatment with CNO compared with pre-treatment with saline. However, all rats showed an increase in premature responses following treatment with MPH compared with saline as observed before both in the present work (Chapter 4) and in the literature (Puumala et al., 1996; Milstein et al., 2010). Surprisingly, Gi-core rats showed a much greater increase in premature responses compared to the other groups following treatment with MPH, regardless of whether they were pre-treated with CNO or saline. This group difference was not present prior to the viral surgery, suggesting that perhaps the presence of a hM4D(Gi) receptor in transfected dopaminergic cells might have altered their DA release dynamics. This is possible considering that adeno-associated viruses have been found to cause immune/inflammatory responses *in vivo* (Karra & Dahm, 2010). Another possibility is that the hM4D(Gi) receptor was constantly activated, regardless of the presence of CNO, and thus constantly reducing the tonic release of DA. A constant reduction in synaptic transmission could lead to an accumulation of DA vesicles that are available for

release (Zucker & Regehr, 2002). During performance of 5CSRTT, phasic activation of DA neurons could override the hM4D(Gi) -induced tonic silencing and cause these DA vesicles to be released at once. MPH would then amplify this effect leading to an increase in premature responses, compared to the other groups.

A final test of whether chemogenetics affected DA release from either the VTA-shell pathway or the VTA-core pathway, despite there not being any behavioural manifestation of this in the 5CSRTT, was carried out by quantifying c-fos expression in the striatum. C-fos is an immediate early gene which is induced following neuronal activation and produces a protein that can be detected with immunohistochemistry (Cruz et al., 2015). In this experiment, all groups of rats (Gi-shell, Gi-core, mcherry) were pre-treated with either saline or CNO, and 25 min after this were treated with 6mg/kg MPH. When delivered systemically, MPH blocks DA reuptake by binding to the DA transporter (Schweri et al., 1985) and increases extracellular levels of DA in the striatum (Bymaster et al., 2002), coinciding in this region with a dose-dependent increase in c-fos expression (Chase et al., 2003). Thus, in the present study, it was hypothesised that if CNO was acting on inhibitory DREADDs and silencing DA cells projecting onto the NAcb, the increase in c-fos expression in the striatum typically observed following administration of MPH should not be as strong. However, there were no meaningful differences in c-fos expression across groups and pre-treatments (CNO and saline). Specifically, across rats c-fos expression, as quantified with antibody staining, was stronger in medial regions of the striatum such as DMS and medial shell, and weaker in more lateral regions such as DLS, core and lateral shell. This distribution of c-fos was in part consistent with that reported in previous studies (Chase et al., 2003; Brandon and Steiner, 2003). For example, both Chase and colleagues (2003) and Brandon and Steiner (2003) found that treatment with MPH dose-dependently increased c-fos expression mostly in dorso-medial regions of the striatum. Brandon and Steiner (2003), in particular, investigated the expression of c-fos with *in situ* hybridization histochemistry and reported very little, if any, c-fos expression in the NAcb. This is different from what observed in the present investigation, where c-fos expression was detected in the medial shell and to an extent in the core of the NAcb. Differences between the present findings and those of Brandon and Steiner (2003) may owe to differences in the techniques used: while *in situ* hybridization is a very reliable technique due to the specificity of nucleotide sequences, it does not reflect protein levels or post-

translational regulation, thus yielding different results from what is detected with immunohistochemistry (see Nakahara et al., 2020 for an example of how c-fos detection can be achieved both with *in situ* hybridization and immunostaining).

Finally, immunostaining in VTA showed that the virus had expressed to an acceptable level in all rats and that ~43% of these fibres co-localised with TH staining, thus indicating that these were likely dopaminergic in nature. Previous studies have also reported a similar, albeit slightly higher, number of TH+ neurons among those projecting to the NAcB (Beier et al., 2015). Differences between experiments in the degree of colocalisation between TH and the mcherry tag of the virus (used to achieve projection-specificity) could be due to (1) limitations of the immunostaining technique either in labelling transfected cells or in revealing whether these are TH+, (2) limitations of the viral strategy used to infect midbrain cells projecting to the NAcB and, finally, (3) a high degree of molecular heterogeneity in midbrain neurons projecting to the NAcB. For example, medial VTA has been found to be enriched in glutamatergic neurons and combinatorial DA neurons, that is those co-releasing DA and glutamate (Morales & Margolis, 2017; Yamaguchi et al., 2011). To date it is unknown what proportion of medial-shell projecting neurons, located in medial VTA, is composed of DA-ergic, glutamatergic or neurons that are combinatorial in nature. In addition, GABA-releasing neurons are also present in the VTA, with some of these regulating, locally, the activity of VTA DA neurons and others having effects on structures that receive input from the midbrain (Morales & Margolis, 2017; Root et al., 2014).

In summary, chemogenetic inhibition of two different sub-populations of the VTA, projecting either to the core or shell of the VS, did not produce any significant changes in performance of the 5CSRRT. With regards to the VTA-shell pathway, the lack of an effect may be due to a failure of the chemogenetic technique to inhibit enough cells to produce a behavioural response. This claim is supported by findings in experiments other than those on the 5CSRRT, where the delivery of the designer drug CNO -which should bind to the hM4D(Gi) receptors and inhibit cellular activity- (1) did not reduce locomotor activity, which is known to be regulated by DA release from VTA-shell cells (pilot experiment 2; Parkinson et al., 1999) and (2) did not reduce c-fos expression in the striatum when delivered prior to MPH. With regards to the second pathway targeted with chemogenetics, that of neurons projecting from the VTA to the core sub-

region of the NAcB, it is more difficult to draw conclusions on the efficacy of the DREADDs. This is because CNO delivery did reduce locomotor activity in Gi-core rats, suggesting that chemogenetics was somewhat effective at inhibiting the pathway, however there were no appreciable reductions of c-fos in the striatum of these rats. Thus, the possible interpretations are that either 1) DREADDs were inhibiting VTA-core projections, and these are simply not involved in performance of the 5CSRTT or 2) DREADDs did not inhibit a sufficient number of cells to have an impact on premature responses on the 5CSRTT. Experiment 1 (this chapter) suggests that DA cells in the VTA are important in the performance of premature responses of the 5CSRTT and there is previous evidence that DA release in the NAcB core modulates the performance of these responses (Moreno et al., 2013), thus it is likely that the second hypothesis is more plausible.

Further research on the circuit dynamics regulating performance on the 5CSRTT should aim to optimize the efficacy of circuit-specific chemogenetics (or other tools used for neuronal manipulation) before ‘asking’ biological questions. This could be done for example by testing the function of DREADDs with other methods that can interrogate neuronal functioning, such as electrophysiology and *in vivo* microdialysis. A rigorous validation of the method used would allow to confidently rule out a failure of the technique in the event of negative findings at the behavioural level. The difficulty in achieving a functional tool to manipulate neuronal functioning at the circuit level, however, should not discourage the use of such techniques in neuroscience. Indeed, given the complexity of the circuitry that regulates behaviour it is of paramount importance that studies on the behavioural implications of neuronal pathways are carried out following a highly specific anatomical (circuit-specific) and molecular (targeting proteins or gene markers of interest) approach. A greater precision in the questions that one can ask will allow greater precision in the claims that can be made, and ultimately a deeper understanding of the brain mechanisms that regulate behaviour.

# Chapter 6 – A comparative study between 5CSRTT and SDT

## 6.1 Introduction

Sustained attention and vigilance are often compromised in different psychiatric disorders including ADHD and schizophrenia (Barkley, 1997; Cornblatt & Malhotra, 2001). In the context of ADHD, for example, one of the tasks widely used to assess impairments in attentional control and inhibition is the continuous performance test (CPT) (Epstein et al., 2003). Many variations of this task have been developed over the years, however generally speaking in most CPT paradigms subjects are presented with a rapid series of stimuli and must withhold responding until a specified (rare) stimulus is presented (Conners et al., 2003). Traditional measures of this task are errors of commission (i.e., the subject responds inappropriately) and errors of omission (i.e., the subject fails to respond during a trial).

Preclinical research on psychological processes common to psychiatric disorders relies on the efficient translation of tasks and procedures between humans and experimental animals. The 5CSRTT was originally developed as a rodent analogue of the human CPT (Robbins, 2002; Rosvold et al., 1965) and the human Leonard Task (Wilkinson, 1963), to enable translational research on visuospatial attention and vigilance. Similar to what the CPT assesses in humans, the 5CSRTT assesses sustained attention in rodents by incorporating elements of spatial and temporal uncertainty. Spatial uncertainty is achieved by having the target stimulus appearing unpredictably in one of five possible holes, while temporal uncertainty is achieved by varying the timing when stimuli are presented (e.g., vITI paradigms, Bizzarro et al., 2004). Both spatial and temporal uncertainty increase attentional load and require the animal to constantly monitor the target stimulus to perform the task successfully. However, what is not explicitly incorporated in the 5CSRTT but instead is integral to the CPT paradigm are trials where the subject needs to withhold responding for the whole duration of the trial to earn a reward. Thus, a variation of the 5CSRTT was developed to specifically incorporate non-target trials, this task was named the 5-

choice continuous performance task (5C-CPT, Tomlinson et al., 2015; Young et al., 2009) and non-target trials were signalled by all apertures illuminating. As noted by Turner and colleagues (2016), however, an additional element that differentiates the 5CSRTT (and to an extent the 5C-CPT) from the human CPT is the fact that the latter ‘requires the maintenance of a rule to determine the correct response based on stimulus properties’ (Turner et al., 2016). The 5CSRTT (and to an extent the 5C-CPT) does not rely on a specific stimulus-based rule, but simply requires the animal to poke into the hole where the light CS is presented. In contrast, the sustained attention task (SAT, Lustig et al., 2013; McGaughy and Sarter, 1995) requires a more complex stimulus-based rule to guide behaviour, similarly to the CPT, and it has been used extensively to investigate sustained attention. More specifically, the SAT requires the rat to monitor a central panel for the presence or absence of a briefly presented visual cue. Following cue presentation, the animal is given the choice to either press one lever to report having seen the light or press another lever to report not having seen the light (Lustig et al., 2013). The fact that animals can respond for non-signal trials allows omissions to be differentiated from misses, which is an important variable to measure in the context of attention and signal detection.

Building on this work, Turner and colleagues (2016) further refined the assessment of sustained attention in rodents. Specifically, they noticed that none of the tasks described above controlled for the body position during performance. Thus, if an animal made an omission or incorrect response during a trial it was difficult to know (except in the case of video tracking) whether this occurred due to a lapse in attention or due to the animal grooming or resting on one side of the operant chamber (Turner et al., 2016; Turner, 2016). This issue does not concern the CPT as here participants are usually tested seated in front of the computerised task and fixate the stimulus stream in front of them. To validate this criticism, Turner and colleagues (2016) showed that in the 5CSRTT more omission responses occurred when the rat was distant and looking away from the frontal panel. Based on this observation, the authors developed a novel SDT, which: 1) controlled for body movements, by placing the CS cue light just above a central hole in which rats are required to poke into to initiate a new trial; 2) was fast paced, by removing delays between self-paced trial initiation and cue presentation. This was achieved by keeping the animal engaged with the task and so inhibit alternative behaviours. During task validation Turner and co-workers (2016) compared this task with the 5CSRTT and noticed that rats trained on the SDT

required fewer training sessions to learn the task, made fewer omissions and could reach levels of task accuracy as high as those observed in rats trained on the 5CSRRT. The authors speculated that the decrease in omissions in the SDT, compared to the 5CSRRT, was probably due to the control for body movement and fast pace of the SDT. Further, Turner and colleagues (2016) showed that poor performance in the SDT task was improved by systemic administration of low-dose amphetamine and was associated with altered DA and 5-HT function in the dorsal striatum (Turner et al., 2017).

Given the plethora of operant tests investigating sustained attention and vigilance, it is important to assess whether these tests assess the same attentional mechanisms or instead depend on different processes. The present investigation was thus aimed at refining the work done by Turner and colleagues (2016) by testing whether the 5CSRRT and the SDT have shared psychological and neural substrates. Specifically, the same rats were tested first on the 5CSRRT and then subsequently on the SDT to assess whether high accuracy in one task was associated with high accuracy on the other task. In the SDT, premature or anticipatory responses are not punished and the ITI is only 1s in duration. This results in animals performing the task at a faster pace than they would in the 5CSRRT and earning the same number of pellets as in the 5CSRRT but in a shorter time window. Impulsive rats on the 5CSRRT (HI rats) have been shown to respond particularly well to conditions of high-event rate, where rapid information processing, including visual attention and action, is required (Chapter 4). I therefore investigated whether HI rats in the 5CSRRT would perform better on the SDT compared to the other impulsivity groups, owing to their predisposition to perform well in fast paced tasks. Corroborating this prediction, systemic administration of low-dose amphetamine (0.1mg/kg) boosts performance in the SDT (Turner et al., 2017). A similar dose of amphetamine, given systemically, improves performance in conditions of high-event rate in the 5CSRRT, mimicking performance of HI rats on this task. This increase in performance, both on the SDT and on the high-event rate condition of the 5CSRRT, by systemic amphetamine is thought to result from greater extracellular DA levels in the striatum, which HI rats have been speculated to exhibit (Dalley and Robbins, 2017). Thus, there are reasons to expect that HI rats on the 5CSRRT will show superior performance on the SDT.

A further objective of the present study was to explore whether performance on the 5CSRTT and the SDT is regulated by the PFC. For example, evidence in rodents indicates that attentional performance is regulated by different subregions of the frontal cortex (Chudasama, 2011). In one of the first studies looking at PFC function on the 5CSRTT, Muir and colleagues (1996) found that lesions of the mPFC, including the anterior cingulate cortex (Cg1), prelimbic cortex (PrL) and secondary motor cortex (M2, nomenclature from Paxinos and Watson, 2007), decreased accuracy on 5CSRTT, increased the latency to make a correct response and increased perseverative responses. Lesions to the more posterior cingulate cortex (Cg1, Cg2) increased behavioural activation, elevating premature responses, and decreasing omissions. Subsequent research confirmed these findings (Pezze et al., 2009) and refined the precise contribution of individual subregions of the PFC. Thus, lesions to more ventral regions of the mPFC (eg. PrL and infralimbic cortex, IL), led predominantly to deficits in inhibition of control, increasing premature and perseverative responses (Chudasama & Robbins, 2003; Chudasama & Muir, 2001) and only when the damage was extensive was discriminative accuracy also impaired (Passetti et al., 2002). Contrasting with these effects, lesions to the dorsal PFC, specifically Cg1, caused a more persistent reduction in accuracy (Chudasama et al., 2005; Chudasama & Robbins, 2003; Passetti et al., 2002), suggesting that this area of the mPFC is more strongly recruited to mediate discriminative performance.

While the role of different regions of the rodent frontal cortex has been characterised in the context of 5CSRTT, there are no published studies to date on the role of the PFC in the SDT. The present investigation was therefore aimed at bridging this gap by testing the effects of excitotoxic lesions of the mPFC on behavioural performance on the 5CSRTT and SDT. This region of frontal cortex was chosen because it has consistently been shown to support performance on the 5CSRTT (Muir et al., 1996; Passetti et al., 2002; Pezze et al., 2009)

## **6.2 Methods and materials**

### **6.2.1 Subjects**

Animals were kept under the conditions specified in Chapter 2 (section 2.1). A total of 36 male Lister-Hooded rats (Charles River, UK) were used for this study.

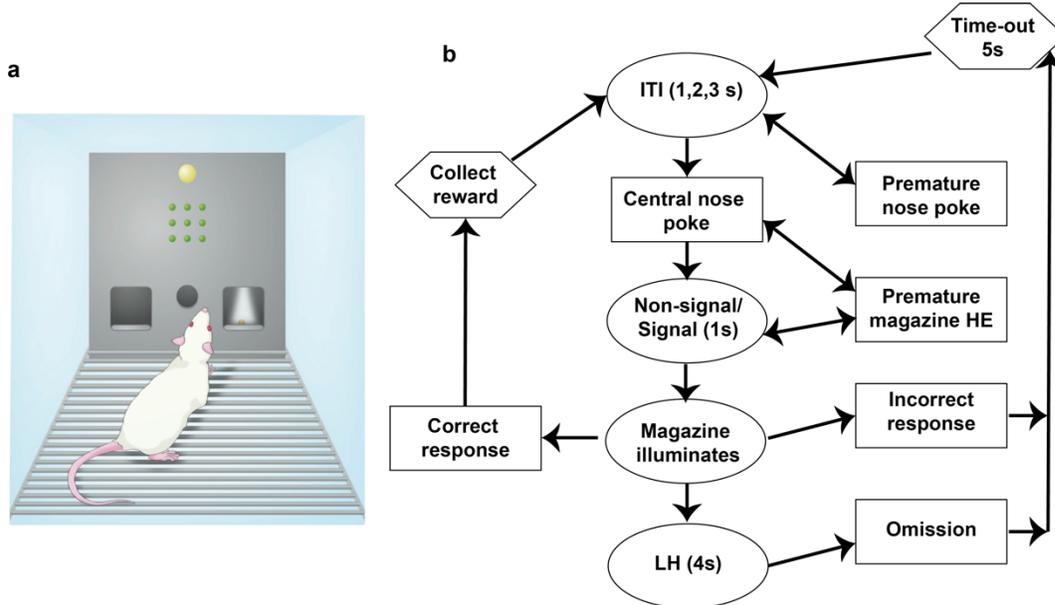
### **6.2.2 5CSRTT training and testing**

Training on the 5CSRTT was conducted as described in Chapter 2 (section 2.3). All animals were tested on at least two baseline sessions consisting of: SD 0.7 s, ITI 5 s and a time-out of 5 s.

Once animals had completed at least two baseline sessions, all thirty-six rats were challenged with two vITI sessions to screen for impulsivity. This followed the procedure as described in Chapter 2 (section 2.3.2).

Following these two vITI sessions and a subsequent baseline session, all rats were challenged with variable SD (vSD) session. The vSD session consisted of a pseudo-random presentation of trials with 0.03 s, 0.06 s, 0.25 s, 0.7 s and 1 s SDs. Each SD was presented at least 50 times and the session ended when animals had completed 250 trials or after 2 h (whichever event occurred first). Animals could not predict which SD was going to be presented on each trial. Time-out (0.5 s) and ITI (5 s) were kept constant at the same level as their baseline training.

### 6.2.3 SDT training and testing



**Figure 6.1 Training on the SDT task.** (a) Schematic representation of the SDT (b) Possible trial sequences of the SDT. Rats on this task were trained as described in Turner and colleagues (2016). Rats were first trained to poke into either of the two illuminated food magazines (left and right) to collect a pellet. This level of training (level 1) lasted until animals had collected 50 pellets from each food magazine. Rats were then trained (level 2) to poke into the central aperture to activate the food magazines, these would then illuminate, and rats could poke into either of the two magazines. This level also lasted until animals collected at least 50 pellets from each food magazine. Level 3 involved rats being exposed to the signal cue and learning the rule of the task. Specifically, rats were trained that poking into the central nose poke activates a light above the central nose poke (central light) which can either turn on, indicating a signal trial, or not, indicating non-signal trials. They also learned that these two events (signal or non-signal) were associated with different contingencies, that is they require entering either in the left or right food magazine to earn a reward. The contingencies dictating which signal cue was associated with which food magazine entry were counterbalanced across the cohort, but constant for an individual. During level 3 training, the signal cue stayed on until the rat had poked into a food magazine. On signal trials, the cue light turned on as soon as the rat poked into the central nose poke. HE = Head Entru; LH = Limited Hold; ITI = inter-trial interval.

Training on the SDT started when testing on the 5CSRTT ended. An overview of the SDT protocol is shown in **Figure 6.1**. Level 4 of the training was used as the ‘baseline’ session and ended when rats had completed 120 trials (60 signal and 60 non-signal trials) or when 30 minutes had elapsed, whichever happened first. Level 4 was identical to level 3 except that the cue (light for signal and no-light for non-signal trials) was presented only for 1s. Responses made before this 1s window of cue presentation had elapsed were marked as anticipatory responses but were not punished. Incorrect and omission responses were always punished with a 5s time-out. The ITI in this task was the time between the end of the previous trial (marked by either the collection of a food pellet after a correct response or the end of a time-out punishment) and the light turning on in the central nose poke aperture indicating that a new trial could be initiated. In all tests of the SDT, a variable ITI was used with values ranging between 1s, 2s or 3s.

The vSD session of the SDT consisted of the presentation of a total of 170 trials. Specifically, 70 of these were non-signal trials (0 s SD), while the other 100 were signal trials. Of signal trials, there were 10 trials of 1 s SD, 30 trials of 0.03 s SD, 30 trials of 0.06 s SD and 30 trials of 0.25 s SD. All trials were pseudo-randomised and the animal could not predict which SD was next presented.

## **6.2.4 Considerations about comparing baseline sessions on the SDT and 5CSRTT**

In the SDT, with a 1 s SD, level 4 was considered the baseline session of this task as chosen by Turner and colleagues (2016). This task is already very different from the 5CSRTT and has not been tested with the rat strain used in this experiment, thus it was decided to adapt what has been designed by Turner and colleagues (2016) to allow comparisons with that experiment. For the 5CSRTT, as well, the baseline level was decided based on previous publications which mostly trained animals to a stimulus duration of 0.7 s-0.5 s SD (Caprioli et al., 2013; Fernando et al., 2012). Thus, the present experiment aimed at comparing baseline sessions of two different tasks as these would normally be run in their standard versions. However, because the 5CSRTT does have sessions of 1 s SD during training, these were also compared against the baseline sessions of the SDT to test the extent to which SD plays a role when comparing accuracy between these tasks.

## **6.2.5 Surgeries**

Following successful training on the 5CSRTT and SDT tasks, rats were divided into a control or sham, group (N=18) and a mPFC lesion group (N=18). The groups were balanced based on performance of the vSD sessions of the 5CSRTT and SDT. All animals underwent a surgical procedure identical to that described in Chapter 2 (section 2.4), however 18 of these animals received bilateral injections of 0.4 µl, 1, 0.09M quinolinic acid (QA, Sigma Aldrich, U.K, dissolved in sterile phosphate buffered saline, pH 7.0–7.2), while the remaining 18 animals only received injections of sterile phosphate buffered saline (pH 7.0–7.2). Infusion of the neurotoxin was made in the following coordinates, from bregma: AP +3.2 mm, L ±0.7 mm, DV–1.5 mm;

AP, +2.7 mm; L,  $\pm 0.7$ ; DV, -2.0 mm and -2.0 mm, AP, +2.2 mm;  $\pm 0.7$  mm; DV -1.5 mm. Coordinates for lesions, preparation and dosage of quinolic acid was taken from Chudasama and colleagues (2005).

## **6.2.6 Performance on the SDT and 5CSRTT tasks following PFC lesions**

Following surgery, rats were allowed to recover for 7 days. One animal was culled due to the occurrence of seizures following surgery. This left the cohort with 18 sham rats and 17 lesioned rats. On the 8th day after surgery, rats were tested again on both tasks. Each animal was tested twice a day, in the morning in one task and in the afternoon in the second task. Pairings of time of day (morning and afternoon) and task (SDT and 5CSRTT) were alternated across the cohort such that rats that were tested in the morning on the 5CSRTT on day 1 were then tested on the 5CSRTT in the afternoon of day 2. All animals were tested on at least two baseline sessions in each task. In addition, sham rats and 12 lesioned rats were also tested on an additional 3rd baseline session, while 16 sham rats and 6 lesioned rats were tested on a fourth baseline session. Only rats that had had at least 3 baseline sessions were further tested on a vSD session both on 5CSRTT and on the SDT. Parameters of the vSD session on the SDT were the same as those used prior to the lesion. On the 5CSRTT, however, less demanding parameters were chosen because, following lesion recovery, lesioned rats showed dramatic accuracy deficits on this task. The new, post-lesion vSD session for the 5CSRTT consisted of 150 trials of different pseudo-randomly interleaved SDs, such as 0.5 s, 1 s and 2 s SD. The session ended when rats completed 150 trials or when 1 h 30 min had elapsed, whichever occurred first.

## **6.2.7 Histology**

For information on transcardial perfusion fixation and tissue preparation see Chapter 2 (section 2.5).

## **6.2.8 Immunohistochemistry**

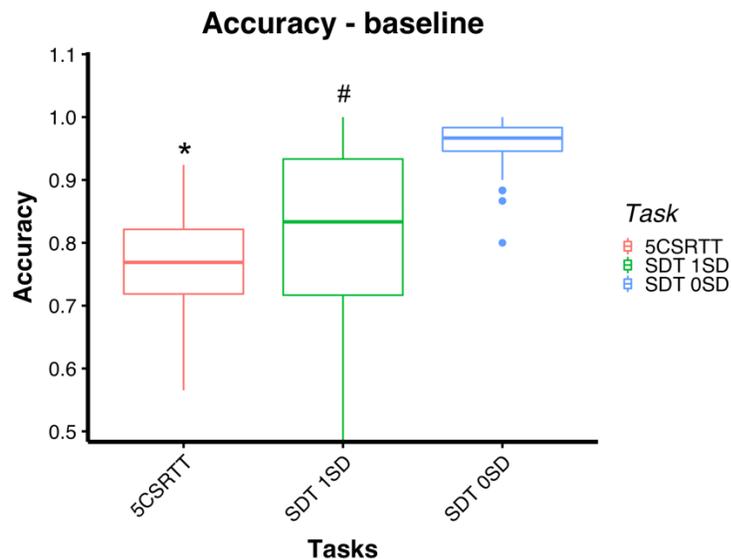
Sections were immunostained with antibodies specific for NeuN, to determine the spread of the neurotoxic lesion. For details on the protocols used see Appendix D (section D1.1).

## **6.2.9 Analyses**

Analyses for baseline, variable SD and post-lesion behaviour were performed as described in Chapter 2 (section 2.6). For details on the fixed factors included in all LMEM models see Appendix A (section D1.2). To compare premature responses between tasks, the proportion of all premature responses made in each trial was calculated by dividing the sum of all premature responses by the total number of trials. In the SDT, premature responses are not punished, thus many are made in each trial, prior to a correct or incorrect response. In the 5CSRTT a premature nosepoke is punished by a 5 s timeout, however the rat still makes some additional nose pokes after performing the first premature response. These additional ‘perseverative’ nose pokes were considered when calculating the ratio of premature responses over total trials in the 5CSRTT. To differentiate them from the traditional way of measuring premature responses in the 5CSRTT (just the first premature nose poke), I will refer to these responses as anticipatory responses.

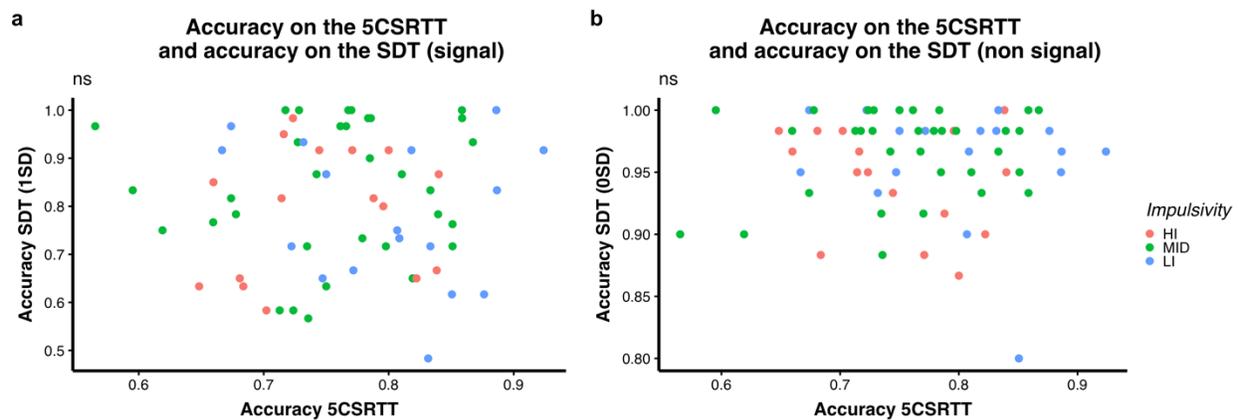
## 6.3 Results

### 6.3.1 Baseline



**Figure 6.2 Accuracy across baseline sessions of 5CSRTT, SDT signal-trials and SDT non-signal trials.** Accuracy on the 5CSRTT was lower than accuracy both on signal and non-signal trials of the SDT,  $*p<0.001$ ; Accuracy on signal trials of the SDT was also lower than accuracy on non-signal trials  $\#p<0.001$ .

Rats needed fewer sessions in the SDT to reach baseline performance compared to the 5CSRTT [ $F(1,61)=220.18$ ,  $p<0.001$ ]; there were no differences between impulsivity groups in sessions required to reach criteria in any of the tasks ( $p=0.327$ ). Accuracy on the SDT was analysed separately for signal and non-signal trials, and then compared with performance on the 5CSRTT. There was a significant difference in accuracy depending on task and SDs [ $F(1,168)=88$ ,  $p<0.001$ ]. **Figure 6.2** shows that accuracy on the 5CSRTT was lower than accuracy both on signal ( $p<0.001$ ) and non-signal trials ( $p<0.001$ ) of the SDT. Accuracy on signal trials of the SDT was also lower than accuracy on non-signal trials ( $p<0.001$ ). There were no differences in accuracy between impulsivity groups ( $p=0.265$ ).



**Figure 6.3** (a) Performance on SDT (signal trials) did not correlate with performance on the 5CSRTT. (b) Performance on SDT (non-signal trials) did not correlate with performance on the 5CSRTT. ns = non significant.

Performance in one task was not associated with performance in the other task, meaning there were no significant correlations between performance on the 5CSRTT and performance both on signal trials ( $p > 0.2$ ) or on non-signal trials of the SDT ( $p > 0.6$ ). See **Figure 6.3a** and **b**. To test the role that duration of the stimulus plays in comparing these two tasks, accuracy on the 5CSRTT on 1 s SD trials (stage 7 of the 5CSRTT training) was compared with accuracy both on signal and non-signal trials of the baseline sessions of the SDT. This analysis revealed that there was a main effect of session [ $F(2,144)=60.86$ ,  $p < 0.001$ ], but no effect of group ( $p = 0.087$ ). Specifically, performance on the 5CSRTT was not different from performance on signal trials of the SDT; however accuracy on both of these measures was lower than that on non-signal trials of the SDT ( $p < 0.001$ , for both comparisons). Again, there was no association between performance on the 5CSRTT and performance on the SDT ( $p > 0.05$ ).

There were other differences between the two tasks, for example on the 5CSRTT an average of 8% of responses (across sessions) were omissions, whereas there were no omissions on the SDT. On the 5CSRTT, impulsivity phenotype influenced the proportion of omission responses [ $F(2,33)=3.31$ ,  $p = 0.049$ ], with LI rats making more omissions than HI rats ( $p = 0.044$ ). Anticipatory responses also differed between the tasks [ $F(1,96)=440$ ,  $p < 0.001$ ], specifically animals made proportionally many more anticipatory responses during the SDT, compared to the 5CSRTT ( $p < 0.001$ ). There were no correlations for anticipatory responses or for perseverative

responses between the two tasks ( $p > 0.05$ ). Response latency for the SDT and 5CSRTT were analysed separately and results are shown **Table 6.1** and **Table 6.2**, respectively.

SDT (baseline)	HI	MID	LI
Central Latency	173.69 (6.85)	203.82 (9.89)	208.05 (10.04)

**Table 6.1 Latencies to initiate a trial in the central nose poke aperture of the SDT, during baseline.** Mean (in ms) and standard error (SE) in brackets. There were no effects of sessions or impulsivity groups on latency to initiate a trial on the SDT.

5CSRTT (baseline)	HI	MID	LI
Correct latency	<b>649.21 (26.30)*#</b>	<b>801.30 (32.06)#</b>	<b>865.75 (36.06)#</b>
Incorrect latency	<b>1480.68 (82.55)*</b>	1658.55 (72.31)	1822.21 (112.67)

**Table 6.2 Latencies to make a correct and incorrect responses on the 5CSRTT, during baseline.** Mean (in ms) and standard error (SE) in brackets. There was an effect of response type [ $F(1,90)=325.38, p < 0.001$ ] and impulsivity group [ $F(2,28)=8.46, p = 0.001$ ] on latency to respond to a signal cue. Specifically, rats were faster at making a correct response compared to an incorrect response ( $p < 0.001$ ) and, regardless of response type, HI were on average faster than MID ( $p = 0.016$ ) and LI ( $p < 0.001$ ) rats. \*significantly different from MID and LI rats. #significantly different from incorrect responses.

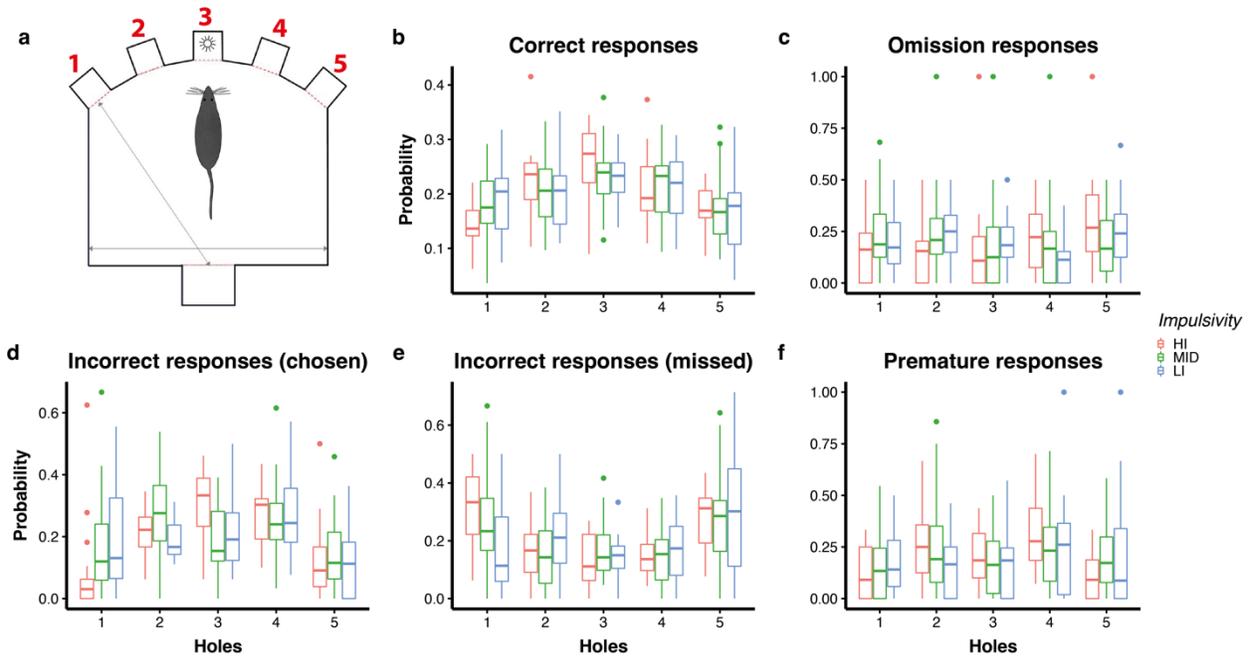
### 6.3.1.1 SDT

With regards to the SDT, the cue and the outcome of the response influenced the rate of anticipatory responses [ $F(1,193)=6.71, p = 0.010$ ]. Specifically, the proportion of anticipatory responses on signal trials that ended up being correct was significantly greater than the proportion of anticipatory responses in signal trials that were incorrectly identified as non-signal ( $p < 0.001$ ). This difference for non-signal trials was only marginally significant ( $p = 0.051$ ).

### 6.3.1.2 5CSRTT

Turner and colleagues (2016) suggested that body position of the animal, with respect to the target cue, determines the likelihood of a correct performance during the 5CSRTT. The following analysis examines whether the CS light appearing in any of the five apertures is more likely to be detected, owing to the spatial configuration of the frontal panel (FP) of the 5CSRTT. For proportion of response types relative to the holes of the 5CSRTT there was a significant Hole x Response Type x Group interaction [ $F(32,1590)=1.80, p = 0.004$ ]. Since the three-way interaction was significant, separate multilevel models were used to further explore the

relationship between Response Type and Hole, in each Group separately. See **Figure 6.4** for a summary of results.



**Figure 6.4 Proportion of responses in each hole of the 5CSRTT for HI, MID and LI rats.** In HI rats the hole location of the CS light determined the response type of the animal [Hole x Response Type  $F(16,820)= 3.40, p<0.001$ ]. HI rats exhibited a higher probability of making an incorrect response when the CS light was presented in holes 1 and 5 compared to hole 3 ( $p<0.02$  all comparisons). In those cases, animals were more likely to make an incorrect response in hole 3 compared to 1 and 5 ( $p<0.02$ ). In cases of an omission response, these happened more frequently when the CS light was presented in hole 5 compared to hole 1, 2 and 3 ( $p<0.05$  all comparisons). Finally, HI rats were less likely to make a premature response in hole 1 and 5 compared to 2 and 4 ( $p<0.02$  all comparisons). In MID rats, the hole location of the CS light also determined the response type of the animal [Hole x Response Type  $F(16,820)= 3.40, p<0.001$ ]. Specifically, MID rats were more likely to make an incorrect response when the CS light was presented in hole 1 compared to hole 4 and in hole 5 compared to hole 2 and 4 ( $p<0.05$  all comparisons). In cases where they made an incorrect response, this was more likely to occur in hole 2 and 4 compared to 1 and 5 ( $p<0.05$ ). There were no other effects of holes on response types. In LI rats the hole location of the CS light also determined the response type of the animal [Hole x Response Type  $F(16,400)= 2.03, p=0.010$ ]. *Post-hoc* contrasts found the only significant effect was that of a higher likelihood to make an incorrect response in hole 4 compared to hole 5 ( $p=0.012$ ).

### 6.3.1.3 In summary

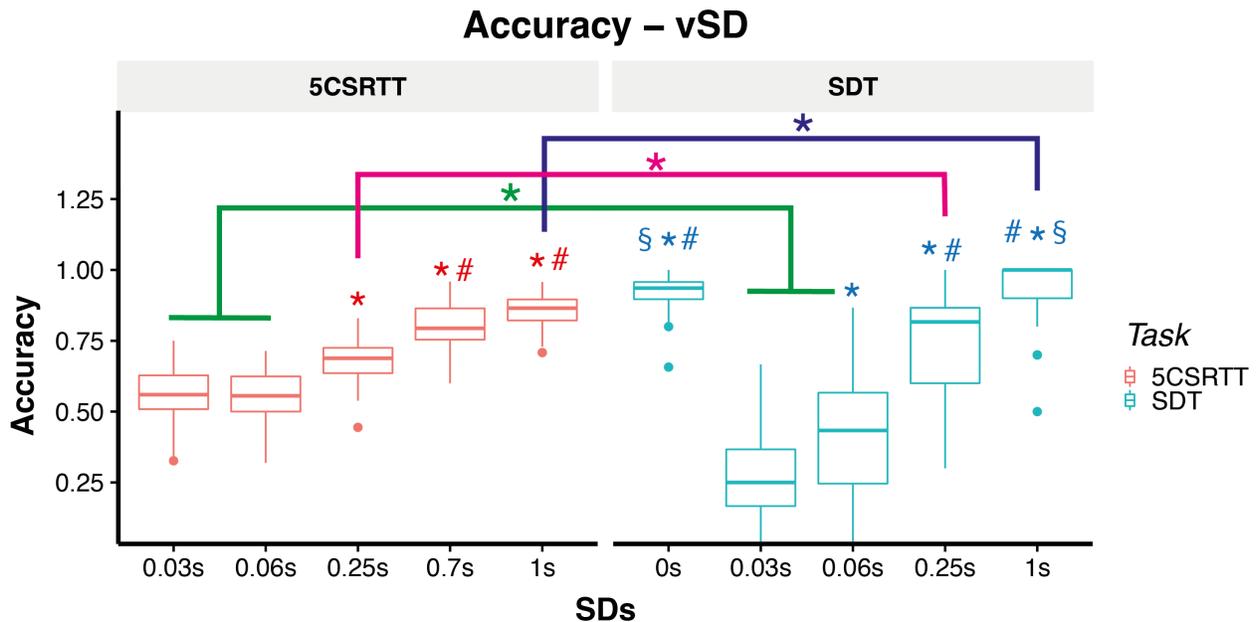
When comparing standard baseline sessions on both tasks, accuracy was on average greater on SDT, both for signal and non-signal trials, compared to the 5CSRTT. However, when comparing the baseline session of the SDT with a session on the 5CSRTT having a SD equal to that of the SDT (i.e., 1s SD), performance did not differ between signal trials of the SDT and 5CSRTT. In contrast, performance on non-signal trials on the SDT was superior both to signal trials on the

SDT and to accuracy on the 5CSRTT with 1s SD. Regardless, there was no association between performance on the two tasks.

During baseline (SD 0.7 s), on the 5CSRTT, the spatial location of the light CS and to an extent the impulsivity phenotype determined the likelihood of making a correct choice. Specifically, HI and MID rats were more likely to miss a CS light when this appeared in the holes at the edges of the array of apertures, (i.e., holes 1 and 5). On the contrary, when a premature or an incorrect response happened this was more likely to occur in the more central holes (2, 3 and 4).

When comparing the baseline sessions of the two tasks, some omission responses (~8% of total trials) were recorded in the 5CSRTT, while there were no omissions in the SDT. Premature responses are not punished in the SDT, contrary to the 5CSRTT, and were indeed much more frequent in the SDT than in the 5CSRTT. In the SDT, the proportion of premature responding was greater during correct trials than incorrect trials, especially in the context of signal trials. If premature responses in this task are a measure of confidence of response choice, rats seemed to be less confident about their choice when they responded 'non-signal' during signal trials. Finally, there were no group differences at initiating a trial in the SDT, however in the 5CSRTT HI rats were faster at responding to the cue signal, compared with MID and LI rats.

### 6.3.2 Variable SD challenge



**Figure 6.5 Accuracy on a vSD challenge both on SDT and 5CSRRT.** Performance on SDT was analysed first to evaluate whether performance on 1 s signal trials was different from performance on non-signal trials (0s). Accuracy on the SDT was influenced by the duration of the stimulus presented [main effect of SD,  $F(4,132)=135.14$ ,  $p<0.001$ ], specifically performance on non-signal-trials was not different from performance on 1 s signal trials ( $p=0.084$ ). However, performance on these trials was superior to all other SDs ( $p<0.001$ ). Performance on the 0.25s, 0.06s and 0.03s were all significantly different from each other ( $p<0.001$  for all comparisons). Because performance on signal and non-signal trials was not significantly different, only signal trials (SD 1s, 0.25s, 0.06s, 0.03s) were considered in the analyses comparing performance on the SDT with that on 5CSRRT. With regards to 5CSRRT, the same SDs as those considered for SDT were included in the analyses thus, SD 1s, 0.25s, 0.06s, 0.03s. Performance on the two tasks was differentially influenced by SD [Task  $\times$  SD,  $F(3,231)=37.73$ ,  $p<0.001$ ]. On the SDT, performance on each SD was significantly different from one another ( $p<0.001$  all comparisons), with the highest accuracy being observed when SD was 1s and the worst accuracy recorded when SD was 0.03s. On the 5CSRRT, performance on SD 0.03 s was not different from performance on 0.06 s ( $p=0.997$ ). However, performance on SD 0.25 s was significantly superior to that on 0.03 s and 0.06 s ( $p<0.01$ ) and performance on 1 s SD was superior to that on 0.25 s SD ( $p<0.001$ ). With regards to performance between tasks for each SD, it was found that performance on SD 1s and SD 0.25s was superior in SDT compared to 5CSRRT (respectively:  $p<0.001$ ,  $p=0.052$  trend level), however performance on SD 0.06s and 0.03s was superior on the 5CSRRT compared to SDT ( $p<0.001$  for both comparisons).

Animals were then exposed to a vSD challenge both in the SDT and in the 5CSRRT. **Figure 6.5** summarises performance accuracy on both tasks.

During performance on the SDT barely any omissions were made (3 in total across all rats), while in the 5CSRRT omissions were influenced by impulsivity phenotype [main effect of Group,  $F(2,33)=4.83$ ,  $p=0.014$ ] and SD [main effect of Group,  $F(4,132)=15.62$ ,  $p<0.001$ ]. Specifically, HI rats made proportionally less omissions than LI rats ( $p=0.012$ ) and, in general, animals made proportionally more omissions during SD 0.03 s, 0.06 s and 0.25 s compared to

0.7 s and 1 s ( $p < 0.015$  all comparisons). Anticipatory responses, instead, differed between tasks and between the baseline and vSD sessions [main effect of Task  $F(1,95)=18.91$ ,  $p < 0.001$ ; main effect of Session  $F(1,95)=961.16$ ,  $p < 0.001$ ]. Specifically, rats made more premature responses in the SDT ( $p < 0.001$ ), and, across tasks, during the baseline session as opposed to the vSD session ( $p < 0.001$ ). In this analysis, impulsivity phenotype did not influence anticipatory responses, however when analyses were restricted to the vSD session of the 5CSRRT, there was a main effect of Group [ $F(2,33)=4.41$ ,  $p=0.020$ ], with HI rats making more anticipatory responses than LI ( $p=0.009$ ) and MID rats ( $p=0.020$ ). With regards to anticipatory responses in the vSD session of the SDT, these were not influenced by Group, but by SD and response type [Response type  $\times$  SD,  $F(4,271)=8.20$ ,  $p < 0.001$ ]. Specifically, rats always made more anticipatory responses during correct trials compared to incorrect trials across all SDs ( $p < 0.001$  for all comparisons), except for non-signal trials ( $p=0.110$ ). In addition to this, there was no difference in anticipatory responses across correct responses. However, across incorrect responses these were greater for the more ambiguous trials, thus for 0 s compared to 0.06 s, 0.25 s and 1 s ( $p < 0.05$  all comparisons), and for 0.03 s compared to 0.25 s ( $p=0.002$ ). Response latency was analysed separately for the two tasks. Results are summarised in **Table 6.3, 6.4 and 6.5**.

<b>SDT (vSD)</b>	<b>HI</b>	<b>MID</b>	<b>LI</b>
Central Latency	204.45 (22.26)	235.54 (24.51)	228.55 (15.10)

**Table 6.3 Latencies to initiate a trial in the central nose poke aperture of the SDT, during baseline.** Mean (in ms) and standard error (SE) in brackets. There were no differences between impulsivity groups on latency to initiate a trial on the SDT.

<b>5CSRTT (vSD)</b>	<b>HI</b>	<b>MID</b>	<b>LI</b>
0.03 s SD	<b>732.63 (72.37)*</b>	904.16 (55.66)	1056.67 (61.18)
0.06 s SD	<b>694.49 (78.83)*</b>	854.67 (33.01)	895.44 (99.62)
0.25 s SD	<b>593.54 (41.46)*</b>	827.94 (48.23)	889.59 (65.64)
0.7 s SD	<b>619.03 (39.77)*</b>	738.43 (39.97)	801.32 (45.55)
1 s SD	<b>632.02 (33.55)*</b>	717.96 (27.48)	847.55 (58.92)

**Table 6.4 Latencies to make a correct response on the 5CSRTT, during the vSD challenge.** Mean (in ms) and standard error (SE) in brackets. The latency to make a correct response was influenced by impulsivity phenotype [ $F(2,33)=8.40$ ,  $p=0.001$ ], with HI rats being faster than MID ( $p=0.015$ ) and LI rats ( $p<0.001$ ). \*significantly different from MID and LI rats.

5CSRRTT (vSD)	HI	MID	LI
0.03 s SD	1747.31 (112.27)	1781.80 (114.93)	2021.57 (110.86)
0.06 s SD	1514.35 (105)	1892.97 (94.95)	1881.70 (169.65)
0.25 s SD	1677.78 (116.90)	1685.32 (99.71)	1597.00 (154.18)
0.7 s SD	1747.09 (131.29)	1769.56 (99.71)	1958.43 (200.33)
1 s SD	1373.08 (205.63)	1740.41 (114.40)	<b>2523.942 (190.98)*</b>

**Table 6.5 Latencies to make an incorrect response on the 5CSRRTT, during the vSD challenge.** Mean (in ms) and standard error (SE) in brackets. The latency to make an incorrect response varied depending on impulsivity group and SD [Group x SD,  $F(8,132)=3.26, p=0.002$ ]. The only differences between groups were observed during 1 s SD trials, where LI rats were slower than MID and HI rats ( $p<0.001$  for both comparisons). \*significantly different from HI and MID rats  $p<0.001$ .

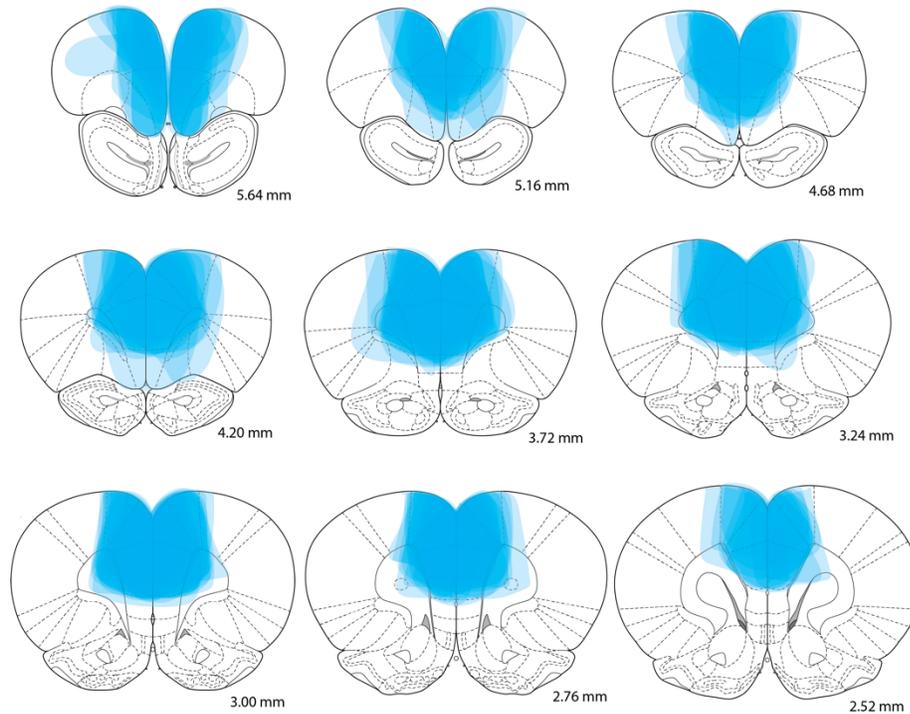
### 6.3.2.1 In summary

Rats responded to a vSD challenge differently in the SDT compared to the 5CSRRTT.

Specifically, in the SDT, performance was lowest when SD was 0.03 s and became progressively better with less ambiguous SDs, being highest with 1 s and 0 s SDs. Importantly, except for accuracy on 0 s and 1 s, performance significantly differed across SDs. In the 5CSRRTT, accuracy levels were also lowest at 0.03 s and 0.06 s SDs, but did not differ between each other, suggesting perhaps a floor effect of performance at this level. Performance was higher in the SDT, compared to 5CSRRTT, for 1 s and 0.25 s SDs, however worsened in the SDT, compared to the 5CSRRTT in SDs 0.03 s and 0.06 s. This suggests that very brief SDs are more challenging on the SDT compared to the 5CSRRTT. Similar to what was observed during the baseline session, rats made many more omissions in the 5CSRRTT compared to the SDT, especially with very brief SDs. Anticipatory responses were more frequent in the SDT, compared to the 5CSRRTT and were predicted by impulsivity phenotype only on the 5CSRRTT. In the SDT, anticipatory responses were proportionally lower for incorrect responses on less ambiguous trials suggesting perhaps that animals were less confident about their choices in these trials. Across tasks, anticipatory responses were fewer in the vSD session compared to the baseline session. Finally, there were no group differences at initiating a trial in the SDT, however in the 5CSRRTT, HI rats were faster at making a correct response compared to MID and LI rats.

### 6.3.3 Effects of mPFC lesions on SDT and 5CSRTT performance

#### 6.3.3.1 Histology

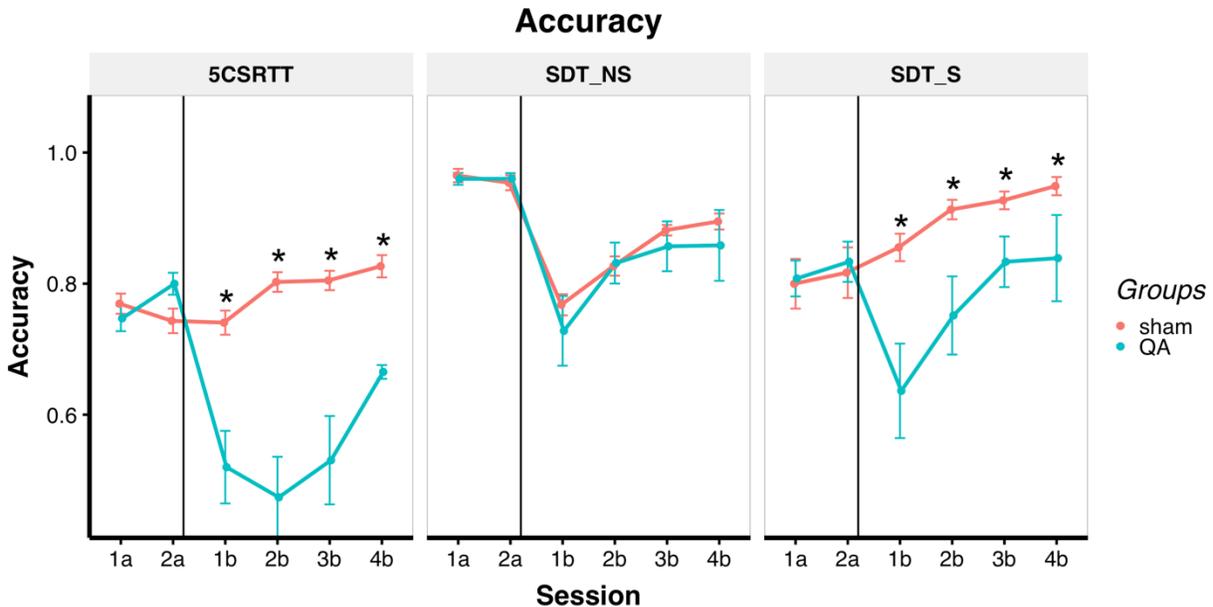


**Figure 6.2** A diagrammatic representation of the extent of the lesion across rats. All animals had damage in the dorsal mPFC, including Cg1 and the secondary motor cortex (M2), which extended more ventrally to the PrL. More anteriorly, damage also encompassed the medial orbitofrontal cortex (mOFC). Lightness of the shade is a function of how many sections share the extent of the lesion with fainter shade indicating that fewer animals have that extent of lesion. Images taken from Paxinos and Watson (2007).

