Serotonergic modulation of cognition

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This dissertation is submitted for the degree of Doctor of Philosophy

PREFACE

This dissertation is submitted for the degree of Doctor of Philosophy.

This dissertation is the result of my own work and includes nothing, which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

It 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 or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text.

It does not exceed the prescribed word limit for the relevant Degree Committee.

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Dedicated to my mother

Chapter 1. General Introduction

Cognitive functions

Cognition can be broadly defined as the sum of mental processes of acquiring knowledge through problem solving, memory, perception and planning. The term 'executive functions' is also often used to denote higher-order cognitive functions, interchangeably with frontal functions, as prefrontal cortical function is considered to serve control over 'lower-level' behaviours (Shallice and Burgess, 1996). This is produced by a synergy of multiple neural circuits (Lawrence et al., 1998).

Distinct cognitive aspects are described in the literature; "cold" cognition refers to information processing in the absence of emotional effect and "hot" cognition refers to affective processing (Roiser and Sahakian, 2013). These two types are measured with different tasks, such as the Wisconsin card sorting test for "cold" cognition and emotional processing tasks for "hot" cognition. "Cold" cognitive impairments, such as attention and memory deficits, are shown in unipolar depression and included among the diagnostic criteria (Diagnostic and statistical manual of mental disorders, American Psychiatric Association, 1994). "Hot" cognitive deficits are also observed with affective biases, corresponding to "hot" cognition, typically seen in depressed patients (Erickson et al., 2005).

Cognitive flexibility is a construct referring to the ability of ignoring irrelevant information in a stable environment and rapidly adjusting behaviour when faced with changes in the environment (den Ouden et al., 2013). This is important for adaptive functioning and can be disrupted in several neuropsychiatric disorders. Reversal learning paradigms are widely used to measure the ability to flexibly change behaviour following reversal of reward contingencies (Izquierdo et al., 2017). Typically in rodent and monkey paradigms, correctly chosen stimuli are paired with delivery of a reward (Boulougouris et al., 2007; Clarke et al., 2005). In human paradigms, which are typically probabilistic in nature with varying reinforcement schedules (Costa et al., 2015), participants receive feedback of choosing the correct or incorrect stimulus (Fellows and Farah, 2003).

Response (or action) inhibition defined as the ability to suppress inappropriate responses (Aron et al., 2004) is essential for goal-directed behaviour (Mostofsky and Simmonds, 2008; Logan, 1994). Impulsive actions are characterized by lack or unsuccessful response inhibition and/or premature responding (actions performed without prior thinking).

Previous studies have segregated action impulsivity into anatomically and pharmacologically distinct behavioural subtypes (Chamberlain and Sahakian, 2007; Eagle et al., 2008a).

Serotonin and cognitive functions

Serotonin (5HT) is implicated in several aspects of behaviour including impulsivity, aggression, mood and attention (Daw et al., 2002; Harrison et al., 1999; Soubrié, 1986; Stahl, 2000). It is also involved in sleep regulation and reproduction. It plays a key role in decision-making, a fundamental cognitive process that entails choice and outcome representation and appraisal, mediated by several brain areas, including the ventromedial prefrontal cortex, basal ganglia, thalamus and amygdala. The role of 5HT in cognitive functions such as learning is fairly complex, unlike the role of dopamine (DA) which is mapped in more detail (Schultz et al., 1997; Montague et al., 1996; de Wit et al., 2012a; Goto and Grace, 2005). The lack of clarity stems from the serotonergic system being more anatomically widespread and showing larger diversities in behavioural output (Daw et al., 2002). A well-established role of 5HT is its involvement with behavioural inhibition (Soubrié, 1986) and aversive processing, formulated by Deakin (1983) and Deakin and Graeff (1991). These two functions are interconnected, as behavioural inhibition is traditionally studied in the context of aversive events (such as shocks applied in experimental studies) (note it is alternatively studied under differential reinforcement of behaviour, Daw et al., 2002) and inhibiting responses is a pre-potent action in light of aversive consequences (LeDoux, 1996). Depletion of brain 5HT levels decreases behavioural suppression in light of aversive cues (Soubrié, 1986) and 5HT-releasing neurons are activated by aversive stimuli (Takase et al., 2004). Distinguishing the exact role of 5HT in mediating aversive processing is essential.

Altered 5HT transmission and deficits in cognitive functions in animal and human studies

5HT is largely implicated in impulsive behaviour. Decreased brain 5HT levels are associated with impulsive aggression (Linnoila et al., 1983), suicidal depression (Coccaro, 1989) and mania (Thakore et al., 1996). Fineberg et al. (2010) defined impulsivity as “a predisposition towards rapid, unplanned reactions to internal or external stimuli with diminished regard to the negative consequences of these reactions to the impulsive individual or to others”. Impulsivity though is not a unitary construct (Evenden, 1999) but it is rather comprised by distinct underlying neural and cognitive mechanisms (Robbins et

al., 2012). Delay-discounting tasks are widely used in order to study impulsive choice, i.e. the selection of an immediate, small reward rather than a delayed, large one (Evenden and Ryan, 1996). Forebrain 5HT depletion in rats was shown to steepen the temporal discounting function resulting in choosing sooner rather than delayed rewards (Bizot et al., 1999; Wogar et al., 1993). Administration of fenfluramine (a 5-HT releasing agent) reduced the number of impulsive choices made by male participants with prior history of conduct disorder compared to matched controls in a delay gratification task (Cherek and Lane, 2000). A previous study had shown reduced impulsive choices in a delayed reward task following dexfenfluramine administration in rats (Poulos et al., 1996). The other aspect of impulsivity is impulsive action, i.e. the inability to withhold a pre-potent response such as in go/no-go design (Harrison et al., 1999), stop-signal tasks (Eagle et al., 2009) and in tasks using differential reinforcement of low-rate schedules (Richards et al., 1993).

Rat studies of selective 5-HT lesions previously showed increased impulsivity in both impulsive choice and action (Harrison et al., 1999, 1997a,b; Fletcher, 1995). Winstanley et al. (2004) showed increased impulsive responding in rats after global brain 5-TH depletion in a delayed gratification task but no effect on sensitivity to probabilistic reinforcement and subsequent choices. Distinct aspects of impulsivity include 'waiting' impulsivity, which is the 'inability to regulate responding in anticipation of reinforcement', and 'stopping', which is the inability to regulate responding after the initiation of a response (Worbe et al., 2014) and several animal and human studies showed clear differential effects of manipulations of serotonergic function on the two aforementioned aspects of impulsivity (Eagle et al., 2008a, Harrison et al., 1997b). Eagle et al. (2009) used the stop-signal task to capture both impulsive choice (through a 'waiting' component introduced in the task similar to the rodent five choice serial reaction time task) and action (by measuring the time taken to stop a pre-potent action) and they found distinct effects of central 5HT depletion with the intra-cerebroventricular injection of the neurotoxin 5,7-dihydroxytryptamine (DHT) in rats (please see Chapter 7 for further details).

5HT polymorphisms and cognitive functions

Insights for the role of 5HT in cognitive functions also arise from genetic studies. Previously shown superior performance in the WCST (Borg et al., 2009) and improved reversal learning (den Ouden, 2013) in the 5HTT s-allele carriers support that down-regulation of 5HTT and increased 5HT levels facilitate tracking down stimulus-response changes. Several attempts are made to reconcile the different effects of 5HT system in the

literature. Homberg (2012) suggested that changes in vigilance behaviour can explain the effects of increased and decreased 5HT in distinct cognitive aspects; as an example, reduced vigilance behaviour can account for “stimulus-bound” responding seen after 5HT depletion.

Lesch et al. (1996) showed that the short variant (s-allele) of 5HTT gene-linked promoter region (5-HTTLPR) polymorphism was associated with higher anxiety-related personality traits. A number of subsequent studies reported on the relationship between 5-HTTLPR and personality traits of neuroticism (Sen et al., 2004; Murakami et al., 1999; Ricketts et al., 1998). Takano et al. (2007) showed an association of higher 5-HTT binding in the thalamus with higher neuroticism levels and reported depressive feelings. The s-allele carriers performed better in an affective Go/No-Go task and showed increased memory recall in an affective directed forgetting test (Roiser et al., 2007) and enhanced, motivationally speeded, action in a cued-reinforcement reaction time task (Roiser et al., 2006). S-allele carriers of the 5-HTTLPR polymorphism showed faster learning to avoid punishing stimuli compared to the long allele carriers in a probabilistic reversal learning task and following tryptophan depletion, the long allele carriers failed to use punishment information to guide appropriate responding (Finger et al., 2007). S-allele carriers though do show cognitive deficits including impaired recall of a noun that preceded another emotionally valenced noun (Strange et al., 2008) and impaired performance in the Iowa gambling task (Holmes et al., 2010). In a more recent overview of these findings, Homberg and Lesch (2010) concluded that s-allele carriers display hyper vigilance, possibly mediated by hyperactivity in corticolimbic structures, which may result in superior performance depending on environmental conditions.