Sections of the rat PFC were defined according to the subdivisions in Paxinos and Watson (2007). A diagrammatic representation of the extent of the lesion across rats is depicted in **Figure 6.6**. To see an exemplar image of the lesion, see **Figure 6.1C** in Appendix C.

## 6.3.3.2 Baseline

### 6.3.3.2.1 Accuracy



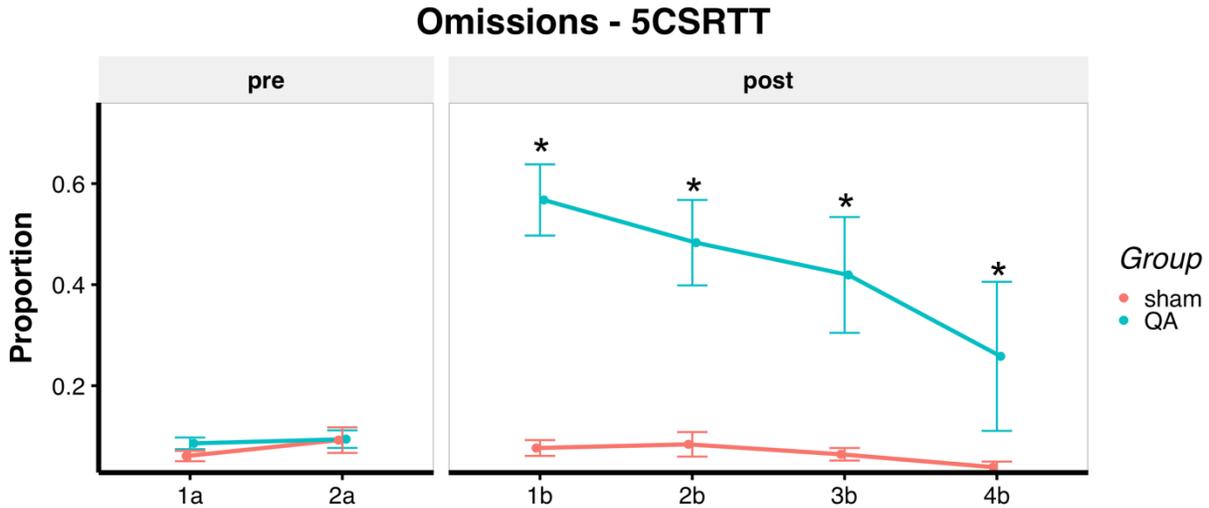
**Figure 6.7 Accuracy on the 5CSRRT and SDT on signal trials (S) and non-signal trials (NS) before and after lesion to the mPFC.** The solid vertical line marks the time point of when the lesion was made. Sessions before the lesion: 1a and 2a; sessions after the lesion: 1b, 2b, 3b, 4b. \*significantly different from sham rats  $p < 0.05$ . QA = quinolinic acid-lesioned rats.

Rats were re-introduced to the baseline schedule for 4 days following post-operative recovery.

Rats with mPFC lesions were profoundly impaired on several behavioural measures. **Figure 6.7** shows how lesions to the mPFC impacted baseline performance on the SDT and 5CSRRT differently. In relation to attentional accuracy, there was a significant Task x Session x Lesion group interaction [ $F(10,497)=2.70$ ,  $p=0.003$ ]. Since the three-way interaction was significant, performance was analysed separately for each session (before vs after lesion). Prior to the lesion, there were no differences in accuracy between lesion groups, in either of the tasks ( $p=0.993$ ). After the lesion, performance differed between lesion groups depending on the task [Task x Lesion,  $F(2,301)=15.89$ ,  $p < 0.001$ ]. Specifically, across baseline sessions, there was no difference between sham and lesioned (QA) rats on non-signal trials in the SDT ( $p=0.763$ ), however on signal trials of the SDT and on the 5CSRRT the accuracy of QA rats was significantly lower than that of sham rats ( $p < 0.001$  for both comparisons). The difference in performance between QA rats and sham rats was greater for the 5CSRRT ( $t=7.07$ ,  $p < 0.001$ ) compared to signal trials of the SDT ( $t=4.82$ ,  $p < 0.001$ ). Sham rats, on the contrary, performed significantly better on signal trials of the SDT, compared to non-signal trials on the same task and to the 5CSRRT ( $p < 0.001$  for both

comparisons). Performance on the 5CSRTT did not correlate with performance neither on signal trials nor on non-signal trials of the SDT, both for sham and QA rats.

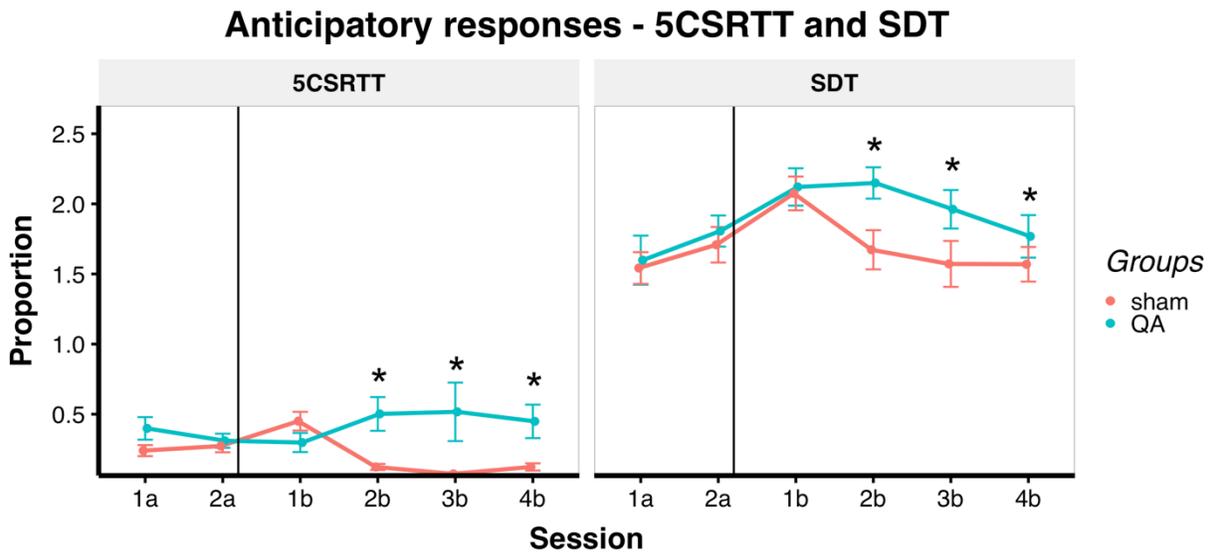
### 6.3.3.2.2 Omissions



**Figure 6.8 Omissions in the 5CSRTT before and after the mPFC lesion.** Following lesioning of the mPFC, omissions in the 5CSRTT were influenced by lesion group and session number. [Lesion x Session,  $F(3,81)=4.42$ ,  $p=0.006$ ]. Across sessions, QA rats made proportionally more omissions than sham rats ( $p<0.02$ ); however the proportion of omissions decreased with testing. Specifically, PFC-lesioned rats made more omissions on the first two days of testing, after the lesions, compared with the last 4<sup>th</sup> day ( $p<0.001$ ). QA rats also made more omissions on day 1 compared with day 3 ( $p=0.045$ ) and on day 3 compared with day 4 ( $p=0.006$ ). \*significantly different from sham rats  $p<0.05$

With regards to the proportion of omission responses during baseline sessions, prior to the lesion, in the 5CSRTT, there was no difference across groups ( $p=0.321$ ), while in the SDT rats did not make any omission responses. Following mPFC lesions, only one rat made 1 omission in three sessions of the SDT. **Figure 6.8** shows that in the 5CSRTT, instead, many more omissions were recorded and these were influenced by lesion group and session number.

### 6.3.3.2.3 Anticipatory responses



**Figure 6.9 Anticipatory responses before and after mPFC lesions on the 5CSRRT and SDT.** Anticipatory responses varied with the number of sessions both depending on task [Session x Task,  $F(5,316)=2.64$ ,  $p=0.023$ ] and on lesion group [Session x Lesion,  $F(1,320)=5.13$ ,  $p<0.001$ ]. Specifically, across tasks there were no differences between groups in the two baseline sessions prior to the lesion and in the first session following the lesion ( $p>0.05$ ). However, in the second, third and fourth session following the lesion, rats made more anticipatory responses than sham rats ( $p<0.01$  for all comparisons). Across sessions, the proportion of anticipatory responses was always greater in the SDT compared to the 5CSRRT ( $p<0.001$ ). \*significantly different from sham rats

Focusing on anticipatory responses, these varied across days of testing and the task, as summarised in **Figure 6.9**.

### 6.3.3.2.4 Response latencies

Response latencies were analysed separately for the two tasks. A summary of the effects of the mPFC lesion on response latency on both tasks can be found in **Table 6.6, 6.7 and 6.8**.

SDT (baseline)	Pre-lesion		Post-lesion			
	1a	2a	1b	2b	3b	4b
<b>QA</b>	190.75 (11.70)	196.00 (13.64)	<b>376.19</b> <b>(29.99)*</b>	<b>320.00</b> <b>(21.61)*</b>	<b>278.58</b> <b>(35.02)*</b>	<b>284.99</b> <b>(30.96)*</b>
<b>sham</b>	209.19 (13.52)	187.16 (9.27)	195.46 (11.34)	234.80 (25.03)	204.80 (13.00)	184.69 (20.02)

**Table 6.6 Latencies to initiate a trial on the SDT, during baseline, before and after mPFC lesions.** Mean (in ms) and standard error (SE) in brackets. The latency to initiate a trial was influenced by session and lesion group [F(5,136)=9.35, p<0.001]. Specifically, there were no differences, on any of the baseline sessions, in latency to initiate a trial between sham and QA rats before the lesion surgery (p>0.05 for all comparisons), however after the lesion, rats were slower at initiating a trial than sham rats on all baseline sessions (p<0.006 for all comparisons) \*significantly different from sham rats p<0.006.

5CSRTT (baseline)	Pre-lesion		Post-lesion			
	1a	2a	1b	2b	3b	4b
<b>QA</b>	770.31 (48.953)	728.06 (41.69)	<b>1445.83</b> <b>(186.32)*</b>	<b>1229.22</b> <b>(118.05)*</b>	<b>1344.91</b> <b>(204.44)*</b>	<b>1051.80</b> <b>(186.44)*</b>
<b>sham</b>	819.53 (41.01)	797.23 (41.38)	677.31 (34.56)	741.86 (55.53)	722.41 (42.00)	707.914 (38.52)

**Table 6.7 Latencies to make a correct response on the 5CSRTT, during baseline, before and after mPFC lesions.** Mean (in ms) and standard error (SE) in brackets. The latency to make a correct response varied depending on lesion group and session [F(5,138)=21.79, p<0.001]. Specifically, before the lesion there were no differences between sham and lesion rats, however after the lesion, rats were slower than sham on all baseline sessions tested (p<0.001 for all comparisons). \*significantly different from sham rats p<0.006.

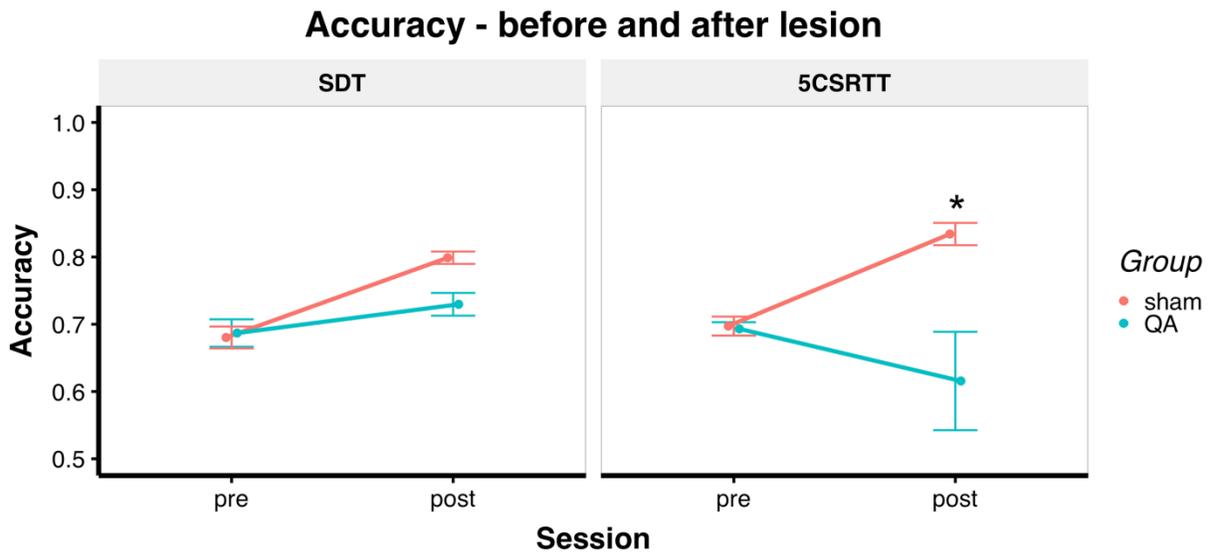
5CSRTT (baseline)	Pre-lesion		Post-lesion			
	1a	2a	1b	2b	3b	4b
<b>QA</b>	1680.59 (121.06)	1740.70 (131.99)	<b>2653.76</b> <b>(181.51)*</b>	<b>2066.90</b> <b>(117.69)*</b>	<b>1999.89</b> <b>(272.33)*</b>	2061.21 (124.72)*
<b>sham</b>	1618.49 (81.05)	1602.66 (87.88)	1257.31 (115.00)	1641.05 (145.35)	1765.52 (116.38)	1678.112 (165.93)

**Table 6.8 Latencies to make an incorrect response on the 5CSRTT, during baseline, before and after mPFC lesions.** Mean (in ms) and standard error (SE) in brackets. The latency to make a correct response varied depending on lesion group and session [F(5,142)=16.35, p<0.001]. Specifically, before the lesion there were no differences between sham and lesionrats, however after the lesion, rats were slower than sham on all baseline sessions (p<0.001 for all comparisons) except the last one (4b, p=0.177). \*significantly different from sham rats p<0.006.

### 6.3.3.3 Variable SD challenge

Following four baseline sessions both sham and QA rats were challenged with a vSD session. On the SDT rats were challenged with similar parameters as used before the lesion. On the 5CSRTT, however, rats were challenged with an less demanding version of the vSD challenge that included both difficult and easy trials, specifically 0.5s, 1s and 2s SDs. For this reason, performance accuracy was compared between tasks as an aggregate of accuracy on different SDs.

### 6.3.3.3.1 Accuracy

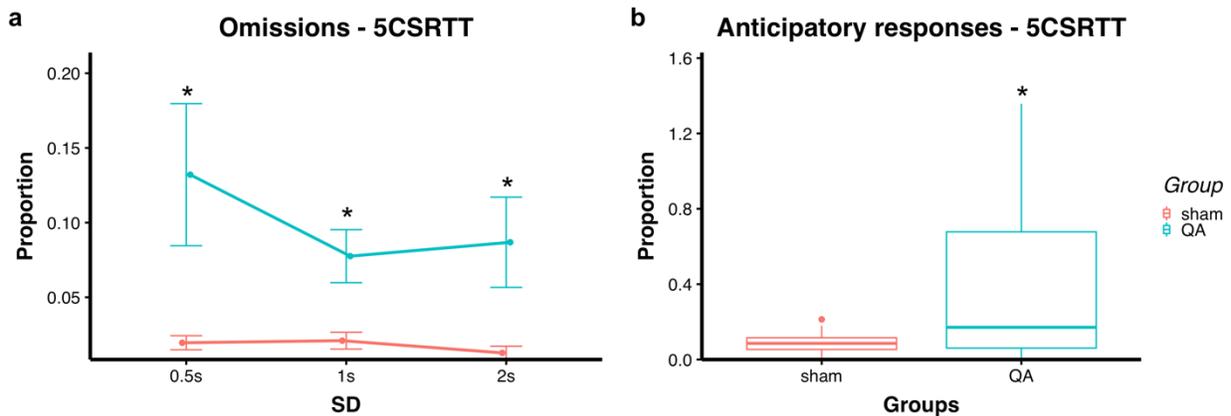


**Figure 6.10** Effects of mPFC lesions on (1) 5CSRTT performance during a vSD challenge and (2) SDT performance (averaged across SDs). Following mPFC lesions, sham rats performed better than lesion rats in the 5CSRTT ( $p < 0.001$ ) and in the SDT ( $p = 0.055$ ). However, in the latter case, this difference was only significant at a trend level. pre = before the lesion; post = after the lesion. \*significantly different from QA (lesion) rats  $p < 0.05$ .

**Figure 6.10** shows how lesions to the mPFC impacted performance on a vSD challenge on both tasks. Performance accuracy varied depending on task, lesion group and session [Session x Task x Lesion,  $F(1,95) = 5.28$ ,  $p = 0.024$ ]. Because there was a three-way significant interaction, performance was analysed separately for each session. Prior to the lesion, there were no differences in accuracy between lesion groups ( $p = 0.924$ ) and between tasks ( $p = 0.542$ ). After the lesion, however, sham rats performed better than QA rats in the 5CSRTT ( $p < 0.001$ ) and in the SDT ( $p = 0.055$ ), however in the latter case, this difference was only significant at a trend level. The difference in performance between QA rats and sham rats was greater for the 5CSRTT ( $t = 5.19$ ,  $p < 0.001$ ) compared to the SDT ( $t = 1.96$ ,  $p < 0.001$ ). Performance of sham rats did not differ across tasks ( $p = 0.160$ ), while QA rats were more impaired in the vSD challenge on the 5CSRTT compared with the SDT ( $p = 0.035$ ). Accuracy performance did not correlate between tasks for either of the lesion groups ( $p > 0.05$ ). To compare performance between tasks on trials with the same SD before and after lesions, 1s SD trials were isolated and compared across sessions and tasks. There was a significant three way interaction between session, task and lesion group [Lesion x Session x Task,  $F(1,111) = 5.84$ ,  $p = 0.017$ ], thus separate models were run for each group separately. Performance of QA rats was modulated by task and session [Task x Session,  $F(1,48) = 5.41$ ,  $p = 0.024$ ]. Specifically, accuracy on 1 s SD trials was not affected by lesion on the

SDT ( $p=0.435$ ); however 5CSRTT performance deteriorated significantly on these trials after the lesion ( $p<0.001$ ). On the contrary, for sham rats performance on these trials only varied between tasks [ $F(1,70)=28.69$ ,  $p<0.001$ ], with accuracy being greater on the SDT compared to the 5CSRTT ( $p<0.001$ ).

### 6.3.3.3.2 Omissions



**Figure 6.11** Effects of mPFC lesions on (a) omissions and (b) premature responses during a vSD challenge on 5CSRTT. mPFC-lesioned rats made more omissions and anticipatory responses than sham rats. \*significantly different from sham rats  $p<0.05$ .

Omission were more frequent in the 5CSRTT compared to the SDT. In the latter task, just 4 animals made 1 omission each (over a total of 170 trials), while in the 5CSRTT, ~9% of responses for lesioned rats were omissions and for sham rats this percentage was roughly 2%.

**Figure 6.11a** shows the number of omissions on the 5CSRTT. In this task, omissions were influenced both by SD [main effect of SD,  $F(2,56)=4.36$ ,  $p=0.017$ ] and lesion group [main effect of Lesion group,  $F(1,28)=10.94$ ,  $p<0.001$ ]. Specifically, a higher proportion of omissions was made during 0.5s SD compared to 2s SD ( $p=0.013$ ), and regardless of the SD presented, lesioned rats always made more omissions than sham rats ( $p<0.001$ ).

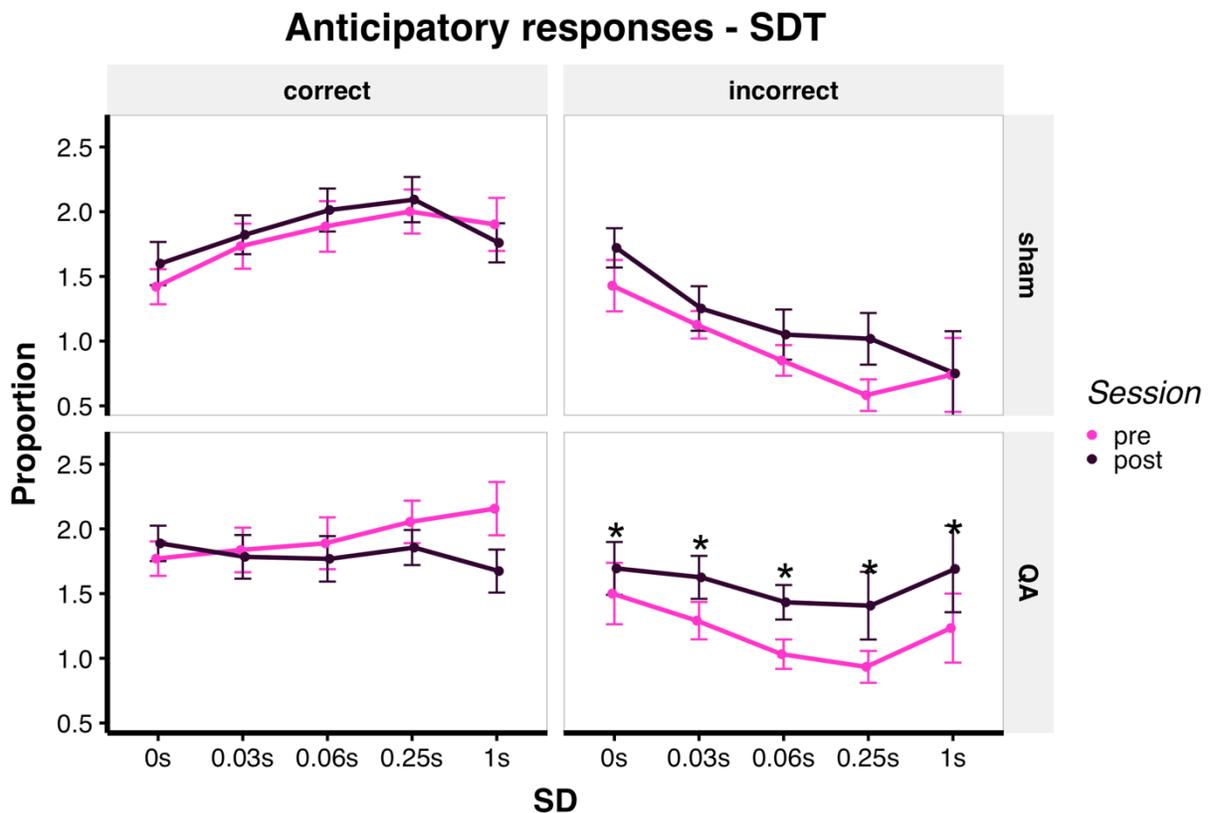
### 6.3.3.3.3 Anticipatory responses

Anticipatory responses varied between tasks depending on lesion group [Lesion x Task,  $F(1,33)=4.47$ ,  $p=0.042$ ]. Specifically, across groups, rats made more anticipatory responses in

the SDT compared to the 5CSRTT ( $p < 0.001$ ), however in the SDT there were no differences between lesion groups ( $p = 0.891$ ), whereas in the 5CSRTT, lesioned rats made proportionally more anticipatory responses than sham rats ( $p = 0.006$ ), as shown in **Figure 6.11b**.

In the SDT, when looking at the proportion of anticipatory responses for each response type (i.e. correct and incorrect) and for each SD (0.03 s, 0.06 s, 0.25 s, 1 s and 0 s), there was a three way interaction between Lesion group x Response type x Session [ $F(4,567) = 4.37$ ,  $p = 0.002$ ]. Because there was a significant three-way interaction, data was analysed separately for each lesion group.

**Figure 6.12** summarises these findings for anticipatory responses on the SDT.



**Figure 6.12 Anticipatory responses on the SDT before and after mPFC lesions.** Anticipatory responses of lesioned rats were influenced by the mPFC lesions and response type [Response Type x Session,  $F(1,261) = 10.09$ ,  $p = 0.002$ ]. Specifically, there was no effect of the mPFC lesions on anticipatory responses prior to correct responses ( $p = 0.099$ ); however, following mPFC lesions, rats made proportionally more premature responses for incorrect responses than prior to the lesion ( $p = 0.007$ ). There was no such effect of mPFC lesions on anticipatory responses prior to incorrect responses for sham rats ( $p = 0.684$ ). Anticipatory responses of sham rats were influenced by response type and SD, both before and after lesion surgery [Response Type x SD,  $F(4,306) = 13.82$ ,  $p < 0.001$ ]. Specifically, anticipatory responses were greater for correct responses compared to incorrect responses for all SDs ( $p < 0.002$ ), except for non-signal (0s) trials ( $p = 0.885$ ). In addition, anticipatory responses for incorrect trials diminished with decreasing ambiguity of the SD, such that responses were greater during 0 s and 0.03 s SDs compared with 0.25 s and 1 s SD ( $p < 0.004$ ). They were also greater during non-signal trials (0 s SD) compared with 0.06 s SD ( $p < 0.001$ ), and during 0.06 s SD compared with 1 s SD ( $p = 0.042$ ). pre = before the lesion; post = after the lesion. \*significantly different from pre-lesion sessions  $p < 0.05$ .

### 6.3.3.3.4 Response Latencies

Response latencies were analysed separately for the two tasks. A summary of the effects of the mPFC lesions on response latency on both tasks can be found in **Table 6.9** and **6.10**.

SDT (vSD)	Pre-lesion	Post-lesion
QA	212.36 (13.91)	<b>234.68 (14.91)*</b>
sham	238.248 (12.04)	187.11 (12.04)

**Table 6.9 Latencies to initiate a trial on the SDT during the vSD challenge, before and after mPFC lesions.** Mean (in ms) and standard error (SE) in brackets. In the SDT, the latency to initiate a trial was influenced by session and lesion group [F(1,32)=6.81, p=0.014]. Specifically, there were no differences in latency to initiate a trial before the lesion surgery, however after the lesion, lesioned rats were slower to respond than sham rats (p=0.017). \*significantly different from sham rats p<0.05.

5CSRTT (vSD)	Correct latency		Incorrect latency	
	pre-lesion	post-lesion	pre-lesion	post-lesion
QA	792.47 (37.61)	<b>1255.27 (142.61)*</b>	1870.71 (72.74)	<b>2101.21 (141.42)*</b>
sham	792.39 (36.00)	785.92 (38.17)	1716.11 (63.59)	1580.983 (150.08)

**Table 6.10 Latencies to make correct and incorrect responses during the vSD challenge, before and after mPFC lesions.** Mean (in ms) and standard error (SE) in brackets. Response latencies on the 5CSRTT were always longer for incorrect responses than correct responses [F(1,89)=264.79, p<0.001]. However, regardless of response type, they also varied according to lesion group and session [Lesion x Session, F(1,101)=13.58, p<0.001]. Specifically, there were no differences between lesion and sham rats in response latencies prior to the lesion surgery (p=0.404). However, after the surgery, lesioned rats were significantly slower at responding than sham rats (p<0.001). \*significantly different from sham rats p<0.05.

### 6.3.3.3.5 In summary

**Table 6.11** shows a summary of how the mPFC lesions affected performance on the two tasks on different indexes of performance. Lesions of the mPFC differentially affected performance on the SDT and 5CSRTT. In both tasks, lesioned rats were impaired compared with sham rats.

However, when comparing accuracy performance between tasks, sham rats performed similarly on both tasks whereas lesioned rats exhibited decreased accuracy on the 5CSRTT compared with the SDT. Performance on the two tasks, however, did not correlate. Omissions were much more

frequent in the 5CSRTT compared to the SDT, with lesioned rats making significantly more omissions than sham rats. In addition, anticipatory responses were more frequent in the SDT compared with the 5CSRTT and only in the latter did the lesion groups differ in performance, with lesioned rats making more anticipatory responses than sham rats. While in the SDT there were no differences between lesion groups in the overall rate of anticipatory responses, there were some group differences when looking specifically at proportion of anticipatory responses relative to correct and incorrect responses. Thus, during less demanding trials (with relatively long SDs), sham rats, both before and after the lesion, made proportionally fewer anticipatory responses during trials that ended as incorrect. After mPFC lesions, rats did not show this reduction in anticipatory responses during incorrect trials. However, prior to correct trials, anticipatory responses were unaffected by the mPFC lesions.

	<b>5CSRTT</b>	<b>SDT</b>
Accuracy (baseline)	↓	↓ (signal trials only)
Accuracy (vSD)	↓	↓ (trend level significance when compared to the 5CSRTT)
Anticipatory responses	↑	↑
Omissions	↑	-
Response latency	↑	↑

**Table 6.11** A summary of the effects of mPFC lesions on performance of the 5CSRTT and SDT. ↑ indicates an increase; ↓ indicates a decrease.

## 6.4 Discussion

### 6.4.1 A comparative analysis of the SDT and 5CSRTT

The 5CSRTT (Robbins, 2002) and the SDT (Turner, et al., 2016) were developed as rodent analogues of the human CPT and have been widely used to assess attention and impulsivity in rodents with the aim to facilitate cross-species translation. This study evaluated whether 1) these two tasks assess the same attentional construct; 2) whether HI rats, who have been shown to have an advantage on a high-event rate version of the 5CSRTT show a similar advantage on the SDT, which is a more fast-paced task; 3) whether these two tasks similarly depend on the functional integrity of the PFC. It was found that: 1) performance does not correlate between tasks, neither on baseline nor during sessions that required greater attentional control; 2) HI rats did not perform better than the other impulsivity groups on the SDT; 3) excitotoxic lesions to the mPFC affected performance on these tasks differently, severely disrupting behaviour on the 5CSRTT but only marginally decreasing accuracy on the SDT. Thus, it appears that these two tasks involve different psychological processes, both at the attentional and motivational level, and that they depend to different extents on the functional integrity of the mPFC.

An obvious difference between the two tasks is that rats require fewer training sessions to reach baseline performance in the SDT (~ 10 sessions), compared to the 5CSRTT (~ 20 sessions). This replicates findings from Turner and colleagues (2016) and speaks to the different learning and attentional processes required to successfully perform the two tasks. Further, when comparing baseline sessions, rats reached higher levels of accuracy in the SDT and, specifically, in non-signal trials of this task. This is inconsistent with what Turner and colleagues (2016) observed, as they reported no difference in accuracy across tasks. However, there are several reasons why the present findings might differ from that study. Firstly, Turner and colleagues (2016) tested a different strain of rats from the one used in this investigation and, importantly, did not test the same rats on both tasks. Thus, their observation of a lack of difference in performance accuracy between tasks could be explained both by differences in strain (between the one presently used and the one they used), and differences between batches, with one batch of animals outperforming the other on the more difficult task. Another important difference between this

study and that by Turner and colleagues (2016), is that in the present investigation the SD of the cue was different between tasks, specifically 0.7 s for the 5CSRTT and 1 s for the SDT. This was implemented with the aim of comparing performance between tasks on their ‘standard’ baseline procedure. Turner and colleagues (2016) instead tested animals on both tasks with the same SD of 1 s. In the present study, animals experienced the 1 s SD during training on the 5CSRTT before achieving their baseline level of 0.7 s. Thus, performance on the level of training with 1 s SD was compared with performance on the SDT (whose baseline sessions are with 1 s SD on signal trials). Accuracy on the 5CSRTT, with 1 s SD, did not differ from accuracy on signal trials on the SDT. However, these measures were significantly lower than accuracy on non-signal trials of the SDT. When accuracy on the SDT was averaged across signal and non-signal trials, performance on the 5CSRTT was indeed impaired relative to the SDT; however, this was the case for HI and MID rats, but not for LI rats. Collectively, these findings show that during standard baseline sessions, accuracy on the 5CSRTT is lower than on the SDT, and even when the duration of the signal trials is equated between 5CSRTT and SDT, performance on the former task is still lower for most animals. Regardless of the relationship between absolute values of accuracy on each task and SD, performance on the 5CSRTT and the SDT did not correlate, suggesting that different psychological processes are at play when performing these two tasks. As mentioned by Turner and colleagues (2016), these two tasks are profoundly different: while they both require sustained attention to identify the visual target, the SDT also relies on the maintenance of a rule (e.g, light -> poke left, no light -> poke right) to select the action associated with the visual stimulus detected. The 5CSRTT, instead, in addition to sustained attention, also relies on elements of spatially divided attention, in that the rat needs to scan all five locations of the visual array to detect the stimulus (Chudasama & Robbins, 2004). Thus, the two tasks involve different cognitive processes, which likely explains why performance on the two tasks did not correlate.

Reductions in SD, which make the visual stimulus more difficult to detect, decreased accuracy in both tasks but in different ways. On the SDT, performance on 1 s SD trials and non-signal trials did not differ, and accuracy decreased monotonically as the SD shortened. On the 5CSRTT, accuracy also decreased as the SD shortened but plateaued on the short 0.03 s and 0.06 s SDs. Between tasks comparisons showed that performance on the ‘easier’ 1 s and 0.25 s trials was

superior in the SDT compared to 5CSRTT. However, performance on the more challenging 0.03 s and 0.06 s SDs was superior on the 5CSRTT compared to the SDT. This could be explained by the fact that in the SDT the presence and absence of a signal are cues that indicate which action should be elicited to earn a reward. Thus, in ambiguous trials, when the SD is difficult to detect, the animal has a 50% chance of getting it correct, thus behaving more ‘randomly’ and having lower accuracy. On the SDT, these more difficult trials do not result in a greater incidence of omissions, indicating perhaps that the animal is willing ‘to gamble’ a response instead of giving up on the trial. This is probably because the SDT is designed to promote rapid responding and to minimize distractibility (see below for a discussion on this), thus discouraging the occurrence of omissions. In the 5CSRTT, instead, there are no ‘non-signal’ trials, thus whenever the rat detects a light stimulus, however brief, there is less ambiguity regarding the nature of the signal (however there is some ambiguity on the location of the signal). Differently from the SDT, in the 5CSRTT, animals make omissions, suggesting they do sometimes prefer not to respond when trial uncertainty is high. When omissions were included in the denominator for accuracy of the 5CSRTT, performance between the SDT and the 5CSRTT ceased to be significantly different on the 0.06 s SD, however accuracy on the 5CSRTT was still superior to that of the SDT on the short 0.03 s SD trials. This suggests that the difference in accuracy on more difficult to discriminate trials (short SDs), between the two tasks, is not simply due to a tendency to make fewer omissions but more incorrect responses on the SDT.

Beyond measures of accuracy, the two tasks differed in other indexes of performance. For example, in the 5CSRTT rats made more omissions, both during baseline and during the vSD challenge, when the stimulus was reduced to very short durations. A higher incidence of omissions on the 5CSRTT compared to the SDT replicated findings by Turner and colleagues (2016). The SDT was designed to minimise the opportunity for distraction by reducing unnecessary ambulation in the chamber and shortening delays between cue presentation and the requirement of a response (Turner, 2016). Thus, for example, in the SDT the delay between trial initiation and cue detection is almost non-existent, while in the 5CSRTT the animal is usually required to sustain attention for the duration of the ITI (usually 5 s) before being able to respond to the cue. In addition, the animal is forced to orient towards the light-cue in the SDT when this is presented because the central aperture where the rat pokes to initiate a trial is located just

below the light-cue. Instead, in the 5CSRTT, trial initiation occurs in the food magazine located opposite the visual array, thus the animal needs to rotate its body position by 180 degrees to attend to a stimulus appearing in one of the five apertures in the frontal panel. Both delays in responding and body position during cue presentation may play a role in inducing a higher degree of omissions in the 5CSRTT compared with the SDT. In line with this, it was observed that omissions and incorrect responses were more likely to occur when the cue signal was presented in the holes at the periphery of the visual array. As expected, during trials with very short SDs rats made more omissions on the 5CSRTT but virtually none in the SDT.

With regards to anticipatory responses, these were much more frequent in the SDT than in the 5CSRTT. This also replicated findings by Turner and colleagues (2016) and is consistent with the design of the tasks, in that anticipatory responses are punished in the 5CSRTT but are not in the SDT. In the SDT anticipatory responses were always more frequent during trials that were correct compared to trials where the magazine was incorrectly chosen, both during baseline and during the vSD challenge. During the vSD challenge, anticipatory responses of choices that were incorrect decreased with increasing discriminability of the stimulus. Thus, anticipatory responses of incorrect choices during trials of 1 s, 0.25 s and 0.06 s were lower than during more ambiguous trials of 0.03 s and 0 s SD. This could perhaps indicate that anticipatory responses reflect the confidence of the choice of the animal, with low anticipatory responses during incorrect responses of unambiguous signal trials.

One of the main aims of this study was to evaluate whether impulsivity on the 5CSRTT, which in this task confers an advantage during high event-rate conditions, was also beneficial on the SDT, which by design has a faster pace than the 5CSRTT. There were, however, no effects of impulsivity phenotype on the SDT. The only effects of impulsivity on accuracy were reported on the 5CSRTT early during training when the SD was 1 s. However, these did not replicate in further sessions on this task with 1 s SD. Effects of impulsivity phenotype were reported on other indexes of performance of the 5CSRTT including omissions, anticipatory responses and response latencies. For example, both during baseline and the vSD, LI rats made more omissions than HI rats. This is in line with evidence in Chapter 4 that LI rats are more prone to make omissions than the other impulsivity groups. Anticipatory responses only differed between impulsivity

groups during the vSD session, with HI rats making more anticipatory responses than the other groups. Finally, HI rats were generally faster than the other groups at responding on the 5CSRTT both during baseline and during the vSD, this also has been observed across paradigms and batches in the present thesis (Chapter 3, 4 and 5).

## **6.4.2 Behavioural effects of mPFC lesions**

Lesions of the mPFC affected attentional performance on the two tasks, albeit in different ways. Specifically, when tested on baseline sessions of the 5CSRTT and SDT over four consecutive days, rats with mPFC lesions showed deficits in performance on both tasks. However, in the SDT, the deficit was restricted to signal trials and did not affect non-signal trials. This suggests that processing of signal and non-signal trials might recruit different attentional and neural mechanisms, such that lesions of the mPFC affect detection of the former rather than the latter. Of some interest, performance of mPFC lesioned rats deviated more from that of sham rats on the 5CSRTT compared to the SDT, suggesting that the lesion affected attentional accuracy on the 5CSRTT more than it did on the SDT. This was true also when the task was made more challenging by introducing a vSD with very short SDs. Impaired accuracy on the 5CSRTT, following lesions to the mPFC, is in line with previous findings (Muir et al., 1996; Passetti et al., 2002; Chudasama et al., 2003, 2005, Pezze et al., 2009), which have implicated predominantly the dorsal portion of the mPFC (e.g., Cg1) in attentional performance. With regards to the SDT, there is only one unpublished experiment on the effects of lesions to the mPFC on performance of the SDT (Turner, 2016). Turner (2016) did not observe a decrease in accuracy following lesions to the mPFC during baseline testing on the SDT; however she did observe a decrement in accuracy when a distractor manipulation was integrated in the task. The present findings, however, reveal a deficit in performance on the SDT following lesions of the mPFC, both during baseline and during the vSD manipulation. These findings, together with a dramatic decrease in accuracy observed on the 5CSRTT, confirm a role of the mPFC in attentional performance. This is in line with recent evidence showing that neurons in the mPFC, both dorsal (Lak et al., 2020; Pinto & Dan, 2015) and ventral (le Merre et al., 2018), encode a variety of signals during sensory discrimination tasks, including sensory cues, motor action, and trial outcomes. In the context of sensory cues, for example, it was shown that sensory signals of responses that are correct elicit stronger activity of mPFC neurons to the signal of responses that are incorrect (Lak et al., 2020;

Le Merre et al., 2018) and that sensory-evoked responses in this region correlate with task performance (Le Merre et al., 2018). This could explain, in part, why lesions of the mPFC did not affect accuracy on non-signal trials on the SDT, given that during these trials there is no ‘signal’ to detect. However, in the present experiment, the accuracy deficit observed on the SDT was not as extreme as that observed in the 5CSRRT. This suggests that the mPFC, while being implicated in sustained attention (necessary to perform both SDT and 5CSRRT), is perhaps more strongly recruited in tasks that have a component of divided attention, such as the 5CSRRT.

Lesions to the mPFC led to a significant increase in omissions in the 5CSRRT, unlike the SDT. This difference is consistent with what discussed above on the design of the SDT, which by minimising waiting times and the opportunity to be distracted, has almost no incidence of omissions. A lesion-dependent increase in omissions on the 5CSRRT was concomitant with an increase in anticipatory responses and in response latency. These findings concur with previous research on the role of the mPFC in performance of the 5CSRRT (Pezze et al., 2009; Passetti et al., 2002; Chudasama et al., 2005). An increase in the latency to initiate a trial and in premature responses was also observed in lesioned animals performing the SDT. With regards to premature responses, an increment of these responses following mPFC lesions is in line with previous evidence suggesting that this region, and especially the more ventral division, plays a role in inhibitory control (Chudasama et al., 2003; Passetti et al., 2002; Donnelly et al., 2015). This is based on evidence that lesions to the ventral portion of the mPFC (PrL, IL) result in an increase in premature and perseverative responses (Chudasama et al., 2003; Passetti et al., 2002). Further work on this has indicated that the ventral mPFC may also be involved in temporal discriminations rather than just response inhibition (Donnelly et al., 2015; Turner & Parkes, 2020). For example, in another task assessing motor impulsivity such as the DRL, Cho and Jeantet (2010) showed that mice with lesions of the ventral mPFC (PrL and IL), with repeated testing, did not exhibit the typical peak in response around the delay time but showed a more flattened and wider peak. This impairment in performance however was rescued by the introduction of a cue that indicated the end of the delay period suggesting that lesions to the mPFC might have altered temporal judgment more than the ability to inhibit responding (Turner and Parkes, 2020; Cho and Jeantet, 2010). Further to this, Donnelly and colleagues (2015) found that, during performance on the 5CSRRT, neurons in the PrL exhibit ramping activity during the

waiting period prior to cue onset (i.e., the ITI). During premature responses, this ramping activity started earlier and was associated with shorter latencies to begin waiting (during the ITI). This led the authors to conclude that premature responses may result from an inability to start the waiting process at the correct time during the task, with the mPFC keeping track of this aberrant timing signal (Donnelly and colleagues, 2015). Ramping activity in mPFC during waiting, however, could also indicate the time in which maximal attention needs to be deployed to attend to an upcoming stimulus or in which maximal control needs to be exerted to constrain a prepotent response. This last interpretation would be in line with recent work on the role of ventral mPFC on inhibitory control (Hardung et al., 2017; Terra et al., 2020). Hardung and colleagues (2017), for example, showed in a response-preparation task, where rats were asked to press a lever until a tone occurred, that PrL neurons suppress activity prior to premature responses and that optical inhibition of these cells led to an increase in premature responses. Somewhat in line with this, Terra and colleagues (2020) showed that chemogenetic inhibition of cells in the mPFC, and specifically those in the dorsal portion projecting onto the DMS, increased the frequency of premature responding. Thus, our observation of increased premature responses may have resulted from the implicated role of the mPFC in timing and response inhibition.

An interesting phenomenon was observed with regards to anticipatory responses in the SDT following mPFC lesions. Thus, sham rats, both before and after the lesion, made proportionally more anticipatory responses during correct responses, compared to incorrect responses. In the case of incorrect responses, the rate of anticipatory responses diminished with decreasing ambiguity of the SD, which as mentioned above suggests that rats can estimate with some degree of confidence they have chosen incorrectly. Lesioned rats, however, did not show this reduction in anticipatory responses during unambiguous incorrect responses. On the contrary, after the mPFC lesion they made proportionally more anticipatory responses during incorrect trials than before the lesion. The lack of a reduction in anticipatory responses during incorrect 'easy' trials could reflect impairments in confidence of perceptual discrimination and indeed neurons in the mPFC have been implicated in sensory discrimination and confidence (Lak et al., 2020). For example, Lak and colleagues (2020), recorded neurons in the mPFC during a visual discrimination task and observed that neuronal activity increased with increasing contrast of the

visual stimulus and correlated with sensory confidence. The latency to make an incorrect response on the 5CSRRT is always longer than latency to make a correct response and could also reflect uncertainty or low confidence regarding the discrimination of the stimulus. In the present study, however, this measure did not differ between mPFC lesioned and sham rats, with both groups taking longer at making an incorrect response compared to a correct response. This perhaps another example of how the mPFC lesions affected performance on the two tasks differentially.

In conclusion, this study showed that two tasks developed as rodent analogues of the human CPT to study sustained attention recruit different attentional mechanisms. This conclusion is supported by evidence of a lack of correlation between performance and the differential sensitivity of the two tasks to excitotoxic lesions of the mPFC.

# Chapter 7

## 7.1 General discussion

Using complementary approaches, this thesis investigated the neurobehavioural substrates of impulsivity in the 5CSRTT. Impulsivity was operationalised as anticipatory (or premature) responses; that is, responses occurring before a prescribed time has elapsed. Previous research from this lab and other labs found that increased demand on waiting prior to operant responding exacerbates premature responses. The key scientific objectives addressed by this work was (1) the elaboration of psychological drivers underlying the manifestation of trait impulsivity, and (2) the extent to which impulsivity depends on the mesolimbic DA system.

In procedures widely used to investigate impulsivity in rodents such as the 5CSRTT and the DRL, premature responses are more likely to occur when the waiting time prior to making an operant response is long or longer than what the animal is trained to normally expect (Bari, et al., 2008; Caprioli et al., 2013; Dalley, et al., 2007a; Pothuizen et al., 2005). In the specific case of the 5CSRTT, an increase in premature responses is usually observed when rats are trained to wait for 5 s prior to the appearance of the light cue and are then challenged -in some sessions- with an increase in waiting time by 2 or more seconds (Dalley, et al., 2007a; Jupp et al., 2020). This increase in premature responses, as a function of waiting time, was indeed replicated in this thesis. Thus, in **Chapter 3, 4 and 5** rats made more premature responses during the 7 s ITI sessions compared to the 5 s ITI sessions and as described in **Chapter 4**, premature responses increased to an even greater extent when rats were challenged with an even longer waiting period of 9 s. The inability to withhold a response made prepotent by its association with reward is a defining feature of waiting impulsivity and trait impulsivity in rodents, at least on the 5CSRTT, is assessed by quantifying the number of premature responses occurring during these long ITI challenges. While differences between impulsivity groups manifest, by definition, during long (>7s) ITI sessions, HI rats tend to make more premature responses than the two other groups of rats (and especially LI rats) already at baseline, that is during standard 5 s ITI sessions. This is

indeed shown in **Chapter 4**, in two separate cohorts of rats, and in **Chapter 5** in a third cohort of rats.

Previous work in the lab, both published (Donnelly et al., 2014) and unpublished (findings reported in this thesis), showed that premature responses in the 5CSRTT, both occurring during a 5 s and a 7 s ITI, tend to occur following a non-rewarded trial. This led to the hypothesis that premature responses on the 5CSRTT occur because of frustration or so-called negative urgency. This hypothesis highlights the potential translational value of the 5CSRTT, as impulsivity in humans is thought to arise, in some circumstances, because of negative affect or frustration (Whiteside and Lynam, 2001; Eben et al., 2020). Specifically, both in humans (Dixon et al., 2013; Yu et al., 2014) and other animals (Amsel and Roussel, 1952; Judice-Daher et al., 2011), the omission of an expected reward has been shown to lead to an invigoration or speeding of behaviour, which can result in impulsive responding (Whiteside and Lynam, 2001). Experiments in **Chapter 3** explored whether frustration-dependent invigoration of behaviour plays a role in the occurrence of premature responses in the 5CSRTT (the ‘frustration’ hypothesis). Frustration is expected to be maximal when there is a violation of expectation with regards to the earning of an appetitive reward (Amsel, 1958). Thus, in **Chapter 3** rats were tested on a continuous and partial reinforcement schedule to assess whether schedules with a greater occurrence of ‘unexpectedly’ omitted rewards (i.e., correct response that were not rewarded) would elicit frustration and thus induce a greater number of premature responses. In this respect, reinforcement omission effects (ROEs) were evaluated both at the macro and micro levels. Specifically, at the macro level, analyses focused on quantifying whether sessions with varying degrees of partial reinforcement schedules (that is 0.8, 0.5, 0.2 reinforcement rate,  $rr$ ) elicited, in accordance, differing degrees of premature responses and, ultimately, whether a continuous reinforcement schedule elicited fewer premature responses. At the micro level, a first-order Markov chain model was fit to a trial-by-trial breakdown of the 5CSRTT performance of each rat, to test whether the probability of making a premature response was higher following a correct non rewarded trial (NR) as opposed to any other trial type. A first-order Markov chain model tests the prediction that actions in the current state  $t$  (in this case a response type in a trial) determines responding in the future state  $t+1$  (Davison, 2003). This implies that the probability of transitioning from one state to the other is different from ‘chance’ or from what would be

observed if there were no dependencies between states (the independence model). Deviation from the independence model is usually assessed by calculating the likelihood ratio statistic  $W$  over the whole matrix of transition probabilities (as explained by Davison, Chapter 6, 2003), while chi-square tests can be used, separately, to test whether transitions between specific states of interest deviate from chance (as explained by Anderson and Goodman, 1957). In the present work, states were represented by response types (that is, correct non rewarded responses, correct rewarded responses, premature responses, omissions, and incorrect responses) and the diagnostic tests mentioned above evaluated whether rats were more likely than chance to transition from a specific response type to another. This micro analysis was aimed not only at validating the frustration hypothesis but also at determining whether, in the event of an increase in premature responses during partial reinforcement, such an increase could not be explained by the incentive hope hypothesis (Anselme, 2015).

The incentive hope hypothesis holds that, during schedules of partial reinforcement, rats come to display greater conditional responding (CR) or ‘wanting’ upon presentation of the CS as this gives them ‘hope’ that it will be paired with a reward. In psychological terms, this ‘hope’ translates into incentive motivation and behaviourally into stronger approach behaviour (Anselme, 2015; Anselme and Robinson, 2019). In the 5CSRTT, facilitated approach behaviour could induce the rat to nose-poke into the front-panel (FP) before the ITI has terminated, thus resulting in a premature response. The incentive hope theory developed from work using Pavlovian conditioned approach (PavCA) tasks whereby during conditions of reward uncertainty (regarding reward magnitude and reward delivery), rats tended to approach a lever predictive of the appetitive reward (CS) to a much greater extent than during conditions of reward certainty. Thus, in the event of an increase in premature responses during partial reinforcement schedules, the micro analysis would reveal whether such premature responses are likely to occur following any response type, thus supporting the incentive hypothesis, or whether they would occur primarily following NR responses, thereby supporting the frustration hypothesis.

Micro analyses of behaviour during partial reinforcement showed that there are dependencies between response types and thus that the outcome of the previous trial influenced performance on the following trial. This goes against the incentive hope hypothesis which would predict that

invigoration of behaviour (resulting in this case into an anticipatory response) does not depend on the outcome of the previous trial, but instead is driven by the ‘hope’ to be rewarded on each trial (Anselme, 2015). It was observed that while rats were more likely than chance to make a premature response following a NR trial, during most behavioural manipulations, they were more likely to make a premature response following another premature response, especially when the frequency of premature responses was higher (Experiment 1, 2, 4, 5b). It is difficult to interpret these findings as supporting the frustration hypothesis because if timeout punishment of making a premature response elicited frustration and, because of this, induced a premature response in the following trial, one would expect premature responses to occur also following incorrect responses and omissions (which are also followed by a timeout punishment). However, that was not the case. The only way to reconcile this under the frustration hypothesis is that when making an incorrect or omission response rats are less confident about their choices and thus less ‘surprised’ -and therefore more frustrated- if they are punished for such choices. Latencies to make incorrect and omission responses did indeed indicate low confidence or uncertainty regarding the choice of action. This interpretation would thus imply that rats either (1) do not ‘realise’ that they are acting prematurely or (2) have difficulties learning that premature responses are punished. In both cases, if they do not expect that they will be punished for their anticipatory responses, then the timeout punishment could generate frustration and lead to another premature response in the next trial. With regards to the first point (1) it is possible that premature responses occur due to poor timing abilities, as suggested before (Donnelly et al., 2015), however premature responses are uncued. Thus, even if rats did perceive that sufficient time had elapsed since the beginning of the trial, it remains unexplained why they would poke ‘uncued’ into any aperture. In line with this, when rats are uncertain about the location of the light cue in the visual array, response latencies are normally longer, as is the case for incorrect responses. The second point also seems difficult to reconcile considering that trait impulsive rats do not show differences in learning to perform an operant task (both 5CSRTT and SDT) compared to the other impulsivity groups, as shown in **Chapter 6**. A final point in relation to the micro analysis is that rats were much less likely than chance to make a premature response after R trials. This could indicate that premature responses are influenced by the post-consummatory pause, which is the suppression of behaviour after reinforcement (Seward, et al., 1957, for a longer discussion on this see the section 3.4 of **Chapter 3**). Thus, when animals have an

‘urgency’ to make a premature response (be this originating from negative or positive affect), this is mitigated by the consumption of reinforcement.

Macro analyses comparing the partial and continuous reinforcement sessions, revealed an opposing trend to that predicted by the frustration and incentive hope hypotheses. Specifically, rats were more likely to make a premature response during schedules of continuous reinforcement as opposed to schedules of partial reinforcement. This was true for premature responses occurring both during a 5 s ITI and a 7 s ITI window, and across impulsivity groups. In addition, premature responses also increased when the magnitude of the appetitive reward increased, in line with previous research (on a different task, King et al., 2016). This suggests that premature responses are influenced by manipulations that increase the motivational properties of a task, such as changes in reinforcement rate or magnitude, rather than by manipulations that increase the frustrative aspect of the task, such as omissions of expected reward. Corroborating this idea is the fact that across cohorts of animals and behavioural manipulations premature responses were associated with a reduced speed of making a correct response. Thus, in all experiments of **Chapter 3** there was a negative correlation between making a premature and the latency to make a correct response, and in **Chapters 3, 4, 5** and **6** HI rats from three different cohorts responded more rapidly than the other impulsivity groups. This was the case during baseline (as described in **Chapter 5** and **6**); during a long and short vITI challenge (as described in **Chapter 4**); during a fixed long ITI session (**Chapter 3** and **5**) and during a vSD challenge (as described in **Chapter 6**).

In **Chapter 4**, the faster reaction times, or increased motor readiness, displayed by HI rats was found to be advantageous during the performance of a high-event rate paradigm of the 5CSRTT. Specifically, when challenged with very short ITIs, HI rats earned more reinforcers and made fewer omissions than the other two impulsivity groups. This is in line with research in humans showing that reinforcement density increases the speed of action of impulsive individuals and that this can be advantageous in some settings (Cools et al., 2005). In a similar fashion, novel and fast-paced activities improve ADHD symptoms among young adults (Lasky et al., 2016). Faster reaction times have been postulated to reflect a greater motivational drive in performing an operant action (Niv et al., 2007), because they require greater energy (i.e., are more costly,

Staddon 2001, Niv, et al., 2005, 2007; Opris et al., 2011) and thus indicate an increased willingness to exert effort to gain a reward. High levels of trait impulsivity on the 5CSRTT have been associated with indices of enhanced subjective utility of appetitive rewards, such as choosing high-reward versus high-risk options (Barrus et al., 2015), escalation and emergence of compulsive intravenous drug self-administration (Belin et al., 2008; Dalley et al., 2007a; Diergaarde et al., 2008; 2009), increased sucrose seeking (Diergaarde et al., 2009) and increased predisposition to develop ST behaviour (Lovic et al., 2011; King et al., 2016). In **Chapter 4**, experiments were carried out to investigate whether HI rats do have a stronger representation of the value of the reinforcer by evaluating their proclivity to invest effort in pursuit of reward. I investigated this idea by testing rats on two different versions of the PR task, the exponential, and the linear version, which required animals to invest increasing amounts of work (changing more or less steeply, respectively) to earn a food pellet. Analyses of this experiment did not reveal a strong difference between impulsivity groups in breakpoint (i.e., the number of lever presses in the last completed trial of the PR task), however it did show that this measure, both on the exponential and linear version of the PR, correlated significantly with premature responses on a vITI challenge. Because of these conflicting findings it is difficult to draw conclusions on whether impulsivity groups differ with regards to willingness to expend energy to obtain a reward. Further experiments should be carried out evaluating performance of HI and LI rats on a variety of tasks that tap into effort-related choices, to illuminate a more complete understanding of whether and how impulsivity is associated with differences in cost/benefit decision-making. Ultimately however, most effort-based tasks do not differentiate between the various components that play a role in cost/benefit decision-making, such as (1) subjective utility and (2) work-related response cost (Salamone, 2006). For this reason, more refined experimental designs are needed to specifically address the question of whether and how impulsivity relates separately to these two components.

Following the evidence and arguments provided above, a further hypothesis for the manifestation of premature responses is that these result from an ‘irresistible’ attraction towards the appetitive cue, that fails to be inhibited due to its intensity. An alternative interpretation of why premature responding is linked to shortened latencies may, instead, reflect increased motor readiness at performing a planned action and, in the case of premature responses, a failure of withholding

such an action until the appropriate time. This second hypothesis need not include abnormalities in reinforcement sensitivities in the aetiology of premature responses, but instead failures in action constraint and perhaps in timing. An increase in premature responses during continuous, as opposed to partial reinforcement, and during a session with increased reward magnitude, supports the first interpretation. However, to date, no experiments have formally tested whether trait impulsive rats have deficits in tracking time or in withholding an action in the absence of an appetitive context. Further research is needed to clarify these possibilities. Finally, these two hypotheses need not to be mutually exclusive. Thus, premature responses could occur because of both a greater drive to respond and an inability to control this drive.

Exploration of the neural bases underlying premature responding may inform the psychological mechanisms underlying maladaptive anticipatory responding. One of the most consistent findings in the literature is the role that DA plays in the occurrence of premature responses (Dalley and Robbins, 2017). For example, some of the early findings showed that systemic administration of AMPH increased premature responses, an effect that was blocked by selective depletion of DA in the NAcB (Cole and Robbins 1989). The effect of AMPH on premature responses was likely due to its action on extracellular levels of DA in the NAcB, since intra-accumbens infusions of AMPH also increased premature responses and this effect was blocked by systemic treatment with a mixed D1/D2 receptor antagonist (alpha-flupenthixol, Cole and Robbins 1987). These early findings have been confirmed and refined over the years (Jupp et al., 2013). For example, Pattij and colleagues (2007), showed that the effects of systemic administration of AMPH were blocked by intra-infusions of a D2, but not a D1, antagonist both in the shell and core sub-regions of the NAcB. In parallel, a growing body of research has focused on the neural bases of innate impulsivity in rodents. In summary, this research showed that trait impulsivity in rodents is associated with reduced density of DAT and DA D2/3 receptors in the ventral striatum (Dalley et al., 2007a) and specifically in the NAcB shell (Jupp et al., 2013). Studies on brain slice studies *in vitro* have found that DA release is increased in the NAcB shell but decreased in the NAcB core of HI rats compared with low-impulsive (LI) rats (Diergarde et al., 2008). Importantly, this research is consistent with findings in humans showing that scoring high on the BIS scale is associated with low D2/3 receptors in the midbrain and that reduced D2 receptor expression in this region predicts AMPH-induced increases of DA release

in the striatum (Buckholtz, et al., 2010). Increased striatal DA release following AMPH administration was associated with a stronger subjective desire (or “wanting”) for AMPH, suggesting a link between impulsivity and increased motivational drive for appetitive rewards. In line with this, two separate reviews of fMRI studies in healthy subjects (Kennis et al., 2013; Plichta & Scheres, 2014) found a positive relationship between blood oxygenation level dependent (BOLD) activity in the VS during reward processing (anticipation and receipt of reward) and impulsivity-related traits measured using a variety of personality questionnaires. Collectively this evidence suggests that impulsivity, across species, is associated with activity in the NAc, and more specifically, with an increase of DA efflux into this region. In the case of rodents, excessive extracellular DA levels may be confined to the NAc shell sub-region and arise from reduced expression of DAT in this region, which in turn downregulates inhibitory D2/3 autoreceptor located on afferent midbrain fibres (Dalley and Robbins, 2017).

**Chapters 4 and 5** investigated the role of mesolimbic DA in the occurrence of premature responses. Replicating previous research (Murphy et al. 2008; Pattij et al. 2007; Milstein et al., 2010), systemic administration of MPH and AMPH, in **Chapter 4**, increased premature responses during the long ITI trials of a vITI challenge on the 5CSRTT. Administration of ATO, on the other hand, reduced anticipatory responses. This also confirmed previous research (Blondeau and Dells-Hagedorn 2007; Baarendse and Vanderschuren 2012; Fernando et al. 2012) and suggested an involvement of striatal DA in the effects of MPH, since the neuropharmacological profiles of ATO and MPH differ primarily on this measure (Bymaster, et al., 2002). A novel finding of the experiment described in **Chapter 4** is that the administration of AMPH and MPH, which as mentioned have a strong influence on extracellular striatal DA levels (Kuczenski & Segal, 1997, 2001), boosted performance on short ITI trials of a vITI session. This mimicked the advantage that HI rats have on these fast-paced trials, once again linking elevated striatal DA levels with trait impulsivity. In addition, these findings suggest that high levels of extracellular DA sub-cortically confer an advantage in contexts where a motor plan needs to be rapidly activated and performed (however they lead to inappropriate actions when such motor plans need to be withheld for an extended period). This is consistent with a plethora of evidence linking increased DA release and signalling to faster and more vigorous responding (Wassum et al., 2012; Hamid et al., 2016; Mohebi et al., 2019). For example, Berke and co-workers (Hamid

et al., 2016; Mohebi et al., 2019) showed that increases in reinforcement rate drove a monotonic increase in DA release in the NAcB (as measured using *in vivo* microdialysis) and a concomitant reduction in response latency. When DA release was monitored on a subsecond scale with fibre photometry (Mohebi et al., 2019), rapidly-evolving ramps of DA were observed that scaled in magnitude with the probability and proximity of the reward (replicating previous findings with voltammetry, Hamid et al., 2016; Howe et al., 2013; Wassum et al., 2012). Importantly, this could explain why in **Chapter 3** when rats were tested under a continuous reinforcement schedule, a greater number of premature responses occurred than when they were tested on a partial reinforcement schedule. This again would support a role of DA in premature responses and a link between these responses and motivated behaviour.

Further evidence for a role of DA in action initiation and vigour comes from work showing that stimulation of midbrain DA cells promotes movement both in the context of instrumental tasks (Saunders et al., 2018) and during spontaneous locomotion (Silva et al., 2018). For example, Saunders and colleagues (2018) showed in the context of a Pavlovian cue conditioning procedure, that optogenetic stimulation of DA cells in the VTA-NAcb core pathway, concomitant with cue presentation (light + tone, 7 s) and substituting for natural reward delivery, elicited strong approach behaviour towards the cue. In these rats, DA neurons in the VTA that received laser stimulation acquired a phasic response at cue onset (prior to laser activation), which positively correlated with the speed of approach behaviour to the cue. The same cue-laser pairing procedure delivered to DA cells in the VTA-NacB medial shell pathway did not elicit CR towards the cue, while stimulation of DA cells projecting from the SNc to the dorsal striatum elicited vigorous movement but not directed towards the cue. A similar pattern was also found in the context of instrumental behaviour, with optogenetic stimulation of VTA-NacB cells speeding the latency to initiate a trial (Hamid et al., 2016). These and other studies led Klaus and colleagues (2019) to outline a theoretical framework whereby DA input is needed to excite neuronal ensembles in the striatum representing the appropriate motor plan for the context, leading to action initiation. According to this framework, the choice of which motor plan to actuate is, instead, informed by cortical input to the striatum. Considering evidence pointing to a role of the DA midbrain-accumbens pathway in cue approach behaviour and response latency, work in **Chapter 5** explored whether reducing activity of midbrain DA cells, more generally and

in a projection-specific way, affected the occurrence of premature responses. In the first experiment of **Chapter 5** quinpirole (0, 0.01, 0.03, 0.3, 1  $\mu\text{g}/\mu\text{l}$ ) was infused in the VTA and rats were tested on a long ITI session. Quinpirole is a D2/D3 receptor agonist that activates D2-autoreceptors located on the soma of DA cells in the midbrain, reducing their firing activity (Lacey, 1993; Werkman et al., 1999). Intra-VTA infusions of a D2/D3 receptor agonist quinpirole, at the highest dose, reduced premature responses and increased response and collection latency. However, infusion of this drug did not affect accuracy or reinforcers earned, suggesting that the action of quinpirole on DA firing did not impair visuo-spatial attention and was not sufficiently disruptive to completely abolish motivation to perform the task. A second experiment in **Chapter 5** attempted to refine the precise contribution of projection-specific circuits in VTA to impulsive behaviour. Specifically, inhibitory chemogenetics (DREADDs) was implemented to silence VTA neurons projecting either to the NAc core or the NAc shell, while rats were challenged on a long ITI session of the 5CSRTT. Before carrying out this experiment, inhibitory DREADDs were validated in a pilot study on motivational arousal, assessed by spontaneous locomotor activity of rats in a novel enclosure, both under baseline testing conditions and following the administration of MPH, a DA and NA reuptake inhibitor shown previously to increase locomotor activity (Gerasimov et al., 2000). The pilot experiment yielded promising preliminary data of a successful reduction in locomotion following the administration of CNO, compared to vehicle, in rats infused with inhibitory DREADDs in the medial NAc shell. Based on this, it was decided to proceed with the main experiment and target VTA-NAc shell and VTA-NAc core with inhibitory chemogenetics. The effect observed in the pilot experiment, of a suppressive action of inhibitory DREADDs on locomotor activity in rats infused with DREADDs in the medial shell, following the administration of CNO, was not replicated in this second experiment. However, CNO administration, compared to vehicle, did reduce locomotor activity in rats expressing inhibitory DREADDs in the VTA-NAc core pathway. Nonetheless there were no effects of inhibitory chemogenetics in any of the virus groups on 5CSRTT impulsivity or behavioural variables associated with attentional performance (discriminative accuracy, response latencies, and errors of omission). A comprehensive immunohistochemical analysis of the activity-related transcription marker c-fos in various regions of the striatum (medial shell, lateral shell, core, DMS and DLS), suggested that the

negative effects above were due to either an inadequate transfection of virus in the intended regions of interest or a failure of the technique in silencing the neurons targeted.

As described above, in **Chapter 3, 4 and 5**, across different behavioural and pharmacological manipulations, premature responses were consistently associated with a reduction in response latencies and in omissions, supporting the idea that -at least in some circumstances- they might index greater motivational drive to engage with the task. In **Chapter 6**, however, excitotoxic lesions of the mPFC disrupted accuracy on the 5CSRTT, slowed performance, increased omissions, and importantly increased premature responses. These findings are compatible with previous research of a similar effect of mPFC lesions on the performance of rats on the 5CSRTT (Passetti et al., 2002). These findings are noteworthy as they present evidence that in some experimental conditions premature responses are not associated with indices of greater motivation to perform the task, but instead are associated with overall poor performance on the 5CSRTT. This may indicate that premature responses can result from impaired sustained attention or deficits in timing. In the context of 5CSRTT, the mPFC has been suggested to regulate different aspects of attention, with the dorsal region (e.g., Cg1) more heavily involved in perceptual discrimination while the ventral portion (PrL, IL) playing a more significant role in inhibitory control (Chudasama, 2011). Adding to the body of literature on the role of mPFC in the 5CSRTT, Donnelly and colleagues (2015) observed that neurons in the mPFC exhibit ramping activity during the ITI, a period during which the animal is withholding the operant response. Specifically, they observed that the onset of ramping activity coincided with the onset of wait-start behaviour, which in premature responses occurred earlier than for trials that ended up as correct responses. Wait-start behaviour was defined as ‘the first time following the start of a trial where the rat’s head left a rectangular area surrounding the food magazine’ (Donnelly, et al., 2015). The fact that wait-start, and ramping activity in mPFC, started earlier in trials ending in premature responses compared to trials ending in correct responses, raised questions on the nature of premature responses and specifically on whether these types of responses result more from a failure in timing the delay-cue period rather than from a failure in waiting (Donnelly, et al., 2015). However, faster wait-start latency could also be explained by eagerness to engage with the task, especially if the trial prior to a premature was not rewarded, as was often the case for premature responses in that experiment (Donnelly, et al., 2014). Donnelly and colleagues

(2015) also showed that when rats were challenged with ITIs of different durations, ramping activity in mPFC began at the start of waiting, reached a maximum level before the earliest time of stimulus presentation, and remained stable until the time of nose-poking. The authors speculated that neurons in the mPFC could signal the moment in which maximum attention needs to be deployed to detect a stimulus or in which top-down control needs to be exerted to either withhold an action or select a motor plan to be executed (Donnelly et al., 2014). Considering this, it is possible that neurotoxic lesions of this area, described in **Chapter 6**, disrupted timing behaviour or inhibitory control, such that premature responses were more frequent and in association with poor performance on the 5CSRTT. These findings are in line with further evidence of a role of the mPFC in inhibitory control. For example, Hardung and colleagues (2017) observed that optical inhibition of cells in the PrL increased premature responses in a response-preparation task, where animals are required to press a lever until a tone is presented to earn an appetitive reward (Hardung et al., 2017). The authors also observed that activity of neurons in this region decreased during trials that ended in premature responses compared to when they ended in correct responses. Along the same lines, Terra and colleagues (2020) showed that chemogenetic inhibition of cells projecting from the mPFC to the DMS led to an increase in the numbers of premature responses.

Taken together, premature responses on the 5CSRTT are most evident when waiting behaviour is challenged and may result from an enhanced (motivational) drive to engage in a learned action or increased motor readiness (void of a value component). This could then be coupled with a deficit in top-down inhibition of the prepotent motor response. The performance of this response thus implicates circuits that regulate (reward-driven) action initiation, such as DA cells of the VTA-NAc pathway, and circuits responsible for action inhibition, such as neurons in the mPFC.

## 7.2 Translational implications and further considerations

The 5CSRTT was originally developed to investigate the neuro-behavioural and neuro-pharmacological bases of sustained attention and impulsivity in rodents (Robbins, 2002). Ultimately the aim of this research was to develop therapies to tackle those psychiatric disorders involving deficits in attention and impulsivity. In **Chapter 4** of this thesis, I described an environmental condition within which trait impulsive rats have an advantage over the other impulsivity groups. Specifically, it was shown that HI rats earn more reinforcers, compared to the other impulsivity groups, during a fast-paced version of the 5CSRTT, where they need to respond rapidly, and remained focused on the cue. Importantly, this is in line with research in humans showing that context plays a fundamental role in dictating whether the proclivity to be impulsive, hyperactive, or risky is beneficial to an individual. For example, highly impulsive subjects on the BIS impulsivity scale were found to have faster latencies in the high-reward condition of a reaction-time paradigm, which gave them an advantage in performance compared to their non-impulsive counterparts (Cools et al., 2005). Further, there is substantial evidence showing that higher-than-average levels of impulsivity and risky-decision making are advantageous in entrepreneurship and represent a positive financial opportunity for society and for entrepreneurs themselves (Lawrence et al., 2008; Rajah et al., 2021). Finally, recent work on whether the environmental context plays a role in the presentation of ADHD showed that individuals with this disease prefer tasks or jobs that are stimulating, novel and fast-paced and, importantly, engaging with such tasks alleviates their symptoms. My work in **Chapter 4** replicated these findings in rodents and, in addition, showed that the administration of drugs typically used for the treatment of ADHD, such as MPH, AMPH and ATO, improved performance, albeit for different reasons, in rats of all impulsivity phenotypes (not just HI rats). Specifically, ATO reduced impulsivity on long ITI sessions, while MPH and AMPH boosted performance on fast-paced trials of the 5CSRTT. The beneficial effect of ATO on waiting impulsivity is in line with previous evidence in rodents on the 5CSRTT (Blondeau and Dellu-Hagedorn 2007; Navarra et al. 2008; Robinson 2012) and with work on ADHD patients tested on measures of response inhibition and working memory (Chamberlain et al., 2007). These findings

highlight the translational value of this task and provide an example of convergence, at the behavioural and neuro-pharmacological levels, between rodents and humans models.

The present findings of MPH and AMPH on improved performance on the 5CSRRT is however more difficult to interpret. For example, findings in **Chapter 4** of reduced response latency following the administration of MPH in rats tested on a vITI paradigm (with short and long ITIs), are in line with evidence in humans that MPH improves performance by speeding reaction times (Coghill et al., 2014). However, experiments described in **Chapter 5**, which tested rats on a fixed 7 s ITI paradigm, did not replicate the effect of MPH on response latency and, instead, revealed an increase in response latency following the administration of this drug. In ADHD patients, the effect of MPH has been found to vary as a function of task difficulty, with administration of the drug slowing responding on complex tasks, while speeding responding on tasks requiring less cognitive resources (Rhodes et al., 2006). It is possible that a similar effect was observed here, with MPH influencing response latency based on task difficulty. Specifically, it is possible that a fixed 7 s ITI paradigm is more challenging for HI rats than a vITI paradigm as it requires greater inhibitory control over prepotent actions. This could explain why MPH would slow down performance during a 7 s ITI session. Another difference between the present work in rodents and work in humans is that, in the latter group, MPH improves response inhibition in ADHD individuals (Coghill et al., 2014; Scheres et al., 2003). On the contrary, in the present work (as described in **Chapter 4** and **5**) the administration of MPH (but also AMPH in **Chapter 4**), increased premature responses. This was true both for rats tested on a vITI session (in the long ITI trials, **Chapter 4**) and for rats tested on a fixed 7 s ITI session (**Chapter 5**). This is perhaps a paradoxical finding that questions the construct validity of the 5CSRRT. However, in humans (healthy controls) MPH has also been shown to increase waiting impulsivity on the human version of the 5CSRRT, i.e. the 4CSRRT (Voon et al., 2016), while medicated ADHD subjects have not been tested on this task. Thus it is unclear how MPH would affect waiting impulsivity measured on this task in ADHD patients and thus whether the 5CSRRT would have translational validity on this measure. There is previous evidence on this task (Caprioli et al., 2015; Tomlinson et al., 2014) that administration of MPH improved waiting impulsivity in HI rats. For example, Caprioli and colleagues (2015) tested the effects of chronic exposure to MPH on performance of long ITI sessions of the 5CSRRT, by administering the

compound orally twice a day, 7 days a week for 4 consecutive weeks. Following this treatment rats were tested on three fixed 7 s ITI sessions, each separated by 5 days of baseline testing. MPH here was found to reduce impulsivity, thus it is possible that under specific experimental manipulations MPH has rate-dependent beneficial effect on waiting impulsivity, as tested in the 5CSRRTT.

While it is unclear as to whether HI rats and ADHD patients have common neural substrates, there is evidence that trait impulsivity in rodents, screened on the 5CSRRTT, engage similar brain mechanisms as those described for high-impulsive individuals. For example, individuals scoring high on the BIS scale of impulsivity were found to have reduced D2/3 receptor availability in the midbrain, which was associated with elevated DA release in the striatum (Buckholtz, et al., 2010). In line with this, HI rats on the 5CSRRTT were found to have reduced D2/3 receptor availability and DAT expression in the VS (Dalley, et al., 2007a; Jupp et al., 2013). This may be mediated by D2/3 autoreceptors on afferent DA fibers based on findings that HI rats were also found to have reduced DA D2R mRNA levels in the midbrain (Besson et al., 2013). Considering these findings, HI rats have been postulated to exhibit greater phasic DA release in the VS (Dalley and Robbins, 2017). While no experiment, to date, has tested this idea *in vivo*, there is evidence *in vitro* that this might be the case (Diergaarde et al., 2008). Parallel to this, there is evidence in humans that high-impulsive individuals exhibit greater AMPH-induced DA release in the VS (Buckholtz, et al., 2010) and greater activity in the VS during anticipation or receipt of reward (Plichta and Scheres, 2014). All together, these findings corroborate the idea that the 5CSRRTT is a useful translational tool for the study of impulsivity, and consistent with recent calls to reinvigorate preclinical research to meet the growing gap in the mechanistic understanding of neuropsychiatric disorders, and with it the development of novel therapies (Tricklebank et al., 2021).

## **7.3 Limitations and alternative approaches**

Several limitations should be considered when interpreting these results. For example, animals underwent different behavioural manipulations which might interfere with each other and confound performance. However, this was done with the aim to compare performance across

different tasks, to develop a deeper understanding of impulsivity on the 5CSRTT. It was also motivated by the aspiration to comply with the three Rs and specifically with the principle of ‘reduction’ which aims at minimising the number of animals used to address specific scientific questions.

In **Chapter 3** the Markov chain model applied to the micro-level analysis of the data was assumed to be homogeneous, meaning that transition probabilities were assumed to be independent of time. However, there may be time-dependent changes in the performance of the 5CSRTT, such as fatigue towards the end of the session or small learning components (especially during the 7 s ITI session) that might affect the transition probabilities between response types. One way to investigate time-dependent effects in the performance of the 5CSRTT could be to break down each session into two halves and look at whether a Markov chain model applied to the first half differs from that applied to the second half. An alternative, more accurate solution, would be to use a non-homogenous Markov Chain model, in which the transition probabilities are time dependent. However, such a model would greatly increase the number of free parameters that need to be inferred from the data. Unfortunately, there was not sufficient data to perform these alternative methods.

In **Chapter 5** the chemogenetic approach was not verified with electrophysiology, but instead was verified with a more indirect measure such as c-fos expression. Ideally, however, techniques that are meant to silence or excite a neural pathway (such as chemogenetics) should be assessed, either *in vitro* or *in vivo*, by inserting a microelectrode in the circuit of interest and recording the activity of neurons before and after the application/delivery of CNO.

In **Chapter 6** the behavioural assessment of the mPFC lesion had to be abruptly curtailed due to the lockdown measures imposed by the Covid-19 pandemic. Had this not been the case I would have collected behavioural data from many more days of baseline testing to investigate whether performance on any of the tasks changed over time and with repeated training.

## 7.4 Future directions

This thesis showed for the first time that premature responses on the 5CSRTT increase with increases in the motivational value of the task and that negative urgency does not seem to play an important role in the occurrence of these responses (**Chapter 3**). In line with this, this thesis also showed that HI rats have an advantage in performance when the task is fast-paced and that administration of stimulants, such as MPH and AMPH, which are known to elevate subcortical DA release, mimic this advantage (**Chapter 4**). On the contrary, administration of ATO, which does not affect striatal DA release, impaired performance on fast-paced trials on the 5CSRTT and increased response latencies (**Chapter 4**). **Chapter 5** of this work showed that infusing a D2/3 receptor agonist, quinpirole, into the midbrain has the effect of decreasing premature responses, confirming a role of midbrain DA release in the genesis of premature responses on the 5CSRTT. Finally, the findings reported in **Chapter 6** this thesis showed that two tasks that have been developed to study sustained attention, the 5CSRTT and the SDT, assess two distinct forms of attention, based on evidence that performance of rats trained on both tasks does not correlate. Lesions of the mPFC were found to disrupt performance in both tasks but to have a greater impact on behaviour of the 5CSRTT.

While this work has revealed some novel findings about the psychological and neural bases of attention and impulsivity in the 5CSRTT, many questions remain unanswered. For example, it is still unclear whether HI rats on the 5CSRTT have a deficit in temporal discrimination, which could explain in part their tendency to make many premature responses during long (un-trained) ITI trials. Different tasks have been developed to measure timing abilities in rats, two of the most used ones are the Peak Interval (PI, Meck, 1986) task and the Duration Discrimination task (Soares et al., 2016). The former task trains animals to press a lever to receive a food reward following a 20-s fixed-interval (FI) schedule. Some trials are reinforced, while in others (probe trials) a food pellet is not delivered upon lever-pressing after 20 s. If the rat has intact timing abilities, they should press vigorously around the time the pellet is expected to be available. This measure is called peak time and is one of the main indexes of performance of this task. The problem with testing HI rats on the PI task is that if rats press a lever earlier-than-optimal it is impossible to resolve whether such response is due to impulsivity or due to a deficit in keeping

time. In this regard, the PI task resembles the 5CSRTT with the only difference that it does not punish premature responses. To overcome this issue, it would be preferable to test HI rats on a temporal discrimination task (Soares et al., 2016). Here subjects are presented with two tones, separated by an interval of a specific duration, and are required to discriminate whether the interval is shorter (poke left) or longer (poke right) than a reference point of choice (e.g., 1.5 s). The task is made more difficult when interval durations close to the reference point are presented. If HI rats do tend to estimate time as passing more quickly, perhaps this ‘error’ would manifest in this task with HI rats choosing the ‘short duration’ option on more ambiguous trials.

Another important question that remains unanswered is whether HI rats exhibit increased phasic DA release in the NAcB shell and core regions, compared with LI rats. This should be tested during performance of the 5CSRTT to answer the question of whether HI rats make more premature responses due to increases in the frequency and magnitude of DA release in the NAcB. This question could be addressed by recording DA release either with fast-scan cyclic voltammetry (FSCV) or fibre photometry, in freely behaving animals. FSCV offers real-time measurements of sub-second changes in ‘absolute’ levels of extracellular DA concentrations, thus allowing between-subject comparisons of DA levels. However, because DA and NA have nearly identical cyclic voltammograms, the chemical sensitivity of FSCV for DA is lower in regions that are densely innervated with NA fibres, such as the caudal NAcB shell (Park et al., 2010). To overcome this problem, while maintaining high spatiotemporal resolution of DA release, a new emerging technique could be used that couples fibre photometry recording with the dLight1 sensor. Fibre photometry is a method to optically record from subcortical regions and consists of an optical fibre which collects bulk fluorescence signals following stimulation of a fluorescent indicator (Sych et al., 2019). The dLight1 indicator, is a genetically encoded fluorescent indicator capable of reporting DA transients in awake, behaving animals (Cosme et al., 2018). The signal collected with fibre photometry does not reflect absolute levels of DA release but can provide information on the relative change between phasic release and baseline levels. A possible experiment would be to infuse the dLight1 sensor either in the NAcB shell or core sub-regions and test HI and LI rats on various manipulations on the 5CSRTT (vITI sessions with long and short ITIs, partial and continuous reinforcement, etc). In this respect it would be interesting to explore whether HI rats have enhanced DA release, relative to baseline levels,

when compared to LI rats and whether a differential increase in DA release is confined to the NAc shell or core sub-region.

Ultimately, pre-clinical research should strive to adopt behavioural paradigms that are closely matched to those of humans and make use of the extraordinary advances in neuroscience to finely dissect the circuit and molecular mechanisms underlying behaviour. This will not only be a necessary step towards discovering new medications for psychiatric disorders but, more generally, will allow us to bridge the intangible gap between neurobiology and behaviour.

# Bibliography

- Aase, H., & Sagvolden, T. (2006). Infrequent, but not frequent, reinforcers produce more variable responding and deficient sustained attention in young children with attention-deficit/hyperactivity disorder (ADHD). *Article in Journal of Child Psychology and Psychiatry*, 47(5), 457–471
- Aberman, J. E., Ward, S. J., & Salamone, J. D. (1998). Effects of dopamine antagonists and accumbens dopamine depletions on time-constrained progressive-ratio performance. *Pharmacology Biochemistry and Behavior*, 61(4), 341–348.
- Adam, R., Bays, P. M., & Husain, M. (2012). Rapid decision-making under risk. *Cognitive Neuroscience*, 3(1), 52–61.
- Aleksandrova, L. R., Creed, M. C., Fletcher, P. J., Lobo, D. S. S., Hamani, C., & Nobrega, J. N. (2013). Deep brain stimulation of the subthalamic nucleus increases premature responding in a rat gambling task. *Behavioural Brain Research*, 245, 76–82.
- Alexander, G. E., & Crutcher, M. D. (1990). Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends in Neurosciences*, 13(7), 266–271.
- Alheid, G. F., & Heimer, L. (1988). New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: The striatopallidal, amygdaloid, and corticopetal components of substantia innominata. *Neuroscience*, 27(1), 1–39.
- Alsiö, J., Phillips, B. U., Sala-Bayo, J., Nilsson, S. R. O., Calafat-Pla, T. C., Rizwand, A., Plumbridge, J. M., López-Cruz, L., Dalley, J. W., Cardinal, R. N., Mar, A. C., & Robbins, T. W. (2019). Dopamine D2-like receptor stimulation blocks negative feedback in visual and spatial reversal learning in the rat: behavioural and computational evidence. *Psychopharmacology*, 8, 2307–2323.

- Ambroggi, F., Ghazizadeh, A., Nicola, S. M., & Fields, H. L. (2011). Roles of nucleus accumbens core and shell in incentive-cue responding and behavioral inhibition. *Journal of Neuroscience*, *31*(18), 6820–6830.
- Amsel, A. (1958). The role of frustrative nonreward in noncontinuous reward situations. *Psychology Bulletin*, *55*, 102–119.
- Amsel, A. (1992). *Frustration theory: An analysis of dispositional learning and memory*. Cambridge University Press.
- Amsel, A., & Roussel, J. (1952). Motivational properties of frustration: I. Effect on a running response of the addition of frustration to the motivational complex. *Journal of Experimental Psychology*, *43*, 363–368.
- Amsel, Abram. (1962). Frustrative nonreward in partial reinforcement and discrimination learning: Some recent history and a theoretical extension. *Psychological Review*, *69*(4), 306–328.
- Anderson, T. W., & Goodman, L. A. (1957). Statistical Inference about Markov Chains. *The Annals of Mathematical Statistics*, *28*(1), 89–110.
- Anker, J. J., Perry, J. L., Gliddon, L. A., & Carroll, M. E. (2009). Impulsivity predicts the escalation of cocaine self-administration in rats. *Pharmacology Biochemistry and Behavior*, *93*(3), 343–348.
- Anokhin, A. P., Golosheykin, S., Grant, J. D., & Heath, A. C. (2011). Heritability of delay discounting in adolescence: A longitudinal twin study. *Behavior Genetics*, *41*(2), 175–183.
- Anselme, P. (2015). Incentive salience attribution under reward uncertainty: A Pavlovian model. *Behavioural Processes*, *111*, 6–18.

- Anselme, P. (2018). Gambling hijacks an ancestral motivational system shaped by natural selection. In A. Tomie & J. Morrow (Eds.), *Sign-tracking and drug addiction* (pp. 1–26). Ann Arbor, MI: University of Michigan Press.
- Anselme, P., & Robinson, M. J. F. (2013). What motivates gambling behavior? Insight into dopamine's role. *Frontiers in Behavioral Neuroscience*, *7*, 1–4.
- Anselme, P., & Robinson, M. J. F. (2019). Evidence for motivational enhancement of sign-tracking behavior under reward uncertainty. *Journal of Experimental Psychology: Animal Learning and Cognition*, *45*(3), 350–355.
- Anselme, P., Robinson, M. J. F., & Berridge, K. C. (2013). Reward uncertainty enhances incentive salience attribution as sign-tracking. *Behavioural Brain Research*, *238*(1), 53–61.
- Anzalone, A., Ramos, M., Mei, C. de, Hopf, F. W., Iaccarino, C., Halbout, B., Jacobsen, J., Kinoshita, C., Welter, M., Caron, M. G., Bonci, A., & Sulzer, D. (2012). *Dual control of dopamine synthesis and release by presynaptic and postsynaptic dopamine D2 receptors*. *32*(26), 9023–9034.
- Arnsten, A. F.T., & Jentsch, J. D. (1997). The alpha-1 adrenergic agonist, cirazoline, impairs spatial working memory performance in aged monkeys. *Pharmacology Biochemistry and Behavior*, *58*(1), 55–59.
- Arnsten, Amy F.T., Mathew, R., Ubriani, R., Taylor, J. R., & Li, B. M. (1999). A-1 noradrenergic receptor stimulation impairs prefrontal cortical cognitive function. *Biological Psychiatry*, *45*(1), 26–31.
- Aron, A. R., & Poldrack, R. A. (2006). Cortical and Subcortical Contributions to Stop Signal Response Inhibition: Role of the Subthalamic Nucleus. *The Journal of Neuroscience*, *26*(9), 2424–2433.

- Aubert, I., Ghorayeb, I., Normand, E., & Bloch, B. (2000). Phenotypical characterization of the neurons expressing the D1 and D2 dopamine receptors in the monkey striatum. *The Journal of Comparative Neurology*, *418*(1), 22–32.
- Audrain-McGovern, J., Rodriguez, D., Epstein, L. H., Cuevas, J., Rodgers, K., & Wileyto, E. P. (2009). Does delay discounting play an etiological role in smoking or is it a consequence of smoking? *Drug and Alcohol Dependence*, *103*(3), 99–106.
- Baarendse, P. J. J., & Vanderschuren, L. J. M. J. (2012). Dissociable effects of monoamine reuptake inhibitors on distinct forms of impulsive behavior in rats. *Psychopharmacology*, *219*(2), 313–326.
- Badiani, A., Belin, D., Epstein, D., Calu, D., & Shaham, Y. (2011). Opiate versus psychostimulant addiction: The differences do matter. *Nature Reviews Neuroscience*, *12*(11), 685–700.
- Ballanger, B., van Eimeren, T., Moro, E., Lozano, A. M., Hamani, C., Boulinguez, P., Pellecchia, G., Houle, S., Poon, Y. Y., Lang, A. E., & Strafella, A. P. (2009). Stimulation of the subthalamic nucleus and impulsivity: Release your horses. *Annals of Neurology*, *66*(6), 817–824.
- Barbera, G., Liang, B., Zhang, L., Gerfen, C. R., Culurciello, E., Chen, R., Li, Y., & Lin, D. T. (2016). Spatially compact neural clusters in the dorsal striatum encode locomotion relevant information. *Neuron*, *92*(1), 202–213.
- Bardgett, M. E., Depenbrock, M., Downs, N., Points, M., & Green, L. (2009). Dopamine Modulates Effort-Based Decision-Making in Rats. *Behav Neurosci*, *123*(2), 242–251.
- Bari, A., Dalley, J. W., & Robbins, T. W. (2008a). The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. *Nature Protocols*, *3*(5), 759–767.

- Bari, A., Dalley, J. W., & Robbins, T. W. (2008b). The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. *Nature Protocols*, 3(5), 759–767.
- Bari, A., & Robbins, T. W. (2013). Inhibition and impulsivity: Behavioral and neural basis of response control. *Progress in Neurobiology*, 108, 44–79.
- Barkley, R. A. (1997). Behavioral inhibition, sustained attention, and executive functions: Constructing a unifying theory of ADHD. *Psychological Bulletin*, 121(1), 65–94.
- Barkley, R. A. (2002). Psychosocial treatments for attention-deficit/hyperactivity disorder. *Journal of Clinical Psychiatry*, 63(12), 36–43.
- Barratt, E. S. (1959). Anxiety and impulsiveness related to psychomotor efficiency. *Perceptual and Motor Skills*, 9, 191–198.
- Barrus, M. M., Hosking, J. G., Zeeb, F. D., Tremblay, M., Winstanley, C. A., & Barrus, M. M. (2015). Disadvantageous decision-making on a rodent gambling task is associated with increased motor impulsivity in a population of male rats. *Journal of Psychiatry Neuroscience*, 40(2), 108–125.
- Barrus, M. M., & Winstanley, C. A. (2017). *Preclinical models and neurocircuitry of gambling and impulsive behavior*.
- Basar, K., Sesia, T., Groenewegen, H., Steinbusch, H. W. M., Visser-Vandewalle, V., & Temel, Y. (2010a). Nucleus accumbens and impulsivity. In *Progress in Neurobiology* (Vol. 92, Issue 4, pp. 533–557).
- Basar, K., Sesia, T., Groenewegen, H., Steinbusch, H. W. M., Visser-Vandewalle, V., & Temel, Y. (2010b). Nucleus accumbens and impulsivity. *Progress in Neurobiology*, 92(4), 533–557.

- Baunez, C., Humby, T., Eagle, D. M., Ryan, L. J., Dunnett, S. B., & Robbins, T. W. (2001). Effects of STN lesions on simple vs choice reaction time tasks in the rat: Preserved motor readiness, but impaired response selection. *European Journal of Neuroscience*, *13*(8), 1609–1616.
- Baunez, C., Nieoullon, A., & Amalric, M. (1995). In a rat model of parkinsonism, lesions of the subthalamic nucleus reverse increases of reaction time but induce a dramatic premature responding deficit. *Journal of Neuroscience*, *15*(10), 6531–6541.
- Baunez, C., & Robbins, T. W. (1997). Bilateral lesions of the subthalamic nucleus induce multiple deficits in an attentional task in rats. *European Journal of Neuroscience*, *9*(10), 2086–2099.
- Bechara, A., Damasio, A. R., Damasio, H., & Anderson, S. W. (1994). Insensitivity to future consequences following damage to human prefrontal cortex. *Cognition*, *50*(1–3), 7–15.
- Bechara, A., Damasio, H., Tranel, D., & Anderson, S. W. (1998). Dissociation of working memory from decision making within the human prefrontal cortex. *Journal of Neuroscience*, *18*(1), 428–437.
- Bechara, A., Dolan, S., Denburg, N., Hinds, A., Anderson, S. W., & Nathan, P. E. (2001). Decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in alcohol and stimulant abusers. *Neuropsychologia*, *39*(4), 376–389.
- Beier, K. T., Steinberg, E. E., Deloach, K. E., Kremer, E. J., Malenka, R. C., Luo, L., Beier, K. T., Steinberg, E. E., Deloach, K. E., Xie, S., Miyamichi, K., Schwarz, L., Gao, X. J., Kremer, E. J., Malenka, R. C., & Luo, L. (2015). Circuit architecture of VTA dopamine neurons revealed by systematic input-output mapping article circuit architecture of VTA dopamine neurons revealed by systematic input-output mapping. *Cell*, *162*(3), 622–634.

- Belin, D., Mar, A. C., Dalley, J. W., Robbins, T. W., & Everitt, B. J. (2008). High impulsivity predicts the switch to compulsive cocaine-taking. *Science*, *320*(5881), 1352–1355.
- Berendse, H. W., Graaf, Y. G., & Groenewegen, H. J. (1992a). Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. *Journal of Comparative Neurology*, *316*(3), 314–347.
- Berendse, H. W., Graaf, Y. G., & Groenewegen, H. J. (1992b). Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. *Journal of Comparative Neurology*, *316*(3), 314–347.
- Berg, J. M., Latzman, R. D., Bliwise, N. G., & Lilienfeld, S. O. (2015). Parsing the heterogeneity of impulsivity: A meta-analytic review of the behavioral implications of the UPPS for psychopathology. *Psychological Assessment*, *27*(4), 1129–1146.
- Berke, J. (2018). What does dopamine mean? Is dopamine a signal for learning, for motivation, or both? *Nature Neuroscience*, *21*(6), 787–793.
- Berridge, C. W., & Waterhouse, B. D. (2003). The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Research Reviews*, *42*(1), 33–84.
- Besson, M., Belin, D., McNamara, R., Theobald, D. E. H., Castel, A., Beckett, V. L., Crittenden, B. M., Newman, A. H., Everitt, B. J., Robbins, T. W., & Dalley, J. W. (2010). Dissociable control of impulsivity in rats by dopamine D2/3 receptors in the core and shell subregions of the nucleus accumbens. *Neuropsychopharmacology*, *35*(2), 560–569.
- Besson, M., Pelloux, Y., Dilleen, R., Theobald, D. E., Lyon, A., Belin-Rauscent, A., Robbins, T. W., Dalley, J. W., Everitt, B. J., & Belin, D. (2013). Cocaine modulation of frontostriatal expression of Zif268, D2, and 5-HT2c receptors in high and low impulsive rats. *Neuropsychopharmacology*, *38*(10), 1963–1973.

- Bezdjian, S., Baker, L. A., & Tuvblad, C. (2011). Genetic and environmental influences on impulsivity: A meta-analysis of twin, family and adoption studies. *Clinical Psychology Review, 31*(7), 1209–1223.
- BioRender (2022). Direct and Indirect Pathways of the Basal Ganglia. Retrieved from <https://app.biorender.com/biorender-templates/t-5f7f90a71892ae00ac869473-direct-and-indirect-pathways-of-the-basal-ganglia>
- Birnbaum, S. G., Yuan, P. X., Wang, M., Vijayraghavan, S., Bloom, A. K., Davis, D. J., Gobeske, K. T., Sweatt, J. D., Manji, H. K., & Arnsten, A. F. T. (2004). Protein kinase C overactivity impairs prefrontal cortical regulation of working memory. *Science, 306*(5697), 882–884.
- Bizarro, L., Patel, S., Murtagh, C., & Stoleran, I. P. (2004). Differential effects of psychomotor stimulants on attentional performance in rats: nicotine, amphetamine, caffeine and methylphenidate. *Behavioral Pharmacology, 15*(3), 195–206.
- Bizarro, L., & Stoleran, I. P. (2003). Attentional effects of nicotine and amphetamine in rats at different levels of motivation. *Psychopharmacology, 170*(3), 271–277.
- Blondeau, C., & Dellu-Hagedorn, F. (2007). Dimensional analysis of ADHD subtypes in rats. *Biological Psychiatry, 61*(12), 1340–1350
- Boekhoudt, L., Voets, E. S., Flores-Dourojeanni, J. P., Luijendijk, M. C., Vanderschuren, L. J., & Adan, R. A. (2017). Chemogenetic activation of midbrain dopamine neurons affects attention, but not impulsivity, in the Five-Choice Serial Reaction time task in rats. *Neuropsychopharmacology, 42*(6), 1315–1325.
- Boender, A. J., de Jong, J. W., Boekhoudt, L., Luijendijk, M. C. M., van der Plasse, G., & Adan, R. A. H. (2014). Combined use of the canine adenovirus-2 and DREADD technology to activate specific neural pathways in vivo. *PLoS ONE, 9*(4), 1–6.

- Botvinick, M., & Braver, T. (2015). Motivation and cognitive control: From behavior to neural mechanism. *Annual Review of Psychology*, *66*, 83–113.
- Brandon, C. L., & Steiner, H. (2003). Repeated methylphenidate treatment in adolescent rats alters gene regulation in the striatum. *European Journal of Neuroscience*, *18*(6), 1584–1592.
- Breen, R. B., & Zuckerman, M. (1999). 'Chasing' in gambling behavior: personality and cognitive determinants. *Personality and Individual Differences*, *27*, 1097–1111.
- Brog, J. S., Salyapongse, A., Deutch, A. Y., & Zahm, D. S. (1993). The patterns of afferent innervation of the core and shell in the Accumbens part of the rat ventral striatum: Immunohistochemical detection of retrogradely transported fluoro-gold. *The Journal of Comparative Neurology*, *338*(2), 255–278.
- Broos, N., Schmaal, L., Wiskerke, J., Kostelijk, L., & Lam, T. (2012). The relationship between impulsive choice and impulsive action: A cross-species translational study. *PLoS ONE*, *7*(5), 36781.
- Buckholtz, J. W., Treadway, M. T., Cowan, R. L., Woodward, N. D., Li, R., Ansari, M. S., Baldwin, R. M., Schwartzman, A. N., Shelby, E. S., Smith, C. E., Kessler, R. M., & Zald, D. H. (2010). Dopaminergic network differences in human impulsivity. *Science*, *329*(5991), 532.
- Buhusi, C. v., & Meck, W. H. (2002). Differential effects of methamphetamine and haloperidol on the control of an internal clock. *Behavioral Neuroscience*, *116*(2), 291–297.
- Burgess, M. A. (1997). Theory and methodology in executive function research. In P. Rabbitt (Ed.), *Methodology of Frontal and Executive Function*. (pp. 81–116). Psychology Press.

- Bymaster, F. P., Katner, J. S., Nelson, D. L., Hemrick-Luecke, S. K., Threlkeld, P. G., Heiligenstein, J. H., Morin, S. M., Gehlert, D. R., & Perry, K. W. (2002). Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: A potential mechanism for efficacy in Attention Deficit/Hyperactivity Disorder. *Neuropsychopharmacology*, *27*(5), 699–711.
- Caballero-Puntiverio, M., Fitzpatrick, C. M., Woldbye, D. P., & Andreasen, J. T. (2017). Effects of amphetamine and methylphenidate on attentional performance and impulsivity in the mouse 5-Choice Serial Reaction Time Task. *Journal of Psychopharmacology*, *31*(2), 272–283.
- Cáceres, P., & Martín, R. S. (2017). Low cognitive impulsivity is associated with better gain and loss learning in a probabilistic decision-making task. *Frontiers in Psychology*, *8*, 1–7.
- Callahan, P. M., Plagenhoef, M. R., Blake, D. T., & Terry Jr, A. v. (2019). Atomoxetine improves memory and other components of executive function in young-adult rats and aged rhesus monkeys. *Neuropharmacology*, *155*, 69–75.
- Caprioli, D., Hong, Y. T., Sawiak, S. J., Ferrari, V., Williamson, D. J., Jupp, B., Adrian Carpenter, T., Aigbirhio, F. I., Everitt, B. J., Robbins, T. W., Fryer, T. D., & Dalley, J. W. (2013). Baseline-dependent effects of cocaine pre-exposure on impulsivity and D<sub>2/3</sub> receptor availability in the rat striatum: Possible relevance to the attention-deficit hyperactivity syndrome. *Neuropsychopharmacology*, *38*(8), 1460–1471.
- Caprioli, D., Jupp, B., Hong, Y. T., Sawiak, S. J., Ferrari, V., Wharton, L., Williamson, D. J., McNabb, C., Berry, D., Aigbirhio, F. I., Robbins, T. W., Fryer, T. D., & Dalley, J. W. (2015). Dissociable rate-dependent effects of oral methylphenidate on impulsivity and D<sub>2/3</sub> receptor availability in the striatum. *Journal of Neuroscience*, *35*(9), 3747–3755.
- Caprioli, D., Sawiak, S. J., Merlo, E., Theobald, D. E. H., Spoelder, M., Jupp, B., Voon, V., Carpenter, T. A., Everitt, B. J., Robbins, T. W., & Dalley, J. W. (2014). Gamma

- aminobutyric acidergic and neuronal structural markers in the nucleus accumbens core underlie Trait-like impulsive behavior. *Biological Psychiatry*, 75(2), 115–123.
- Carboni, E., Silvagni, A., Vacca, C., & di Chiara, G. (2006). Cumulative effect of norepinephrine and dopamine carrier blockade on extracellular dopamine increase in the nucleus accumbens shell, bed nucleus of stria terminalis and prefrontal cortex. *Journal of Neurochemistry*, 96(2), 473–481.
- Cardinal, R. N. (2006). Neural systems implicated in delayed and probabilistic reinforcement. *Neural Networks*, 19(8), 1277–1301.
- Cardinal, R. N., & Aitken, M. R. F. (2010). Whisker: A client-server high-performance multimedia research control system. *Behavior Research Methods*, 42(4), 1059–1071.
- Carli, M., Robbins, T. W., Evenden, J. L., & Everitt, B. J. (1983). Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. *Behavioural Brain Research*, 9(3), 361–380.
- Carli, Mirjana, & Samanin, R. (1992). Serotonin<sub>2</sub> receptor agonists and serotonergic anorectic drugs affect rats' performance differently in a five-choice serial reaction time task. *Psychopharmacology*, 106(2), 228–234
- Carlson, S. R., Pritchard, A. A., & Dominelli, R. M. (2013). Externalizing behavior, the UPPS-P Impulsive Behavior scale and Reward and Punishment Sensitivity. *Personality and Individual Differences*, 54(2), 202–207
- Carlsson, A., Lindqvist, M., Magnusson, T., & Waldeck, B. (1958). On the presence of 3-hydroxytyramine in brain. *Science*, 127(3296), 471–471.
- Carver, C. S. (2006). Approach, avoidance, and the self-regulation of affect and action. *Motivation and Emotion*, 30(2), 105–110.

- Castellanos-Ryan, N., O'Leary-Barrett, M., Sully, L., & Conrod, P. (2013). Sensitivity and Specificity of a Brief Personality Screening Instrument in Predicting Future Substance Use, Emotional, and Behavioral Problems: 18-Month Predictive Validity of the Substance Use Risk Profile Scale. *Alcoholism: Clinical and Experimental Research*, *37*, 281–290.
- Castellanos-Ryan, N., Rubia, K., & Conrod, P. J. (2011). Response Inhibition and Reward Response Bias Mediate the Predictive Relationships Between Impulsivity and Sensation Seeking and Common and Unique Variance in Conduct Disorder and Substance Misuse. *Alcoholism: Clinical and Experimental Research*, *35*(1), 140–155.
- Chamberlain, S. R., del Campo, N., Dowson, J., Müller, U., Clark, L., Robbins, T. W., & Sahakian, B. J. (2007). Atomoxetine improved response inhibition in adults with Attention Deficit/Hyperactivity Disorder. *Biological Psychiatry*, *62*(9), 977–984.
- Chamberlain, S. R., Robbins, T. W., Winder-Rhodes, S., Miller, U., Sahakian, B. J., Blackwell, A. D., & Barnett, J. H. (2011). Translational approaches to frontostriatal dysfunction in attention-deficit/hyperactivity disorder using a computerized neuropsychological battery. *Biological Psychiatry*, *69*(12), 1192–1203.
- Chase, T. D., Brown, R. E., Carrey, N., & Wilkinson, M. (2003). Daily methylphenidate administration attenuates c-fos expression in the striatum of prepubertal rats. *NeuroReport*, *14*(5), 769–772.
- Cho, Y. H., & Jeantet, Y. (2010). Differential involvement of prefrontal cortex, striatum, and hippocampus in DRL performance in mice. *Neurobiology of Learning and Memory*, *93*(1), 85–91.
- Christakou, A., Robbins, T. W., & Everitt, B. J. (2001). Functional disconnection of a prefrontal cortical-dorsal striatal system disrupts choice reaction time performance: Implications for attentional function. *Behavioral Neuroscience*, *115*(4), 812–825.