Anatomy of the brain 5HT system

The forebrain 5HT system consists of ascending projections from dorsal raphe nucleus (DRN) and medial raphe nucleus (MRN) located in the brainstem. Projections from both areas do not overlap but do not exclude each other (Vertes et al., 1999; Vertes, 1991). Specifically, DRN neurons project through the ventrolateral aspect of the medial forebrain bundle and innervate predominantly the nucleus accumbens, caudate-putamen, the substantia nigra, the globus pallidus and the amygdala (Bobillier et al., 1976; Azmitia and Segal, 1978). A large part of DRN projections innervate the frontal cortex (O’Hearn and Molliver, 1984). The neurons in the MRN project through the ventromedial aspect of the medial forebrain bundle to the cingulate cortex, the hippocampus and the septal nuclei

(Azmitia and Segal, 1978). The projection areas of the DRN also constitute targets of the DA system, namely the amygdala and striatum in contrast to the projections sites from the MRN (Figure 1).

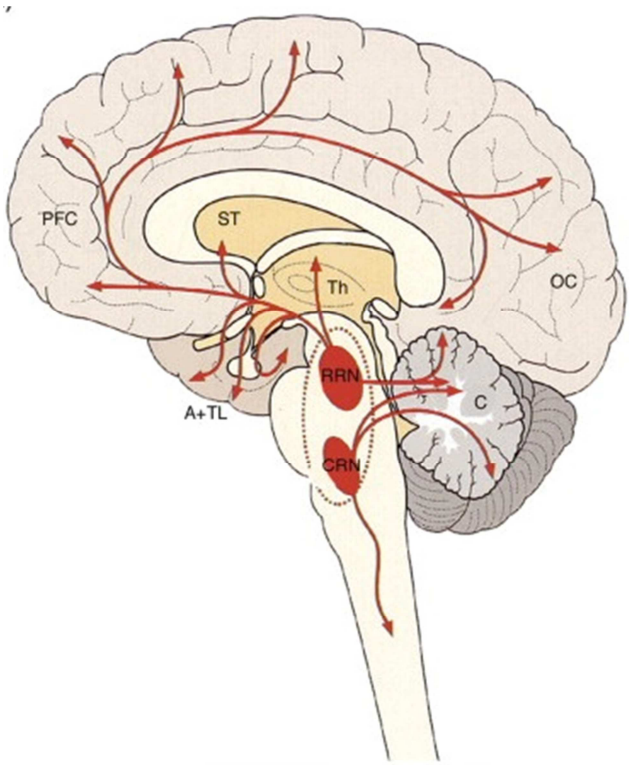


Figure 1: The anatomy of brain 5HT system. From Cools et al. 2008b

In terms of 5HT receptors, several subtypes are identified with distinct pattern of expression and distribution in brain (Martin et al., 1998) and differential interaction with other neurotransmitter systems (Homber, 2014). As an example, somatodendritic $5HT_{1A}$ receptors are found (Pazos et al., 1988) in the raphe nuclei where they act as presynaptic receptors (Sprouse and Aghajanian, 1986). Traditionally, serotonergic manipulations in experimental settings include tryptophan depletion (ATD), a dietary intervention which reduces availability of the 5HT precursor tryptophan and results in global brain 5HT depletion, and the agonists or antagonists of 5HT receptors. The latter affects receptors to a different extent, dependent on selectivity, and it may also include effect on other neurotransmitter systems, such as DA or noradrenaline. Application of serotonergic agents in experimental settings has frequently yielded contrasting findings, which could be – partially- explained by effect on distinct receptor sub-types in different brain areas (Carli and Samani, 2000). Serotonin reuptake inhibitors are also used with a suggested effect of increasing 5HT concentration in the synaptic cleft (Stahl, 1996). Largely, one limitation to

delineating the precise mechanisms of 5HT function in various brain processes lies in the contrasting effects between different methods used and/or confounding effects to other neurotransmitter systems.

Serotonin is largely implicated in emotional processing with agents that inhibit 5HT reuptake in the synaptic cleft being the first line treatment for mood disorders (NICE clinical guidelines 2011; 2009). Their effect though is not shown immediately, but after two weeks of treatment in therapeutic dosages, thus posing the question of underlying mechanisms mediating their effects. Interestingly, 5HT reuptake inhibitors show effects on several cognitive domains and it is suggested that their effect on sustained improvement in mood might be mediated by learning mechanisms, specifically "re-appraisal" and "re-evaluation" of emotions (Robinson and Sahakian, 2008; Harmer and Cowen, 2013).