- Christakou, A., Robbins, T. W., & Everitt, B. J. (2004). Prefrontal cortical-ventral striatal interactions involved in affective modulation of attentional performance: Implications for corticostriatal circuit function. *The Journal of Neuroscience*, *24*(4), 773–780.
- Chudasama, Y., Nathwani, F., & Robbins, T. W. (2005). d-Amphetamine remediates attentional performance in rats with dorsal prefrontal lesions. *Behavioural Brain Research*, *158*(1), 97–107.
- Chudasama, Y., & Robbins, T. W. (2003). Dissociable contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. *The Journal of Neuroscience*, *23*(25), 8771–8780.
- Chudasama, Y. (2011). Animal models of prefrontal-executive function. *Behavioral Neuroscience*, *125*(3), 327–343.
- Chudasama, Y., Baunez, C., & Robbins, T. W. (2003). Functional disconnection of the medial prefrontal cortex and subthalamic nucleus in attentional performance: Evidence for corticosubthalamic interaction. *Journal of Neuroscience*, *23*(13), 5477–5485.
- Chudasama, Y., & Muir, J. L. (2001). Visual attention in the rat: A role for the prelimbic cortex and thalamic nuclei? *Behavioral Neuroscience*, *115*(2), 417–428.
- Chudasama, Y., & Robbins, T. W. (2004). Dopaminergic modulation of visual attention and working memory in the rodent prefrontal cortex. *Neuropsychopharmacology*, *29*(9), 1628–1636.
- Chuhma, N., Mingote, S., Kalmbach, A., Yetnikoff, L., & Rayport, S. (2017). Heterogeneity in dopamine neuron synaptic actions across the striatum and its relevance for schizophrenia. *Biological Psychiatry*, *81*(1), 43–51.

- Clark, L., Robbins, T. W., Ersche, K. D., & Sahakian, B. J. (2006). Reflection Impulsivity in Current and Former Substance Users. *Biological Psychiatry*, *60*(5), 515–522.
- Coghill, D. R., Seth, S., Pedroso, S., Usala, T., Currie, J., & Gagliano, A. (2014). Effects of methylphenidate on cognitive functions in children and adolescents with attention-deficit/hyperactivity disorder: Evidence from a systematic review and a meta-analysis. *Biological Psychiatry*, *76*(8), 603–615.
- Cohen, J. Y., Haesler, S., Vong, L., Lowell, B. B., & Uchida, N. (2012). Neuron-type-specific signals for reward and punishment in the ventral tegmental area. *Nature*, *482*(7383), 85–88.
- Cohen, S., & Greenberg, M. E. (2008). Communication between the synapse and the nucleus in neuronal development, plasticity, and disease. *Annual Review of Cell and Developmental Biology*, *24*, 183–209.
- Colder, C. R., Hawk, L. W., Lengua, L. J., Wiezcorek, W., Eiden, R. das, & Read, J. P. (2013). Trajectories of reinforcement sensitivity during adolescence and risk for substance use. *Journal of Research on Adolescence*, *23*(2), 345–356.
- Cole, B. J., & Robbins, T. W. (1989). Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi on performance of a 5-choice serial reaction time task in rats: Implications for theories of selective attention and arousal. *Behavioural Brain Research*, *33*(2), 165–179.
- Conners, C. K., Epstein, J. N., Angold, A., & Klaric, J. (2003). Continuous performance test performance in a normative epidemiological sample. *Annals of Operations Research*, *31*(5), 555–562.
- Conrod, P. J., Pihl, R. O., Stewart, S. H., & Dongier, M. (2000). Validation of a system of classifying female substance abusers on the basis of personality and motivational risk factors for substance abuse. *Psychology of Addictive Behaviors*, *14*(3), 243–256.

- Conrod, Patricia J., Petersen, J. B., & Pihl, R. O. (1997). Disinhibited personality and sensitivity to alcohol reinforcement: Independent correlates of drinking behavior in sons of alcoholics. *Alcoholism: Clinical and Experimental Research*, 21(7), 1320–1332.
- Cools, R., Blackwell, A., Clark, L., Menzies, L., Cox, S., & Robbins, T. W. (2005). Tryptophan depletion disrupts the motivational guidance of goal-directed behavior as a function of trait impulsivity. *Neuropsychopharmacology*, 30(7), 1362–1373.
- Cope, Z. A., Halberstadt, A. L., van Enkhuizen, J., Flynn, A. D., Breier, M., Swerdlow, N. R., Geyer, M. A., & Young, J. W. (2016). Premature responses in the five-choice serial reaction time task reflect rodents' temporal strategies: evidence from no-light and pharmacological challenges. *Psychopharmacology*, 233(19–20), 3513–3525.
- Corbit, L. H., Muir, J. L., & Balleine, B. W. (2001). The role of the nucleus accumbens in instrumental conditioning: Evidence of a functional dissociation between accumbens core and shell. *The Journal of Neuroscience*, 21(9), 3251–3260.
- Cornblatt, B. A., & Malhotra, A. K. (2001). Impaired attention as an endophenotype for molecular genetic studies of schizophrenia. *American Journal of Medical Genetics - Neuropsychiatric Genetics*, 105(1), 11–15
- Corr, P. J. (2002). J.A. Gray's reinforcement sensitivity theory and frustrative nonreward: A theoretical note on expectancies in reactions to rewarding stimuli. *Personality and Individual Differences*, 32(7), 1247–1253.
- Corr, P. J., Gray, J. A., & Pickering, A. D. (1997). Personality, punishment, and procedural learning: A test of J. A. gray's anxiety theory. *Journal of Personality and Social Psychology*, 73(2), 337–344.
- Cosme, C. v., Palissery, G. K., & Lerner, T. N. (2018). A dLight-ful new view of neuromodulation. *Trends in Neurosciences*, 41(9), 566–568.

- Costa, P. T., & McCrae, R. R. (1992). Normal personality assessment in clinical practice: The NEO Personality Inventory. *Psychological Assessment*, 4(1), 5–13.
- Costikyan, G. (2013). *Uncertainty in Games*. (MIT Press Eds)
- Cousins, M. S., Atherton, A., Turner, L., & Salamone, J. D. (1996). Nucleus accumbens dopamine depletions alter relative response allocation in a T-maze cost/benefit task. *Behavioural Brain Research*, 74(1–2), 189–197.
- Cruz, B. F., Soares, S., & Paton, J. J. (2020). Striatal circuits support broadly opponent aspects of action suppression and production. *BioRxiv*, 1–40.
- Cruz, F. C., Javier Rubio, F., & Hope, B. T. (2015). Using c-fos to study neuronal ensembles in corticostriatal circuitry of addiction. *Brain Research*, 1628, 157–173.
- Cui, G., Jun, S. B., Jin, X., Pham, M. D., Vogel, S. S., Lovinger, D. M., & Costa, R. M. (2013). Concurrent activation of striatal direct and indirect pathways during action initiation. *Nature*, 494(7436), 238–242.
- Cyders, M. A., & Coskunpinar, A. (2011). Measurement of constructs using self-report and behavioral lab tasks: Is there overlap in nomothetic span and construct representation for impulsivity? *Clinical Psychology Review*, 31, 965–982.
- Cyders, M. A., & Smith, G. T. (2007). Mood-based rash action and its components: Positive and negative urgency. *Personality and Individual Differences*, 43(4), 839–850.
- Cyders, M. A., & Smith, G. T. (2008). Emotion-based Dispositions to Rash Action: Positive and Negative Urgency. *Psychological Bulletin*, 134(6), 807–828.

- Cyders, M., Smith, G. T., Spillane, N. S., Fischer, S., Annus, A. M., & Peterson, C. M. (2007). Integration of Impulsivity and Positive Mood to Predict Risky Behavior: Development and Validation of a Measure of Positive Urgency. *Psychological Assessment, 19*(1), 107–118.
- da Silva, J., Tecuapetla, F., Paixão, V., & Costa, R. M. (2018). Dopamine neuron activity before action initiation gates and invigorates future movements. *Nature, 554*(7691), 244–248.
- Dalley, J. W., & Ersche, K. D. (2019). Neural circuitry and mechanisms of waiting impulsivity: relevance to addiction. *Philosophical Transactions of the Royal Society B: Biological Sciences, 374*(1766), 20180145.
- Dalley, J. W., Everitt, B. J., & Robbins, T. W. (2011). Impulsivity, compulsivity, and top-down cognitive control. *Neuron, 69*(4), 680–694.
- Dalley, J. W., Fryer, T. D., Brichard, L., Robinson, E. S. J., Theobald, D. E. H., Lääne, K., Peña, Y., Murphy, E. R., Shah, Y., Probst, K., Abakumova, I., Aigbirhio, F. I., Richards, H. K., Hong, Y., Baron, J., Everitt, B. J., & Robbins, T. W. (2007a). Nucleus Accumbens D2/3 Receptors Predict Trait Impulsivity and Cocaine Reinforcement. *Science, 315*(5816), 1267–1270.
- Dalley, J. W., Lääne, K., Theobald, D. E. H., Pěa, Y., Bruce, C. C., Huszar, A. C., Wojcieszek, M., Everitt, B. J., & Robbins, T. W. (2007b). Enduring deficits in sustained visual attention during withdrawal of intravenous methylenedioxymethamphetamine self-administration in rats: Results from a comparative study with d-amphetamine and methamphetamine. *Neuropsychopharmacology, 32*(5), 1195–1206.
- Dalley, J. W., & Robbins, T. W. (2017). Fractionating impulsivity: Neuropsychiatric implications. *Nature Reviews Neuroscience, 18*(3), 158–171.

- Dalley, J. W., Theobald, D. E., Eagle, D. M., Passetti, F., & Robbins, T. W. (2002). Deficits in impulse control associated with tonically-elevated serotonergic function in rat prefrontal cortex. *Neuropsychopharmacology*, *26*(6), 716–728.
- Dang, J., King, K. M., & Inzlicht, M. (2020). Why are self-report and behavioral measures weakly correlated? *Trends in Cognitive Sciences*, *24*(4), 267–269.
- Davison, A. C. (2003). *Statistical Models* (Cambridge University Press, Ed.).
- Day, D. v. (2011). Integrative perspectives on longitudinal investigations of leader development: From childhood through adulthood. *Leadership Quarterly*, *22*(3), 561–571.
- de Wit, H. (2009). Impulsivity as a determinant and consequence of drug use: A review of underlying processes. *Addiction Biology*, *14*(1), 22–31.
- DeLong, M. R. (1990). Primate models of movement disorders of basal ganglia origin. *Trends in Neurosciences*, *13*(7), 281–285.
- Deutch, A. Y., & Cameron, D. S. (1992). Pharmacological characterization of dopamine system in the nucleus accumbens core and shell. *Neuroscience*, *46*, 49-56.
- di Ciano, P., Cardinal, R. N., Cowell, R. A., Little, S. J., & Everitt, B. J. (2001). Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. *Journal of Neuroscience*, *21*(23), 9471–9477.
- Dickman, S. (1985). Impulsivity and perception. Individual differences in the processing of the local and global dimensions of stimuli. *Journal of Personality and Social Psychology*, *48*(1), 133–149.

- Dickman, S. J. (1990). Functional and dysfunctional impulsivity: Personality and cognitive correlates. *Journal of Personality and Social Psychology*, *58*(1), 95–102.
- Dickman, S. J. (2000). Impulsivity, arousal and attention. *Personality and Individual Differences*, *28*(3), 563–581.
- Dickman, S. J., & Meyer, D. E. (1988). Impulsivity and speed-accuracy tradeoffs in information processing. *Journal of Personality and Social Psychology*, *54*(2), 274–290.
- Diergaarde, L., Pattij, T., Nawijn, L., Schoffemeer, A. N. M., & de Vries, T. J. (2009). Trait impulsivity predicts escalation of sucrose seeking and hypersensitivity to sucrose-associated. *Behavioral Neuroscience*, *123*(4), 794–803
- Diergaarde, L., Pattij, T., Poortvliet, I., Hogenboom, F., de Vries, W., Schoffemeer, A. N. M., & de Vries, T. J. (2008). Impulsive choice and impulsive action predict vulnerability to distinct stages of nicotine seeking in rats. *Biological Psychiatry*, *63*(3), 301–308.
- Diergaarde, L., van Mourik, Y., Pattij, T., Schoffemeer, A. N. M., & de Vries, T. J. (2012). Poor impulse control predicts inelastic demand for nicotine but not alcohol in rats. *Addiction Biology*, *17*(3), 576–587.
- Dixon, M. J., MacLaren, V., Jarick, M., Fugelsang, J. A., & Harrigan, K. A. (2013). The frustrating effects of just missing the jackpot: Slot machine near-misses trigger large skin conductance responses, but no post-reinforcement pauses. *Journal of Gambling Studies*, *29*(4), 661–674.
- Dixon, M. R., Jacobs, E. A., & Sanders, S. (2006). Contextual control of delay discounting by pathological gamblers. *Journal of Applied Behavior Analysis*, *39*(4), 413–422.
- Dodson, P. D., Dreyer, J. K., Jennings, K. A., Syed, E. C. J., Wade-Martins, R., Cragg, S. J., Bolam, J. P., & Magill, P. J. (2016). Representation of spontaneous movement by

dopaminergic neurons is cell-type selective and disrupted in parkinsonism. *Proceedings of the National Academy of Sciences*, *113*(15), E2180–E2188.

Donnelly, N A, Holtzman, T., Rich, P. D., Nevado-Holgado, A. J., & Fernando, A. B. P. (2014). Oscillatory Activity in the Medial Prefrontal Cortex and Nucleus Accumbens Correlates with Impulsivity and Reward Outcome. *PLoS ONE*, *9*(10), 111300.

Donnelly, Nicholas A., Paulsen, O., Robbins, T. W., & Dalley, J. W. (2015). Ramping single unit activity in the medial prefrontal cortex and ventral striatum reflects the onset of waiting but not imminent impulsive actions. *European Journal of Neuroscience*, *41*(12), 1524–1537.

Dougherty, D. M., Bjork, J. M., Harper, R. A., Marsh, D. M., Moeller, F. G., Mathias, C. W., & Swann, A. C. (2003). Behavioral impulsivity paradigms: A comparison in hospitalized adolescents with disruptive behavior disorders. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *44*(8), 1145–1157.

Dougherty, D. M., Bjork, J. M., Huckabee, H. C. G., Moeller, F. G., & Swann, A. C. (1999). Laboratory measures of aggression and impulsivity in women with borderline personality disorder. *Psychiatry Research*, *85*(3), 315–326.

Eagle, D. M., & Robbins, T. W. (2003). Inhibitory Control in Rats Performing a Stop-Signal Reaction-Time Task: Effects of Lesions of the Medial Striatum and d-Amphetamine. *Behavioral Neuroscience*, *117*(6), 1302–1317.

Eagle, Dawn M., Bari, A., & Robbins, T. W. (2008). The neuropsychopharmacology of action inhibition: Cross-species translation of the stop-signal and go/no-go tasks. *Psychopharmacology*, *199*(3), 439–456.

- Eagle, Dawn M., & Baunez, C. (2010). Is there an inhibitory-response-control system in the rat? Evidence from anatomical and pharmacological studies of behavioral inhibition. *Neuroscience and Biobehavioral Reviews*, 34(1), 50–72.
- Eben, C., Billieux, J., & Verbruggen, F. (2020). Clarifying the role of negative emotions in the origin and control of impulsive actions. *Psychologica Belgica*, 60(1), 1–17.
- Economidou, D., Theobald, D. E. H., Robbins, T. W., Everitt, B. J., & Dalley, J. W. (2012). Norepinephrine and dopamine modulate impulsivity on the five-choice serial reaction time task through opponent actions in the shell and core sub-regions of the nucleus accumbens. *Neuropsychopharmacology*, 37(9), 2057–2066.
- Eisenberg, D. T. A., MacKillop, J., Modi, M., Beauchemin, J., Dang, D., Lisman, S. A., Lum, J. K., & Wilson, D. S. (2007). Examining impulsivity as an endophenotype using a behavioral approach: A DRD2 TaqI A and DRD4 48-bp VNTR association study. *Behavioral and Brain Functions*, 3, 1–14.
- Epstein, J. N., Erkanli, A., Conners, C. K., Klaric, J., Costello, J. E., & Angold, A. (2003). Relations between continuous performance test performance measures and ADHD behaviors. *Journal of Abnormal Child Psychology*, 31(5), 543-554
- Ersche, K. D., Jones, P. S., Williams, G. B., Smith, D. G., Bullmore, E. T., & Robbins, T. W. (2013). Distinctive personality traits and neural correlates associated with stimulant drug use versus familial risk of stimulant dependence. *Biological Psychiatry*, 74(2), 137–144.
- Ersche, K. D., Turton, A. J., Pradhan, S., Bullmore, E. T., & Robbins, T. W. (2010). Drug addiction endophenotypes: Impulsive versus sensation-seeking personality traits. *Biological Psychiatry*, 68(8), 770–773.
- Eshel, N., Tian, J., Bukwich, M., & Uchida, N. (2016). Dopamine neurons share common response function for reward prediction error. *Nature Neuroscience*, 19(3), 479–486.

- Everitt, B. J., Parkinson, J. A., Olmstead, M. C., Arroyo, M., Robledo, P., & Robbins, T. W. (1999). Associative processes in addiction and reward the role of amygdala-ventral striatal. *Annals of the New York Academy of Sciences*, 877(1), 412–438.
- Evren, C., Durkaya, M., Evren, B., Dalbudak, E., & Cetin, R. (2012). Relationship of relapse with impulsivity, novelty seeking and craving in male alcohol-dependent inpatients. *Drug and Alcohol Review*, 31(1), 81–90.
- Eysenck, S. B. G., & Eysenck, H. J. (1968). The measurement of psychoticism: a study of factor stability and reliability. *British Journal of Social and Clinical Psychology*, 7(4), 286–294.
- Eysenck, S. B. G., Eysenck, H. J., & Barrett, P. (1985). A revised version of the psychoticism scale. *Personality and Individual Differences*, 6(1), 21–29
- Feja, M., Hayn, L., & Koch, M. (2014). Nucleus accumbens core and shell inactivation differentially affects impulsive behaviours in rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 54, 31–42
- Fellows, L. K., & Farah, M. J. (2005). Different underlying impairments in decision-making following ventromedial and dorsolateral frontal lobe damage in humans. *Cerebral Cortex*, 15(1), 58–63.
- Fernando, A. B. P., Economidou, D., Theobald, D. E., Zou, M.-F., Newman, A. H., Spoelder, M., Caprioli, D., Moreno, M., Hipólito, L., Aspinall, A. T., Robbins, T. W., & Dalley, J. W. (2012). Modulation of high impulsivity and attentional performance in rats by selective direct and indirect dopaminergic and noradrenergic receptor agonists. *Psychopharmacology*, 219(2), 341–352.
- Fineberg, N. A., Potenza, M. N., Chamberlain, S. R., Berlin, H. A., Menzies, L., Bechara, A., Sahakian, B. J., Robbins, T. W., Bullmore, E. T., & Hollander, E. (2010). Probing

compulsive and impulsive behaviors, from animal models to endophenotypes: A narrative review. *Neuropsychopharmacology*, 35(3), 591–604.

Fiorillo, C. D., Tobler, P. N., & Schultz, W. (2003). Discrete coding of reward dopamine neurons. *Science*, 299, 1898–1902

Flagel, S. B., Clark, J. J., Robinson, T. E., Mayo, L., Czuj, A., Willuhn, I., Akers, C. A., Clinton, S. M., Phillips, P. E. M., & Akil, H. (2011). A selective role for dopamine in stimulus-reward learning. *Nature*, 469(7328), 53–59.

Flaherty, C. F. (1999). *Incentive relativity* (Cambridge University Press, Ed.).

Floresco, S. B., McLaughlin, R. J., & Haluk, D. M. (2008). Opposing roles for the nucleus accumbens core and shell in cue-induced reinstatement of food-seeking behavior. *Neuroscience*, 154(3), 877–884.

Floresco, Stan B. (2015). The nucleus accumbens: An interface between cognition, emotion, and action. *Annual Review of Psychology*, 66, 25–32.

Floresco, Stan B, Yang, C. R., Phillips, A. G., & Blaha, C. D. (1998). Basolateral amygdala stimulation evokes glutamate receptor-dependent dopamine efflux in the nucleus accumbens of the anaesthetized rat. *European Journal of Neuroscience*, 10(4), 1241–1251.

Forder, L., & Dyson, B. J. (2016). Behavioural and neural modulation of win-stay but not lose-shift strategies as a function of outcome value in Rock, Paper, Scissors. *Scientific Reports*, 6, 1–8

Frank, M. J. (2006). Hold your horses: A dynamic computational role for the subthalamic nucleus in decision making. *Neural Networks*, 19(8), 1120–1136.

- Frank, M. J., Santamaria, A., O'Reilly, R. C., & Willcutt, E. (2007). Testing computational models of dopamine and noradrenaline dysfunction in attention deficit/hyperactivity disorder. *Neuropsychopharmacology*, *32*(7), 1583–1599.
- Franken, I. H. A., van Strien, J. W., Nijs, I., & Muris, P. (2008). Impulsivity is associated with behavioral decision-making deficits. *Psychiatry Research*, *158*(2), 155–163.
- Frijda, N. H. (2010). Impulsive action and motivation. *Biological Psychology*, *84*(3), 570–579.
- Gabriel, D. B. K., Freels, T. G., Setlow, B., & Simon, N. W. (2019). Risky decision-making is associated with impulsive action and sensitivity to first-time nicotine exposure. *Behavioural Brain Research*, *359*, 579–588.
- Gerard Moeller, F., Barratt, E. S., Dougherty, D. M., Schmitz, J. M., & Swann, A. C. (2001). Psychiatric Aspects of Impulsivity. *American Journal of Psychiatry*, *158*, 1783–1793
- Gerasimov, N. R., Franceschi, M., Nora, D. v, Gifford, A., Gatley, J. S., Marsteller, D., Molina, P. E., & Dewey, S. L. (2000). Comparison between Intraperitoneal and oral methylphenidate administration: A microdialysis and locomotor activity study. *The Journal of Pharmacology and Experimental Therapeutics*, *295*, 51–57.
- Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J., & Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science*, *250*(4986), 1429–1432.
- Gerfen, C. R., & Surmeier, D. J. (2011). Modulation of striatal projection systems by dopamine. *Annual Review of Neuroscience*, *34*(1), 441–466.
- Gerfen, C., & Wilson, C. (1996). The basal ganglia. In S. Swanson, A. Bjorklund, & T. Hokfelt (Eds.), *Handbook of Chemical Neuroanatomy* (pp. 365–62).

- Gipson, C. D., Beckmann, J. S., Adams, Z. W., Marusich, J. A., Nesland, T. O., Yates, J. R., Kelly, T. H., & Bardo, M. T. (2012). A translational behavioral model of mood-based impulsivity: Implications for substance abuse. *Drug and Alcohol Dependence, 122*(1–2), 93–99
- Gomez, J. L., Bonaventura, J., Lesniak, W., Mathews, W. B., Sysa-Shah, P., Rodriguez, L. A., Ellis, R. J., Richie, C. T., Harvey, B. K., Dannals, R. F., Pomper, M. G., Bonci, A., & Michaelides, M. (2017). Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. *Science, 357*(6350), 503–507.
- Gomez, R., Cooper, A., McOrmond, R., & Tatlow, S. (2004). Gray's reinforcement sensitivity theory: Comparing the separable and joint subsystems. Hypotheses in the predictions of pleasant and unpleasant emotional information processing. *Personality and Individual Differences, 37*(2), 289–305.
- Granon, S., Passetti, F., Thomas, K. L., Dalley, J. W., Everitt, B. J., & Robbins, T. W. (2000). Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. *The Journal of Neuroscience, 20*(3), 1208–1215
- Gray, J. (1987). Perspectives on anxiety and impulsivity: A commentary. *Journal of Research in Personality, 21*(4), 493–509.
- Gray, J. C., MacKillop, J., Weafer, J., Hernandez, K. M., Gao, J., Palmer, A. A., & de Wit, H. (2018). Genetic analysis of impulsive personality traits: Examination of a priori candidates and genome-wide variation. *Psychiatry Research, 259*, 398–404.
- Graybiel, A. M. (2000). The Basal Ganglia. *Current Biology, 10*, R509–R511.
- Groenewegen, H. J., Wright, C. I., Beijer, A. v., & Voorn, P. (1999). Convergence and segregation of ventral striatal inputs and outputs. *Annals of the New York Academy of Sciences, 877*(1), 49–63.

- Grottick, A. J., & Higgins, G. A. (2000). Effect of subtype selective nicotinic compounds on attention as assessed by the five-choice serial reaction time task. *Behavioural Brain Research, 117*(1–2), 197–208.
- Grottick, Andrew J., & Higgins, G. A. (2002). Assessing a vigilance decrement in aged rats: Effects of pre-feeding, task manipulation, and psychostimulants. *Psychopharmacology, 164*(1), 33–41.
- Guiard, B. P., Mansari, M. el, & Blier, P. (2008). Cross-talk between dopaminergic and noradrenergic systems in the rat ventral tegmental area, locus ceruleus, and dorsal hippocampus. *Molecular Pharmacology, 74*(5), 1463–1475.
- Haber, S., Fudge, J., & McFarland, N. (2000). Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *Journal of Neuroscience, 20*, 2369–2382.
- Haber, S. N., & Knutson, B. (2010). The reward circuit: Linking primate anatomy and human imaging. *Neuropsychopharmacology, 35*(1), 4–26.
- Hahn, B., Shoaib, M., & Stoleran, I. P. (2002). Nicotine-induced enhancement of attention in the five-choice serial reaction time task: The influence of task demands. *Psychopharmacology, 162*(2), 129–137.
- Hailwood, J. M., Heath, C. J., Robbins, T. W., Saksida, L. M., & Bussey, T. J. (2018). Validation and optimisation of a touchscreen progressive ratio test of motivation in male rats. *Psychopharmacology, 235*, 2739–2753.
- Hamid, A. A., Pettibone, J. R., Mabrouk, O. S., Hetrick, V. L., Schmidt, R., vander Weele, C. M., Kennedy, R. T., Aragona, B. J., & Berke, J. D. (2016). Mesolimbic dopamine signals the value of work. *Nature Neuroscience, 19*(1), 117–126.

- Hamilton, K. R., Littlefield, A. K., Anastasio, N. C., Cunningham, K. A., Fink, L. H., Wing, V. C., Mathias, C. W., Lane, S. D., Schutz, C., Swann, A. C., Lejuez, C. W., Clark, L., Moeller, F. G., & Potenza, M. N. (2015a). Rapid-response impulsivity: Definitions, measurement issues, and clinical implications. *Personality Disorders, 6*(2), 168–181.
- Hamilton, K. R., Mitchell, M. R., Wing, V. C., Balodis, I. M., Bickel, W. K., Fillmore, M., & Lane, S. D. (2015b). Choice impulsivity: Definitions, measurement issues, and clinical implications. *Personality Disorders, 6*(2), 182–198.
- Hardung, S., Epple, R., Jäckel, Z., Eriksson, D., Uran, C., Senn, V., Gibor, L., Yizhar, O., & Diester, I. (2017). A functional gradient in the rodent prefrontal cortex supports behavioral inhibition. *Current Biology, 27*(4), 549–555.
- Harrison, A. A., Everitt, B. J., & Robbins, T. W. (1999). Central serotonin depletion impairs both the acquisition and performance of a symmetrically reinforced go/no-go conditional visual discrimination. *Behavioural Brain Research, 100*(1–2), 99–112.
- Harrison, Amanda A., Everitt, B. J., & Robbins, T. W. (1997). Central 5-HT depletion enhances impulsive responding without affecting the accuracy of attentional performance: Interactions with dopaminergic mechanisms. *Psychopharmacology, 133*(4), 329–342.
- Hart, A. S., Clark, J. J., & Phillips, P. E. M. (2015). Dynamic shaping of dopamine signals during probabilistic Pavlovian conditioning. *Neurobiology of Learning and Memory, 117*, 84–92.
- Hasbi, A., O’Dowd, B. F., & George, S. R. (2011). Dopamine D1-D2 receptor heteromer signaling pathway in the brain: Emerging physiological relevance. *Molecular Brain, 4*(1), 26.
- Heal, D. J., Smith, S. L., Gosden, J., & Nutt, D. J. (2013). Amphetamine, past and present—a pharmacological and clinical perspective. *Journal of Psychopharmacology, 27*(6), 479–496.

- Heidbreder, C. A., & Groenewegen, H. J. (2003). The medial prefrontal cortex in the rat: Evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. *Neuroscience and Biobehavioral Reviews*, *27*(6), 555–579.
- Heimer, L., Zahm, D. S., Churchill, L., Kalivas, P. W., & Wohltmann, C. (1991). Specificity in the projection patterns of accumbal core and shell in the rat. *Neuroscience*, *41*(1), 89–125.
- Heimer, Lennart, & van Hoesen, G. W. (2006). The limbic lobe and its output channels: Implications for emotional functions and adaptive behavior. *Neuroscience and Biobehavioral Reviews*, *30*(2), 126–147.
- Hikosaka, O., Takikawa, Y., & Kawagoe, R. (2000). Role of the basal ganglia in the control of purposive saccadic eye movements. *Physiological Reviews*, *80*(3), 953–978.
- Hofmann, W., Friese, M., & Strack, F. (2009). Impulse and self-control from a dual-systems perspective. *Perspectives on Psychological Science*, *4*(2), 162–176.
- Hosking, J. G., Floresco, S. B., & Winstanley, C. A. (2015). Dopamine antagonism decreases willingness to expend physical, but not cognitive, effort: A comparison of two rodent cost/benefit decision-making tasks. *Neuropsychopharmacology*, *40*(4), 1005–1015.
- Howe, M., & Dombeck, D. (2016). Rapid signalling in distinct dopaminergic axons during locomotion and reward. *Nature*, *535*(7613), 505–510.
- Howe, M. W., Tierney, P. L., Sandberg, S. G., Phillips, P. E. M., & Graybiel, A. M. (2013). Prolonged dopamine signalling in striatum signals proximity and value of distant rewards. *Nature*, *500*(7464), 575–579.

- Huang-Pollock, C. L., Karalunas, S. L., Tam, H., & Moore, A. N. (2012). Evaluating Vigilance Deficits in ADHD: A Meta-Analysis of CPT Performance. *Journal of Abnormal Psychology, 121*(2), 360–371.
- Hughes, L. F., Dunlap, W. P., & Dachowski, L. (1974). Reward magnitude and partial reinforcement effects in a single runway. *Journal of Comparative and Physiological Psychology, 87*, 563–570.
- Ikemoto, S. (2007). Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Research Reviews, 56*(1), 27–78.
- Ikemoto, S., & Panksepp, J. (1999). The role of nucleus accumbens dopamine in motivated behavior: A unifying interpretation with special reference to reward-seeking. *Brain Research Reviews, 31*, 6–41.
- Ioannidis, K., Hook, R., Wickham, K., Grant, J. E., & Chamberlain, S. R. (2019). Impulsivity in gambling disorder and problem gambling: a meta-analysis. *Neuropsychopharmacology, 44*, 1354–1361.
- Ito, R., & Hayen, A. (2011). Opposing roles of nucleus accumbens core and shell dopamine in the modulation of limbic information processing. *Journal of Neuroscience, 31*(16), 6001–6007.
- Izquierdo, A., & Jentsch, J. D. (2012). Reversal learning as a measure of impulsive and compulsive behavior in addictions. *Psychopharmacology, 219*, 607–620.
- Jensen, C., & Fallon, D. (1973). Behavioral aftereffects of reinforcement and its omission as a function of reinforcement magnitude. *Journal of the Experimental Analysis of Behavior, 19*(3), 459–468.

- Jentsch, J. D., & Taylor, J. R. (1999). Impulsivity resulting from frontostriatal dysfunction in drug abuse: Implications for the control of behavior by reward-related stimuli. *Psychopharmacology*, *146*(4), 373–390.
- Jin, X., & Costa, R. M. (2010). Start/stop signals emerge in nigrostriatal circuits during sequence learning. *Nature*, *466*(7305), 457–462.
- Jin, X., Tecuapetla, F., & Costa, R. M. (2014). Basal ganglia subcircuits distinctively encode the parsing and concatenation of action sequences. *Nature Neuroscience*, *17*(3), 423–430.
- Jones, J. L., Day, J. J., Wheeler, R. A., & Carelli, R. M. (2010). The basolateral amygdala differentially regulates conditioned neural responses within the nucleus accumbens core and shell. *Neuroscience*, *169*(3), 1186–1198.
- Jongen-Rêlo, A. L., Voorn, P., & Groenewegen, H. J. (1994). Immunohistochemical characterization of the shell and core territories of the nucleus accumbens in the rat. *European Journal of Neuroscience*, *6*(8), 1255–1264.
- Joseph, D. L., & Newman, D. A. (2010). Emotional Intelligence: An Integrative Meta-Analysis and Cascading Model. *Journal of Applied Psychology*, *95*(1), 54–78.
- Judice-Daher, Danielle M, Tavares, T. F., & Bueno, L. O. (2012). Involvement of the basolateral complex and central nucleus of amygdala in the omission effects of different magnitudes of reinforcement. *Behavioural Brain Research*, *233*, 149–156.
- Judice-Daher, Danielle Marcilio, Tavares, T. F., & Bueno, J. L. O. (2011). Influence of the reinforcement magnitude on omission effects. *Behavioural Processes*, *88*(1), 60–62.
- Jupp, B., Caprioli, D., & Dalley, J. W. (2013). Highly impulsive rats: modelling an endophenotype to determine the neurobiological, genetic and environmental mechanisms of addiction. *Disease Models & Mechanisms*, *6*(2), 302–311.

- Jupp, Bianca, Caprioli, D., Saigal, N., Reverte, I., Shrestha, S., Cumming, P., Everitt, B. J., Robbins, T. W., & Dalley, J. W. (2013). Dopaminergic and GABA-ergic markers of impulsivity in rats: evidence for anatomical localisation in ventral striatum and prefrontal cortex. *European Journal of Neuroscience*, *37*(9), 1519–1528.
- Jupp, Bianca, Sawiak, S. J., van der Veen, B., Lemstra, S., Toschi, C., Barlow, R. L., Pekcec, A., Bretschneider, T., Nicholson, J. R., Robbins, T. W., & Dalley, J. W. (2020). Diminished myoinositol in ventromedial prefrontal cortex modulates the endophenotype of impulsivity. *Cerebral Cortex*, *30*(5), 3392–3402.
- Kagan, J. (1966). The generality and dynamics of conceptual tempo. *Journal of Abnormal Psychology*, *71*(1), 17–24.
- Karra, D., & Dahm, R. (2010). Transfection techniques for neuronal cells. *Journal of Neuroscience*, *30*(18), 6171–6177.
- Kayir, H., Semenova, S., & Markou, A. (2014). Baseline impulsive choice predicts the effects of nicotine and nicotine withdrawal on impulsivity in rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *48*, 6–13.
- Kello, J. E. (1972). The reinforcement-omission effect on fixed-interval schedules: frustration or inhibition? *Learning and Memory*, *3*, 138–147.
- Kennis, M., Rademaker, A. R., & Geuze, E. (2013). Neural correlates of personality: An integrative review. *Neuroscience and Biobehavioral Reviews*, *37*(1), 73–95.
- Kimberg, D. Y., & Farah, M. J. (1993). A unified account of cognitive impairments following frontal lobe damage: The role of working memory in complex, organized behavior. *Journal of Experimental Psychology: General*, *122*(4), 411–428.

- King, C. P., Palmer, A. A., Woods, L. C. S., Hawk, L. W., Richards, J. B., & Meyer, P. J. (2016). Premature responding is associated with approach to a food cue in male and female heterogeneous stock rats. *Psychopharmacology*, *233*(13), 2593–2605.
- Kirby, K. N., Petry, N. M., & Bickel, W. K. (1999). Heroin addicts have higher discount rates for delayed rewards than non-drug-using controls. *Journal of Experimental Psychology: General*, *128*(1), 78–87.
- Kirmizi-Alsan, E., Bayraktaroglu, Z., Gurvit, H., Keskin, Y. H., Emre, M., & Demiralp, T. (2006). Comparative analysis of event-related potentials during Go/NoGo and CPT: Decomposition of electrophysiological markers of response inhibition and sustained attention. *Brain Research*, *1104*(1), 114–128.
- Klaus, A., Alves da Silva, J., & Costa, R. M. (2019). What, if, and when to move: basal ganglia circuits and self-paced action initiation. *Annual Review of Neuroscience*, *42*(1), 459–483.
- Klingberg, T., Fernell, E., Olesen, P. J., Johnson, M., Gustafsson, P., Dahlström, K., Gillberg, C. G., Forssberg, H., & Westerberg, H. (2005). Computerized training of working memory in children with ADHD - A randomized, controlled trial. *Journal of the American Academy of Child and Adolescent Psychiatry*, *44*(2), 177–186.
- Koda, K., Ago, Y., Cong, Y., Kita, Y., Takuma, K., & Matsuda, T. (2010). Effects of acute and chronic administration of atomoxetine and methylphenidate on extracellular levels of noradrenaline, dopamine and serotonin in the prefrontal cortex and striatum of mice. *Journal of Neurochemistry*, *114*(1), 259-270.
- Kollins, S. H., McClernon, F. J., & Fuemmeler, B. F. (2005). Association between smoking and attention-deficit/hyperactivity disorder symptoms in a population-based sample of young adults. *Archives of General Psychiatry*, *62*(10), 1142–1147.

- Koskinen, T., Haapalinna, A., & Sirviö, J. (2003).  $\alpha$ -adrenoceptor-mediated modulation of 5-HT<sub>2</sub> receptor agonist induced impulsive responding in a 5-choice serial reaction time task. *Pharmacology and Toxicology*, *92*(5), 214–225.
- Kreek, M. J., Nielsen, D. A., Butelman, E. R., & Laforge, S. K. (2005). Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. *Nature Neuroscience*, *8*(11), 1450–1457.
- Kropotov, J. D., & Etlinger, S. C. (1999). Selection of actions in the basal ganglia-thalamocortical circuits: Review and model. *International Journal of Psychophysiology*, *31*(3), 197–217.
- Kuczenski, R., & Segal, D. S. (1997). Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: Comparison with amphetamine. *Journal of Neurochemistry*, *68*(5), 2032–2037
- Kuczenski, R., & Segal, D. S. (2001). Locomotor effects of acute and repeated threshold doses of amphetamine and methylphenidate: relative roles of dopamine and norepinephrine. *The Journal of Pharmacology and Experimental Therapeutics*, *296*(3), 876–883.
- Kupchik, Y. M., Brown, R. M., Heinsbroek, J. A., Lobo, M. K., Schwartz, D. J., & Kalivas, P. W. (2015). Coding the direct/indirect pathways by D1 and D2 receptors is not valid for accumbens projections. *Nature Neuroscience*, *18*(9).
- Lacey, M. G. (1993). Neurotransmitter receptors and ionic conductances regulating the activity of neurones in substantia nigra pars compacta and ventral tegmental area. *Progress in Brain Research*, *99*, 251–276.
- Lak, A., Okun, M., Moss, M. M., Gurnani, H., Farrell, K., Wells, M. J., Reddy, C. B., Kepecs, A., Harris, K. D., & Carandini, M. (2020). Dopaminergic and prefrontal basis of learning from sensory confidence and reward value. *Neuron*, *105*(4), 700-711.e6.

- Lammel, S., Hetzel, A., Häckel, O., Jones, I., Liss, B., & Roeper, J. (2008). Unique properties of mesoprefrontal neurons within a dual mesocorticolimbic dopamine system. *Neuron*, *57*(5), 760–773.
- Lasky, A. K., Weisner, T. S., Jensen, P. S., Hinshaw, S. P., Hechtman, L., Arnold, L. E., Murray, D. W., & Swanson, J. M. (2016). ADHD in context: Young adults' reports of the impact of occupational environment on the manifestation of ADHD. *Social Science and Medicine*, *161*, 160–168.
- Latzman, R. D., Chan, W. Y., & Shishido, Y. (2013). Impulsivity moderates the association between racial discrimination and alcohol problems. *Addictive Behaviors*, *38*(12), 2898–2904.
- Lawrence, A., Clark, L., Labuzetta, J. N., Sahakian, B., & Vyakarnum, S. (2008). The dark side? *Nature*, *456*(7219), 168–169
- le Merre, P., Esmaili, V., Charrière, E., Galan, K., Salin, P. A., Petersen, C. C. H., & Crochet, S. (2018). Reward-based learning drives rapid sensory signals in medial prefrontal cortex and dorsal hippocampus necessary for goal-directed behavior. *Neuron*, *97*(1), 83-91.e5.
- Lee, B., London, E. D., Poldrack, R. A., Farahi, J., Nacca, A., Monterosso, J. R., Mumford, J. A., Bokarius, A. v., Dahlbom, M., Mukherjee, J., Bilder, R. M., Brody, A. L., & Mandelkern, M. A. (2009). Striatal dopamine D2/D3 receptor availability Is reduced in methamphetamine dependence and is linked to impulsivity. *Journal of Neuroscience*, *29*(47), 14734–14740.
- Lejuez, C. W., Aklin, W. M., Zvolensky, M. J., & Pedulla, C. M. (2003). Evaluation of the Balloon Analogue Risk Task (BART) as a predictor of adolescent real-world risk-taking behaviours. *Journal of Adolescence*, *26*(4), 475–479.

- Lerner, D. A., Verheul, I., & Thurik, R. (2019). Entrepreneurship and attention deficit/hyperactivity disorder: a large-scale study involving the clinical condition of ADHD. *Small Business Economics*, *53*(2), 381–392.
- Lhommée, E., Klinger, H., Thobois, S., Schmitt, E., Ardouin, C., Bichon, A., Kistner, A., Fraix, V., Xie, J., Aya Kombo, M., Chabards, S., Seigneuret, E., Benabid, A. L., Mertens, P., Polo, G., Carnicella, S., Quesada, J. L., Bosson, J. L., Broussolle, E., ... Krack, P. (2012). Subthalamic stimulation in Parkinson's disease: Restoring the balance of motivated behaviours. *Brain*, *135*(5), 1463–1477.
- Li, C. S. R., Yan, P., Sinha, R., & Lee, T. W. (2008). Subcortical processes of motor response inhibition during a stop signal task. *NeuroImage*, *41*(4), 1352–1363.
- Lieb, A., Weston, M., & Kullmann, D. M. (2019). Designer receptor technology for the treatment of epilepsy. *EBioMedicine*, *43*, 641–649.
- Lijffijt, M., Kenemans, J. L., Verbaten, M. N., & van Engeland, H. (2005). A meta-analytic review of stopping performance in attention-deficit/ hyperactivity disorder: Deficient inhibitory motor control? *Journal of Abnormal Psychology*, *114*(2), 216–222.
- Linnet, J., Mouridsen, K., Peterson, E., Møller, A., Doudet, D. J., & Gjedde, A. (2012). Striatal dopamine release codes uncertainty in pathological gambling. *Psychiatry Research - Neuroimaging*, *204*(1), 55–60.
- Lipszyc, J., & Schachar, R. (2010). Inhibitory control and psychopathology: A meta-analysis of studies using the stop signal task. *Journal of the International Neuropsychological Society*, *16*(6), 1064–1076.
- Logan, G. D., & Cowan, W. B. (1984). On the ability to inhibit thought and action: General and special theories of an act of control. *Psychological Review*, *91*(3), 295–327.

- Long, E. C., Kaneva, R., Vasilev, G., Gerard Moeller, F., & Vassileva, J. (2018). Neurocognitive and psychiatric markers for addiction: Common vs. specific (endo)phenotypes for opiate and stimulant dependence. bioRxiv.
- Loos, M., Staal, J., Smit, A. B., de Vries, T. J., & Spijker, S. (2013). Enhanced alcohol self-administration and reinstatement in a highly impulsive, inattentive recombinant inbred mouse strain. *Frontiers in Behavioral Neuroscience*, 7(151).
- Loree, A. M., Lundahl, L. H., & Ledgerwood, D. M. (2015). Impulsivity as a predictor of treatment outcome in substance use disorders: Review and synthesis. *Drug and Alcohol Review*, 34(2), 119–134.
- Lovic, V., Saunders, B. T., Yager, L. M., & Robinson, T. E. (2011). Rats prone to attribute incentive salience to reward cues are also prone to impulsive action. *Behavioural Brain Research*, 223(2), 255–261.
- Luman, M., Oosterlaan, J., & Sergeant, J. A. (2005). The impact of reinforcement contingencies on AD/HD: A review and theoretical appraisal. *Clinical Psychology Review*, 25(2), 183–213.
- Lustig, C., Kozak, R., Sarter, M., Young, J. W., & Robbins, T. W. (2013). CNTRICS final animal model task selection: Control of attention. *Neuroscience and Biobehavioral Reviews*, 37, 2099–2110.
- Lyvers, M. (2000). “Loss of control” in alcoholism and drug addiction: A neuroscientific interpretation. *Experimental and Clinical Psychopharmacology*, 8(2), 225–249.
- Ma, C. L., Qi, X. L., Peng, J. Y., & Li, B. M. (2003). Selective deficit in no-go performance induced by blockade of prefrontal cortical  $\alpha$ 2-adrenoceptors in monkeys. *Neuroreport*, 14(7), 1013–1016.

- Ma, C.-L., Arnsten, A. F. T., & Li, B.-M. (2005). Locomotor hyperactivity induced by blockade of prefrontal cortical 2-adrenoceptors in monkeys. *Biological Psychiatry*, *57*(2), 192–195.
- Ma, I., van Duijvenvoorde, A., & Scheres, A. (2016). The interaction between reinforcement and inhibitory control in ADHD: A review and research guidelines. *Clinical Psychology Review*, *44*, 94–111.
- Magid, V., & Colder, C. R. (2007). The UPPS Impulsive Behavior Scale: Factor structure and associations with college drinking. *Personality and Individual Differences*, *43*(7), 1927–1937.
- Mai, B., Sommer, S., & Hauber, W. (2012). Motivational states influence effort-based decision making in rats: The role of dopamine in the nucleus accumbens. *Cognitive, Affective and Behavioral Neuroscience*, *12*(1), 74–84.
- Mao, Z. M., Arnsten, A. F. T., & Li, B. M. (1999). Local infusion of an  $\alpha$ -1 adrenergic agonist into the prefrontal cortex impairs spatial working memory performance in monkeys. *Biological Psychiatry*, *46*(9), 1259–1265.
- Marczinski, C. A., & Fillmore, M. T. (2003). Preresponse cues reduce the impairing effects of alcohol on the execution and suppression of responses. *Experimental and Clinical Psychopharmacology*, *11*(1), 110–117.
- Marusich, J. A., & Bardo, M. T. (2009). Differences in impulsivity on a delay discounting task predict self-administration of a low unit dose of methylphenidate in rats. *Behavioural Pharmacology*, *20*(5–6), 447–454.
- McCrae, R. R., & Costa, P. T. J. (1990). *Personality in adulthood*. (G. Press, Ed.).
- McGaughy, J., & Sarter, M. (1995). Behavioral vigilance in rats: task validation and effects of age, amphetamine, and benzodiazepine receptor ligands. *Psychopharmacology*, *117*(3), 340–357.

- McHose, J. H., & Gavelek, J. R. (1969). The frustration effect as a function of training magnitude: Within-and between-Ss designs. *Psychonomic Science*, *17*(5), 261–262.
- McNamara, R., Dalley, J. W., Robbins, T. W., Everitt, B. J., & Belin, D. (2010). Trait-like impulsivity does not predict escalation of heroin self-administration in the rat. *Psychopharmacology*, *212*(4), 453–464.
- Mechelmans, D. J., Strelchuk, D., Doñamayor, N., Banca, P., Robbins, T. W., Baek, K., Voon, V., & Leuven -, K. (2017). Reward sensitivity and waiting impulsivity: shift towards reward valuation away from action control. *International Journal of Neuropsychopharmacology*, *20*(12), 971–978.
- Meck, W. H. (1986). Affinity for the dopamine D2 receptor predicts neuroleptic potency in decreasing the speed of an internal clock. *Pharmacology, Biochemistry and Behavior*, *25*(6), 1185–1189.
- Meredith, G. M. (1999). The synaptic framework for chemical signaling in nucleus accumbens. *Annals-New York Academy of Sciences*, *877*, 140–156.
- Meyer, P. J., Cogan, E. S., & Robinson, T. E. (2014). The form of a conditioned stimulus can influence the degree to which it acquires incentive motivational properties. *PLoS ONE*, *9*(6).
- Milstein, J A, Dalley, J., & Robbins, T. (2010). Methylphenidate-induced impulsivity: pharmacological antagonism by  $\beta$ -adrenoreceptor blockade. *Journal of Psychopharmacology*, *24*(3), 309–321.
- Milstein, Jean A, Lehmann, O., Theobald, D. E. H., Dalley, J. W., & Robbins, T. W. (2007). Selective depletion of cortical noradrenaline by anti-dopamine beta-hydroxylase-saporin

impairs attentional function and enhances the effects of guanfacine in the rat. *Psychopharmacology*, *190*, 51–63.

Mirza, N. R., & Stolerman, I. P. (1998). Nicotine enhances sustained attention in the rat under specific task conditions. *Psychopharmacology*, *138*, 266–274.

Moeller, F. G., Dougherty, D. M., Barratt, E. S., Schmitz, J. M., Swann, A. C., & Grabowski, J. (2001). The impact of impulsivity on cocaine use and retention in treatment. *Journal of Substance Abuse Treatment*, *21*(4), 193–198.

Moffitt, T. E., Arseneault, L., Belsky, D., Dickson, N., Hancox, R. J., Harrington, H., Houts, R., Poulton, R., Roberts, B. W., Ross, S., Sears, M. R., Thomson, W. M., & Caspi, A. (2011). A gradient of childhood self-control predicts health, wealth, and public safety. *Proceedings of the National Academy of Sciences*, *108*(7), 2693–2698.

Mogenson, G. J., Jones, D. L., & Yim, C. Y. (1980). From motivation to action: functional interface between the limbic system and the motor system. *Progress in neurobiology*, *14*(2-3), 69-97.

Mohebi, A., Pettibone, J. R., Hamid, A. A., Wong, J.-M. T., Vinson, L. T., Patriarchi, T., Tian, L., Kennedy, R. T., & Berke, J. D. (2019). Dissociable dopamine dynamics for learning and motivation. *Nature*, *570*(7759), 65–70.

Montague, P. (1997). Biological substrates of predictive mechanisms in learning and action choice. *Advances in Psychology*, *121*, 406–421.

Morales, M., & Margolis, E. B. (2017). Ventral tegmental area: Cellular heterogeneity, connectivity and behaviour. *Nature Reviews Neuroscience*, *18*(2), 73–85.

Moreno, M., Economidou, D., Mar, A. C., López-Granero, C., Caprioli, D., Theobald, D. E., Fernando, A., Newman, A. H., Robbins, T. W., & Dalley, J. W. (2013). Divergent effects of

D2/3 receptor activation in the nucleus accumbens core and shell on impulsivity and locomotor activity in high and low impulsive rats. *Psychopharmacology*, 228(1), 19–30.

Morgan, J. I., & Curran, T. (1991). Stimulus-transcription coupling in the nervous system: Involvement of the inducible proto-oncogenes fos and jun. *Annual Review of Neuroscience*, 14, 421–451.

Morin, J.-F. G., Afzali, M. H., Bourque, J., Stewart, S. H., Séguin, J. R., O’leary-Barrett, M., & Conrod, P. J. (2019). A population-based analysis of the relationship between substance use and adolescent cognitive development. *American Journal of Psychiatry*, 176(2), 98–106.

Morris, L. S., Kundu, P., Baek, K., Irvine, M. A., Mechelmans, D. J., Wood, J., Harrison, N. A., Robbins, T. W., Bullmore, E. T., & Voon, V. (2016). Jumping the gun: Mapping neural correlates of waiting impulsivity and relevance across alcohol misuse. *Biological Psychiatry*, 79(6), 499–507.

Mott, A. M., Nunes, E. J., Collins, L. E., Port, R. G., Sink, K. S., Hockemeyer, J., Müller, C. E., & Salamone, J. D. (2009). The adenosine A2A antagonist MSX-3 reverses the effects of the dopamine antagonist haloperidol on effort-related decision making in a T-maze cost/benefit procedure. *Psychopharmacology*, 204(1), 103–112.

Muir, J. L., Everitt, B. J., & Robbins, T. W. (1996). The cerebral cortex of the rat and visual attentional function: Dissociable effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task. *Cerebral Cortex*, 6(3), 470–481.

Murphy, B. A., & Lilienfeld, S. O. (2019). Are self-report cognitive empathy ratings valid proxies for cognitive empathy ability? Negligible meta-analytic relations with behavioral task performance. *Psychological Assessment*, 31(8), 1062–1072.

- Murphy, E. R., Robinson, E. S. J., Theobald, D. E. H., Dalley, J. W., & Robbins, T. W. (2008). Contrasting effects of selective lesions of nucleus accumbens core or shell on inhibitory control and amphetamine-induced impulsive behaviour. *European Journal of Neuroscience*, *28*(2), 353–363.
- Nakahara, T. S., Carvalho, V. M. de A., Souza, M. A. de A., Trintinalia, G. Z., & Papes, F. (2020). Detection of activated mouse neurons with temporal resolution via dual c-Fos staining. *STAR Protocols*, *1*(3), 100153.
- Nambu, A., Tokuno, H., Hamada, I., Kita, H., Imanishi, M., Akazawa, T., Ikeuchi, Y., & Hasegawa, N. (2000). Excitatory conical inputs to pallidal neurons via the subthalamic nucleus in the monkey. *Journal of Neurophysiology*, *84*(1), 289–300.
- Navarra, R., Graf, R., Huang, Y., Logue, S., Comery, T., Hughes, Z., & Day, M. (2008). Effects of atomoxetine and methylphenidate on attention and impulsivity in the 5-choice serial reaction time test. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *32*(1), 34–41.
- Nemeth, C. L., Paine, T. A., Rittiner, J. E., Béguin, C., Carroll, F. I., Roth, B. L., Cohen, B. M., & Carlezon, W. A. (2010). Role of kappa-opioid receptors in the effects of salvinorin A and ketamine on attention in rats. *Psychopharmacology*, *210*(2), 263–274.
- Nicola, S. M. (2010). The flexible approach hypothesis: unification of effort and cue-responding hypotheses for the role of nucleus accumbens dopamine in the activation of reward-seeking behavior. *The Journal of Neuroscience*, *30*(49), 16585–16600.
- Nilsson, S. R. O., Alsiö, J., Somerville, E. M., & Clifton, P. G. (2015). The rat's not for turning: Dissociating the psychological components of cognitive inflexibility. *Neuroscience and Biobehavioral Reviews*, *56*(October 1980), 1–14.