Opponency with dopamine

Deakin (1983) proposed the activation of the DRN serotonergic system by conditioned aversive stimuli. This activation may be derived from projections from the hippocampus or amygdala and from the frontal cortex. Behavioural output can be differentially mediated by release of 5HT in the terminal areas (Deakin and Graeff, 1991). The function of serotonergic system is suggested to oppose the dopaminergic one (Deakin, 1983), which is implicated in appetitive processing and approach behaviour (Ikemoto and Panksepp, 1999), motivation and psychomotor arousal (Cannales and Iversen, 2000). Midbrain dopaminergic neurons are activated in anticipation of rewards (Schult et al., 1997). 5HT is suggested to play a key role in avoidance behaviour and fight/flight responses driving adaptive ones in light of aversive situations, thus contrasting the role of DA in approach behaviour. Behavioural inhibition output (approach and avoidance) is suggested to arise from a balance between 5HT and DA release in ventral striatal areas (Deakin and Graeff, 1991). Experimental studies show that 5HT agonists oppose behaviours (conditioned ones, such as responding for conditioned rewards, and unconditioned ones, such as feeding) brought into action by DA (Fletcher et al., 1995a; 1993). Studies also show direct opposition between DA and 5HT in neural areas including the ventral tegmental area, the substantia nigra, nucleus accumbens and the striatum (Kapur and Remington, 1996). Contrasting effects though on aversive processing have been shown with 5HT depletion producing a deficit in aversive conditioning to a context whereas increasing aversive conditioning for explicit conditioned stimulus (Wilkinson et al., 1995). Daw et al. (2002) supported the opposition between DA and 5HT from a reinforcement learning perspective suggesting

that tonic 5HT signals the long-run average reward rate within the framework of average-case reinforcement learning and tonic DA signals, in the same framework, the long-run average punishment rate. A phasic 5HT signal is proposed to account for prediction errors for aversive events or punishments (Daw et al., 2002).

Other neurotransmitter systems affecting cognitive functions

It is worth noting that other neurotransmitter systems are implicated in several of these cognitive functions. Mesolimbic noradrenaline is described to fulfil many functions ascribed to mesolimbic dopamine implicated in goal-directed behaviour and motivation (Cools and Tuinstra, 2002). Norepinephrine directly affects dopamine in the dopaminergic nigrostriatal fibres and indirectly by changing 5HT activity in raphe-nigral or raphe-neostriatal fibres (Cools and Tuinstra, 2002). D-amphetamine, modafinil, and methylphenidate administration show baseline-dependent effects on SSRT (Robinson et al., 2007; Eagle and Robbins, 2003a; Eagle et al, 2007). Atomoxetine was previously shown to improve SSRT in a human volunteer study (Chamberlain et al., 2006). Increased noradrenergic transmission, both systemically and localised in the amygdala, is shown to enhances emotional memory (Phelps and LeDoux, 2005). The PFC is the main neural substrate supporting cognitive flexibility. Working memory is dependent upon prefrontal cortex, and moderate levels of noradrenaline are thought to facilitate working memory whereas higher levels during extreme stress may impair working memory and engage more posterior cortical and sub-cortical circuitry (Chamberlain et al., 2006). Atomoxetine administration produces differential effects on distinct forms of impulsivity by speeding Stop-signal reaction time in a Stop-signal task in a dose-dependent manner, selectively decreasing premature responding the five-choice serial reaction time task and increasing preference for large-value rewards in a delay discounting task (Robinson et al., 2008).

Cognitive deficits in psychiatric disorders

Cognitive dysfunction is systematically reported in both first-episode (Lee et al., 2014) and recurrent depression (Conradi et al., 2011) with impairments in memory, attention and executive functions (Bora et al., 2013). Interestingly, cognitive dysfunction persists in remitted depressed patients (Rock et al., 2012; Hasselbalch et al., 2010) with significant deficits in attention and executive functions and a tendency towards memory impairment (Rock et al., 2012). Attention biases in information processing have been consistently reported in depression (Erickson et al., 2005; Murphy et al., 1999), mania (Murphy et al.,

1999) and anxiety disorders (Mogg et al., 1995). The cognitive domains that show impairments are fairly wide and include both “cold” and “hot” cognition (Roiser and Sahakian, 2013). Depressed patients show enhanced memory recall of sad facial expressions (Gilboa-Schechtman et al., 2002) and neural markers of reward processing deficits in neuroimaging studies (Steele et al., 2014; Gradin et al., 2011). Changes in emotional processing occur early in treatment with antidepressants prior to clinically observed improvement in mood (Harmer and Cowen, 2013). Acute administration of a clinically relevant dosage of citalopram increases recognition of happy faces and attention to socially relevant stimuli (Harmer et al., 2003) and improves recollection of positive memories (Harmer et al., 2004). This has led to the suggestion of reversal of negative biases with antidepressant treatment acting as a potential biomarker predicting overall antidepressant therapeutic response (Shiroma et al., 2014; Harmer et al., 2009). Emotional “blunting” is also reported in patients receiving SSRIs (Opbroek et al., 2002).