- Niv, Y., Daw, N. D., & Dayan, P. (2005). How fast to work: Response vigor, motivation and tonic dopamine. In W. Y. S. B. & J. Platt (Eds.), *Advances in neural information processing systems* (pp. 1019–1026). MIT Press.
- Niv, Y., Daw, N. D., Joel, D., & Dayan, P. (2007). Tonic dopamine: Opportunity costs and the control of response vigor. *Psychopharmacology*, *191*(3), 507–520.
- Nock, M. K., & Prinstein, M. J. (2004). A functional approach to the assessment of self-mutilative behavior. *Journal of Consulting and Clinical Psychology*, *72*(5), 885–890.
- Oberlin, B. G., & Grahame, N. J. (2009). High-alcohol preferring mice are more impulsive than low-alcohol preferring mice as measured in the delay discounting task. *Alcoholism: Clinical and Experimental Research*, *33*(7), 1294–1303.
- Odum, A. L. (2011). Delay discounting: Trait variable? *Behavioural Processes*, *87*(1), 1–9.
- Opris, I., Lebedev, M., & Nelson, R. J. (2011). Motor planning under unpredictable reward: Modulations of movement vigor and primate striatum activity. *Frontiers in Neuroscience*, *5*, 1–12.
- Ouzir, M. (2013). Impulsivity in schizophrenia: A comprehensive update. *Aggression and Violent Behavior*, *18*(2), 247–254.
- Paine, T. A., Tomasiwicz, H. C., Zhang, K., & Carlezon, W. A. (2007). Sensitivity of the five-choice serial reaction time task to the effects of various psychotropic drugs in sprague-dawley rats. *Biological Psychiatry*, *62*(6), 687–693.
- Pais-Vieira, M., Lima, D., & Galhardo, V. (2007). Orbitofrontal cortex lesions disrupt risk assessment in a novel serial decision-making task for rats. *Neuroscience*, *145*(1), 225–231.
- Papini, M. R. (2003). Comparative psychology of surprising nonreward. *Brain, Behaviour and Evolution*, *62*, 83–95.

- Papini, M. R. (2006). Role of surprising nonreward in associative learning. *The Japanese Journal of Animal Psychology*, *56*(1), 35–54.
- Papini, M. R., & Dudley, T. r. (1997). Consequences of Surprising Reward Omissions. *Review of General Psychology*, *1*(2), 175–197.
- Parent, A., & Hazrati, L. N. (1995). Functional anatomy of the basal ganglia. II. The place of subthalamic nucleus and external pallidum in basal ganglia circuitry. *Brain Research Reviews*, *20*(1), 128–154.
- Park, J., Aragona, B. J., Kile, B. M., Carelli, R. M., & Wightman, R. M. (2010). In vivo voltammetric monitoring of catecholamine release in subterritories of the nucleus accumbens shell. *Neuroscience*, *169*(1), 132–142.
- Parkinson, J. A., Willoughby, P. J., Robbins, T. W., & Everitt, B. J. (2000). Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs pavlovian approach behavior: Further evidence for limbic cortical-ventral striatopallidal systems. *Behavioral Neuroscience*, *114*(1), 42–63.
- Passetti, F., Chudasama, Y., & Robbins, T. W. (2002). The frontal cortex of the rat and visual attentional performance: Dissociable functions of distinct medial prefrontal subregions. *Cerebral Cortex*, *12*(12), 1254–1268.
- Paterson, N. E., Ricciardi, J., Wetzler, C., & Hanania, T. (2011). Sub-optimal performance in the 5-choice serial reaction time task in rats was sensitive to methylphenidate, atomoxetine and d-amphetamine, but unaffected by the COMT inhibitor tolcapone. *Neuroscience Research*, *69*(1), 41–50.

- Pattij, T., Janssen, M. C. W., Vanderschuren, L. J. M. J., Schoffelmeer, A. N. M., & van Gaalen, M. M. (2007). Involvement of dopamine D1 and D2 receptors in the nucleus accumbens core and shell in inhibitory response control. *Psychopharmacology*, *191*(3), 587–598.
- Pattij, T., Schetters, D., Schoffelmeer, A. N. M., & van Gaalen, M. M. (2012). On the improvement of inhibitory response control and visuospatial attention by indirect and direct adrenoceptor agonists. *Psychopharmacology*, *219*(2), 327–340.
- Pattij, T., van Mourik, Y., Diergaarde, L., & de Vries, T. J. (2020). The role of impulsivity as predisposing behavioural trait in different aspects of alcohol self-administration in rats. *Drug and Alcohol Dependence*, *212*, 107984.
- Patton, J. H., Stanford, M. S., & Barratt, E. S. (1995). Factor structure of the barratt impulsiveness scale. *Journal of Clinical Psychology*, *51*(6), 768–774.
- Paxinos, G., & Watson, C. (2007). *The Rat Brain in Stereotaxis Coordinates, Sixth Edition* (Elsevier).
- Peckham, R. H., & Amsel, A. (1967). Within-subject demonstration of a relationship between frustration and magnitude of reward in a differential magnitude of reward discrimination. *Journal of Experimental Psychology*, *73*, 187–195.
- Peña Oliver, Y., Ripley, T. L., & Stephens, D. N. (2009). Ethanol effects on impulsivity in two mouse strains: similarities to diazepam and ketamine. *Psychopharmacology*, *204*, 679–692.
- Peña-Oliver, Y., Giuliano, C., Economidou, D., Goodlett, C. R., Robbins, T. W., Dalley, J. W., & Everitt, B. J. (2015). Alcohol-preferring rats show goal oriented behaviour to food incentives but are neither sign-trackers nor impulsive. *PLoS ONE*, *10*(6), e0131016.

- Perry, J. L., Nelson, S. E., & Carroll, M. E. (2008). Impulsive choice as a predictor of acquisition of IV cocaine self-administration and reinstatement of cocaine-seeking behavior in male and female rats. *Experimental and Clinical Psychopharmacology*, *16*(2), 165–177.
- Peters, D. P., & McHose, J. H. (1974). Effects of varied preshift reward magnitude on successive negative contrast effects in rats. *Journal of Comparative and Physiological Psychology*, *86*(1), 85–95.
- Peters, H., Hunt, M., & Harper, D. (2010). An animal model of slot machine gambling: The effect of structural characteristics on response latency and persistence. *Journal of Gambling Studies*, *26*(4), 521–531.
- Pezze, M. A., Dalley, J. W., & Robbins, T. W. (2009). Remediation of attentional dysfunction in rats with lesions of the medial prefrontal cortex by intra-accumbens administration of the dopamine D2/3 receptor antagonist sulpiride. *Psychopharmacology*, *202*(1–3), 307–313.
- Phillips, B. U., Heath, C. J., Ossowska, Z., Bussey, T. J., & Saksida, L. M. (2017). Optimisation of cognitive performance in rodent operant (touchscreen) testing: Evaluation and effects of reinforcer strength. *Learning and Behaviour*, *45*, 252–262.
- Phillips, P. E. M., Robinson, D. L., Stuber, G. D., Carelli, R. M., & Wightman, R. M. (2003). Real-time measurements of phasic changes in extracellular dopamine concentration in freely moving rats by fast-scan cyclic voltammetry. *Methods in Molecular Medicine*, *79*, 443–464.
- Piantadosi, P. T., Yeates, D. C. M., & Floresco, S. B. (2018). Cooperative and dissociable involvement of the nucleus accumbens core and shell in the promotion and inhibition of actions during active and inhibitory avoidance. *Neuropharmacology*, *138*, 57–71.

- Piantadosi, P. T., Yeates, D. C. M., Wilkins, M., & Floresco, S. B. (2017). Contributions of basolateral amygdala and nucleus accumbens subregions to mediating motivational conflict during punished reward-seeking. *Neurobiology of Learning and Memory*, *140*, 92–105.
- Pietrzak, R. H., Mollica, C. M., Maruff, P., & Snyder, P. J. (2006). Cognitive effects of immediate-release methylphenidate in children with attention-deficit/hyperactivity disorder. *Neuroscience and Biobehavioral Reviews*, *30*(8), 1225–1245.
- Pinto, L., & Dan, Y. (2015). Cell-type-specific activity in prefrontal cortex during goal-directed behavior. *Neuron*, *87*(2), 437–450.
- Plichta, M. M., & Scheres, A. (2014). Ventral-striatal responsiveness during reward anticipation in ADHD and its relation to trait impulsivity in the healthy population: A meta-analytic review of the fMRI literature. *Neuroscience and Biobehavioral Reviews*, *38*, 125–134.
- Pothuizen, H. H. J., Jongen-Rêlo, A. L., Feldon, J., & Yee, B. K. (2005). Double dissociation of the effects of selective nucleus accumbens core and shell lesions on impulsive-choice behaviour and salience learning in rats. *European Journal of Neuroscience*, *22*(10), 2605–2616.
- Poulos, C. X., Le, A. D., & Parker, J. L. (1995). Impulsivity predicts individual susceptibility to high levels of alcohol self-administration. *Behavioural Pharmacology*, *6*, 810–814).
- Radwanska, K., & Kaczmarek, L. (2012). Characterization of an alcohol addiction-prone phenotype in mice. *Addiction Biology*, *17*(3), 601–612.
- Raio, C. M., Konova, A. B., & Ross Otto, A. (2020). trait impulsivity and acute stress interact to influence choice and decision speed during multi-stage decision-making. *Scientific Reports*, *10*(7754), 1–12.

- Rajah, N., Bamiatzi, V., & Williams, N. (2021). How childhood ADHD-like symptoms predict selection into entrepreneurship and implications on entrepreneurial performance. *Journal of Business Venturing*, 36(3), 106091.
- Randall, P. A., Pardo, M., Nunes, E. J., Ló Pez Cruz, L., Vemuri, V. K., Makryannis Alex, Baqi Younis, Muller Christa, Correa Merce, & Salomone John D. (2012). Dopaminergic Modulation of Effort-Related Choice Behavior as Assessed by a Progressive Ratio Chow Feeding Choice Task: Pharmacological Studies and the Role of Individual Differences. *Plos One*, 7(10), 1–10.
- Rescorla R. A., & Wagner A. R. (1972). A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement. In Black A., & Prokasy W. R. (Eds.) *Classical Conditioning II*, (pp. 64–99). New York, NY: Academic Press.
- Redgrave, P., Prescott, T. J., & Gurney, K. (1999). The basal ganglia: A vertebrate solution to the selection problem? *Neuroscience*, 89(4), 1009–1023.
- Reynolds, B., Ortengren, A., Richards, J. B., & de Wit, H. (2006). Dimensions of impulsive behavior: Personality and behavioral measures. *Personality and Individual Differences*, 40(2), 305–315.
- Reynolds, S. M., & Berridge, K. C. (2002). Positive and negative motivation in nucleus accumbens shell: Bivalent rostrocaudal gradients for GABA-elicited eating, taste “liking”/ “disliking” reactions, place preference/avoidance, and fear. *The Journal of Neuroscience*, 22(16), 7308–7320.
- Rhodes, S. M., Coghill, D. R., & Matthews, K. (2006). Acute neuropsychological effects of methylphenidate in stimulant drug-naïve boys with ADHD II - Broader executive and non-executive domains. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 47(11), 1184–1194.

- Richards, J. B., Sabol, K. E., & Seiden, L. S. (1993). DRL Interresponse-time distributions: Quantification by peak deviation analysis. *Journal of the Experimental Analysis of Behavior*, *60*(2), 361–385.
- Rivalan, M., Ahmed, S. H., & Dellu-Hagedorn, F. (2009). Risk-prone individuals prefer the wrong options on a rat version of the Iowa Gambling Task. *Biological Psychiatry*, *66*(8), 743–749.
- Robbins, T., & Cole, B. (1987). Amphetamine impairs the discriminative performance of rats with dorsal noradrenergic bundle lesions on a 5-choice serial reaction time task: new evidence for central dopaminergic-noradrenergic interactions. *Psychopharmacology*, *91*(4), 458–466.
- Robbins, T. W. (2002). The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. *Psychopharmacology*, *163*, 362–380.
- Robbins, T. W., & Everitt, B. J. (2007). A role for mesencephalic dopamine in activation: Commentary on Berridge (2006). *Psychopharmacology*, *191*(3), 433–437.
- Robbins, Trevor W., Gillan, C. M., Smith, D. G., de Wit, S., & Ersche, K. D. (2012). Neurocognitive endophenotypes of impulsivity and compulsivity: Towards dimensional psychiatry. *Trends in Cognitive Sciences*, *16*(1), 81–91.
- Roberts, B. W., & Jackson, J. J. (2008). Sociogenomic personality psychology. *Journal of Personality*, *76*(6), 1523–1544.
- Roberts, D. C. S., & Richardson, N. R. (1992). Self-administration of psychomotor stimulants using progressive ratio schedules of reinforcement. In A. Boulton, G. Baker, & P. Wu (Eds.), *Animal Models of Drug Addiction. Neuromethods* (pp. 233–269). Humana Press.

- Robinson, E. S.J., Eagle, D. M., Economidou, D., Theobald, D. E. H., Mar, A. C., Murphy, E. R., Robbins, T. W., & Dalley, J. W. (2009). Behavioural characterisation of high impulsivity on the 5-choice serial reaction time task: Specific deficits in “waiting” versus “stopping.” *Behavioural Brain Research*, *196*(2), 310–316.
- Robinson, Emma S J. (2012). Blockade of noradrenaline re-uptake sites improves accuracy and impulse control in rats performing a five-choice serial reaction time tasks. *Psychopharmacology*, *219*(2), 303–312.
- Robinson, Emma S.J., Eagle, D. M., Mar, A. C., Bari, A., Banerjee, G., Jiang, X., Dalley, J. W., & Robbins, T. W. (2008). Similar effects of the selective noradrenaline reuptake inhibitor atomoxetine on three distinct forms of impulsivity in the rat. *Neuropsychopharmacology*, *33*(5), 1028–1037.
- Robinson, M. J. F., & Anselme, P. (2019). How uncertainty sensitizes dopamine neurons and invigorates amphetamine-related behaviors. *Neuropsychopharmacology*, *44*(2), 237–238.
- Robinson, M. J. F., Anselme, P., Fischer, A. M., & Berridge, K. C. (2014). Initial uncertainty in Pavlovian reward prediction persistently elevates incentive salience and extends sign-tracking to normally unattractive cues. *Behavioural Brain Research*, *266*, 119–130.
- Robinson, T. E., & Flagel, S. B. (2009). Dissociating the predictive and incentive motivational properties of reward-related cues through the study of individual differences. *Biological Psychiatry*, *65*(10), 869–873.
- Robinson, T. E., Yager, L. M., Cogan, E. S., & Saunders, B. T. (2014). On the motivational properties of reward cues: Individual differences. *Neuropharmacology*, *76*, 450–459.
- Rogers, R. D., Baunez, C., Everitt, B. J., & Robbins, T. W. (2001). Lesions of the medial and lateral striatum in the rat produce differential deficits in attentional performance. *Behavioral Neuroscience*, *115*(4), 799–811.

- Romer, D., Betancourt, L., Giannetta, J. M., Brodsky, N. L., Farah, M., & Hurt, H. (2009). Executive cognitive functions and impulsivity as correlates of risk taking and problem behavior in preadolescents. *Neuropsychologia*, *47*(13), 2916–2926.
- Roos, L. E., Pears, K., Bruce, J., Kim, H. K., & Fisher, P. A. (2015). Impulsivity and the association between the feedback-related negativity and performance on an inhibitory control task in young at-risk children. *Physiology*, *52*(5), 704–713.
- Root, D. H., Mejias-Aponte, C. A., Zhang, S., Wang, H. L., Hoffman, A. F., Lupica, C. R., & Morales, M. (2014). Single rodent mesohabenular axons release glutamate and GABA. *Nature Neuroscience*, *17*(11), 1543–1551.
- Rosval, L., Steiger, H., Bruce, K., Israel, M., Richardson, J., & Aubut, M. (2006). Impulsivity in women with eating disorders: Problem of response inhibition, planning, or attention? *International Journal of Eating Disorders*, *39*, 590–593.
- Rosvold, H. E., Mirsky, A. F., Sarason, I., Bransome, E. D., Jr., & Beck, L. H. (1965). A continuous performance test of brain damage. *Journal of Consulting Psychology*, *20*, 343–350.
- Salamone, J D, Correa, M., Farrar, A., & Mingote, S. M. (2007). Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. *Psychopharmacology*, *191*, 461–482.
- Salamone, J D, Correa, M., Mingote, S. M., & Weber, S. M. (2005). Beyond the reward hypothesis: alternative functions of nucleus accumbens dopamine. *Current Opinion in Pharmacology*, *5*, 34–41.
- Salamone, J. D., Steinpreis, R. E., McCullough, L. D., Smith, P., Grebel, D., & Mahan, K. (1991). Haloperidol and nucleus accumbens dopamine depletion suppress lever pressing for food but increase free food consumption in a novel food choice procedure. *Psychopharmacology*, *104*(4), 515–521.

- Salamone, John D. (2006). Will the last person who uses the term “reward” please turn out the lights? Comments on processes related to reinforcement, learning, motivation and effort. *Addiction Biology*, *11*(1), 43–44.
- Salamone, John D., & Correa, M. (2002). Motivational views of reinforcement: Implications for understanding the behavioral functions of nucleus accumbens dopamine. *Behavioural Brain Research*, *137*(1–2), 3–25.
- Salamone, John D., & Correa, M. (2012). The mysterious motivational functions of mesolimbic dopamine. *Neuron*, *76*(3), 470–485.
- Salamone, John D., Correa, M., Farrar, A. M., Nunes, E. J., & Pardo, M. (2009). Dopamine, behavioral economics, and effort. *Frontiers in Behavioral Neuroscience*, *3*, 1–12.
- Salamone, John D., Correa, M., Nunes, E. J., Randall, P. A., & Pardo, M. (2012). The behavioural pharmacology of effort related choice behaviour: dopamine, adenosine and beyond. *Journal of the Experimental Analysis of Behavior*, *97*(1), 125–146.
- Salamone, John D., Cousins, M. S., & Bucher, S. (1994). Anhedonia or anergia? Effects of haloperidol and nucleus accumbens dopamine depletion on instrumental response selection in a T-maze cost/benefit procedure. *Behavioural Brain Research*, *65*(2), 221–229.
- Sanchez-Roige, S., Baro, V., Trick, L., Peña-Oliver, Y., Stephens, D. N., & Duka, T. (2014). Exaggerated waiting impulsivity associated with human binge drinking, and high alcohol consumption in mice. *Neuropsychopharmacology*, *39*(13), 2919–2927.
- Saunders, B. T., Richard, J. M., Margolis, E. B., & Janak, P. H. (2018a). Dopamine neurons create Pavlovian conditioned stimuli with circuit-defined motivational properties. *Nature Neuroscience*, *21*(8), 1072–1083.

- Saunders, B. T., Richard, J. M., Margolis, E. B., & Janak, P. H. (2018b). Dopamine neurons create pavlovian conditioned stimuli with circuit-defined motivational properties. *Nature Neuroscience*, *21*(8), 1072–1083.
- Saunders, B. T., & Robinson, T. E. (2010). A cocaine cue acts as an incentive stimulus in some but not others: Implications for addiction. *Biological Psychiatry*, *67*(8), 730–736.
- Saunders, B. T., & Robinson, T. E. (2012). The role of dopamine in the accumbens core in the expression of pavlovian-conditioned responses. *European Journal of Neuroscience*, *36*(4), 2521–2532.
- Scheres, A., Oosterlaan, J., Swanson, J., Morein-Zamir, S., Meiran, N., Schut, H., Vlasveld, L., & Sergeant, J. A. (2003). The effect of methylphenidate on three forms of response inhibition in boys with AD/HD. *Journal of Abnormal Child Psychology*, *31*(1), 105–120.
- Schippers, M. C., Binnekade, R., Schoffelmeer, A. N. M., Pattij, T., & de Vries, T. J. (2012). Unidirectional relationship between heroin self-administration and impulsive decision-making in rats. *Psychopharmacology*, *219*(2), 443–452.
- Schmitz, Y., Schmauss, C., & Sulzer, D. (2002). Altered dopamine release and uptake kinetics in mice lacking D2 receptors. *Journal of Neuroscience*, *22*(18), 8002–8009.
- Schultz, W., Dayan, P., & Montague, P. R. (1997). A neural substrate of prediction and reward. *Science*, *275*(5306), 1593–1599.
- Schultz, Wolfram. (2007). Multiple dopamine functions at different time courses. *Annual Review of Neuroscience*, *30*, 259–288.
- Schweri, M. M., Skolnick, P., Rafferty, M. F., Rice, K. C., Janowsky, A. J., & Paul, S. M. (1985). [3H]Threo-(±)-methylphenidate binding to 3,4-dihydroxyphenylethylamine uptake

- sites in corpus striatum: Correlation with the stimulant properties of ritalinic acid esters. *Journal of Neurochemistry*, 45(4), 1062–1070.
- Semenova, S., & Markou, A. (2007). The effects of the mGluR5 antagonist MPEP and the mGluR2/3 antagonist LY341495 on rats' performance in the 5-choice serial reaction time task. *Neuropharmacology*, 52(3), 863–872.
- Seward, J. P., Pereboom, A. C., Butler, B., & Jones, R. B. (1957). The role of prefeeding in an apparent frustration effect. *Journal of Experimental Psychology*, 54, 445–450.
- Shao, R., Read, J., Behrens, T. E., & Rogers, R. D. (2013). Shifts in reinforcement signalling while playing slot-machines as a function of prior experience and impulsivity. *Translational Psychiatry*, 3, e213.
- Sharma, L., Markon, K. E., & Clark, L. A. (2014). Toward a theory of distinct types of “impulsive” behaviors: A meta-analysis of self-report and behavioral measures. *Psychological Bulletin*, 140(2), 374–408.
- Sheffer, C. E., Bickel, W. K., Brandon, T. H., Franck, C. T., Deen, D., Panissidi, L., Abdali, S. A., Pittman, J. C., Lunden, S. E., Prashad, N., Malhotra, R., & Mantovani, A. (2018). Preventing relapse to smoking with transcranial magnetic stimulation: Feasibility and potential efficacy. *Drug and Alcohol Dependence*, 182(May 2017), 8–18.
- Shettleworth, S., & Nevin, J. A. (1965). Relative rate of response and relative magnitude of reinforcement in multiple schedules. *Journal of the Experimental Analysis of Behavior*, 8, 199-2-2.
- Shima, K., & Tanji, J. (2006). Binary-coded monitoring of a behavioral sequence by cells in the pre-supplementary motor area. *Journal of Neuroscience*, 26(9), 2579–2582.

- Simon, N. W., Gilbert, R. J., Mayse, J. D., Bizon, J. L., & Setlow, B. (2009). Balancing risk and reward: A rat model of risky decision making. *Neuropsychopharmacology*, *34*(10), 2208–2217.
- Sirviö, J., Jäkälä, P., Mazurkiewicz, M., Haapalinna, A., Riekkinen, P., & Riekkinen, P. J. (1993). Dose- and parameter-dependent effects of atipamezole, an  $\alpha$ 2-antagonist, on the performance of rats in a five-choice serial reaction time task. *Pharmacology, Biochemistry and Behavior*, *45*(1), 123–129.
- Sirviö, J., Mazurkiewicz, M., Haapalinna, A., Riekkinen, P., Lahtinen, H., & Riekkinen, P. J. (1994). The effects of selective alpha-2 adrenergic agents on the performance of rats in a 5-choice serial reaction time task. *Brain Research Bulletin*, *35*(5–6), 451–455.
- Smillie, L. D., & Jackson, C. J. (2006). Functional impulsivity and reinforcement sensitivity theory. *Journal of Personality*, *74*(1), 47–83.
- Smith, G. T. (2005). On construct validity: Issues of method and measurement. *Psychological Assessment*, *17*(4), 396–408.
- Smith, G. T., & Cyders, M. A. (2016). Integrating affect and impulsivity: The role of positive and negative urgency in substance use risk. *Drug and Alcohol Dependence*, *163*, S3–S12.
- Smith, Y., Raju, D. v., Pare, J. F., & Sidibe, M. (2004). The thalamostriatal system: A highly specific network of the basal ganglia circuitry. *Trends in Neurosciences*, *27*(9), 520–527.
- Soares, S., Atallah, B. v., & Paton, J. J. (2016). Midbrain dopamine neurons control judgment of time. *Science*, *354*(6317), 1273–1277.
- Soares-Cunha, C., Coimbra, B., Sousa, N., & Rodrigues, A. J. (2016). Reappraising striatal D1- and D2-neurons in reward and aversion. *Neuroscience and Biobehavioral Reviews*, *68*, 370–386.

- Solanto, M. V. (2002). Dopamine dysfunction in AD/HD: Integrating clinical and basic neuroscience research. *Behavioural Brain Research*, 130(1–2), 65–71.
- Solanto, M. V. (1998). Neuropsychopharmacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: a review and integration. *Behavioural Brain Research*, 94(1), 127–152.
- Staddon, J. E. R. (2001). *Adaptive Dynamics*. The MIT Press.
- Stanford, M. S., Mathias, C. W., Dougherty, D. M., Lake, S. L., Anderson, N. E., & Patton, J. H. (2009). Fifty years of the Barratt Impulsiveness Scale: An update and review. *Personality and Individual Differences*, 47(5), 385–395.
- Stein, J. S., Renda, C. R., Barker, S. M., Liston, K. J., Shahan, T. A., & Madden, G. J. (2015). Impulsive choice predicts anxiety-like behavior, but not alcohol or sucrose consumption, in male long-evans rats. *Alcoholism: Clinical and Experimental Research*, 39(5), 932–940.
- Steinberg, E. E., Keiflin, R., Boivin, J. R., Witten, I. B., Deisseroth, K., & Janak, P. H. (2013). A causal link between prediction errors, dopamine neurons and learning. *Nature Neuroscience*, 16(7), 966–973.
- Steinberg, L., Albert, D., Cauffman, E., Banich, M., Graham, S., & Woolard, J. (2008). Age differences in sensation seeking and impulsivity as indexed by behavior and self-report: Evidence for a dual systems model. *Developmental Psychology*, 44(6), 1764–1778.
- Stojek, M. M., Fischer, S., Murphy, C. M., & MacKillop, J. (2014). The role of impulsivity traits and delayed reward discounting in dysregulated eating and drinking among heavy drinkers. *Appetite*, 80, 81–88.

- Stout, S. C., Boughner, R. L., & Papini, M. R. (2003). Reexamining the frustration effect in rats: Aftereffects of surprising reinforcement and nonreinforcement. *Learning and Motivation*, *34*, 437–456.
- Stricklan, J., & Johnson, M. W. (2021). Rejecting impulsivity as a psychological construct: A theoretical, empirical, and sociocultural argument. *Psychological Review*, *128*(2), 336–361.
- Sugam, J. A., Day, J. J., Wightman, R. M., & Carelli, R. M. (2012). Phasic nucleus accumbens dopamine encodes risk-based decision-making behavior. *Biological Psychiatry*, *71*(3), 199–205
- Sun, H., Cocker, P. J., Zeeb, F. D., & Winstanley, C. A. (2012). Chronic atomoxetine treatment during adolescence decreases impulsive choice, but not impulsive action, in adult rats and alters markers of synaptic plasticity in the orbitofrontal cortex. *Psychopharmacology*, *219*(2), 285–301.
- Swann, A. C., Steinberg, J. L., Lijffijt, M., & Moeller, F. G. (2008). Impulsivity: Differential relationship to depression and mania in bipolar disorder. *Journal of Affective Disorders*, *106*(3), 241–248.
- Swanson, C. J., Perry, K. W., Koch-Krueger, S., Katner, J., Svensson, K. A., & Bymaster, F. P. (2006). Effect of the attention deficit/hyperactivity disorder drug atomoxetine on extracellular concentrations of norepinephrine and dopamine in several brain regions of the rat. *Neuropharmacology*, *50*(6), 755–760.
- Sych, Y., Chernysheva, M., Sumanovski, L. T., & Helmchen, F. (2019). High-density multi-fiber photometry for studying large-scale brain circuit dynamics. *Nature Methods*, *16*(6), 553–560.

- Syed, E. C. J., Grima, L. L., Magill, P. J., Bogacz, R., Brown, P., & Walton, M. E. (2016). Action initiation shapes mesolimbic dopamine encoding of future rewards. *Nature Neuroscience*, *19*(1), 34–36.
- Terra, H., Bruinsma, B., de Kloet, S. F., van der Roest, M., Pattij, T., & Mansvelder, H. D. (2020). Prefrontal cortical projection neurons targeting dorsomedial striatum control behavioral inhibition. *Current Biology*, *30*(21), 4188-4200.e5.
- Threlfell, S., Lalic, T., Platt, N. J., Jennings, K. A., Deisseroth, K., & Cragg, S. J. (2012). Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons. *Neuron*, *75*(1), 58–64.
- Tomlinson, A., Grayson, B., Marsh, S., Harte, M. K., Barnes, S. A., Marshall, K. M., & Neill, J. C. (2014). Pay attention to impulsivity: Modelling low attentive and high impulsive subtypes of adult ADHD in the 5-choice continuous performance task (5C-CPT) in female rats. *European Neuropsychopharmacology*, *24*(8), 1371–1380.
- Tomlinson, A., Grayson, B., Marsh, S., Hayward, A., Marshall, K. M., & Neill, J. C. (2015). Putative therapeutic targets for symptom subtypes of adult ADHD: D4 receptor agonism and COMT inhibition improve attention and response inhibition in a novel translational animal model. *European Neuropsychopharmacology*, *25*(4), 454–467.
- Treadway, M. T., Buckholtz, J. W., Schwartzman, A. N., Lambert, W. E., & Zald, D. H. (2009). Worth the “EEfRT”? The effort expenditure for rewards task as an objective measure of motivation and anhedonia. *PLoS ONE*, *4*(8), 1–9.
- Tricklebank, M. D., Robbins, T. W., Simmons, C., & Wong, E. H. F. (2021). Time to re-engage psychiatric drug discovery by strengthening confidence in preclinical psychopharmacology. *Psychopharmacology*.

- Tripathi, A., Prensa, L., Cebrián, C., & Mengual, E. (2010). Axonal branching patterns of nucleus accumbens neurons in the rat. *The Journal of Comparative Neurology*, *518*(22), 4649–4673.
- Turner, Karly M., & Parkes, S. L. (2020). Prefrontal regulation of behavioural control: Evidence from learning theory and translational approaches in rodents. *Neuroscience and Biobehavioral Reviews*, *118*(July), 27–41.
- Turner, Karly M., Peak, J., & Burne, T. H. J. (2016). Measuring attention in rodents: comparison of a modified signal detection task and the 5-Choice serial reaction time task. *Frontiers in Behavioral Neuroscience*, *9*, 370.
- Turner, Karly M., Peak, J., & Burne, T. H. J. (2017). Baseline-dependent effects of amphetamine on attention are associated with striatal dopamine metabolism OPEN. *Scientific Reports*, *7*(297), 1–10.
- Turner, Karly Maree. (2016). *Establishing a novel cognitive task in rats of relevance to schizophrenia*.
- Uebel, H., Albrecht, B., Asherson, P., Börger, N. A., Butler, L., Chen, W., Christiansen, H., Heise, A., Kuntsi, J., Schäfer, U., Andreou, P., Manor, I., Marco, R., Miranda, A., Mulligan, A., Oades, R. D., van der Meere, J., Faraone, S. v., Rothenberger, A., & Banaschewski, T. (2010). Performance variability, impulsivity errors and the impact of incentives as gender-independent endophenotypes for ADHD. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *51*(2), 210–218.
- Upadhyaya, H. P., Desai, D., Schuh, K. J., Bymaster, F. P., Kallman, M. J., Clarke, D. O., Durell, T. M., Trzepacz, P. T., Calligaro, D. O., Nisenbaum, E. S., Emmerson, P. J., Schuh, L. M., Bickel, W. K., & Allen, A. J. (2013). A review of the abuse potential assessment of atomoxetine: a nonstimulant medication for attention-deficit/hyperactivity disorder. *Psychopharmacology*, *226*, 189–200.

- Valjent, E., & Gangarossa, G. (2020). The Tail of the Striatum: From Anatomy to Connectivity and Function. *Trends in Neurosciences*, 1–12.
- Vallone, D., Picetti, R., & Borrelli, E. (2000). Structure and function of dopamine receptors. *Neuroscience and Biobehavioral Reviews*, 24(1), 125–132.
- van den Bergh, F., Spronk, M., Ferreira, L., Bloemarts, E., Groenink, L., Olivier, B., & Oosting, R. (2006). Relationship of delay aversion and response inhibition to extinction learning, aggression, and sexual behaviour. *Behavioural Brain Research*, 175(1), 75–81.
- van den Bos, R., Lasthuis, W., den Heijer, E., van der Harst, J., & Spruijt, B. (2006). Toward a rodent model of the Iowa gambling task. *Behavior Research Methods*, 38(3), 470–478.
- van Gaalen, M. M., van Koten, R., Schoffelmeer, A. N. M., & Vanderschuren, L. J. M. J. (2006). Critical Involvement of Dopaminergic Neurotransmission in Impulsive Decision Making. *Biological Psychiatry*.
- van Mourik, R., Oosterlaan, J., & Sergeant, J. A. (2005). The Stroop revisited: A meta-analysis of interference control in AD/HD. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 46(2), 150–165.
- Vassileva, J., & Conrod, P. J. (2019). Impulsivities and addictions: a multidimensional integrative framework informing assessment and interventions for substance use disorders. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 374(1766), 20180137.
- Verbruggen, F., Chambers, C. D., Lawrence, N. S., & McLaren, I. P. L. (2017). Winning and losing: Effects on impulsive action. *Journal of Experimental Psychology: Human Perception and Performance*, 43(1), 147–168.

- Verheul, I., Block, J., Burmeister-Lamp, K., Thurik, R., Tiemeier, H., & Turturea, R. (2015). ADHD-like behavior and entrepreneurial intentions. *Small Business Economics*, *45*(1), 85–101.
- Voon, V. (2014). Models of impulsivity with a focus on waiting impulsivity: translational potential for neuropsychiatric disorders. *Current Addiction Reports*, *1*, 281–288.
- Voon, V., Chang-Webb, Y. C., Morris, L. S., Cooper, E., Sethi, A., Baek, K., Grant, J., Robbins, T. W., & Harrison, N. A. (2016). Waiting impulsivity: The influence of acute methylphenidate and feedback. *International Journal of Neuropsychopharmacology*, *19*(1), 1–10.
- Voon, V., Irvine, M. A., Derbyshire, K., Worbe, Y., Lange, I., Abbott, S., Morein-Zamir, S., Dudley, R., Caprioli, D., Harrison, N. A., Wood, J., Dalley, J. W., Bullmore, E. T., Grant, J. E., & Robbins, T. W. (2014). Measuring “waiting” impulsivity in substance addictions and binge eating disorder in a novel analogue of rodent serial reaction time task. *Biological Psychiatry*, *75*(2), 148–155.
- Walker, S. E., Peña-Oliver, Y., & Stephens, D. N. (2011). Learning not to be impulsive: Disruption by experience of alcohol withdrawal. *Psychopharmacology*, *217*(3), 433–442.
- Wallace, J. F., & Newman, J. P. (1990). Differential effects of reward and punishment cues on response speed in anxious and impulsive individuals. *Personality and Individual Differences*, *11*(10), 999–1009.
- Walton, M. E., & Bouret, S. (2019). What Is the Relationship between Dopamine and Effort? *Trends in Neurosciences*, *42*(2), 79–91.
- Wardle, M. C., Treadway, M. T., Mayo, L. M., Zald, D. H., & de Wit, H. (2011). Amping up effort: effects of d-amphetamine on human effort-based decision-making. *The Journal of Neuroscience*, *31*(46), 16597–16602.

- Wassum, K. M., Ostlund, S. B., & Maidment, N. T. (2012). Phasic mesolimbic dopamine signaling precedes and predicts performance of a self-initiated action sequence task. *Biological Psychiatry, 71*, 846–854.
- Werkman, T. R., Kruse, C. G., Nievelstein, H., Long, S. K., & Wadman, W. J. (1999). Neurotensin attenuates the quinpirole-induced inhibition of the firing rate of dopamine neurons in the rat substantia nigra pars compacta and the ventral tegmental area. *Neuroscience, 95*(2), 417–423.
- Whelan, R., Conrod, P. J., Poline, J. B., Lourdasamy, A., Banaschewski, T., Barker, G. J., Bellgrove, M. A., Büchel, C., Byrne, M., Cummins, T. D. R., Fauth-Bühler, M., Flor, H., Gallinat, J., Heinz, A., Ittermann, B., Mann, K., Martinot, J. L., Lalor, E. C., Lathrop, M., ... Garavan, H. (2012). Adolescent impulsivity phenotypes characterized by distinct brain networks. *Nature Neuroscience, 15*(6), 920–925.
- White, H. A., & Shah, P. (2011). Creative style and achievement in adults with attention-deficit/hyperactivity disorder. *Personality and Individual Differences, 50*(5), 673–677.
- Whiteside, S. P., & Lynam, D. R. (2001). The five factor model and impulsivity: Using a structural model of personality to understand impulsivity. *Personality and Individual Differences, 30*(4), 669–689.
- Wilkinson, R. T. (1963). Interaction of noise with knowledge of results and sleep deprivation. *Journal of Experimental Psychology, 66*(4), 332–337.
- Willcutt, E. G., Doyle, A. E., Nigg, J. T., Faraone, S. v., & Pennington, B. F. (2005). Validity of the executive function theory of Attention-Deficit/Hyperactivity Disorder: A meta-analytic review. *Biological Psychiatry, 57*, 1136–1346.

- Williams, J., & Dayan, P. (2005). Dopamine, learning and impulsivity : A biological account of Attention-Deficit / Hyperactivity Disorder. *Journal of Child and Adolescent Psychopharmacology*, *15*(2), 160–179.
- Wilton, R. N., Strongmiiian, K. T., & Nerenberg, A. (1969). Some effects of frustration in a free responding operant situation. *Quarterly Journal of Experimental Psychology*, *21*, 367–380.
- Winstanley, C. A. (2011). The utility of rat models of impulsivity in developing pharmacotherapies for impulse control disorders. *British Journal of Pharmacology*, *164*(4), 1301–1321.
- Winstanley, C. A., Baunez, C., Theobald, D. E. H., & Robbins, T. W. (2005). Lesions to the subthalamic nucleus decrease impulsive choice but impair autoshaping in rats: The importance of the basal ganglia in Pavlovian conditioning and impulse control. *European Journal of Neuroscience*, *21*(11), 3107–3116.
- Winstanley, C. A., Dalley, J. W., & Robbins, T. W. (2005). Interactions between serotonin and dopamine in the control of impulsive choice in rats: Therapeutic implications for impulse control disorders article. *Neuropsychopharmacology*, *30*, 669-682
- Winstanley, C. A., Eagle, D. M., & Robbins, T. W. (2006). Behavioral models of impulsivity in relation to ADHD: Translation between clinical and preclinical studies. *Clinical Psychology Review*, *26*(4), 379–395.
- Woicik, P. A., Stewart, S. H., Pihl, R. O., & Conrod, P. J. (2009). The substance use risk profile scale: A scale measuring traits linked to reinforcement-specific substance use profiles. *Addictive Behaviors*, *34*(12), 1042–1055.
- Worbe, Y., Savulich, G., Voon, V., Fernandez-Egea, E., & Robbins, T. W. (2014). Serotonin depletion induces “waiting impulsivity” on the human four-choice serial reaction time task: Cross-species translational significance. *Neuropsychopharmacology*, *39*(6), 1519–1526.

- Wylie, S. A., Richard Ridderinkhof, K., Elias, W. J., Frysinger, R. C., Bashore, T. R., Downs, K. E., van Wouwe, N. C., & van den Wildenberg, W. P. M. (2010). Subthalamic nucleus stimulation influences expression and suppression of impulsive behaviour in Parkinson's disease. *Brain*, *133*(12), 3611–3624.
- Wyvell, C. L., & Berridge, K. C. (2001). Incentive sensitization by previous amphetamine exposure: Increased cue-triggered “wanting” for sucrose reward. *Journal of Neuroscience*, *21*(19), 7831–7840.
- Yamaguchi, T., Wang, H., Li, X., Ng, T. H., & Morales, M. (2011). *Mesocorticolimbic glutamatergic pathway*. *31*(23), 8476–8490.
- Yavich, L., Sirviö, J., Haapalinna, A., Ylinen, A., & Männistö, P. T. (2003). Atipamezole, an  $\alpha$ -2-adrenoceptor antagonist, augments the effects of L-DOPA on evoked dopamine release in rat striatum. *European Journal of Pharmacology*, *462*(1–3), 83–89.
- Yen, S., Shea, M. T., Sanislow, C. A., Skodol, A. E., Grilo, C. M., Edelen, M. O., Stout, R. L., Morey, L. C., Zanarini, M. C., Markowitz, J. C., McGlashan, T. H., Daversa, M. T., & Gunderson, J. G. (2009). Personality traits as prospective predictors of suicide attempts. *Acta Psychiatrica Scandinavica*, *120*(3), 222–229.
- Yohn, S. E., Errante, E. E., Rosenbloom-Snow, A., Somerville, M., Rowland, M., Tokarski, K., Zafar, N., Correa, M., & Salamone, J. D. (2016). Blockade of uptake for dopamine, but not norepinephrine or 5-HT, increases selection of high effort instrumental activity: Implications for treatment of effort-related motivational symptoms in psychopathology. *Neuropharmacology*, *109*, 270–280.
- Yohn, S. E., Santerre, J. L., Nunes, E. J., Kozak, R., Podurgiel, S. J., Correa, M., & Salamone, J. D. (2015a). The role of dopamine D1 receptor transmission in effort-related choice behavior: Effects of D1 agonists. *Pharmacology Biochemistry and Behavior*, *135*, 217–226.

- Yohn, S. E., Thompson, C., Randall, P. A., Lee, C. A., Müller, C. E., Baqi, Y., Correa, M., & Salamone, J. D. (2015b). The VMAT-2 inhibitor tetrabenazine alters effort-related decision making as measured by the T-maze barrier choice task: Reversal with the adenosine A2A antagonist MSX-3 and the catecholamine uptake blocker bupropion. *Psychopharmacology*, *232*(7), 1313–1323.
- Young, J. W., Light, G. A., Marston, H. M., Sharp, R., & Geyer, M. A. (2009). The 5-Choice continuous performance test: Evidence for a translational test of vigilance for mice. *PLoS ONE*, *4*(1), e4227.
- Yttri, E. A., & Dudman, J. T. (2016). Opponent and bidirectional control of movement velocity in the basal ganglia. *Nature*, *533*(7603), 402-406.
- Yu, R., Mobbs, D., Seymour, B., Rowe, J. B., & Calder, A. J. (2014). The neural signature of escalating frustration in humans. *Cortex*, *54*(1), 165–178.
- Zahm, D. (1999). Functional-anatomical Implications of the Nucleus Accumbens Core and Shell Subterritories. *Annals of the New York Academy of Sciences*, *877*(1 ADVANCING FRO), 113–128.
- Zahm, D. S., & Brog, J. S. (1992). On the significance of subterritories in the “accumbens” part of the rat ventral striatum. *Neuroscience*, *50*(4), 751–767.
- Zalocusky, K. A., Ramakrishnan, C., Lerner, T. N., Davidson, T. J., Knutson, B., & Deisseroth, K. (2016). Nucleus accumbens D2R cells signal prior outcomes and control risky decision-making. *Nature*, *531*(7596), 642–646.
- Zeeb, F. D., Robbins, T. W., & Winstanley, C. A. (2009). Serotonergic and dopaminergic modulation of gambling behavior as assessed using a novel rat gambling task. *Neuropsychopharmacology*, *34*(10), 2329–2343.

Zucker, R. S., & Regehr, W. G. (2002). Short-term synaptic plasticity. *Annual Review of Physiology*, 64, 355–405.

# Appendix A - Chapter 3

## A1.1 Analyses

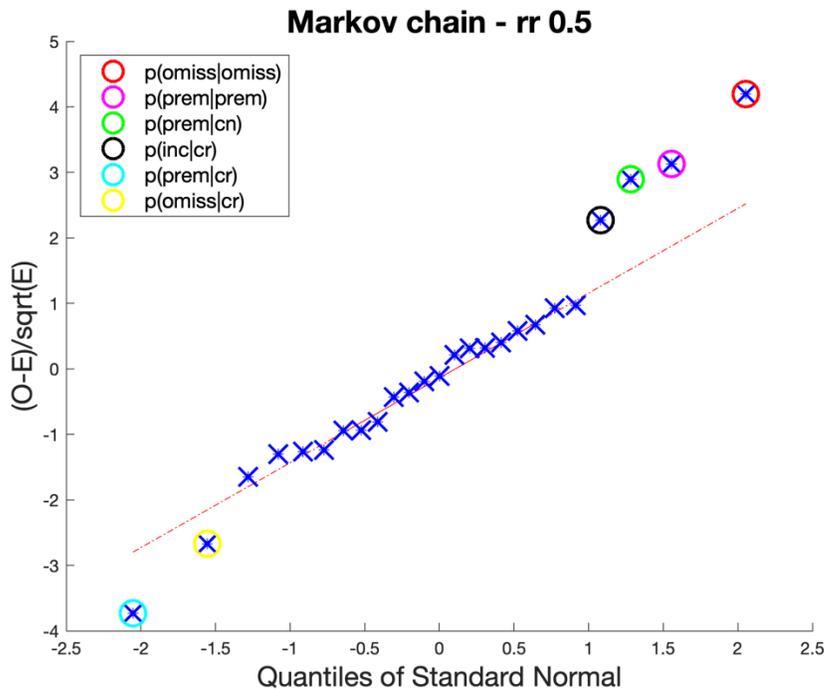
For most of the analyses of effects of rr on behaviour the model contained two fixed factors (rr and impulsivity) and one factor (subject) modelled as a random slope to account for individual differences between rats across manipulations. Where more factors were included in the model, for example in the case of effects of trial outcome on latency to perform the subsequent trial, there are specified in the results section. For example, when analysing latency to make a correct response as function of trial outcome, the model contained three fixed factors (rr, impulsivity and trial outcome) and one factor (subject) modelled as a random slope. Wherever significant three-way interactions were found, further analysis was performed by conducting separate multilevel models on a chosen factor of interest. For cohort 1, two animals were removed due to unstable performance (i.e., low, unstable levels of performance on two days of the five days of Latin square design).

## A1.2 Results

### A1.2.1 Experiment 1

#### A1.2.1.1 Consequences of a rewarded or nonrewarded trial

##### A1.2.1.1.1 Cohort 1



**Figure 3.1A Experiment 1. Fit of the independence model to performance of cohort 1 on 5CSRTT with rr 0.5.** The panel shows normal probability plots of the signed contributions  $(O - E)/E^{1/2}$  made by the 25 cells of the transition matrix under the independence model. The dots show the null line  $x = y$ . Values deviating from the null line show deviation from the independence model.

A chi-square test considering frequencies of one-step transitions starting from NR trials gave a marginally significant result  $X^2=9.54$   $p<0.05$  (under the chi-squared distribution with four degrees of freedom), while that considering transitions starting from R trials gave a more strongly significant result  $X^2=27.24$ ,  $p<0.001$  (under the chi-squared distribution with four degrees of freedom). See **Figure 3.2A** and **3.3A** for a graphical representation of the frequencies of one-step transitions and transition probabilities (respectively) with correct non-rewarded (NR) and correct rewarded (R) as the starting state.

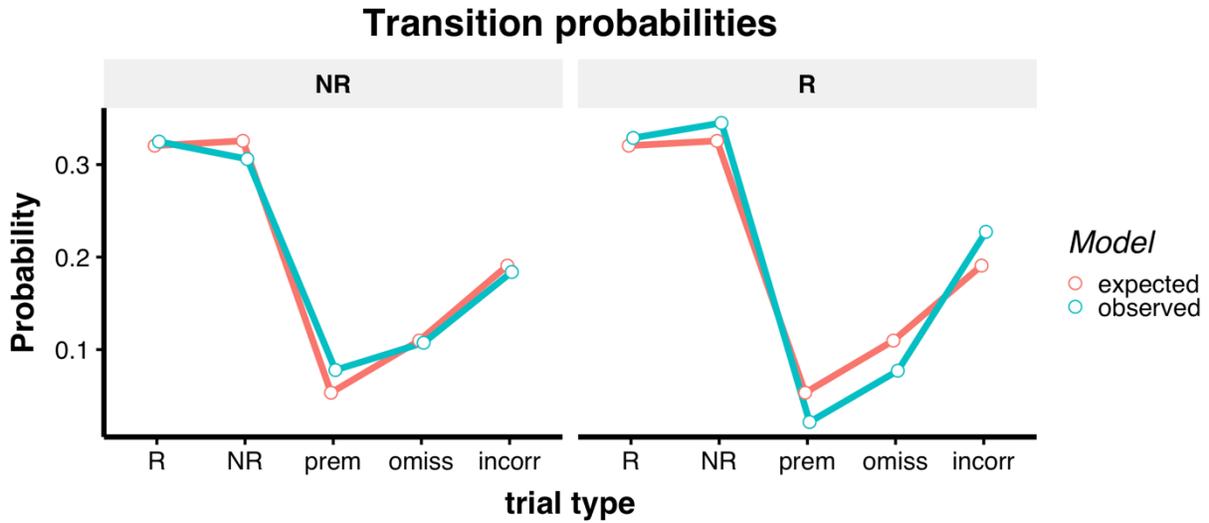


Figure 3.2A Experiment 1. Cohort 1. Transition probabilities starting from either correct non rewarded (NR) or correct rewarded (R) (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

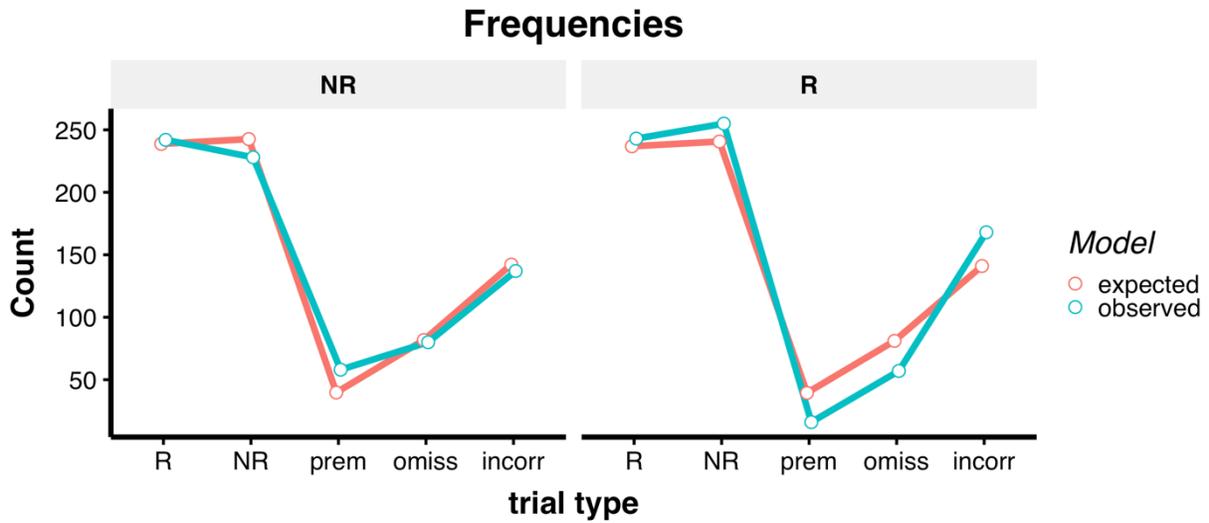
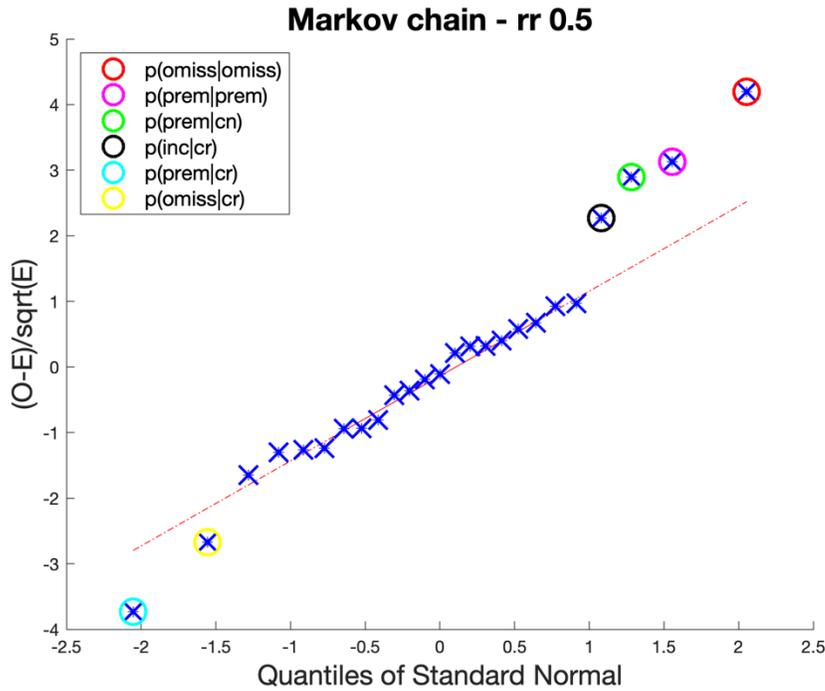


Figure 3.3A Experiment 1. Cohort 1. Frequencies of one-step transitions starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

### A1.2.1.1.2 Cohort 2



**Figure 3.4A Experiment 1. Fit of the independence model to performance of cohort 2 on 5CSRTT with rr 0.5, 5 s ITI.** The panel shows normal probability plots of the signed contributions  $(O - E)/E^{1/2}$  made by the 25 cells of the transition matrix under the independence model. The dots show the null line  $x = y$ . Values deviating from the null line show deviation from the independence model.

A chi-square test considering frequencies of one-step transitions starting from NR trials gave a marginally significant result  $X^2=10.70$   $p<0.05$ , while that starting from R trials gave a more strongly significant result  $X^2=21.44$   $p<0.001$ . See **Figure 3.5A** and **3.6A** for a graphical representation of the frequencies of one-step transitions and transition probabilities (respectively) with NR and R as the starting state.

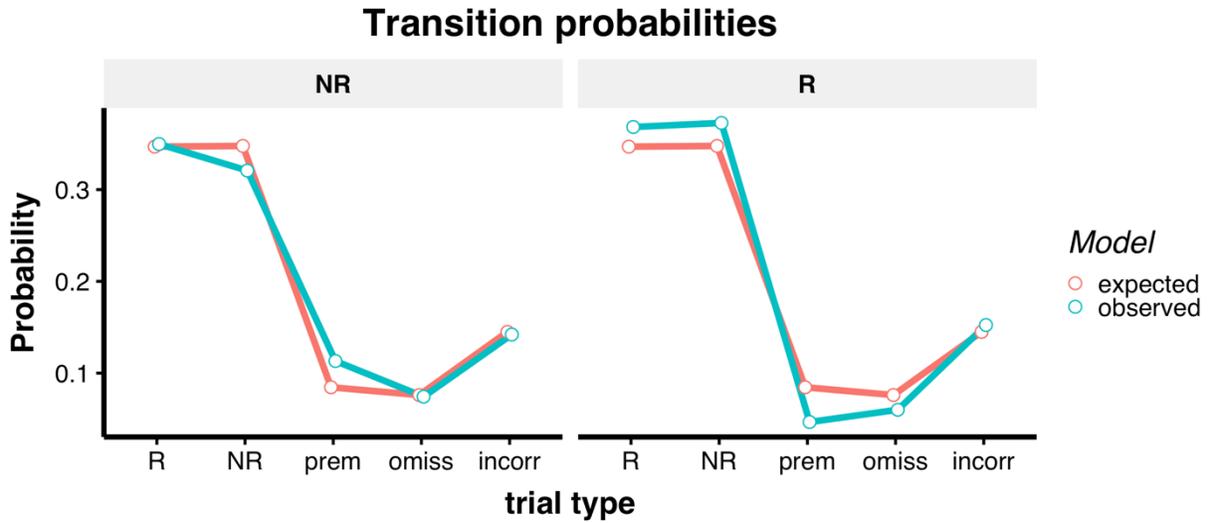


Figure 3.5A Experiment 1. Cohort 2. Transition probabilities starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

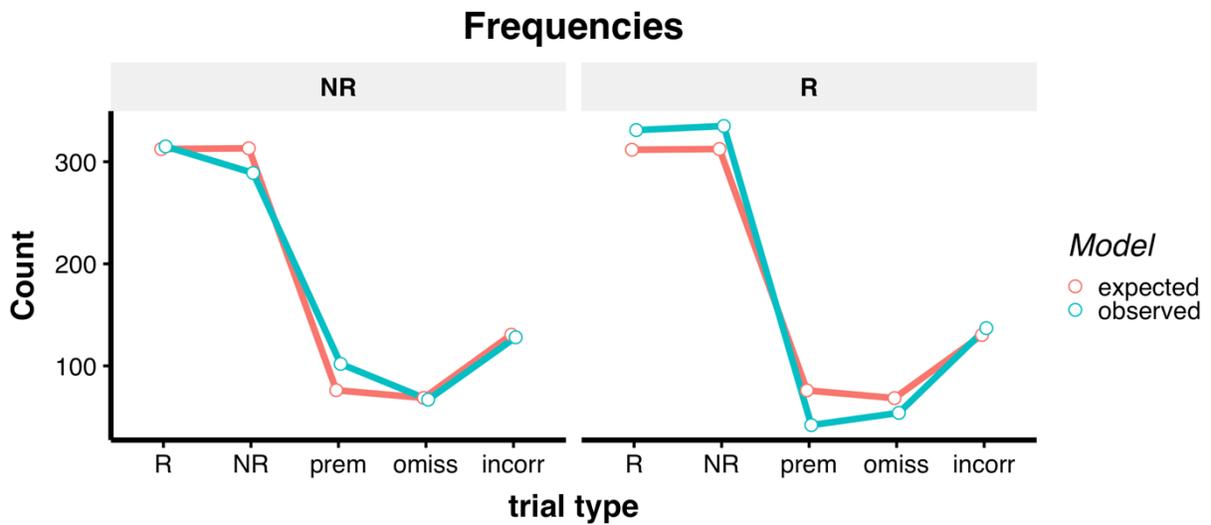
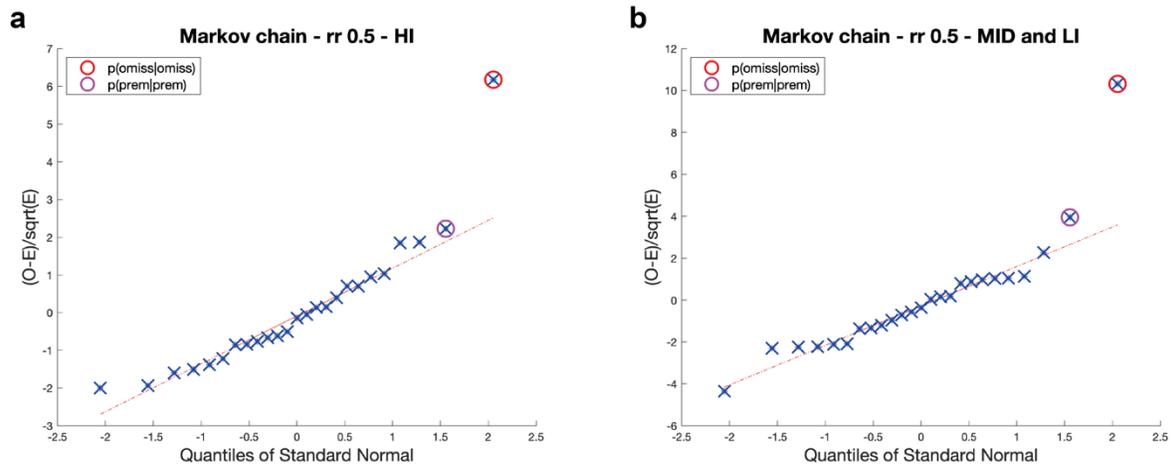


Figure 3.6A Experiment 1. Cohort 2. Frequencies of one-step transitions starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

## A1.2.2 Experiment 2

### A1.2.2.1 Consequences of a rewarded or non-rewarded trial



**Figure 3.7A Experiment 2. Cohort 1. Fit of the independence model to performance of Cohort 1 on 5CSRTT with rr 0.5, 7 s ITI.** The panel shows normal probability plots of the signed contributions  $(O - E)/E^{1/2}$  made by the 25 cells of the transition matrix under the independence model. The dots show the null line  $x = y$ . Values deviating from the null line show deviation from the independence model. (a) HI rats (b) MID and LI rats

A chi-square test considering frequencies of one-step transitions starting from NR trials did not reveal any significant results neither for HI ( $X^2=3.09$   $p>0.05$ ) nor for the combined group of MID and LI ( $X^2=6.26$   $p>0.05$ ). Finally, a chi-square test considering frequencies of one-step transitions starting from R trials gave significant results only for the combined group ( $X^2=28.26$   $p<0.05$ ) but not for HI rats ( $X^2=7.53$   $p>0.05$ ). See **Figure 3.8A** and **3.9A** for a graphical representation of the frequencies of one-step transitions and transition probabilities (respectively), for HI rats, with NR and R as the starting state. See **Figure 3.10A** and **3.11A** for a graphical representation of the frequencies of one-step transitions and transition probabilities (respectively), for the combined group, with NR and R as the starting state.

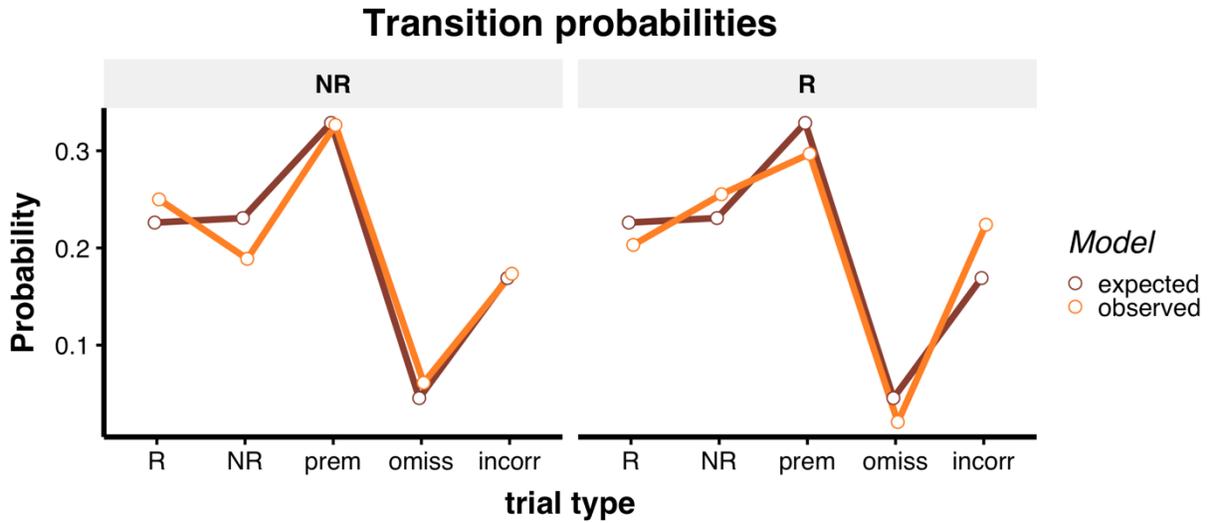


Figure 3.8A Experiment 2. Cohort 1. HI rats. Transition probabilities starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

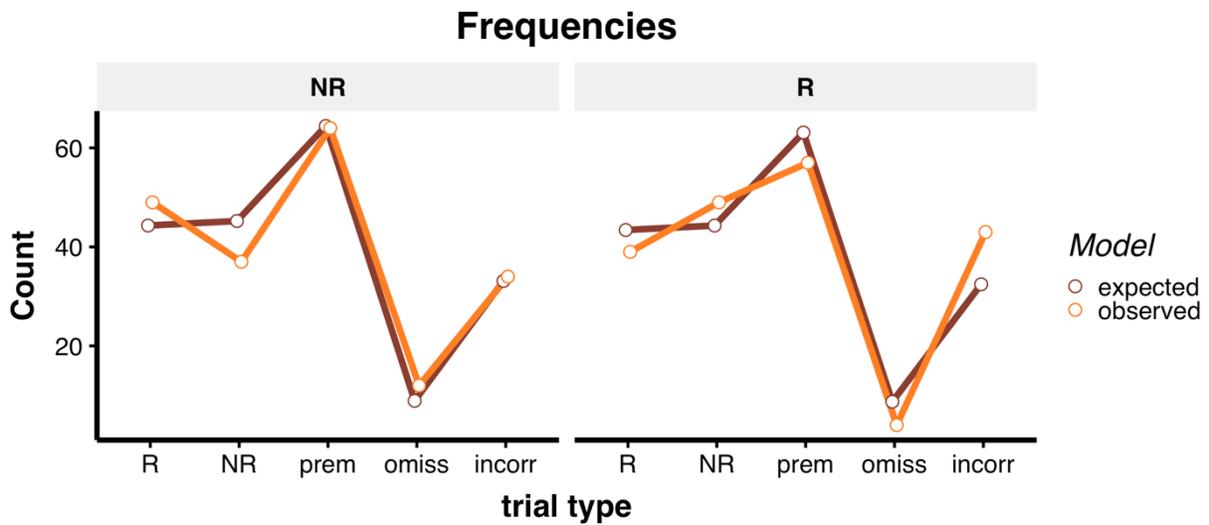


Figure 3.9A Experiment 2. Cohort 1. HI rats. Frequencies of one-step transitions starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

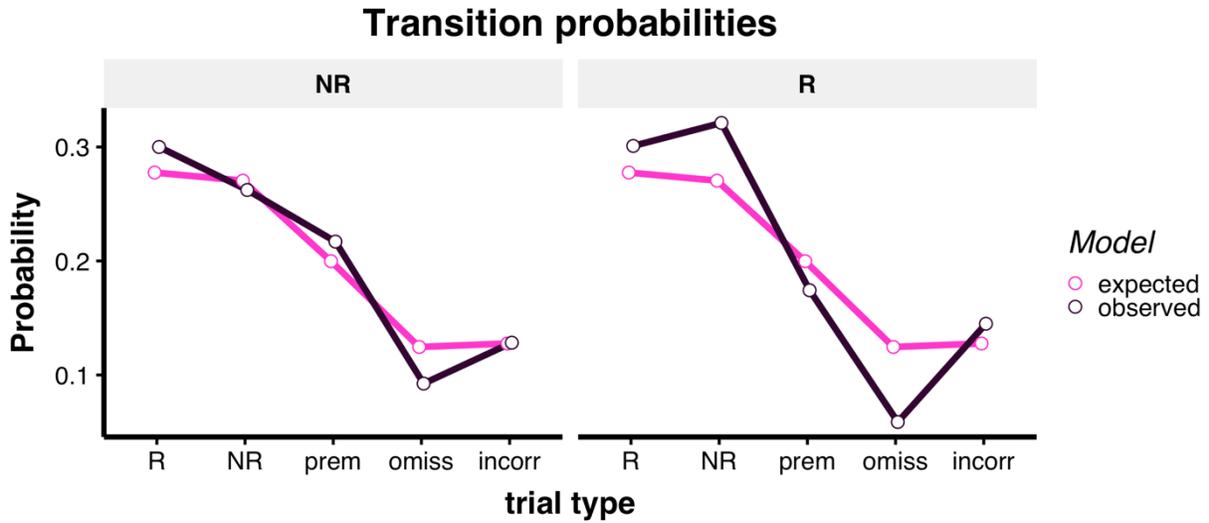


Figure 3.10A Experiment 2. Cohort 1. MID and LI rats. Transition probabilities starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

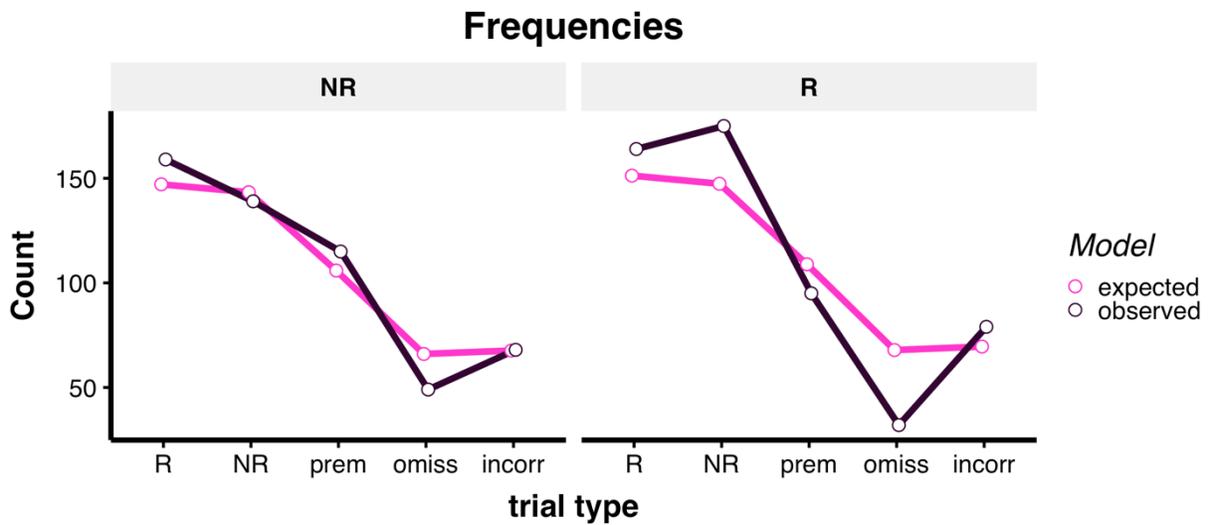
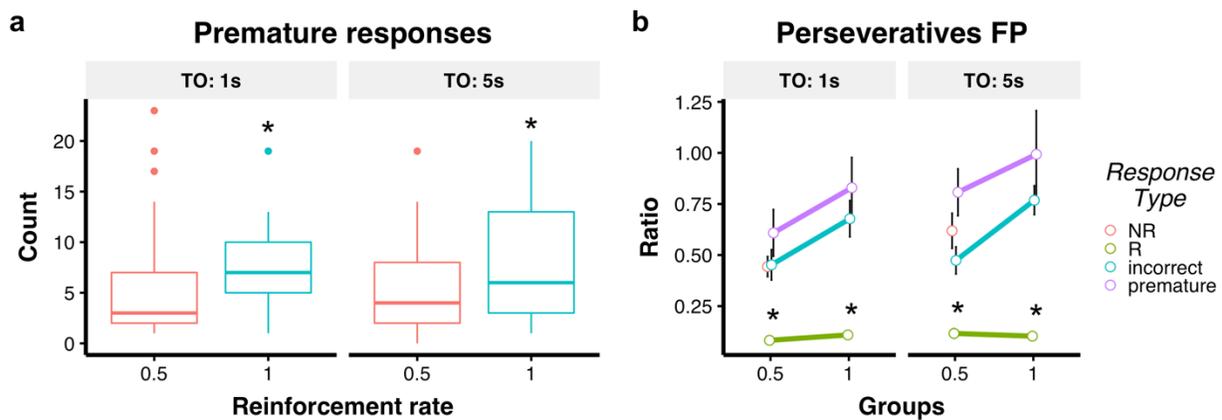


Figure 3.11A Experiment 2. Cohort 1. MID and LI rats. Frequencies of one-step transitions starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

### A1.2.3 Experiment 3

In Experiment 3, a group of rats (cohort 1) were tested with a reduced time-out of 1 s, both on rr 1 and on rr 0.5. Performance on this challenge was compared with the previous Latin square design that was run with these animals that included conditions of rr 1 and rr 0.5 with a time-out of 5s.

#### A1.2.3.1 Results:

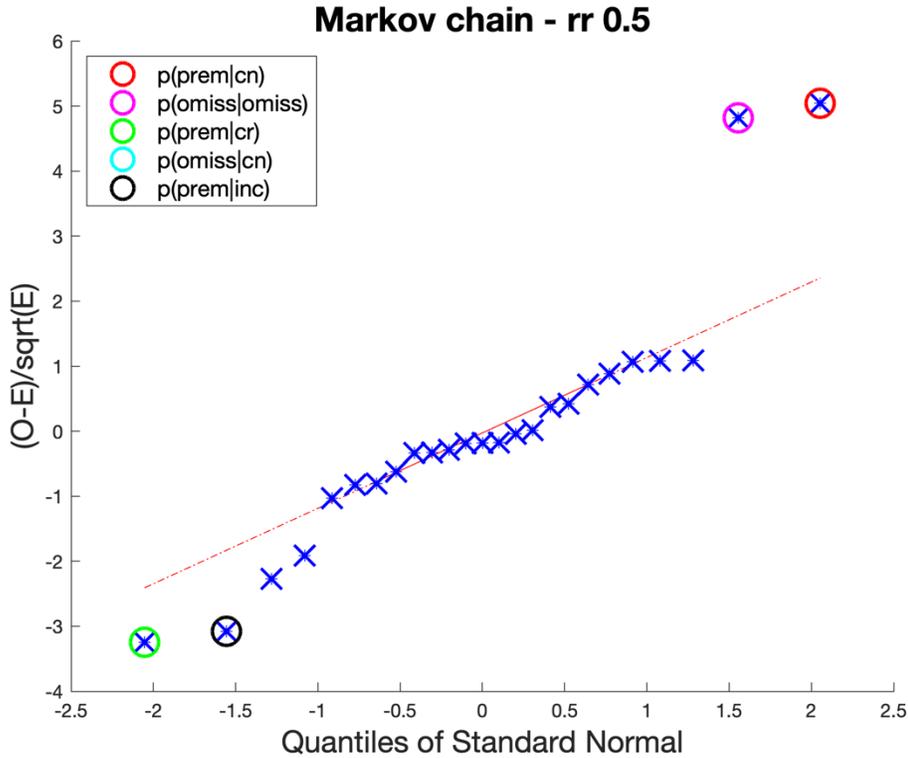


**Figure 3.12A** Effects of rr and time-out on (a) premature responses and (b) ratio perseverative responses in the FP. (a) \*statistically significant difference with rr 0.5,  $p < 0.05$ . (b) For the ratio of perseveratives in the FP there was a main effect of response type [ $F(3,226)=58.37$ ,  $p < 0.001$ ], with rats making proportionally less responses during R trials compared to all other trial types ( $p < 0.001$ ). TO = time-out. \*statistically significant difference of perseveratives in R vs those in all other response types  $p < 0.05$ .

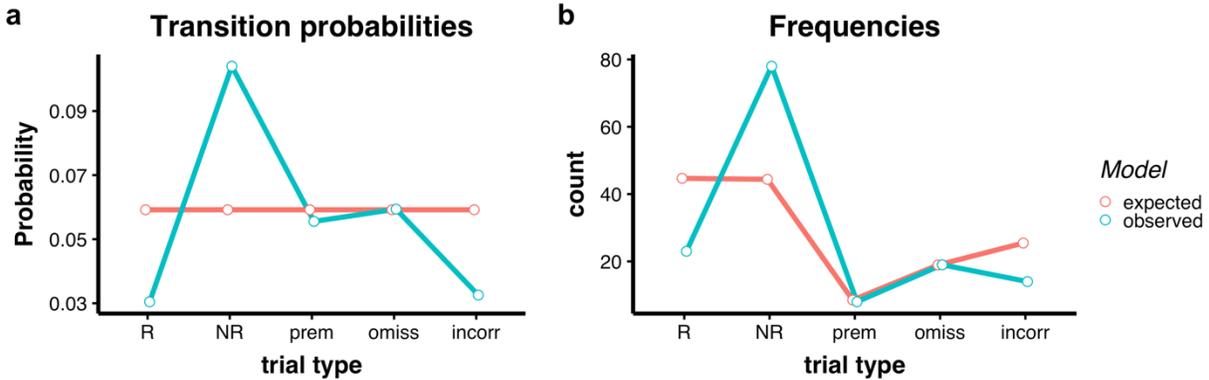
When comparing the four different sessions there was a main effect of rr on latency to make a correct response [ $F(1,52)=10.11$ ,  $p=0.02$ ], with animals being faster in the rr 1 condition compared to the rr 0.5 ( $p=0.003$ ). Timeout had no effect on this measure [ $F(1,64)=2.0$ ,  $p=0.27$ ].

**Figure 3.12Aa** shows that there was a main effect of rr on premature responses [ $F(1,50)=5.06$ ,  $p=0.028$ ], with rats making more premature responses during rr 1 compared to rr 0.5, again there was no effect of timeout on this measure [ $F(1,62)=0.312$ ,  $p=0.578$ ]. When looking at the correlation between the latency to make a correct response in the 1s timeout sessions and number of premature responses, the only significant negative correlation was during rr 1,  $r=-0.44$ ,  $p=0.037$ . **Figure 3.12Ab** summarises results for ratio FP perseverative responses. Focusing just on the rr 0.5, the latency between making a correct response in trial  $t$  and making a premature

response in trial t+1 did not significantly differ between response types and between timeout manipulations, however slightly more time elapsed between premature responses and NR trials.



**Figure 3.13A Experiment 3. Cohort 1. Fit of the independence model to performance of cohort 1 on 5CSRTT with rr 0.5, 5 s ITI, time-out 1 s.** The panel shows normal probability plots of the signed contributions  $(O - E)/E^{1/2}$  made by the 25 cells of the transition matrix under the independence model. The dots show the null line  $x = y$ . Values deviating from the null line show deviation from the independence model.



**Figure 3.14A Experiment 3. Cohort 1. (a) Transition probabilities and (b) frequencies of one-step transitions leading to a premature response (end state). x axis shows starting states. Red = independence model; Blue = observed data.**

A Markov chain model was then fit on data from the rr 0.5 session with 1s time-out. This was found to violate the independence model,  $W=81.49$   $p<0.001$ . **Figure 3.13A** shows the transition probabilities deviating the most from the independence model, when considering all states of the matrix. A chi-square test on the frequencies of one-step transitions leading to a premature response revealed a significant difference between the independence model and the observed data,  $X^2=41.15$ ,  $p<0.001$ . **Figure 3.14Aa** and **b** show how the transition probabilities and frequencies of one-step transitions, respectively, leading to a premature response deviated from the independence model. The largest deviations from the independence model were a higher-than-expected probability to make a premature after a NR trial ( $Y=5.04$ ) and a lower-than-expected probability to make a premature after a R trial ( $Y=-3.24$ ). A chi-square test found a significant difference from the independence model also for frequencies of one-step transitions starting from NR trials,  $X^2=37.25$   $p<0.001$  and one-step transitions starting from R trials,  $X^2=12.25$   $p<0.05$ . See **Figure 3.15A** and **3.16A** for a graphical representation of the frequencies of one-step transitions and transition probabilities (respectively) with NR and R as the starting state.

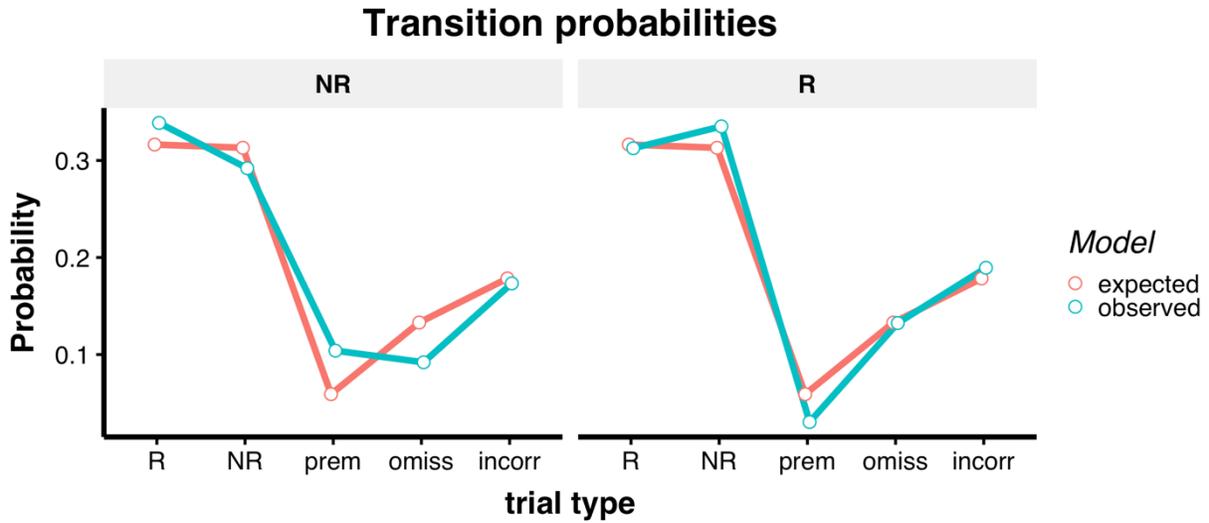


Figure 3.15A Experiment 3. Cohort 1. Transition probabilities starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

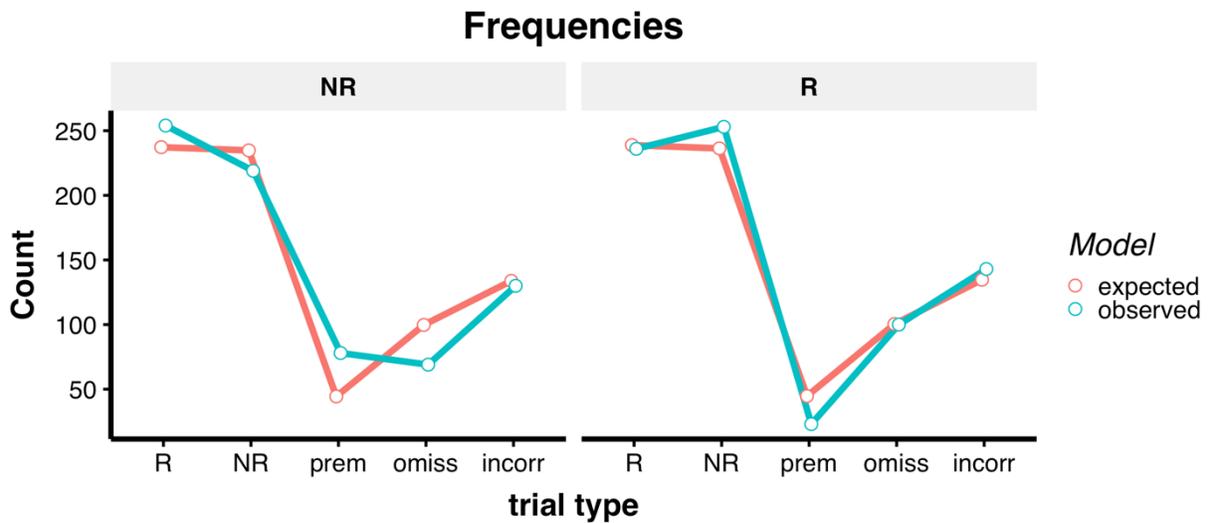


Figure 3.16A Experiment 3. Cohort 1. Frequencies of one-step transitions starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

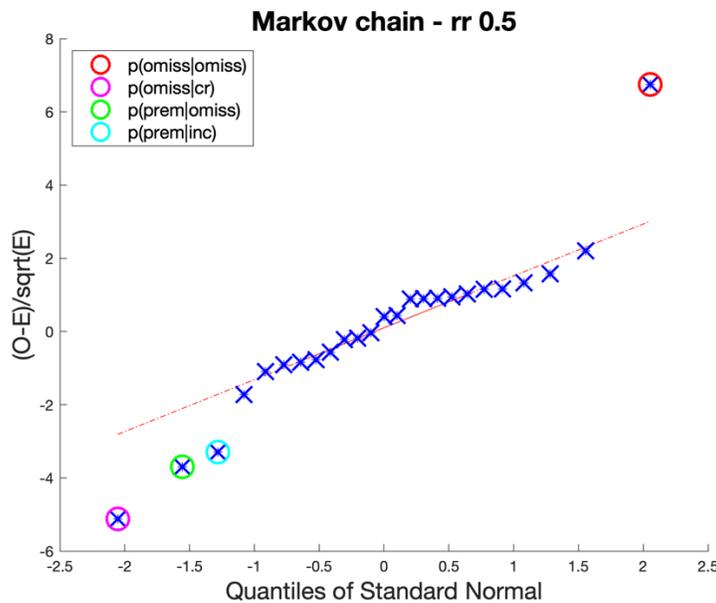
### In Summary

Reducing the timeout had no significant impact on the motivation to perform the task and on the likelihood to make premature responses. In line with previous results, the only parameter that influenced motivation to perform the task, as indexed by latency to make a correct response, was

rr. Thus, rats were faster at making a correct response when the rr was continuous and under this paradigm rats also made more premature responses. Reducing the time-out however does seem to affect the transition probabilities leading to a premature response, when compared to a session with 5 s time-out. Specifically, in the 1 s time-out condition, animals were more likely to transition to a premature response after a NR trial as opposed to after a premature trial. This however did not lead to more premature responses in this condition compared to the 5 s time-out rr 0.5 condition.

## A1.2.4 Experiment 4

### A1.2.4.1 Consequences of a rewarded or non-rewarded trial



**Figure 3.17A Experiment 4. Fit of the independence model to performance of cohort 1 on 5CSRTT with rr 0.5, 7 s ITI, 1 s time-out.** The panel shows normal probability plots of the signed contributions  $(O - E)/E^{1/2}$  made by the 25 cells of the transition matrix under the independence model. The dots show the null line  $x = y$ . Values deviating from the null line show deviation from the independence model.

A chi-square test for frequencies of one-step transitions starting from NR trials did not reveal these to be significantly different from the independence model  $X^2=7.60$ ,  $p>0.05$ . On the contrary, a chi-square test for one-step transitions starting from R trials did reveal a strong difference from the independence model  $X^2=33.70$   $p<0.001$ . See **Figure 3.18A** and **3.19A** for a

graphical representation of the frequencies of one-step transitions and transition probabilities (respectively) with NR and R as the starting state.

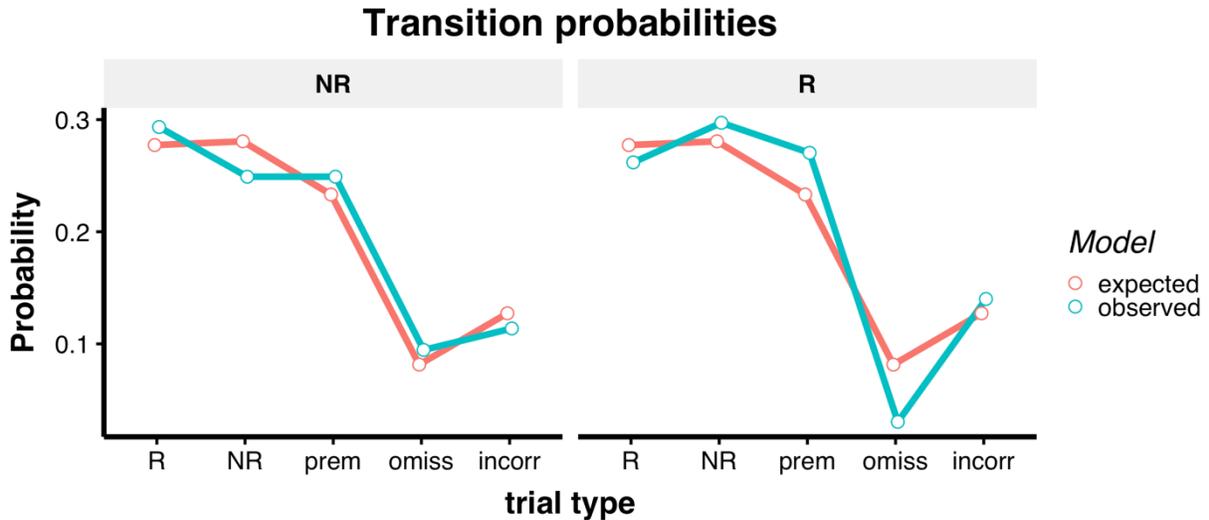


Figure 3.18A Experiment 4. Cohort 1. Transition probabilities starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

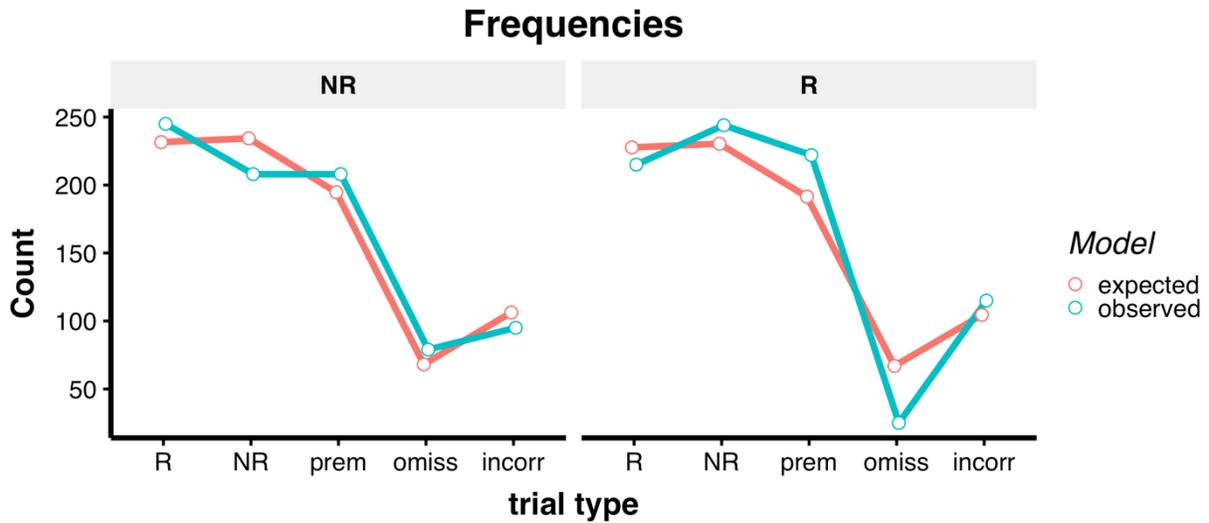


Figure 3.19A Experiment 4. Cohort 1. Frequencies of one-step transitions starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

## **A1.2.5 Experiment 5**

### **A1.2.5.1 – Effects of rr and reward magnitude on indexes of motivation**

To further investigate the slowing of responses in the 3-pellet condition I looked at the number of correct responses and omissions per session. There was a main effect of session both for correct responses [ $F(2,66)=12.06$ ,  $p<0.001$ ] and for omissions [ $F(2,66)=13.26$ ,  $p<0.001$ ]. Specifically, animals made fewer correct responses ( $p<0.001$ ) and more omissions ( $p<0.05$ ) in the 3-pellet condition compared with the 1-pellet condition with rr 0.5 and the 1-pellet condition with rr 1. For omissions there was also a main effect of impulsivity groups [ $F(2,33)=3.64$ ,  $p=0.037$ ], with LI rats making more omissions than HI rats ( $p=0.029$ ), across sessions.

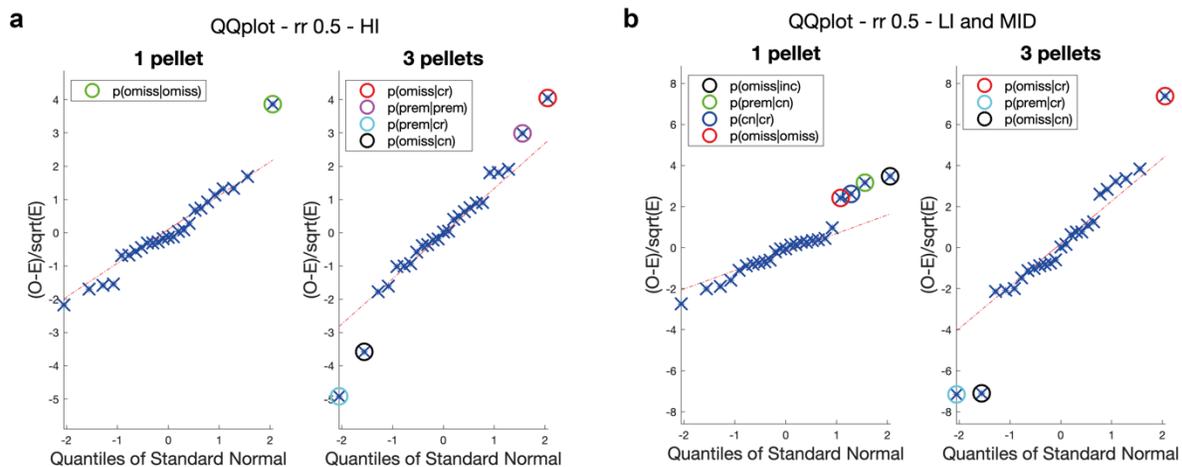
Analyses were then restricted to the two partial reinforcement conditions, where it was evaluated whether responses following a R or NR trial differed in latency depending on the size of the reward (1 vs 3 pellets). This was implemented to test the hypothesis that 3 pellets might induce a stronger post-reinforcement pause than 1 pellet, during rr 0.5. Latency of each response type (i.e., premature responses, correct and incorrect responses) in trial  $t$  following a R or NR trial (in  $t-1$ ) was compared across the two pellets conditions (from the time the animal pokes into the food magazine in trial  $t-1$ ). There was a significant 3-way interaction between outcome of the previous trial, pellet and response type [ $F(2,327)=4.45$ ,  $p=0.012$ ]. Three separate models were run for each response type, revealing that for correct [ $F(1,99)=23.60$ ,  $p<0.001$ ] and for incorrect responses [ $F(1,97)=9.63$ ,  $p=0.003$ ] there was an interaction between outcome and pellet. Specifically, in both cases, responses following a R trial were slower in the 3-pellet condition compared with the 1-pellet condition ( $p<0.001$  for all comparisons). With regards to premature responses, there was only an effect of outcome [ $F(1,69)=11.15$ ,  $p=0.001$ ], that is: across pellet conditions, premature responses occurred later in the trial after a R response compared to a NR response. In the context of premature responses, when looking at the time window between making a correct response in trial  $t-1$  and making a premature response in trial  $t$ , there were no differences in latency to make a premature as a function of the reinforcement outcome of the correct response [ $F(1,65)=1.41$ ,  $p=0.239$ ].

To investigate whether delays in performing responses following a R trial were due to animals spending time in the food magazine eating the reward, the number of perseverative responses in the rear panel (RP) following either R or NR trials was analysed. There was an interaction between pellets and response types [ $F(1,99)=9.25$ ,  $p=0.003$ ]. Specifically, there was no difference in RP perseverative responses between R or NR trials in the 1 pellet condition, however in the 3-pellet condition there was a significant difference, with rats making many more perseverative responses following a R response as opposed to a NR response ( $p=0.017$ ). The increase in RP perseverative responses following a R trial in the 3-pellets condition was also significantly greater than that recorded in the 1-pellet condition ( $p<0.001$ ), however there was no difference across pellets for perseverative responses following NR trials. Finally, it was important to test whether rats, when making many RP perseverative responses following a rewarded trial  $t$ , leave the food magazine late into the ITI of trial  $t+1$  and thus have little time to orient towards the FP and respond. To do this time-in-trial of the last RP perseverative response following either R or NR was compared across these two trial types and across the different pellet conditions. There was an interaction between pellets and outcome [ $F(1,99)=33.85$ ,  $p<0.001$ ]. Specifically, there was no difference across pellet conditions for time-in-trial of the last RP perseverative response following NR trials, with these happening around 970ms since the start of a new trial. However, there was a significant difference across pellet conditions for time-in-trial of the last RP perseverative following R trials, with these happening  $\sim 1100$ ms since the start of the new trial in the 1-pellet condition and at  $\sim 2924$ ms in the 3-pellets conditions ( $p<0.001$ ). When the average latency for leaving the RP was analysed, including trials with and without RP perseverative response, there was an interaction between pellets and outcome [ $F(1,99)=29.02$ ,  $p<0.001$ ]. As per above, the latency to exit the food magazine after a R trial was longer after the 3-pellet as opposed to the 1-pellet condition ( $p<0.001$ ). Specifically, the average latency for leaving the RP following a R trial in the 1-pellet condition was  $\sim 608$ ms since the start of the new trial, while in the 3-pellets condition it was  $\sim 1871$ ms. There were no differences across pellets conditions for latency to leave the RP following a NR trial.

## A1.2.5.2 Consequences of a rewarded or non-rewarded trial

### A1.2.5.2.1 One pellet condition

Both for HI rats and the combined group, the transition probability matrices violated the independence model: HI rats had a  $W$  of 34.79 ( $p < 0.01$ ) while the combined group had a  $W$  of 55.84 ( $p < 0.001$ ). **Figure 3.20A** shows which transition probabilities deviated the most from the independence model.



**Figure 3.20A** Experiment 5a. Fit of the independence model to performance of cohort 2 on 5CSRTT with rr 0.5. The panel shows normal probability plots of the signed contributions  $(O - E)/E^{1/2}$  made by the 25 cells of the transition matrix under the independence model. The dots show the null line  $x = y$ . Values deviating from the null line show deviation from the independence model. (a) HI rats (b) MID and LI rats

A chi-square test on the frequencies of one-step transitions leading to a premature response did not reveal, for HI rats, a significant difference from the independence model,  $X^2 = 2.05$ ,  $p > 0.05$ .

**Figure 3.21A (a and b)** show how for HI rats the transition probabilities and frequencies of one-step transitions, respectively, leading to a premature response deviated from the independence model.

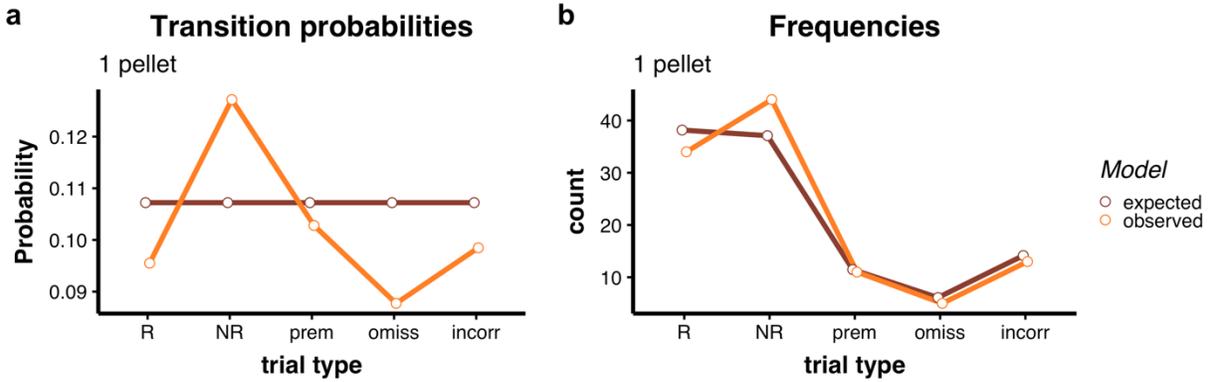


Figure 3.21A Experiment 5a. Cohort 2. HI rats (a) Transition probabilities and (b) frequencies of one-step transitions leading to a premature response (end state). x axis shows starting states. Red = independence model; Blue = observed data.

A chi-square test considering frequencies of one-step transitions starting either from NR trials or from R trials also did not yield any significant effects:  $X^2=5.17$  and  $X^2=6.96$ , respectively ( $p>0.05$  in both cases). For HI rats, see **Figure 3.22A** and **3.23A** for a graphical representation of the frequencies of one-step transitions and transition probabilities (respectively) with NR and R as the starting state.

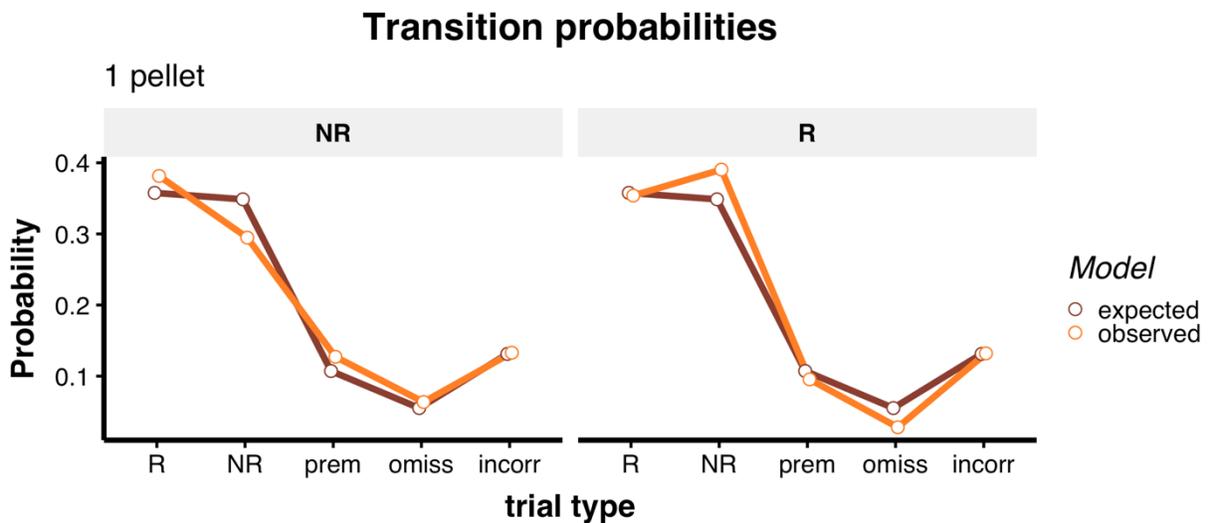


Figure 3.22A Experiment 5s. Cohort 2. Transition probabilities starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

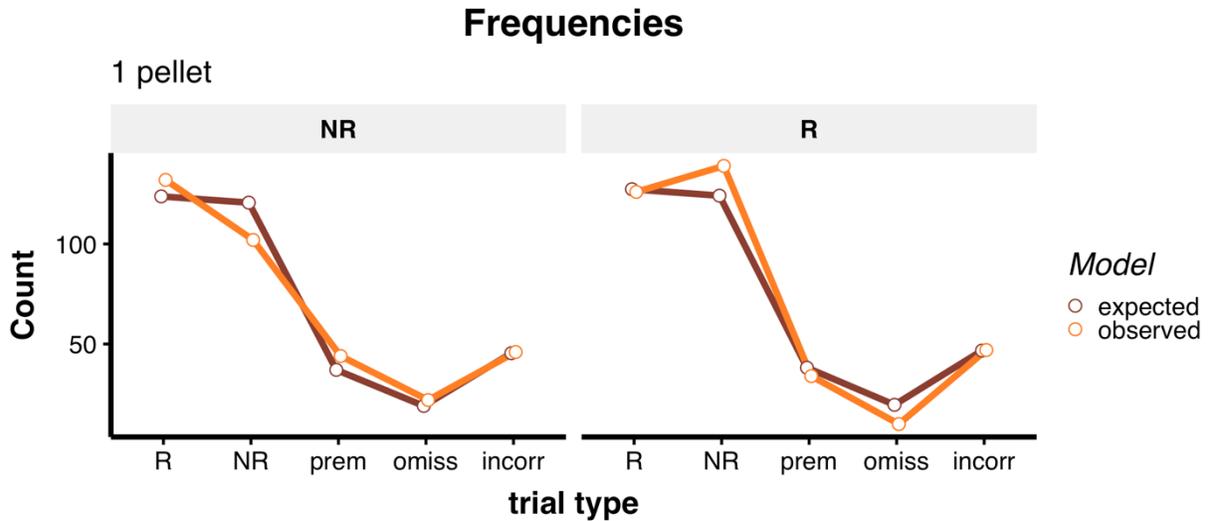


Figure 3.23A Experiment 5s. Cohort 2. Frequencies of one-step transitions starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

For the combined group, there was a significant difference from the independence model for frequencies of one-step transitions leading to a premature response  $X^2=17.91$  ( $p<0.01$ ).

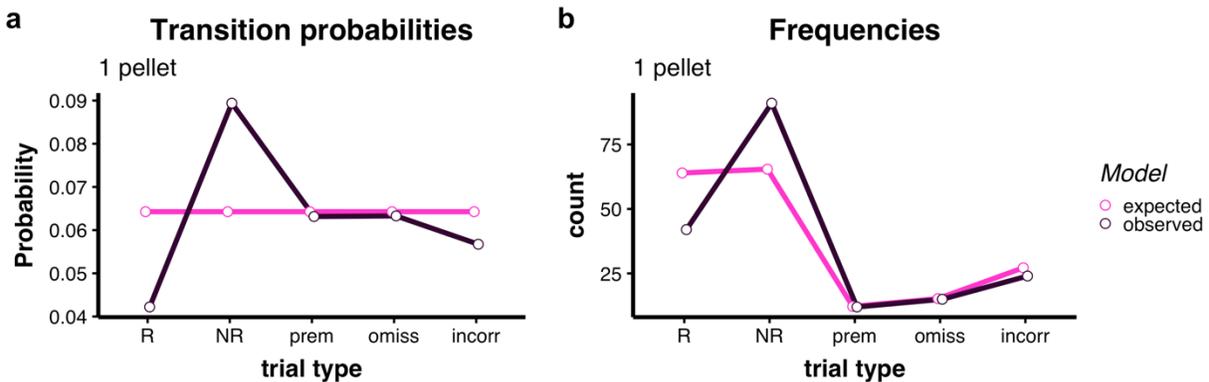
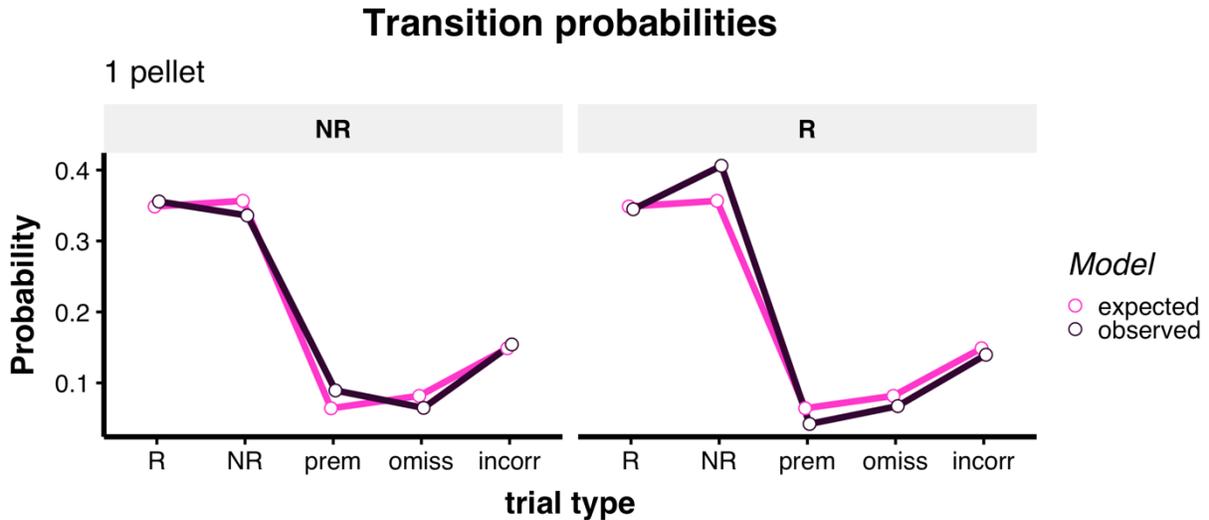


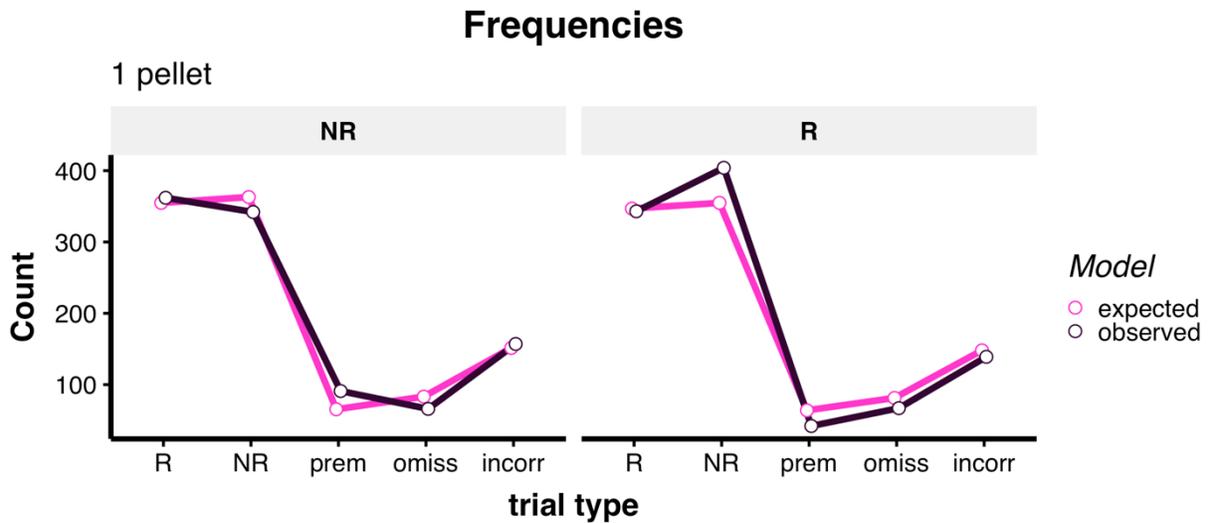
Figure 3.24A Experiment 5a. Cohort 2. HI rats (a) Transition probabilities and (b) frequencies of one-step transitions leading to a premature response (end state). x axis shows starting states. Red = independence model; Blue = observed data.

Figure 3.24A (a and b) show how for the combined group the transition probabilities and frequencies of one-step transitions, respectively, leading to a premature response deviated from the independence model. The largest deviations from the independence model were a higher likelihood to make a premature response after a NR trial ( $Y=3.16$ ) and a lower likelihood to make a premature response after a R trial ( $Y=-2.74$ ). Another chi-square test showed that frequencies of one-step transitions starting either from NR trials significantly deviated from the independence model  $X^2=15.11$  ( $p<0.01$ ), and so did one-step transitions starting from R trials

$X^2=17.46$  ( $p<0.01$ ). For the combined group, see **Figure 3.25A** and **3.26A** for a graphical representation of the frequencies of one-step transitions and transition probabilities (respectively) with NR and R as the starting state.



**Figure 3.25A** Experiment 5s. Cohort 2. MID and LI rats. Transition probabilities starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.



**Figure 3.26A** Experiment 5s. Cohort 2. MID and LI rats. Frequencies of one-step transitions starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

### A1.2.5.2.2 Three pellets condition

Chi-square tests considering frequencies of one-step transitions starting from NR trials revealed significant results for HI rats:  $X^2=16.79$  ( $p<0.01$ ) and for the combined group:  $X^2=73.89$  ( $p<0.001$ ). This was the case also for a chi-square test on one-step transitions starting from R trials, for HI:  $X^2=46$  ( $p<0.001$ ) and for the combined group:  $X^2=121.61$  ( $p<0.001$ ). For HI rats, see **Figure 3.27A** and **3.28A** for a graphical representation of the frequencies of one-step transitions and transition probabilities (respectively) with NR and R as the starting state. For the combined group, see **Figure 3.29A** and **3.30A** for the same graphical representation.

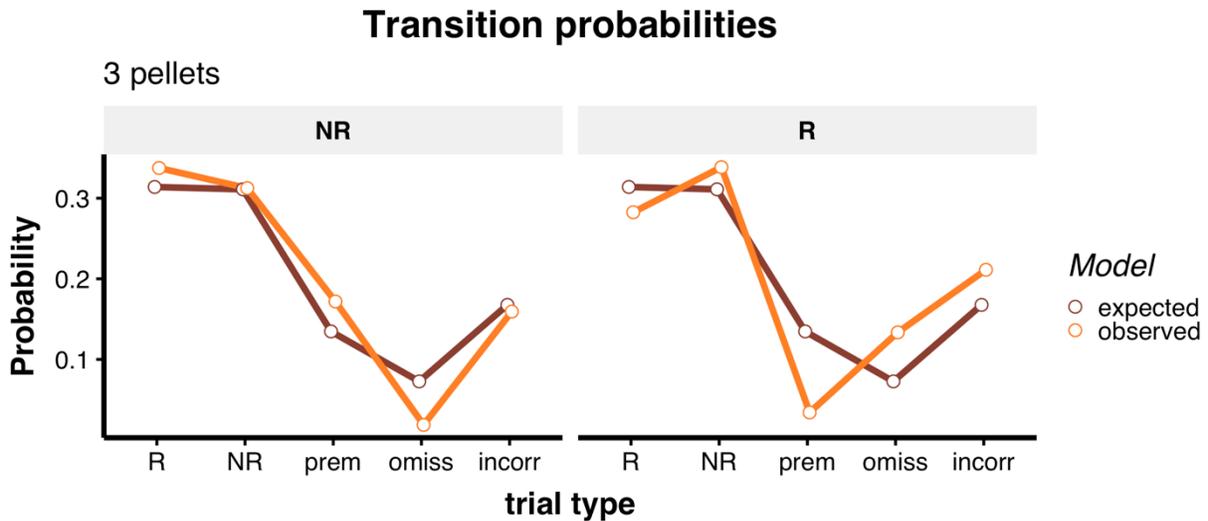


Figure 3.27A Experiment 5b. Cohort 2. HI rats. Transition probabilities starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

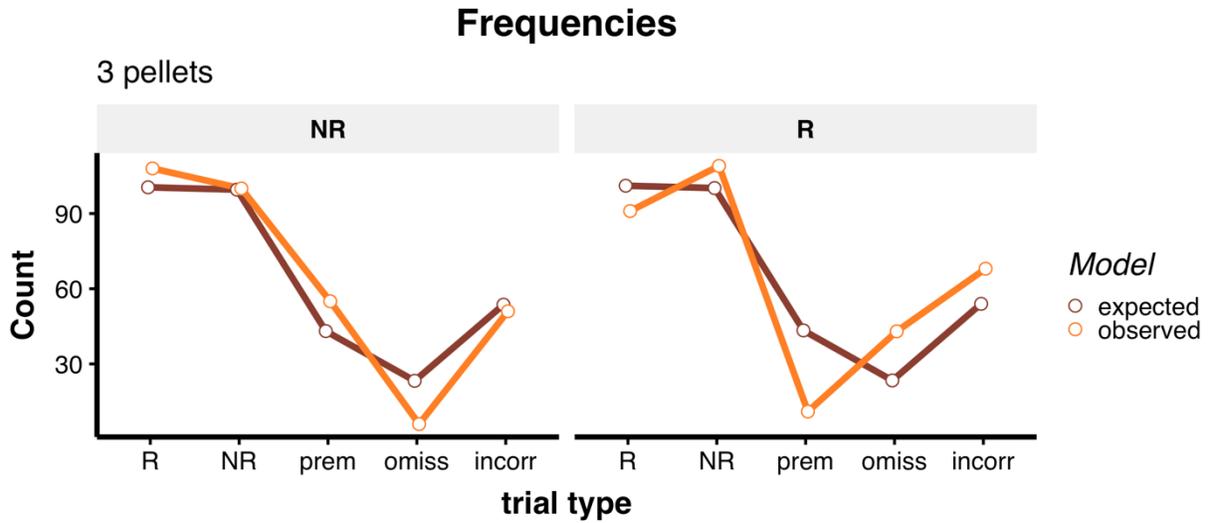


Figure 3.28A Experiment 5b. Cohort 2. HI rats. Frequencies of one-step transitions starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

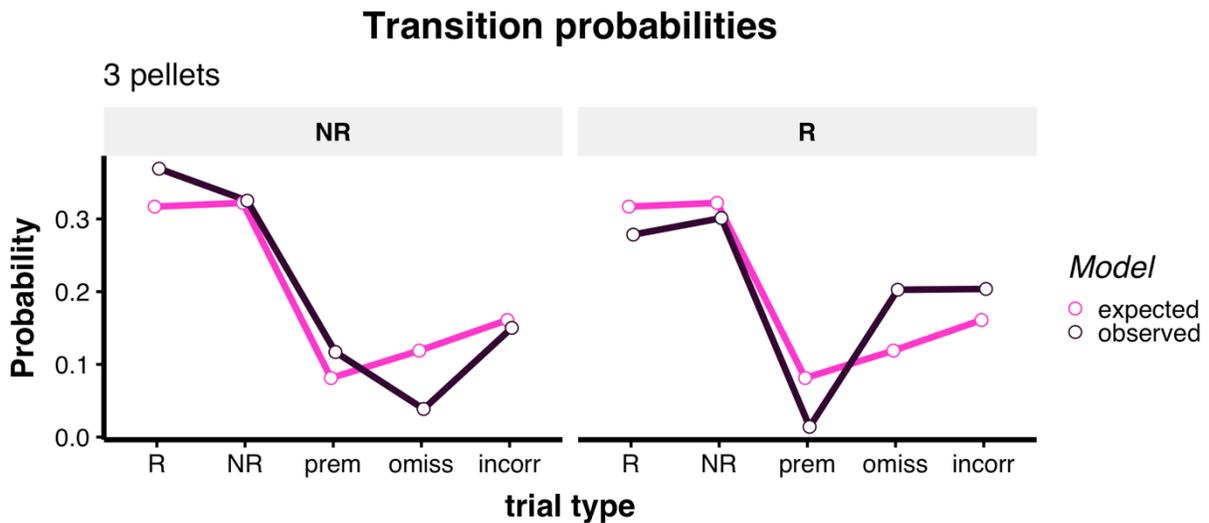


Figure 3.29A Experiment 5b. Cohort 2. MID and LI rats. Transition probabilities starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

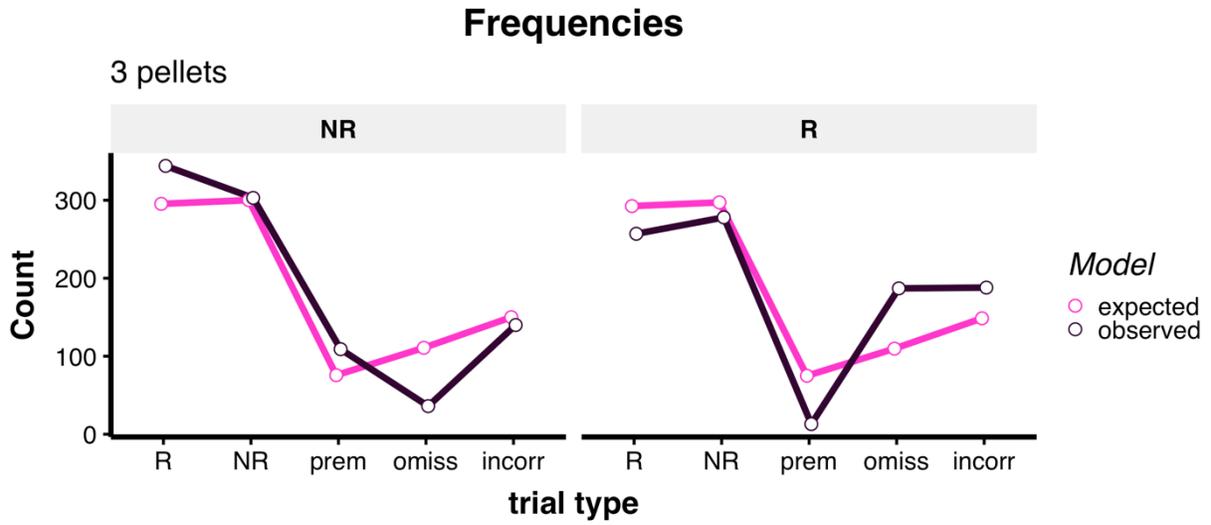


Figure 3.30A Experiment 5b. Cohort 2. MID and LI rats. Frequencies of one-step transitions starting from either NR or R (starting states) and ending in the end states represented in the x axis. Red = independence model; Blue = observed data.

# Appendix B - Chapter 4

## B1.1 Data Analysis

The main dependent variables were percentage of premature responses, percentage of omissions, the number of reinforcers earned and response latencies to make correct, incorrect or premature responses. To assess the temporal profile of responses, we divided the vITI sessions into 5 min bins. Each bin required responses from at least three animals from each impulsivity group to be included in the analyses. The 5 min bins satisfying this criterion were from 5 to 55 min (i.e., 11 bins).

For analyses of behaviour prior to any drug manipulation the model contained three fixed factors (day, ITI and impulsivity) and one factor (subject) modelled as a random slope to account for individual differences between rats across testing days. When significant three-way interactions were found, further analysis was performed by conducting separate multilevel models on “day”. For analyses of drug interventions, the model contained three fixed factors (ITI, impulsivity and drug) and one factor (subject). When significant three-way interactions were found, further analysis was performed by conducting separate multilevel models on “impulsivity”. For drug manipulations post-hoc testing was used to compare drug effects with the vehicle condition.

## B1.2 Progressive ratio

### B1.2.1 Apparatus

All sessions were performed in eight operant chambers (Med Associates, Georgia, TV) controlled by two computers and Whisker Control software (Cardinal & Aitken, 2010). Each chamber had two retractable levers, positioned 60 mm above the chamber floor and 50 mm to either side of a food magazine. A house light in the roof of the chambers remained on throughout the session. Only one lever, the active lever, was used for each rat and its left or right position was counterbalanced across rats. The active lever was extended at the beginning of each session

and retracted at the end. A pellet dispenser delivered 45 mg food pellets (Noyes dustless pellets, Research Diets, UK) in the food magazine located on the side of the active lever.

### **B1.2.2 Training**

As described previously (Eagle et al., 1999), rats were initially habituated to the boxes and trained on a simple, continuous reinforced lever-pressing schedule in daily sessions of 15-30 minutes. Every lever press was occasioned by the onset of a light located above the magazine and the delivery of a food pellet (FR-1). Additional lever presses had no consequence until the rat's nose was detected in the magazine. The session ended when 100 pellets were delivered, or 30 min had elapsed. After 4 sessions under this schedule, all rats were moved to an FR-5 schedule where 5 lever presses were required for a sugar pellet to be delivered. Once the lever-pressing requirement was achieved the light above the magazine would illuminate and a pellet would be delivered. The light above the magazine remained on until the rat's nose was detected in the magazine. The session ended when 100 pellets were delivered, or 30 min had elapsed. After 4 sessions under this schedule, all rats were tested on two different kinds of PR schedule.

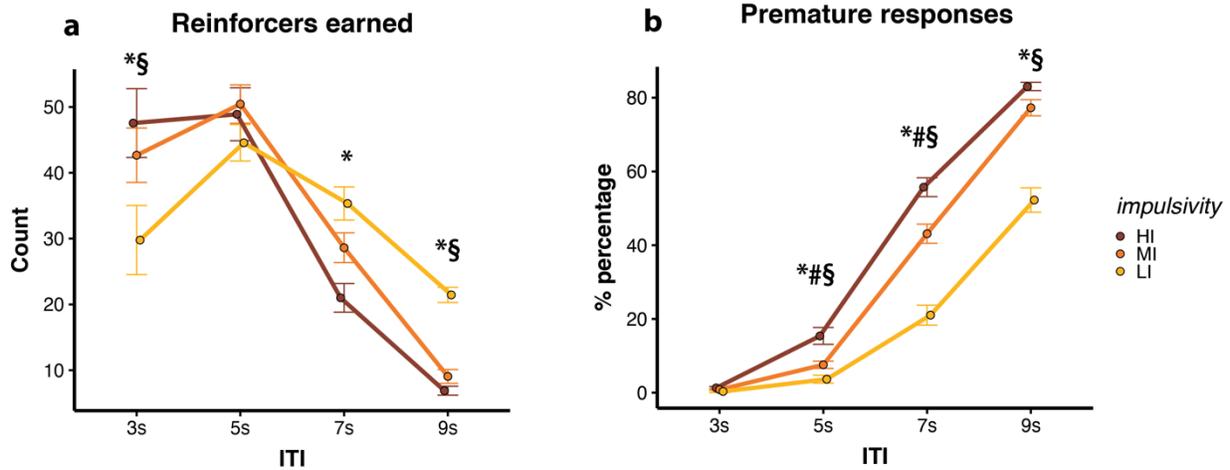
## **B2.1 Results**

### **B2.1.1 Experiment 1**

#### **B21.1.1 Baseline**

Analysis of the last baseline session prior to the vITI challenge revealed that, in Experiment 1, HI rats had elevated premature responses in this session compared to LI and MID rats. Specifically, premature responses differed between impulsivity groups [ $F(2,33)=5.09$   $p=0.012$ ], with HI rats making more premature responses than MID rats ( $p=0.004$ ) and LI rats ( $p=0.028$ ).

### B2.1.1.2 Day 1 of the vITI challenge



**Figure 4.1B** Trait impulsivity modulated (a) reinforcers earned and (b) percentage of premature responses on a vITI paradigm on 5CSRTT. Group differences for Day 1. \*HI vs LI  $p < 0.05$ ; #HI vs MI  $p < 0.05$ ; §MI vs LI  $p < 0.05$

As shown in **Figure 4.1B (a)**, on Day 1, impulsivity phenotypes differed in the number of reinforcers earned, depending on which ITI was presented [ $F(6,99) = 9.69$ ,  $p < .001$ ]. Specifically, LI earned fewer reinforcers than HI ( $p = 0.004$ ) and MID ( $p = 0.016$ ) on the 3 s ITI trials, while HI earned fewer reinforcers than LI during the 7 s ( $p = 0.010$ ) and 9 s ( $p < .001$ ) ITI trials. LI also earned more reinforcers than MID on the 9 s ITI trials ( $p < .001$ ). With regards to premature responses, On Day 1 there was a difference between groups in the number of premature responses made, as a function of ITI [see **Figure 4.1B (b)**,  $F(6,99) = 10.70$ ,  $p < .001$ ]. Specifically, HI had a higher probability of making a premature response than LI and MID on the 5 s ITI ( $p < .001$ ;  $p = 0.002$ , respectively) and on the the 7 s ITI ( $p < .001$ ;  $p = 0.003$ , respectively). On the 9 s ITI HI had a higher probability of making a premature response than LI ( $p < .001$ ). Similarly, MID also had a higher probability of making a premature response than LI on the 5 s ITI ( $p = 0.027$ ), the 7s ITI ( $p < .001$ ) and the 9s ITI ( $p < .001$ ).

### B2.1.1.3 Activity per unit of time

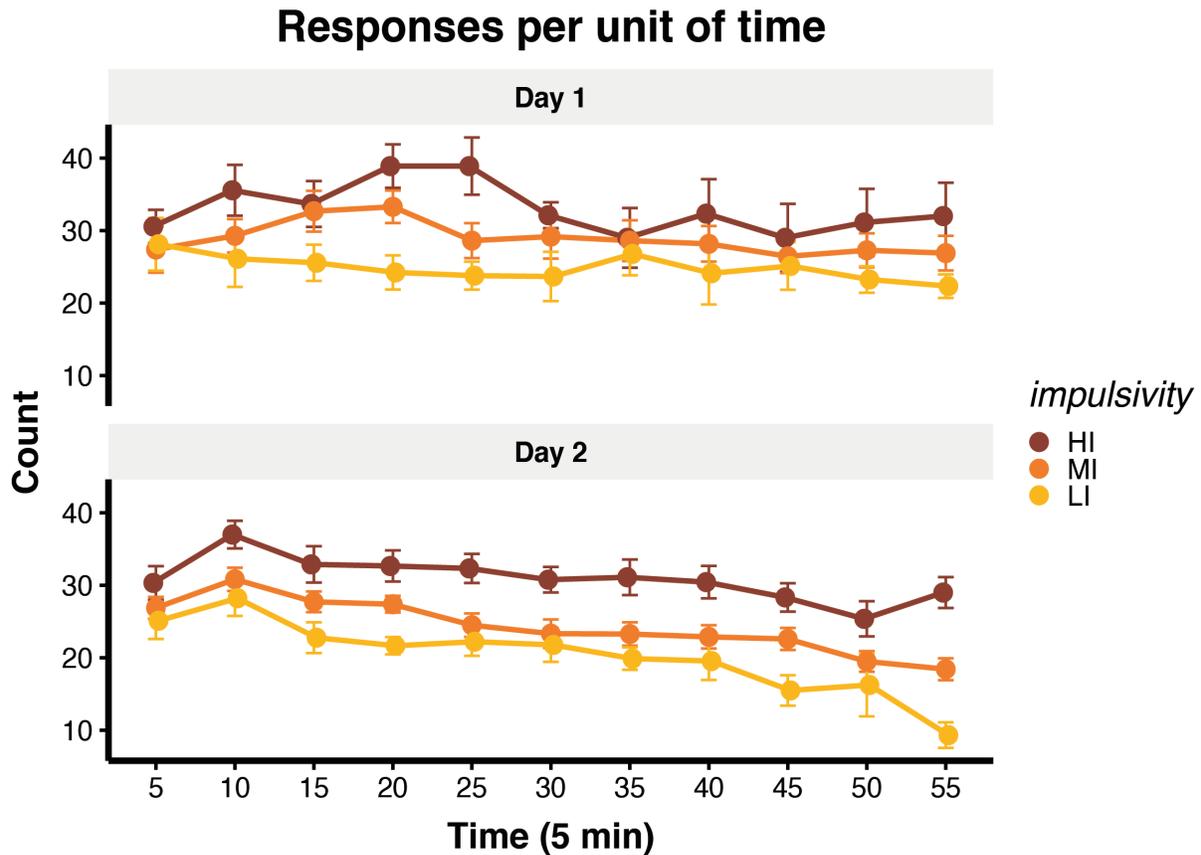


Figure 4.2B Experiment 1. Active responses (premature, correct and incorrect) per unit of time, organised by impulsivity groups and days of testing.

As shown in **Figure 4.2B**, rate of responding changed as a function of time in session ( $F(10, 654) = 4.59, p < .001$ ); impulsivity groups ( $F(2,33) = 6.32, p = 0.005$ ) and day of testing ( $F(1,654) = 12.80, p < .001$ ). Specifically, HI were significantly more active than LI ( $p = 0.003$ ), and in general animals were more active on Day 1 compared to Day 2 ( $p < .001$ ).

### B2.1.1.4 First encounter with and exposure to the long ITIs

A linear mixed model showed that there was a main effect of ITI [ $F(1, 99) = 12.86, p < .001$ ], a main effect of Day [ $F(1, 99) = 6.15, p = 0.015$ ] and a main effect of group [ $F(2,33) = 4.22, p = 0.023$ ]. *Post-hoc* comparisons showed that animals encounter the 7s ITI earlier in the session compared to the 9s ITI ( $p < .001$ ) and encounter these long trials later in the session on Day 1 compared to Day 2 ( $p = 0.015$ ). Finally, HI rats encounter these long ITIs later in the session compared to LI ( $p = 0.023$ ). When focusing on Day 1 only, however, there was no difference

between groups but there was still a main effect of ITI,  $[F(1, 33)=4.74, p=0.037]$ , with animals encountering the 9s ITI later in the session compared to the 7s ITI ( $p=0.037$ ).

I was also interested in exploring whether there was a difference across days and groups in the number of positively reinforced trials with these delayed contingencies. This was done to investigate the extent to which animals across groups and days were equally presented with the same learning signals (i.e., correct responses for the 7s and 9s ITIs). For this analysis, I ran a linear mixed model and found a significant three-way interaction between ITI x Impulsivity x Day  $[F(2, 99)= 3.57, p=0.032]$ , thus each day was analysed separately.

On **Day 1** there was an interaction between ITI and impulsivity  $[F(2, 33)= 4.80, p=0.015]$ . Specifically, across days, HI rats made significantly fewer correct responses during the 7s ITI compared to LI ( $p<.001$ ) and MID rats ( $p=0.046$ ). On the 9s ITI both HI ( $p<.001$ ) and MID rats ( $p<.001$ ) made significantly fewer correct responses than LI. A similar pattern was observed on **Day 2**: an interaction between ITI and impulsivity  $[F(2, 33)= 8.46, p=0.001]$ . Again, across days, HI rats made significantly fewer correct responses during the 7s ITI compared to LI ( $p= 0.003$ ) and MID rats ( $p=0.002$ ). Similarly, during the 9s ITI, HI rats made significantly fewer correct responses than LI ( $p<.001$ ) and MID rats ( $p<.001$ ).

### **B2.1.1.5 Latencies for the short vITI challenge**

Latencies to make a correct response varied significantly across different ITIs  $[F(1,33) = 69.29, p<0.001]$  and impulsivity groups  $[F(2,33) = 4.17, p=0.024]$ . Latencies to make an incorrect response also varied depending on ITI  $[F(1,33) = 6.52, p=0.015]$ . **Table 4.1B** summarises these findings.

	Correct responses		Incorrect responses	
	2s	3s	2s	3s
HI	1120±166.4*	800.2±60.1*	3227.1±197.2	2871±169.3
MI	1318.6±71.9	1009.8±64	3213.2±151.1	2596.7±131.2
LI	1616.3±221.6*	1169.1±119.2*	2994.5±170	2852.2±106.8

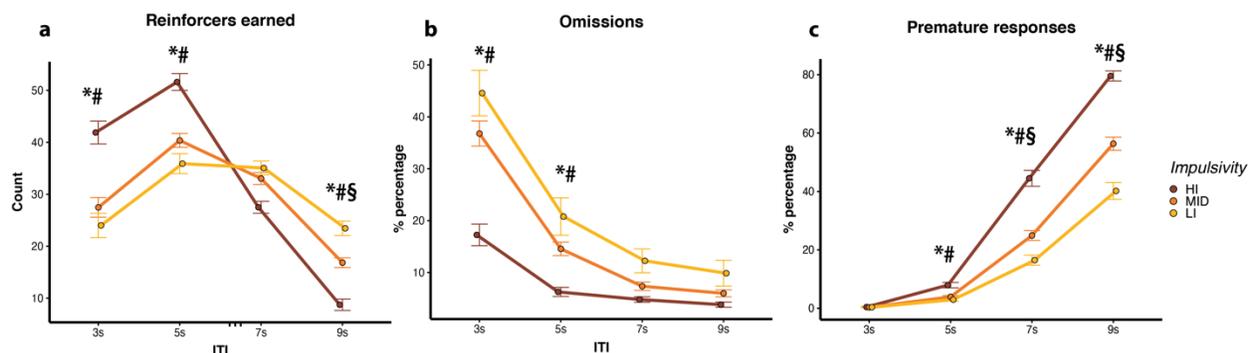
**Table 4.1B Experiment 1, short vITI challenge.** Latencies for correct and incorrect responses. \*HI vs LI  $p < 0.05$ ; ° HI vs MID  $p < 0.05$ .

## B2.1.2 Experiment 2

### B2.1.2.1 Baseline

Analysis of the last baseline session prior to the vITI challenge revealed that in Experiment 2, similar to Experiment 1, HI rats made more premature responses compared with LI and MID rats. Specifically, the percentage of premature responses differed between impulsivity groups in the baseline session prior to the vITI challenge of Experiment 2 [ $F(2,20)=4.16$ ,  $p=0.031$ ], with HI rats making more premature responses than LI rats ( $p=0.024$ ). In this cohort of animals, impulsivity phenotype influenced performance accuracy during the baseline session [ $F(2,20)=3.75$ ,  $p=0.041$ ], with HI rats being slightly less accurate than LI rats ( $p=0.036$ ).

### B2.1.2.2 vITI challenge



**Figure 4.3B Trait impulsivity modulates performance on a vITI paradigm n 5CSRTT (Day 3).** Group differences in (a) reinforcers earned; (b) % omissions; (c) % premature responses. \*HI vs LI  $p < 0.05$ ; #HI vs MI  $p < 0.05$ ; §MI vs LI  $p < 0.05$

As shown in **Figure 4.3B (a)**, there was a difference between impulsivity groups on the number of reinforcers earned depending on the ITI of the trial [ $F(6,231)=29.87, p<.001$ ]. Specifically, on the short 3s and 5s ITI trials HI earned significantly more pellets than LI ( $p<.001$  both ITIs) and MID ( $p<.001; p=0.006$ , respectively). On the contrary, during long 9s ITI trials, LI earned more food rewards than HI ( $p<.001$ ) and MID ( $p=0.016$ ). MID also earned more food rewards in this ITI than HI ( $p<.001$ ).

The probability of making a premature response varied as a function of Day [see **Figure 4.3B (b)**  $F(2,231)=13.63, p<.001$ ]; and between groups depending on which ITI was presented [ $F(6,231)=23.12, p<.001$ ]. Overall, the probability of making a premature response was higher on Day 1 compared to Day 3 ( $p<.001$ ), and on Day 2 compared to Day 3 ( $p<.001$ ). On the 5s ITI, HI had a higher probability than MID ( $p=0.015$ ) and LI ( $p=0.007$ ) of making a premature response. On the 7s and 9s ITI HI had a higher probability than MID ( $p<.001$  for both ITIs) and LI ( $p<.001$  for both ITIs) of making a premature response. MID also had a higher probability of making a premature response than LI both on 7 s ( $p=0.007$ ) and 9 s ITIs ( $p<.001$ ).

The probability of making an omission varied as a function of Day [ $F(2,231)=4.43, p=0.013$ ]; and between groups depending on which ITI was presented [ $F(6,231)=6.33, p=0.007$ ]. Overall, probability of making an omission response was higher on Day 3 compared to Day 2 ( $p=0.009$ ). On the 3 s ITI, MID ( $p<.001$ ) and LI ( $p<.001$ ) had a higher probability than HI of making an omission response. During the 5 s ITI, MID ( $p=0.026$ ) and LI ( $p=0.003$ ) rats also exhibited a higher probability than HI of making an omission. See **Figure 4.3B (c)**.

	Correct responses				Incorrect responses				Premature responses	
	3s	5s	7s	9s	3s	5s	7s	9s	7s	9s
HI	1145± 67.9	685.3± 24.2 <sup>o*</sup>	567.4± 16.8 <sup>o*</sup>	622.1 ±40.7	2668.3 ±66.4	1690± 97.9	1039.8 ±104	1369.6 ±202.3	5823.3 ±49 <sup>o*</sup>	6786± 74.3 <sup>o*</sup>
MID	1367. 2±77. 9	930.9± 42.7 <sup>o</sup>	794.6± 35.4 <sup>o</sup>	770.3 ±33.4	3267.8 ±79.2	2114± 84.4	1426.1 ±89.7	1262.5 ±100.6	5911.1 ±48.9 <sup>o</sup>	7123.2 ±52.8 <sup>o</sup>
LI	1109. 9±63. 8	909.5± 41.8*	790.9± 33*	704.5 ±31.7	3049.7 ±125.7	2281.2 ±136.6	1542.3 ±159.5	1335.1 ±178.8	6039.9 ±81.6*	7242.1 ±67.9*

**Table 4.2B Experiment 2, vITI challenge.** Latencies for correct, incorrect and premature responses. \*HI vs LI  $p < 0.05$ ; <sup>o</sup> HI vs MID  $p < 0.05$

### B2.1.2.3 First encounter with and exposure to the long ITIs

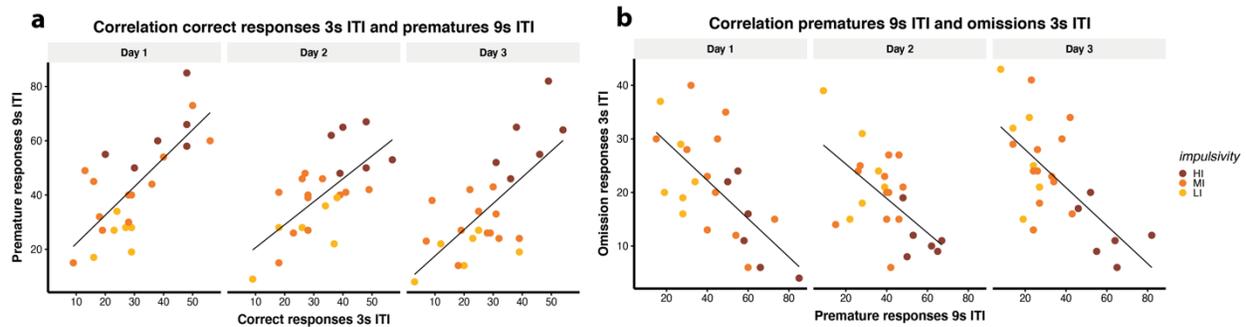
I looked at whether there was a difference in the trial number at which animals encounter the 7s and 9s ITI for the first time, across days. There was a main effect of day [ $F(2,105)=3.54$ ,  $p=0.033$ ] and a main effect of ITI, [ $F(1,105)=27.76$ ,  $p < .001$ ], but no effect of impulsivity group ( $p=0.122$ ). Post-hoc comparisons showed that animals encountered the long ITIs earlier in the session on Day 3 compared to Day 2 ( $p=0.044$ ). In addition, animals encounter the 7s ITI earlier in the session compared to the 9s ITI ( $p < .001$ ). When focusing on Day 1 only, I observed a similar pattern: there was no difference across groups in trial number of first presentation of the 7s and 9s ITI ( $p=0.626$ ), but there was a main effect of ITI ( $p < .001$ ). Again, animals encountered the 7s ITI trial earlier in the session than the 9s ITI ( $p < .001$ ).

I also looked at whether there was a difference across days and groups in the frequency of correct (and therefore rewarded) responses made with longest 7s and 9s ITIs. This was done to explore the extent to which animals across groups and across days were equally being presented with the same learning signals (i.e., correct responses for the 7s and 9s ITIs). This analysis revealed an interaction between ITI and impulsivity [ $F(2,105)=13.94$ ,  $p < .001$ ]. *Post-hoc*

comparisons revealed that, across days, HI rats made significantly fewer correct responses during the 7s ITI compared with LI rats ( $p=0.026$ ). A similar effect was observed with the 9s ITI with HI rats making significantly fewer correct responses than LI ( $p<.001$ ) and MID ( $p<.001$ ) rats. MID rats also made significantly fewer correct responses during the 9s ITI than LI ( $p<.001$ ).

Finally, I looked at the correlation between the trial number within the session at which either the 7s or 9s ITI was encountered for the first time and how many correct responses were made with that ITI within the session. This relationship was calculated for each day pooling all animals together. The only significant negative correlation between these two variables was observed on Day 3,  $r=-.46$ ,  $p=0.024$ .

### B2.1.2.4 Correlations



**Figure 4.4B Experiment 2 correlations between behavioural variables (a)** relationship between premature responses on 9 s ITI trials and correct responses on 3 s ITI, on the three days of testing ( $p<0.01$  in all three days). **(b)** relationship between premature responses on 9 s ITI trials and omission responses on 3 s ITI trials, on the three days of testing ( $p<0.01$  in all three days).

There was a positive correlation ( $r=0.74$ ,  $p<.001$ ) between making a correct response on the 3 s ITI trials and making a premature response on the 9 s ITI trials on Day 1. This was still significant both on Day 2 ( $p<.001$ ) and on Day 3 ( $p<.001$ ). There was also a strong negative relationship ( $r=-0.67$ ,  $p<.001$ ) between making an omission on ITI 3s and making a premature response on ITI 9 s on Day 1. This again held constant across the days (Day2,  $p=0.002$ ; Day3,  $p<.001$ ).

### B2.1.2.5 Activity per unit of time

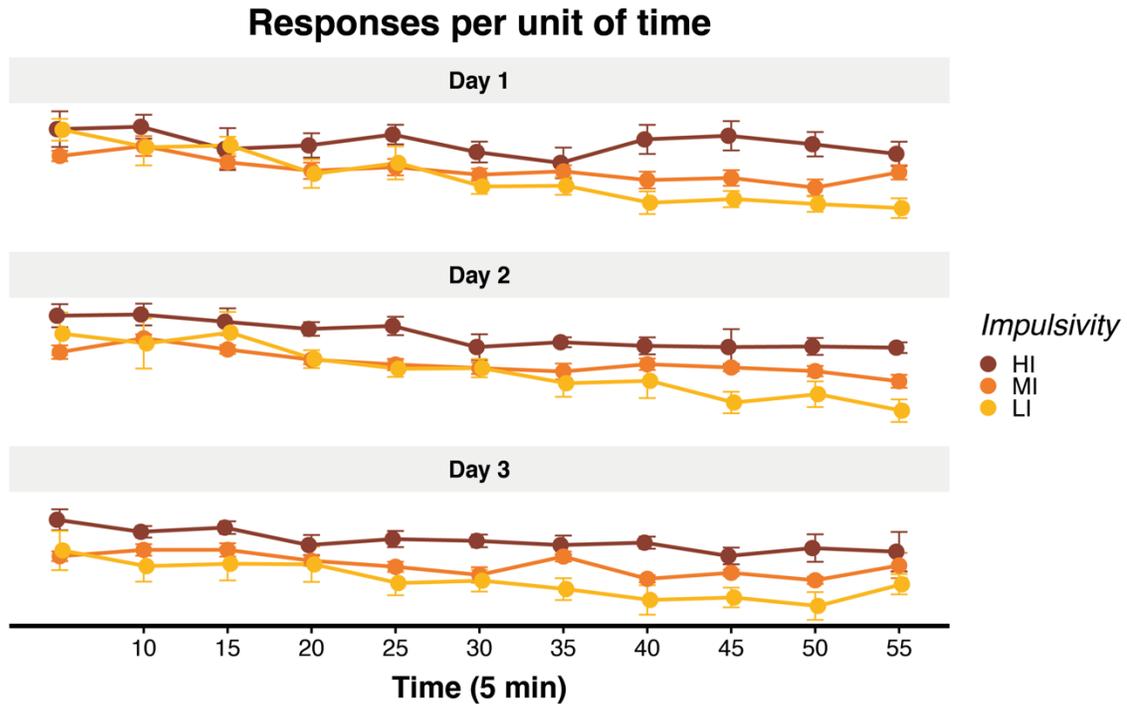


Figure 4.5B Experiment 2. Active responses (premature, correct, and incorrect) per unit of time, organised by impulsivity groups and days of testing.

As shown by **Figure 4.5B**, responding differed between impulsivity groups, depending both on Day ( $F(4,645)=2.79$ ,  $p=0.025$ ) and time ( $F(20,645)=2.87$ ,  $p<.001$ ). Specifically, differences in response rate between HI and LI emerged 25 min within the session ( $p=0.011$ ), with HI rats making significantly more responses per unit time than LI rats. These differences continued for the entirety of the session. HI were also significantly more active than MID at different time points: in the first 5 min ( $p=0.039$ ); after 25 min ( $p=0.023$ ), after 40 min ( $p=0.019$ ) and after 50 min ( $p=0.012$ ). Forty minutes within the session MID were also more active than LI ( $p=0.021$ ); this difference lasted until the end of the session, however at time point 50 min ( $p=0.065$ ) and 55 min ( $p=0.064$ ) it was no longer significant.

## B2.1.2.6 Effects of methylphenidate and atomoxetine

### B2.1.2.6.1 Effects of MPH and ATO as a function of the impulsivity phenotype

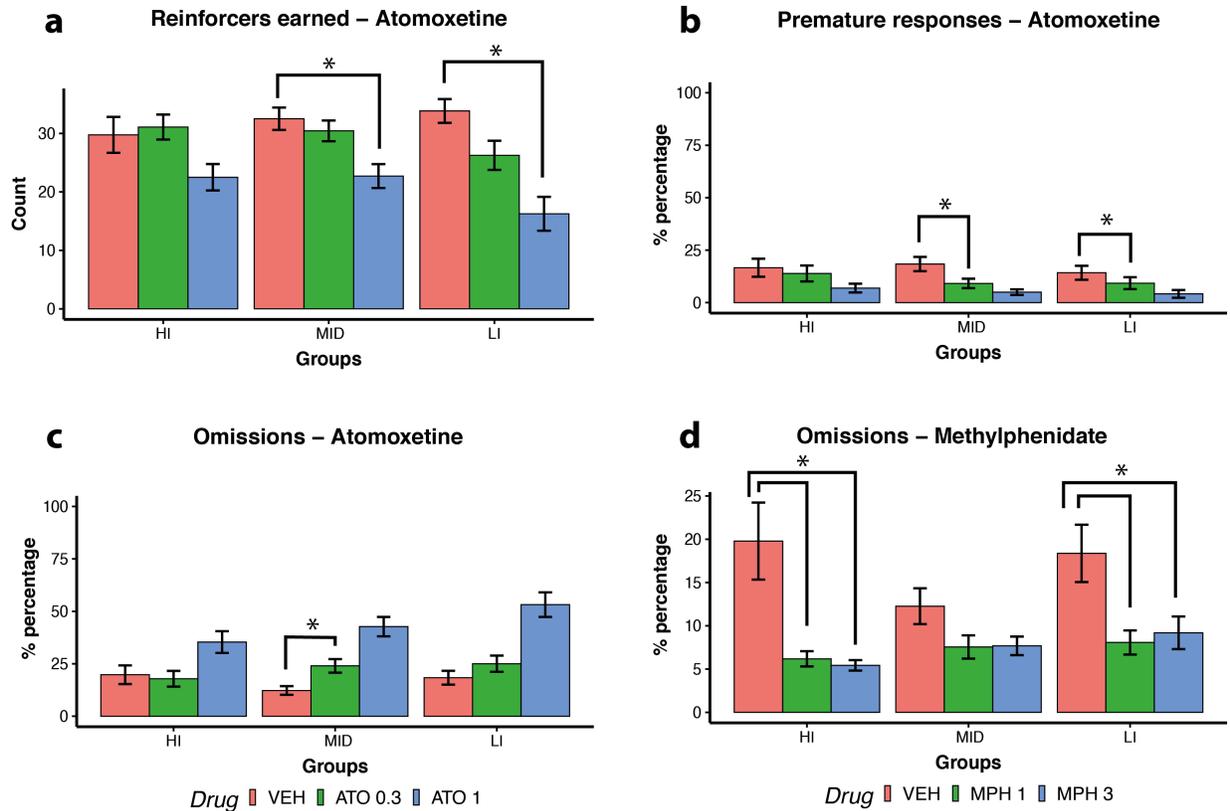


Figure 4.6B Experiment 2 (a-d) Interaction between drugs and trait impulsivity. Effects of ATO and MPH on reinforcers earned, percentages of premature responses and percentages of omissions. \* $p < 0.05$

#### B2.1.2.6.1.1 Reinforcers delivered

ATO affected performance differently depending on the impulsivity phenotype [Drug x Group interaction,  $F(8,380)=2.31$ ,  $p=0.020$ ]. Specifically, ATO (1 mg/kg) impaired the performance mostly of MID ( $t=-5.72$ ,  $p < 0.001$ ) and LI ( $t=-7.66$ ,  $p < 0.001$ ) rats, with a non-significant decrement in performance of HI rats ( $t=-2.37$ ,  $p=0.063$ , see **Figure 4.6B (a)**).

#### B2.1.2.6.1.2 Omissions

Both ATO and MPH modulated performance differently depending on the impulsivity phenotype [Group x Drug,  $F(8,380)=2.42$ ,  $p=0.015$ ]. Specifically, low-dose ATO (0.3 mg/kg) increased the proportion of omission solely for MID rats ( $t=4.30$ ,  $p < 0.001$ ) while high-dose ATO (3 mg/kg)

and low-dose (1 mg/kg) MPH decreased the proportion of omissions for HI ( $t=-3.66$ ,  $p=0.001$ ;  $t=3.96$ ,  $p<0.001$ ; respectively) and LI rats ( $t=-3.30$ ,  $p=0.004$ ;  $t=-2.94$ ,  $p=0.013$ ; respectively; see **Figure 4.6B (c and d)**).

#### **B2.1.2.6.1.3 Premature responses**

ATO modulated performance differently depending on impulsivity phenotype [Group x Drug,  $F(12,380)=18.23$ ,  $p<0.001$ ]. Specifically, low-dose ATO (0.3 mg/kg) decreased the probability of making a premature response compared with vehicle in MID ( $t=-5.20$ ,  $p<.001$ ) and LI ( $t=-2.70$ ,  $p=0.027$ ) but not in HI rats ( $t=-0.86$ ,  $p=0.764$ ; see **Figure 4.6B (b)**).

#### **B2.1.2.6.1.4 Latencies**

Latencies of correct, incorrect and premature responses following administration of ATO and MPH are shown in **Table 4.3B - 4.5B**.

	Correct responses				Incorrect responses				Premature responses	
	3s	5s	7s	9s	3s	5s	7s	9s	7s	9s
Veh	1159.5 ±53.5	858± 58.2	669.6± 30.3	689.5± 43.3	3294.8± 119.8	2167.7± 149.5	1494.6± 179.5	1005.4± 126.1	5997.5± 51.4	7341.8 ±55
ATO 0.3m g/kg	1175.5 ±75.1	893± 46.6	715.2± 26.5	665.5± 26.8	3272.1± 172.1	2313.2± 153.2	1611.9± 128.4	1098.6± 81.4	6226.7± 87.8	7472.8 ±93.6
ATO 1mg/ kg	1451±1 34.5*	1046 ± 61.6*	812.4± 44.7*	756.2± 33	3420.0± 227.9	2718.8± 207.2	2254.9± 163.3*	1477.9± 102.4*	6349.1± 103.3*	7584.9 ±89

**Table 4.3B Experiment 2, effects of ATO on a vITI challenge.** Latencies for correct, incorrect and premature responses. \* p<0.05 compared to vehicle.

	Correct responses				Incorrect responses			
	3s	5s	7s	9s	3s	5s	7s	9s
Veh	1159.5±5 3.5	858±58.2	669.6±3 0.3	689.5±4 3.3	3294.8±11 9.8	2167.7±149 .5	1494.7±17 9.5	1005.4±126 .1
Mph 1mg/ kg	802±33.1 *	638.6±21. 1*	581.5±2 4	595±43. 6	2633.4±15 1.1	1792.8±115 .3	1146.2±12 5	1166.8±123 .8
Mph 3mg/ kg	763.6±29. 8*	653.9±26. 4*	675.5±4 3.9	678.7±6 2.3	2417.7±10 9	1433.9±118 .9*	1548±210. 3	1617.7±235 .3*

**Table 4.4B Experiment 2, effects of MPH on behavioural performance during a vITI session.** Latencies for correct and incorrect. \* p<0.05 compared to vehicle.

	Veh		ATO 0.3mg/kg		ATO 1mg.kg		MPH 1mg/kg		MPH 3mg/kg	
	7s	9s	7s	9s	7s	9s	7s	9s	7s	9s
HI	5812.94 ± 93.19	7342.90 ± 157.16	6184.22 ± 168.09	7351.37 ± 217.73	6460.30 ± 23.77*	7667.88 ± 151.02	5589± 153.09	6486.39 ± 198.79*	5189.33 ± 82.84*	5544.52 ± 74.31*
MI D	6041.73 ± 71.67	7369.17 ± 75.23	6402.32 ± 101.18	7409.92 ± 119.22	6186.54 ± 166.99*	7551.02 ± 144.81	5715.71 ± 147.41	7009.12 ± 138.06*	5648.50 ± 127.49*	6451.02 ± 203.75*
LI	6070.19 ± 92.96	7290.62 ± 69.19	5895.34 ± 164.21	7709.53 ± 180.33	4756.56 ± 1867.97 *	7520.42 ± 166.57	5577.75 ± 237.71	6976.86 ± 223.81	5355.53 ± 330.74	6278.83 ± 524.27

**Table 4.5B Experiment 2.** Effects of ATO and MPH on the latency to make a premature response during a vITI session depended on the impulsivity group. \* p<0.05 compared to vehicle.

### B2.1.2.7 Effects of amphetamine, atipamezole and phenylephrine

	Correct responses				Incorrect responses				Premature responses	
	3s	5s	7s	9s	3s	5s	7s	9s	7s	9s
Veh	981.8± 46.6	714.8 ±29	564.3± 17	548.9± 22.3	3076.7± 157	1974.3± 163.6	1246.6± 106.6	1045±1 27	6104.8± 61.4	7305.2± 72.4
Amp h 0.2m g/kg	763.8± 31.6*	633.3 ±28.1	549±24 .8	641.1± 42.6	2827.1± 139.2	1410.1± 129.8	1051±1 17.3	1038±1 33.2	5630±7 9.9*	6590.5± 104.5*
Ati 0.3m g/kg	842.4± 32.2*	670±3 9.6	568±22 .6	553.8± 35.9	3120±1 42.3	1537.4± 162.9	1190.6± 120.6	1215.4± 146.2	5860.3± 63.3*	6997.1± 103.1*
Phen 1mg/ kg	999.9± 42.5	737.4 ±21.6	608.31 ±18.8	635.6± 33.5*	3488.9± 123.4	2060.4± 141.7	1127.9± 135.5	917.6±9 8.4	6114.2± 75.1	7377.2± 59.3

**Table 4.6B Experiment 2, effects of AMPH, ATI and PHEN on behavioural performance during a vITI session.** Latencies for correct, incorrect and premature responses. \* p<0.05 compared to vehicle.

# **Appendix C - Chapter 5**

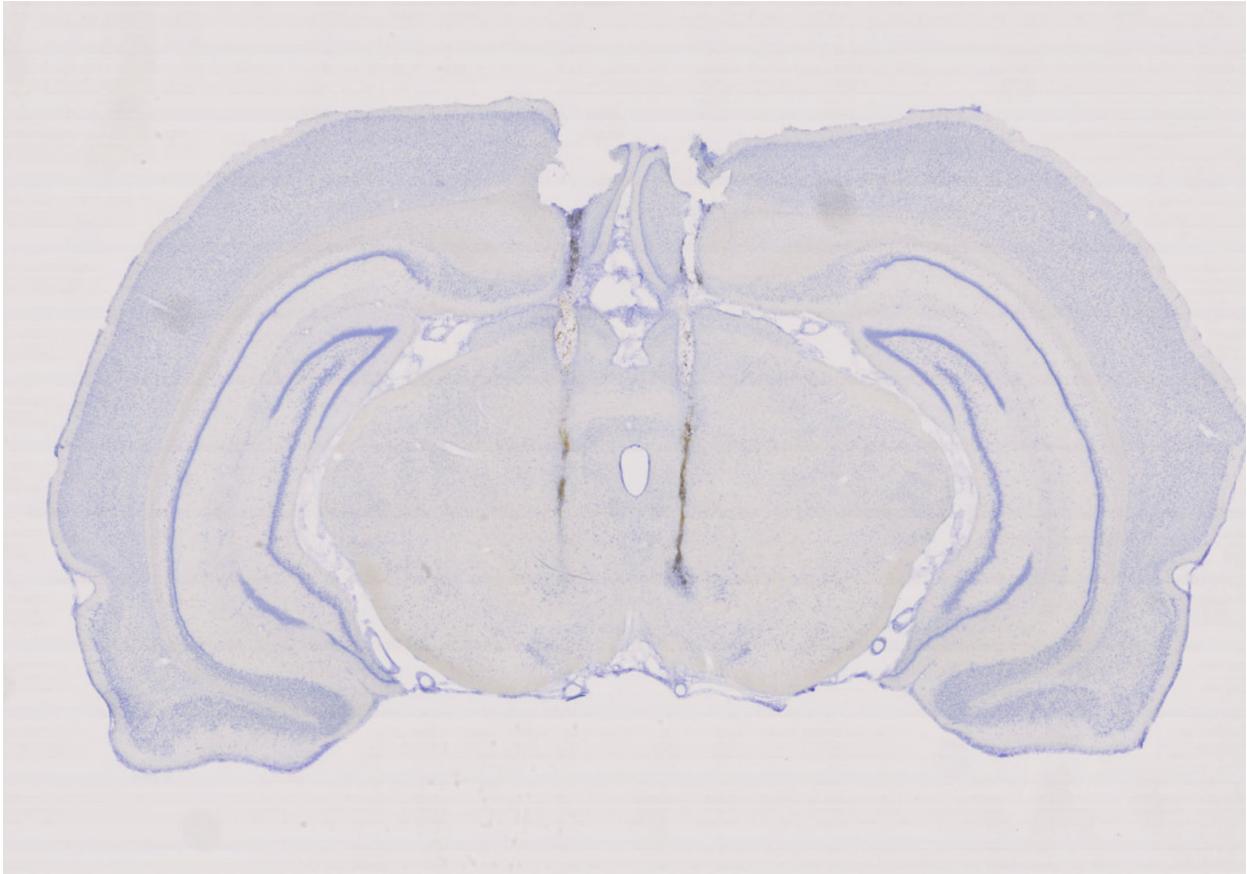
## **C1 Experiment 1**

### **C1.1 Data Analysis**

All LMEM models included the following factors: impulsivity (HI, MID and LI) and dose (veh, 0.01, 0.03, 0.3 and 1 ug/ul).

## **C1.2 Results**

### **C1.2.1 Histology**



**Figure 5.1C Exemplar image of a VTA cannulation**

## **C2 Experiment 2 - circuit-specific interventions**

### **C2.1 Pilot experiment**

This pilot experiment examined whether DREADDs is a reliable technique to silence cells of the VTA-striatal pathway. More specifically, inhibitory DREADDs (hM4D(Gi)) were expressed in cells projecting from VTA to the medial NAc shell 1) to validate with immunohistochemical techniques whether expression of the virus in this pathway was successful and 2) to investigate whether silencing these cells through delivery of the designer drug clozapine-N-oxide (CNO) significantly affects premature responding and other behavioural variables in the 5CSRTT.

## **C2.1.1 Materials and methods**

### **C2.1.1.1 Animals**

Nineteen outbred male Lister Hooded rats (Charles River, Margate, UK) weighing 280–300 g at the beginning of the experiments were used. Animals were kept under the conditions specified in Chapter 2 (see section 2.1).

### **C2.1.1.2 Intracranial Surgery**

All rats were anaesthetised with isoflurane and secured in a stereotaxic frame. To induce DREADD expression selectively in midbrain neurons projecting to medial NAcB shell we used a Cre-dependent DREADD combined with canine-adenovirus2 expressing Cre recombinase (CAV2Cre).

#### AAV5- hSyn-DIO-hM4D(Gi)-mCherry and AAV5- hSyn-DIO-mCherry

Animals were divided into two groups: the DREADD group (n=10) was injected with 1ul of AAV5- hSyn-DIO- hM4D(Gi)-mCherry (Addgene, 1,00E+09pp/ul) in VTA (coordinates in mm relative to Bregma): AP -5.6, ML at +/-1.82 (angle of 10°), DV -7.8 (below dura). Another group served as control (n=9) and was injected with 1ul of AAV5-hSyn-DIO-mCherry (Addgene, 1,00E+09pp/ul) in VTA (coordinates in mm relative to Bregma): AP -5.6, ML at +/-1.82 (angle of 10°), DV -7.8 (below dura).

#### CAV2-Cre

All animals were bilaterally injected with CAV2-Cre (PVM, France) into the medial NAcB shell. To target different areas of the medial NAcB shell both the DREADD (DIO-hM3D(Gq)-mCherry) and the control (DIO-mCherry) groups received bilateral injections of CAV2-Cre virus into different regions of the medial shell. Specifically, 5 animals of the DREADD group were injected with CAV2-Cre (3,33E+09pp/ul) in (coordinates in mm relative to Bregma): AP +1.2, ML at +/-0.75, and the 3 DV coordinates -5.4; -5.9; and -6.9 (below dura). Another 5 animals of the DREADD group were injected with CAV2-Cre (3,33E+09pp/ul) in (coordinates in mm relative to Bregma): AP +1.7, ML at +/-0.75, and the 3 DV coordinates -5.4; -5.9; and -6.9 (below dura).

Of the control group, 3 animals were injected with CAV2-Cre ( $3.33 \times 10^9$  pp/ul) in (coordinates in mm relative to Bregma): AP +1.2, ML at  $\pm 0.75$ , and the 3 DV coordinates -5.4; -5.9; and -6.9 (below dura). Another 5 animals of the control group were injected with CAV2-Cre ( $3.33 \times 10^9$  pp/ul) in (coordinates in mm relative to Bregma): AP +1.7, ML at  $\pm 0.75$ , and the 3 DV coordinates -5.4; -5.9; and -6.9 (mm below dura). Finally, 1 control animal (DIO-mCherry) was injected with CAV2-Cre ( $1.67 \times 10^9$  pp/ul) in two AP targets: 1.7 and 1.2, ML at  $\pm 0.75$ , and the 3 DV coordinates -5.4; -5.9; and -6.9 (below dura).

Infusion volumes varied according to DV target: DV -5.4 and -5.9, a volume of 0.15 ul of virus was injected at a rate of 0.1 ul; at DV -6.9 a volume of 0.30 ul was injected at a rate of 0.1 ul/min. The needle was left in place for 1 min between the first two most dorsal DV targets and 10 min after the last DV target, to allow for diffusion of the virus in the intended brain region.

### **C2.1.1.3 Immunohistochemistry**

Rats were perfused and brains were extracted as described in Chapter 2 (Section 2.5). For this study, immunostaining for mcherry and tyrosine hydroxylase (TH) was performed on midbrain ( $\sim 5.4$  to  $\sim 6.00$  mm posterior to bregma). Staining for mcherry was necessary to assess the expression of the virus, while staining for TH enabled the determination of the degree of colocalisation between TH and mcherry and thus the extent to which VTA-shell cells positive for mcherry were dopaminergic in phenotype. Sections were kept in a cryoprotectant solution (prepared as described by Watson et al., 1986) and stored at  $-20^\circ\text{C}$  prior to immunostaining. The immunohistochemistry protocol used to amplify the fluorescent signal of mcherry and TH was followed as described by de Backer and colleagues (2010). Free floating sections were washed 3 times with PBS and blocked for 2 h in PBS with 3% normal goat serum (NGS) and 0.3% triton X- at RT. For double staining of TH and mcherry, sections were incubated for 24 h at  $4^\circ\text{C}$  in PBS supplemented with chicken polyclonal anti-mcherry (1:1000, Abcam) and mouse monoclonal anti-TH (1:1000, Millipore). The next morning sections were washed 3 times for 10 min with PBS and incubated for 2 h with secondary antibodies ALEXA 568 conjugated goat anti chicken (1:1000) and ALEXA 488 conjugated goat anti mouse (1:1000, both Abcam) in PBS with 3% NGS and 0.3% triton X at RT. After 3, 10 min washes in PBS, the sections were

transferred to microscope slides and left in the dark to dry. Once dried they were kept in a closed box and stored at 4°C.

## **C2.1.1.4 Behavioural Measures**

### **C2.1.1.4.1 Locomotor Task**

Locomotor activity was assessed using 10 activity chambers (MedAssociates; 29.5 x 32.5 x 23.5 cm, USA), equipped with infrared photocell beams and controlled by a PC. Locomotor activity was measured as the number of photocell beam breaks every 5 min for a period of 2 h following the beginning of the session. Different drugs were administered in two separate tests (Test 1 and Test 2), on the same rats. In Test 1, rats were treated with an IP injection of either clozapine (0.1 mg/kg, SigmaAldrich), CNO (3mg/kg, HelloBio, UK), or saline (IP) injections and immediately placed into the activity cages. This test aimed to assess whether CNO and clozapine, administered in isolation, exerted a similar effect on rats transfected with DREADDs. CNO was the only drug that was found to reduce locomotor activity in Gi-rats (see results section), thus Test 2 further explored whether this effect could be amplified by co-treatment between CNO and MPH. Thus, in Test 2 rats, were pre-treated with an IP injection of either CNO (3mg/kg, HelloBio, UK), or saline (IP) injections and immediately placed into the activity cages. After 25 min, animals received injections of methylphenidate (MPH) (3 or 6 mg/kg, IP) or saline (3 mg/kg). In both Test 1 and 2 injections followed a Latin-square design. Test 1 and 2 were separated by one day of inactivity to allow for the drug effects to wane. Inadvertently, all CNO-pre-treated animals were given a second dose of CNO (3mg/kg) instead of the saline control, hence control conditions without MPH were removed from statistical analyses.

#### **C2.1.1.4.1.1 Data Analysis**

A linear mixed model compared differences in locomotor activity between test groups and across treatments, both in Test 1 and Test 2. In Test 1 the model contained four fixed factors: bin (5min-120min), treatment (clozapine, saline, CNO), test group (Gi and mcherry), AP coordinate (1.7 and 1.2). In Test 2 the model contained five fixed factors: bin (5 min-120 min), pre-treatment (CNO and saline), treatment (MPH 6 mg/kg and MPH 3 mg/kg), test group (Gi and mcherry), AP coordinate (1.7 mm and 1.2 mm). All models also included one factor (subject) modelled as a random slope to account for individual differences between rats across sessions. When significant three-way interactions were found, further analyses were performed by conducting separate multilevel models.

## **C2.1.1.4.2 Progressive Ratio Task**

### **C2.1.1.4.2.1 Apparatus**

Testing took place using computer touchscreen operant chambers (Campden Instruments Ltd., Loughborough, U.K.) described by (Horner et al., 2013). The chambers were constructed of black Perspex in a trapezoidal shape (20 cm x 33 cm x 25 cm width at screen and 13 cm width at magazine) and were contained within light and sound-attenuating boxes. Each chamber was fitted with a 15-inch touch sensitive LCD screen. On the opposing side was a magazine connected to a pellet dispenser that delivered 45 mg dustless pellets (TestDiet, Indiana, USA). The food tray was fitted with a light and an infrared (IR) beam that registered magazine entries. Front and rear IR beams were fitted to monitor the rats' activity within the chamber. Black Perspex masks were fitted to the touchscreens that had five equally-sized and equally-spaced response windows.

### **C2.1.1.4.2.2 Pretraining**

Sixteen animals underwent behavioural testing, which consisted of one session per day (5 days per week). All animals were initially given a 20 min habituation period within the chambers. During this session, no stimuli were presented but the house-light was kept on. Following this session, subjects underwent one day of touchscreen press training where a white square stimulus was presented in the central aperture for 30 s. A single response to this stimulus resulted in three food pellets being delivered. Stimulus offset and a short tone (1000 ms, 3 kHz) accompanied reward delivery. Following a 5 s ITI, the stimulus was presented on the screen. If no response was made within 30 s the trial ended and a single food pellet was delivered with stimulus offset and accompanying tone. Each session was terminated following 100 rewards being delivered or after 45 min had elapsed.

Subjects then underwent fixed ratio (FR) 1 training. During these sessions, a single response to the stimulus was required for a single pellet to be delivered, which was again accompanied by the tone. A 5 s ITI was employed. Each session was terminated following 45 min or 100 trials being completed. All animals were required to complete 100 trials within a 45min session before moving on to the next stage of training. The subsequent training stage consisted of FR5 responding, where five responses were required for each reward delivery. The fifth response to

the stimulus completed the trial and resulted in reward delivery. All other parameters were identical to the FR1 stage of training. Each session was terminated following 100 trials (i.e., 500 target responses) or after 45 min. Each animal was required to complete 100 trials for two consecutive sessions before being placed on a PR schedule of reinforcement.

#### **C2.1.1.4.2.3 Test session**

Twenty-five minutes before the start of the test session, subjects were injected with either saline or CNO (3mg/kg, HelloBio, UK). All injections followed a Latin square design. Under this schedule, the number of target responses required for the delivery of the appetitive reward increased in each trial, according to the following formula ( $5 * e^{(0.2*n)} - 5$ ), where n is the trial number. This schedule yielded ratios of ratio requirements of 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145 etc. If no response was made on the touchscreen within 180 s the session was terminated, otherwise sessions ended after 45 min had elapsed.

#### **C2.1.1.4.2.4 Data Analysis**

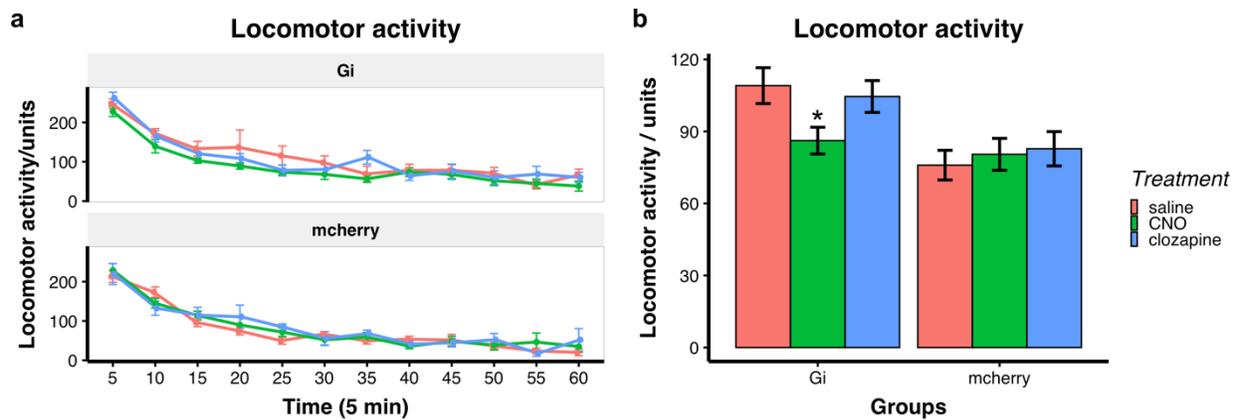
The behavioural variables of interest were total trials, that is the number of trials completed before the animal stopped responding; breakpoint (BP), defined as the number of target responses made in the last successfully completed trial for each subject; the total number of nose-pokes, including those in trials not completed

## C2.1.2 Results

### C2.1.2.1 Histology

Due to technical problems, it was not possible to measure the degree of colocalisation between TH and mcherry, however a qualitative analysis of brain slices showed that all animals expressed mcherry in the regions of interest.

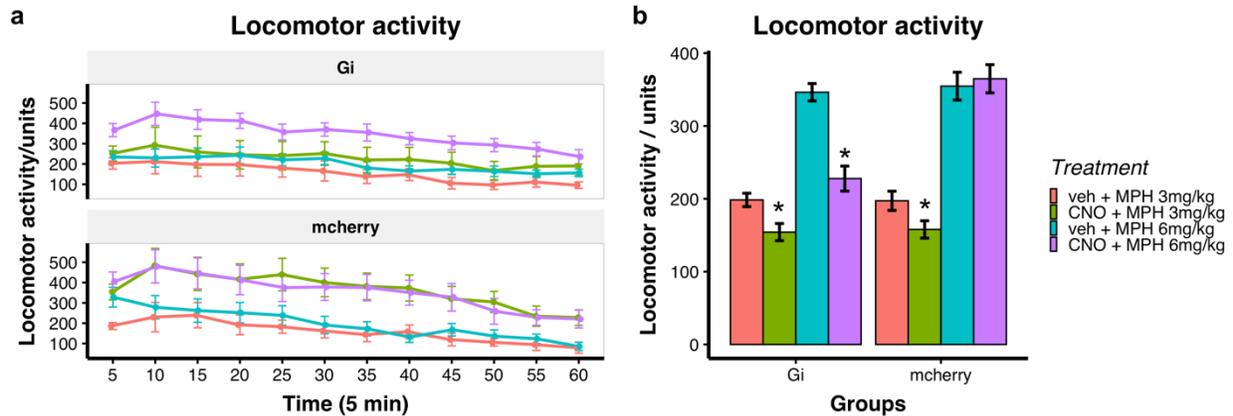
### C2.1.2.2 Effects of DREADDs on locomotor activity - Test 1



**Figure 5.2C Effects of DREADDs on locomotor activity. Test 1.** Gi-shell animals were significantly less active after injection of CNO compared to saline \* $p < 0.05$ .

As shown in **Figure 5.3a** and **b**, locomotor activity was influenced by the administration of drugs depending on the test group [Treatment x Group,  $F(2,1207)=6.99$ ,  $p < 0.001$ ]. Specifically, neither CNO nor clozapine had any effects on locomotor activity of mcherry animals ( $p > 0.9$ ), however Gi-shell animals were significantly less active after injection of CNO compared to vehicle ( $p < 0.001$ ). Clozapine also reduced locomotor activity in the Gi-shell group compared to vehicle, however this was significant only at a trend level ( $p = 0.066$ ).

### C2.1.2.3 Effects of DREADDs on locomotor activity - Test 2



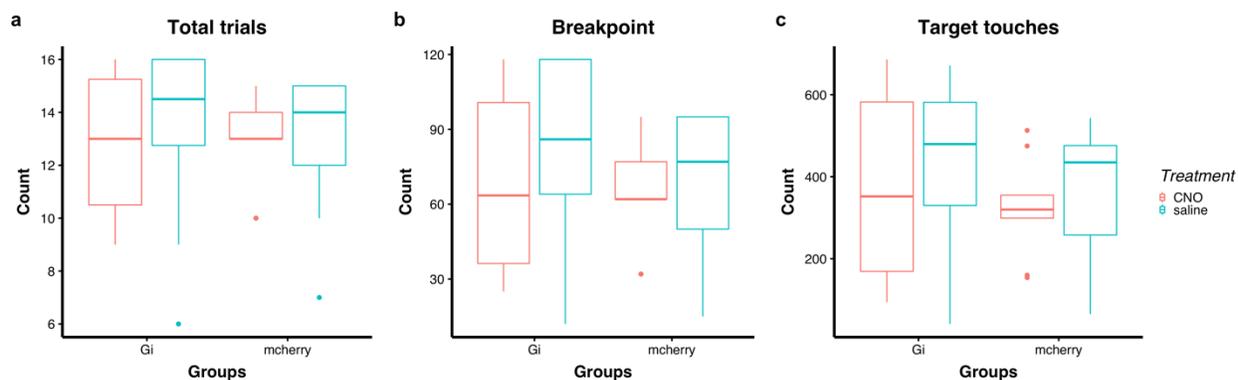
**Figure 5.3C Effects of DREADDs on locomotor activity. Test 2. Gi shell group:** Locomotor activity was greater when rats were pre-treated with saline compared to when they were pre-treated with CNO, for both treatment doses of MPH 3 mg/kg and 6 mg/kg. **mcherry group:** locomotor activity of control rats was greater when rats were pre-treated with saline compared to when they were pretreated with CNO for treatment doses of MPH 3 mg/kg. \*compared to saline  $p < 0.05$ .

For locomotor activity in Test 2 there was a significant three way interaction between test group, pre-treatment and treatment [Group x Pre-treatment x Treatment,  $F(1,1615)=19.89$ ,  $p < 0.001$ ].

Because there was a significant three-way interaction, effects were analysed for each test group separately. As shown in **Figure 5.4a** and **b**, locomotor activity of Gi-shell rats varied depending on pre-treatment and treatment [Pre-treatment x Treatment,  $F(1,855)=11.22$ ,  $p < 0.001$ ].

Specifically, locomotor activity was greater when rats were pre-treated with saline compared to when they were pre-treated with CNO, for both treatment doses of MPH 3 mg/kg and 6 mg/kg ( $p < 0.001$  both comparisons). Locomotor activity of mcherry rats also varied depending on pre-treatment and treatment [Pre-treatment x Treatment,  $F(1,760)=8.81$ ,  $p = 0.003$ ]. Specifically, locomotor activity of control rats was greater when rats were pre-treated with saline compared to when they were pretreated with CNO for treatment doses of MPH 3 mg/kg ( $p < 0.001$ ). However, there was no difference in locomotion between the saline and CNO pre-treatment, when rats were treated with MPH 6 mg/kg ( $p = 0.906$ ).

### C2.1.2.4 Effects of DREADDs on a progressive ratio test



**Figure 5.4C** Effects of DREADDs and treatment with CNO (and saline) on the PR task. (a) Total trials: no effects; (b) breakpoint: no effect; (c) target touches: no effect.

There were no differences between groups and across treatments for total trials, breakpoint, and target touches on the PR ( $p > 0.700$  for all comparisons). See **Figure 5.5a,b,c**. Both virus groups completed around 12 trials, both when administered CNO and saline; they had an average breakpoint of 70 and total target touches of 370.

### C2.1.2.5 In summary

Injection of CNO reduced locomotor activity in rats expressing DREADDs, compared to control rats (expressing mcherry), when administered in isolation and in combination with MPH 6 mg/kg. CNO injection however did not have any effects on motivation as assessed by performance on a PR task in rats expressing DREADDs or in control rats. In addition, clozapine had no significant effects on locomotor activity.

## **C2.2 Chemogenetics intervention in the 5CSRTT**

### **C2.2.1 Methods and Materials**

#### **C2.2.1.1 Immunohistochemistry**

After collection, sections were kept in a cryoprotectant solution (prepared as described by Watson et al., 1986) and stored at -20° C prior to immunostaining. The immunohistochemistry protocol used to amplify the fluorescent signal of mcherry, cfos and TH was followed as described above for the pilot experiment, taken from de Backer and colleagues (2010). Free floating sections were washed 3 times with PBS and blocked for 2 h in PBS with 3% normal goat serum (NGS) and 0.3% triton X- at RT. For double staining of TH and mcherry, sections were incubated for 24 h at 4°C in PBS supplemented with chicken polyclonal anti-mcherry (1:1000, Abcam) and mouse monoclonal anti-TH (1:1000, Millipore). The next morning sections were washed 3 times for 10 min with PBS and incubated for 2 h with secondary antibodies ALEXA 568 conjugated goat anti chicken (1:1000) and ALEXA 488 conjugated goat anti mouse (1:1000, both Abcam) in PBS with 3% NGS and 0.3% triton X at RT. After 3, 10 min washes in PBS, the sections were transferred to microscope slides and left in the dark to dry. Once dried they were kept in a closed box and stored at 4° C. For the staining of cfos, sections were incubated for 24 h at 4°C in PBS supplemented with rabbit polyclonal anti-cfos (1:1000, Millipore). The next morning sections were washed 3 times for 10 min with PBS and incubated for 2 h with the secondary antibody ALEXA 488 conjugated goat anti rabbit (1:1000, both Abcam) in PBS with 3% NGS and 0.3% triton X at RT. After 3 x 10 min washes in PBS, the sections were transferred to microscope slides and left in the dark to dry. Once dried they were kept in a closed box and stored at 4° C.

#### **C2.2.1.2 Data Analysis**

For all analyses, the control groups of rats with mcherry expression in the VTA-Core pathway (N=5) and rats with mcherry expression in the VTA-Shell pathway (N=4) were merged into one group to increase statistical power and based on no evidence of a statistical difference between the groups.

For performance on the 5CSRTT prior to the viral infusion during baseline and during the 7s ITI sessions of the impulsivity screening, separate LMEM tested whether rats later assigned to different viral manipulations (virus groups, model 1) and those sorted based on their impulsivity profile (model 2) differed with regards to accuracy, reinforcers earned, number of omissions, number of premature responses, latency to make correct and incorrect responses. Model 1 contained two fixed factors: virus group (Gi core, Gi shell and mcherry), and ITI (5s and 7s). Model 2 also contained two fixed factors: impulsivity group (HI, MID and LI) and ITI (5s and 7s). Similarly, for performance on the 5CSRTT following virus infusion separate linear mixed models compared differences between virus groups (model 1) and impulsivity groups (model 2), across treatments, for accuracy, reinforcers earned, number of omissions, number of premature responses and response latency (latency for correct and incorrect responses). Model 1 contained three fixed factors: pre-treatment (vehicle and CNO), treatment (vehicle and MPH 6mg/kg) and virus group (Gi core, Gi shell and mcherry). Model 2 contained three fixed factors: pre-treatment (vehicle and CNO), treatment (vehicle and MPH 6 mg/kg) and impulsivity (HI, MID and LI).

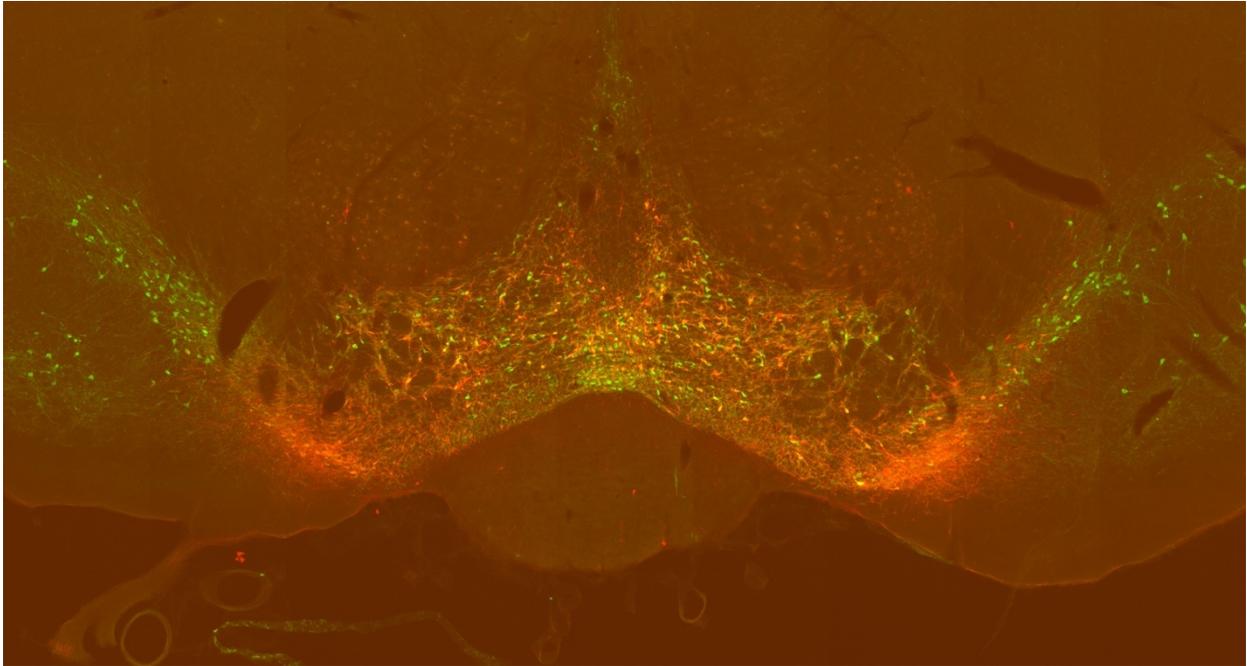
For locomotor activity a linear mixed model compared differences between test groups and treatments. The model contained three fixed factors: bin (5min-120min), treatment (vehicle + MPH 6mg/kg, CNO + MPH 6mg/kg), test group (Gi core, Gi shell and mcherry).

For c-fos immunoreactivity, a linear mixed model compared differences between test groups, treatments and regions. The model contained three fixed factors: region (DMS, DLS, medial shell, lateral shell and core), AP (anterior and posterior), treatment (vehicle + MPH 6 mg/kg and CNO + MPH 6 mg/kg), test group (Gi core, Gi shell and mcherry). In each test group, locomotor activity under the two different treatments, in the first 30 min following the delivery of MPH, was correlated (Pearson correlation) with c-fos activation in each region. For this analysis only the first 30 min following MPH administration was tested as this was the most valid time window coincided with the expected peak in cfos expression 90-60 min later. Because locomotor activity was compared with cfos count in 10 different regions, Bonferroni corrections were applied to control for multiple comparisons, restricting the significance level at  $\alpha = 0.05/10 = 0.005$ .

All models also included one factor (subject) modelled as a random slope to account for individual differences between rats across sessions. When significant three-way interactions were found, further analyses were performed by conducting separate multilevel models.

## **C2.2.2 Results**

### **C2.2.2.1 mcherry expression**



**Figure 5.5C** Representative image of colocalization between mcherry staining and TH staining. Green = TH; red = mcherry; yellow = colocalisation between mcherry and TH.

### C2.2.2.2 c-fos expression

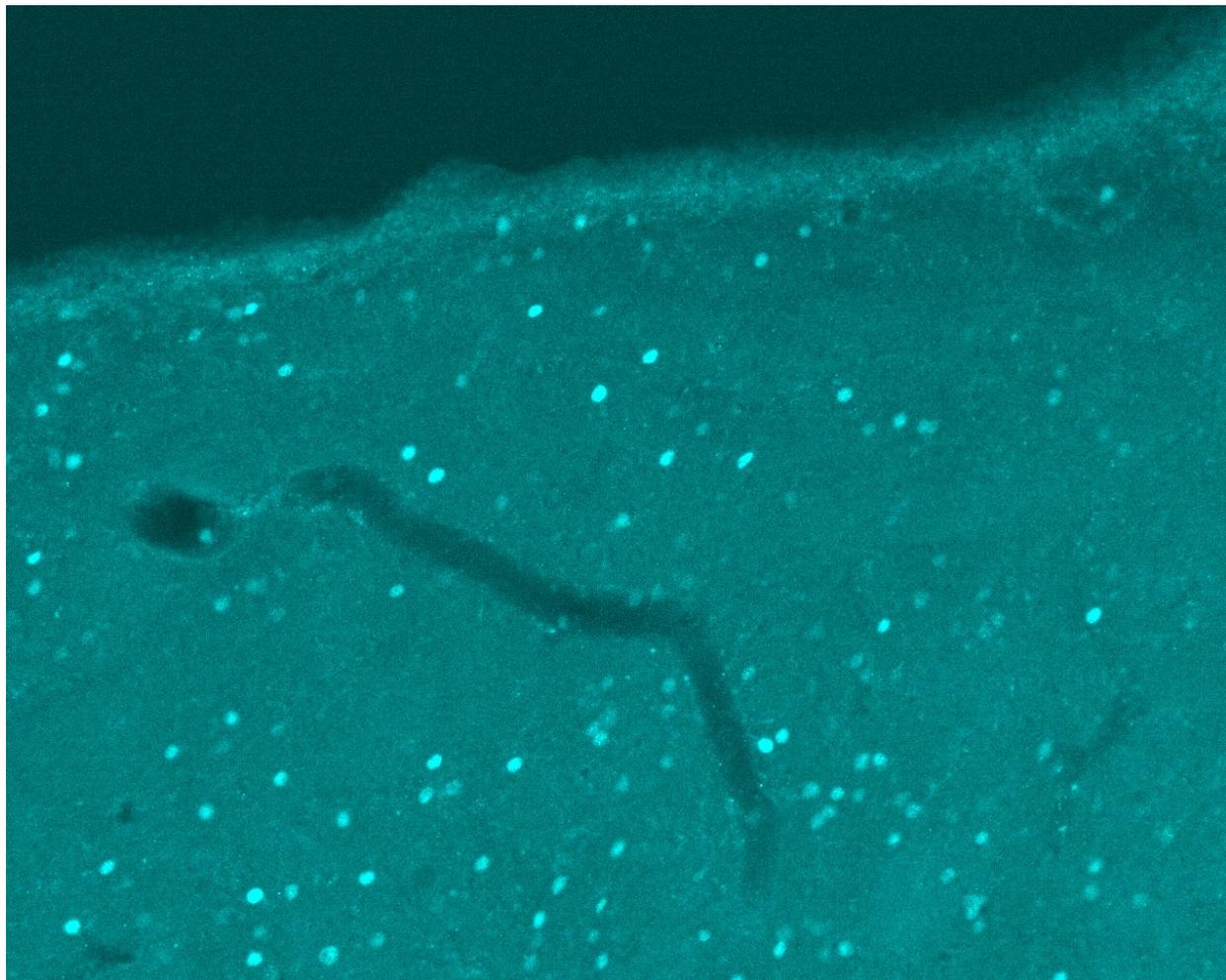


Figure 5.6C Representative image of c-fos expression in the DMS

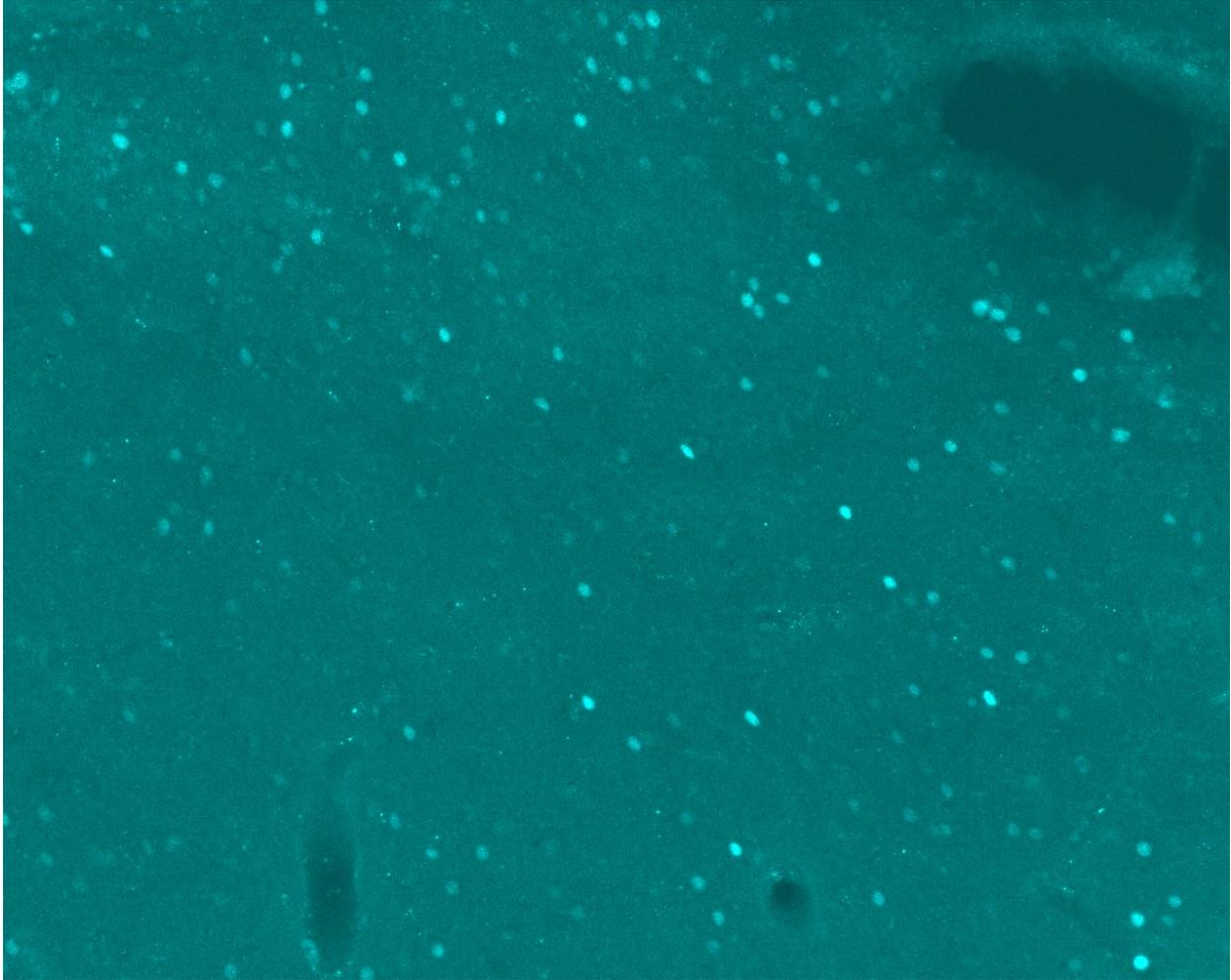


Figure 5.7C Representative image of c-fos expression in the medial shell

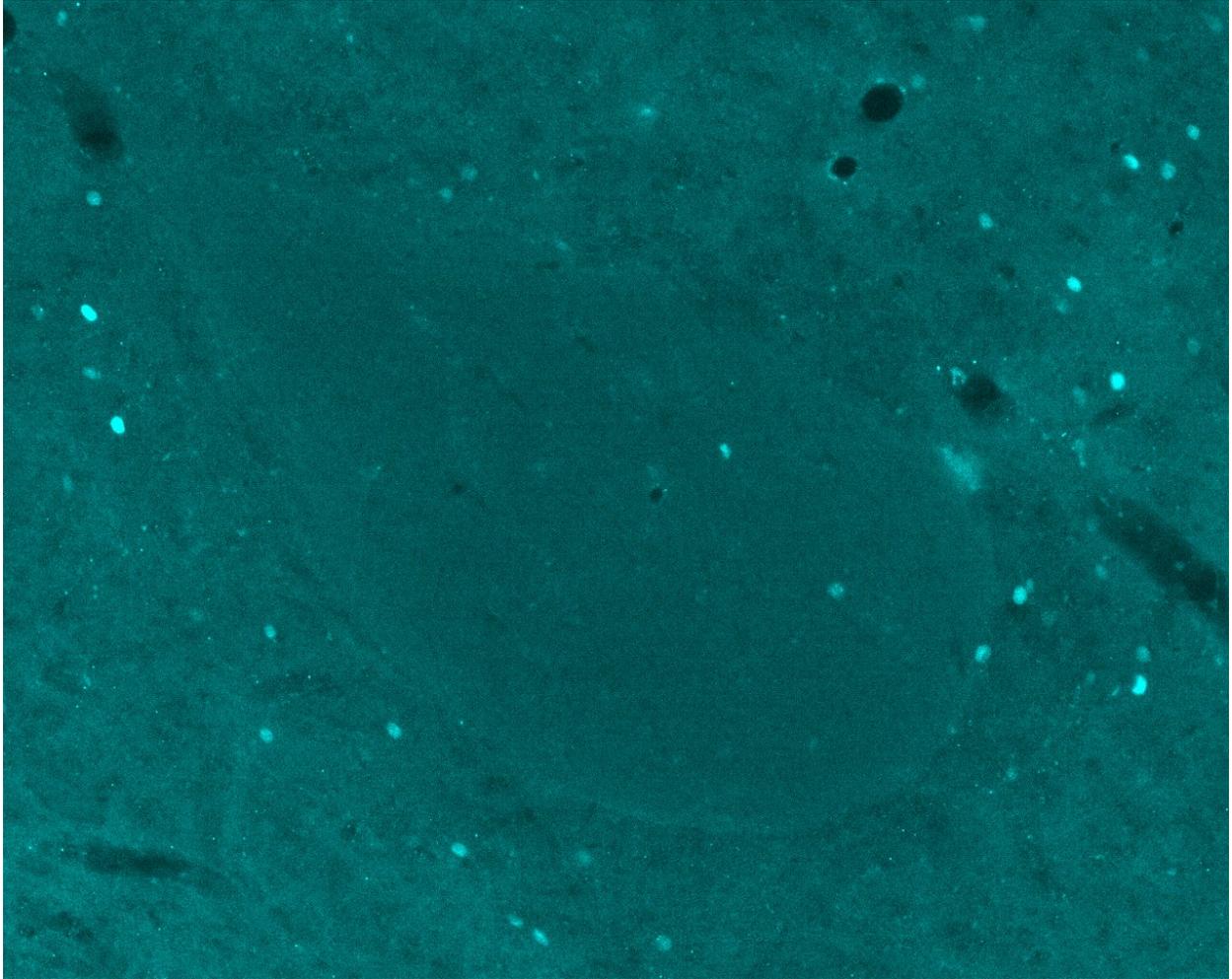


Figure 5.8C Representative image of c-fos expression in the core



Figure 5.9C Representative image of c-fos expression in the lateral shell

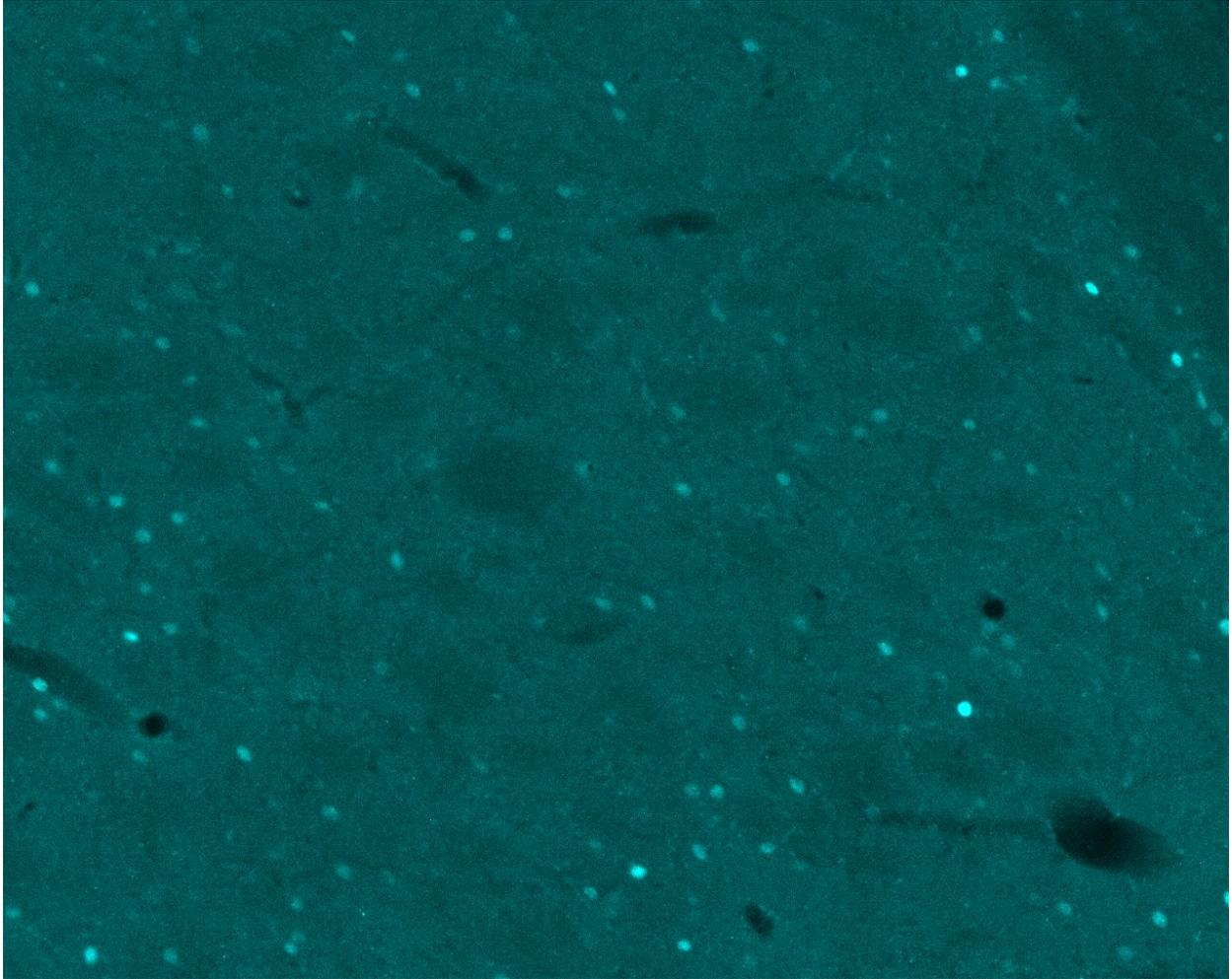


Figure 5.10C Representative image of c-fos expression in the DLS

# Appendix D - Chapter 6

## D1 Methods and materials

### D1.1 Immunohistochemistry

Free-floating 60  $\mu\text{m}$  sections were rinsed for  $3 \times 10$  min in 0.01M phosphate buffered saline (PBS, pH 7.4) and placed into primary antibody solution (NeuN monoclonal mouse anti-neuronal nuclear protein, Millipore MAB377) on a rotatory shaker at room temperature overnight. The primary antibody was made to a concentration of 1:1000 in 0.01M PBS containing the non-ionic detergent 0.3% Triton X-100 (Sigma) to solubilise the protein.

After incubation in primary antibody, the sections were washed for  $3 \times 10$  min in 0.01M PBS with 0.3% TX (PBS-TX) and incubated for 2 h in biotin-conjugated secondary antibody diluted 1:1000 in PBS-TX. Following this, sections were washed for  $3 \times 10$  min in 0.01M PBS-TX and incubated for 1 h in ImmPACT DAB Peroxidase (HRP) (Vector labs SK4105) diluted 1:1000. Sections were again washed  $3 \times 10$  min in 0.01M PBS with 0.3% TX (PBS-TX) and  $1 \times 10$  min in 0.01M PBS. Following this, sections were developed in ImmPACT™ DAB for approximately 10 min.

The visualisation reaction was stopped by transferring the sections rapidly into cold 0.01M PBS and washing thoroughly in 0.01M PBS. Sections were mounted on gelatinised slide, dried, dehydrated for 2 min in 100% ethanol, transferred to 50% ethanol/50% xylene for 2 min and finally delipidated in xylene for 2 min, and finally coverslipped with DePex.

## D1.2 Data Analysis

### D1.2.1 Baseline

The number of sessions to reach criteria were analysed for both tasks. The linear mixed model analysing this considered two fixed factors: task (SDT and 5CSRTT) and impulsivity group (HI, MID and LI). Once criteria were reached, two sessions with the final SD parameters were used to compare baseline performance in the 5CSRTT and SDT. A linear mixed model compared accuracy in the 5CSRTT against accuracy on the SDT, both on signal trials and on non-signal trials. This model contained three fixed factors: session number (1 and 2), task (5CSRTT; SDT signal trials; SDT non-signal trials) and impulsivity group (HI, MID and LI). The same model was applied when comparing baseline performance on the SDT against performance on 1 s SD trials on the 5CSRTT (stage 7 of 5CSRTT training). The linear mixed model analysing the percentage of omission responses in the 5CSRTT considered two fixed factors: session number (1 and 2) and impulsivity group (HI, MID and LI).

Distribution of response types (correct, incorrect, omissions, premature responses) in the different holes of the 5CSRTT were analysed. The model included three fixed factors: response type (correct, incorrect missed, incorrect chosen, premature response, omission missed), hole (1-5), and impulsivity group (HI, MID, LI). In the SDT, additional analyses were run comparing the proportion of anticipatory responses relative to correct and incorrect trials and to signal and non-signal trials. These were performed by dividing anticipatory responses made prior to each response type (signal correct trials, non-signal correct trials, signal incorrect trials, non-signal incorrect trials) by the total number of trials of each response type. Response latencies were also analysed for tasks. However, due to a technical error it was not possible to retrieve latencies for correct and incorrect responses on the SDT. Thus, for the SDT, the only response latency that was possible to analyse was latency to initiate a trial (poking in the central aperture). In contrast, for the 5CSRTT, latencies for correct and incorrect responses were analysed. The linear mixed model analysing latency to initiate a trial in the SDT considered two fixed factors: session number (1 and 2) and impulsivity group (HI, MID and LI). The linear mixed model analysing

response latency in the 5CSRTT included three fixed factors: session number (1 and 2), impulsivity group (HI, MID and LI) and response type (correct and incorrect response).

All models also included one factor (subject) modelled as a random slope to account for individual differences between rats across sessions. When significant three-way interactions were found, further analyses were performed by conducting separate multilevel models.

### **D1.2.2 Variable SD**

Before comparing performance on a vSD between the two tasks, accuracy on the SDT between 1 s and 0 s trials was compared to reveal whether there were any differences in performance between these trial types. Because there were no significant differences in accuracy between these trial types (reported on page 190 of the results section), only SDs common to both tasks were considered for further analyses on accuracy. Thus, the model analysing accuracy for the vSD session prior to the lesion contained three fixed factors. These were: SD (0.03s, 0.06s, 0.25s and 1s), task (5CSRTT; SDT) and impulsivity groups (HI, MID and LI). More details on this on page 190 of the Results section. The linear mixed model analysing the percentage of omission responses in the 5CSRTT included two fixed factors: SD (0.03 s, 0.06 s, 0.25 s, 0.7 s, 1 s) and impulsivity group (HI, MID and LI). The overall proportion of anticipatory responses in the vSD session was analysed similarly to what was done for the baseline sessions and was compared to the proportion of anticipatory responses during the two baseline sessions in a model that included three fixed factors: session (baseline, vSD), task (5CSRTT, SDT) and impulsivity group.

Anticipatory responses were also analysed separately in the vSD sessions of the 5CSRTT and the SDT. In the SDT, the proportion of anticipatory responses were again compared relative to each trial type and SD, in a linear mixed model that included: SD (0s, 0.03s, 0.06s, 0.25s and 1s), response type (correct and incorrect trials) and impulsivity group. The linear mixed model analysing response latency in the 5CSRTT included two fixed factors: impulsivity group (HI, MID and LI) and response type (correct and incorrect response). The linear model analysing response latency in the SDT only included one factor, that is impulsivity group (HI, MID and LI). As for analyses of the baseline sessions, the linear mixed models also included one factor (subject) modelled as a random slope to account for individual differences between rats across

sessions. When significant three-way interactions were found, further analyses were performed by conducting separate multilevel models.

### **D1.2.3 Post-lesion data analysis**

Both baseline sessions and the vSD session conducted after the lesion surgery were analysed in the same way as before the lesion. However, rather than including *impulsivity group* as one of the fixed factors, models for post-lesion analyses included *lesion group* as a fixed factor.

Impulsivity group was not included in the post-lesion analyses because the number of sham and lesioned rats in each impulsivity group was too low to be considered meaningful for statistical comparisons. For analyses comparing baseline sessions between tasks and lesions groups, the model included the following fixed factors: session (pre-lesion, post-lesion), task (5CSRTT; SDT signal trials; SDT non-signal trials) and lesion group (sham and lesioned animals, QA). The fixed factor 'session', for all animals and across tasks, included at least 4 levels: two sessions prior to the lesion (1a and 2a) and at least two sessions following lesion (1b, 2b). In addition to this, twelve lesioned rats and all sham rats also had a third baseline session (3b), and six lesioned rats and sixteen sham rats also had a fourth baseline session (4b). The linear mixed model analysing the percentage of omission responses in the 5CSRTT included two fixed factors: session (1a, 2a, 1b, 2b, 3b, 4b) and lesion group (sham and QA). The linear mixed model analysing latency to initiate a trial in the SDT considered two fixed factors: session number (1a, 2a, 1b, 2b, 3b, 4b) and lesion group (sham and QA). The linear mixed model analysing response latency in the 5CSRTT included three fixed factors: session number (1a, 2a, 1b, 2b, 3b, 4b), lesion group (sham and QA) and response type (correct and incorrect response).

The vSD session of the 5CSRTT, after the lesion, was not the same as that run prior to the lesion but was a less demanding version. For this reason, the linear mixed model analysing differences in performance between tasks and lesion groups, did not include SD as a fixed factor, but instead, accuracy was analysed as an average across SDs. Thus, the model comparing accuracy performance between tasks, lesion groups and sessions included the following fixed factors: task (5CSRTT, SDT), lesion group (sham, QA) and session (pre-lesion, post-lesion). An additional analysis was carried out on trials in both tasks and sessions (before and after lesion) with the

same 1s SD. The model for this analysis included: session (pre-lesion, post-lesion) and lesion group (sham, QA). The model analysing omission responses in the 5CSRTT included two fixed factors: SD (0.5 s, 1 s, 2 s) and lesion group (sham and QA rats). Anticipatory responses were also compared across tasks, during the vSD challenge, in an analysis that included two fixed factors: task (5CSRTT and SDT) and lesion group (sham and QA rats). An additional model was run exclusively for anticipatory responses in the SDT with factors: session (before and after lesion), lesion group (sham and QA rats), response type (correct and incorrect responses) and SD (0.03 s, 0.06 s, 0.25 s, 0.7 s, 1 s). The latency to initiate a trial in the SDT was analysed between lesion groups (sham and QA rats) and sessions (before and after lesion), while response latencies in the 5CSRTT were analysed in a model that included session (before and after lesion), lesion group (QA and sham) and response type (correct and incorrect). As for the analysis of pre-lesion performance, linear mixed models also included one factor (subject) modelled as a random slope to account for individual differences between rats across sessions. When significant three-way interactions were found, further analyses were performed by conducting separate multilevel model.

## D1.3 Results

### D1.3.1 Histology

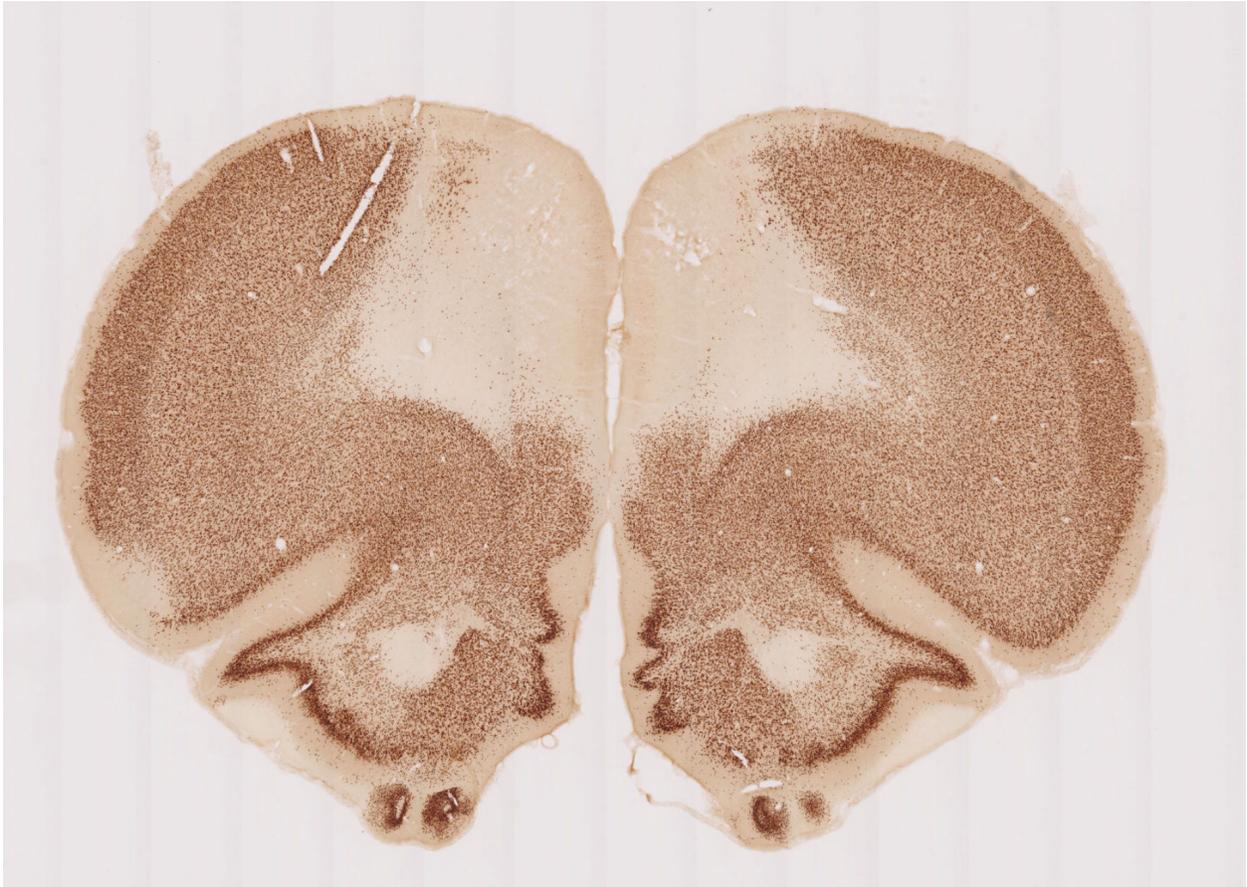


Figure 6.1D Representative image of the extent of the lesion in mPFC