Selective serotonin reuptake inhibitors

Selective-serotonin reuptake inhibitors (SSRIs) are the first-line treatment for major depression and generalised anxiety disorder (GAD) (NICE clinical guidelines 2011; 2009) and approved for the management of obsessive-compulsive disorder (British National Formulary, 2016). This is attributed to evidence-based efficacy and a better side effect profile compared to other available medication such as the tricyclic antidepressants (Stahl et al., 1998). Improvement in anxiety symptoms is reported in GAD patients as early as the first week of treatment with the SSRI escitalopram (Davidson et al., 2004). Although clinically meaningful change in mood takes several weeks to manifest, SSRIs are suggested to exert their effects primarily through blocking the re-absorption of 5HT into the presynaptic neuron thus leading to higher 5HT availability in the synaptic cleft (Stahl, 1998). Neuroadaptive effects feature as another mechanism possibly mediating antidepressant effect. Repeated treatment with SSRIs causes desensitization of serotonergic auto-receptors in pre-synaptic neurons thus leading to a net increase in 5HT in the synaptic cleft (Blier et al., 1990).

The clinical effect of treatment with antidepressants, and SSRIs in particular, on cognitive functions is far from clear. Data appears inconclusive but suggests that in late-life depression, cognitive dysfunction does not improve beyond the level of practice effect after adequate treatment with SSRIs (Culang et al., 2009). Citalopram administration in geriatric depressed patients shows that those responding to treatment improve more on

psychomotor speed measurements than non-responders but this is not significant compared to placebo (Culang et al., 2009). Subjective cognitive difficulties in memory and concentration in depressed patients are reported to improve in a 12-week treatment with escitalopram compared to baseline (Jeon et al., 2014). Acute and chronic treatment of rats with citalopram reversed the cognitive deficit previously induced by chronic intermittent cold stress, which can model cognitive dysfunction in depression (Danet et al., 2010). Vortioxetine, an atypical antidepressant with combined effect on serotonergic receptors' activity and 5HT reuptake inhibition, is shown to have pro-cognitive effects in depressed patients (Katona et al., 2012) separate from its effect on mood (McIntyre et al., 2014). The wake-promoting agent modafinil also shows direct positive effects on cognition in studies with current (De Battista et al., 2004) and remitted depressed patients (Kaser et al., 2017).
The majority of studies investigating the cognitive effects of SSRIs included patient groups, where confounding factors (additional medication, severity of disease, comorbidities) need to be taken into consideration when interpreting the findings.

Distinct effects of acute and chronic SSRI administration on cognition

The majority of aforementioned studies on patient groups refer to chronic treatment with SSRIs. Differential effects of acute *versus* chronic treatment with SSRIs is shown. Contextual processing was impaired after acute citalopram administration in healthy males but this effect was abolished following chronic treatment (Almeida et al., 2010). Acute citalopram administration increased acquisition of auditory fear conditioning which was then reduced after chronic treatment (Burghardt et al., 2004). In the same study, acute treatment with the tricyclic antidepressant tianeptine had no effect but chronic treatment reduced the acquisition of tone conditioning similar to citalopram. From a clinical perspective, 5HT effects also pose an intriguing paradox as benzodiazepines (which reduce 5HT transmission) reduce anxiety whereas long-term SSRI treatment (which increase 5HT transmission) improve anxiety symptoms in both anxiety disorders and depression.

Study rationale

Decision-making, a fundamental cognitive function, is essential for optimal functioning and is disrupted in neuropsychiatric disorders, including depression, anxiety disorders, schizophrenia and substance abuse (Murphy et al., 2001; Everitt and Robbins, 2011; Ernst and Paulus, 2005). It constitutes of several processes including attention set shifting and cognitive flexibility, response inhibition and reward processing as well as emotional

processing (Bechara et al., 2002). Response (or action) inhibition defined as the ability to suppress inappropriate responses (Aron et al., 2004) is essential for goal-directed behaviour (Mostofsky and Simmonds, 2008; Logan, 1994).

We attempted to further delineate the behaviourally observed effects of altered serotonergic transmission on distinct aspects of cognition by administering an acute, clinically relevant dosage of the SSRI escitalopram in order to increase brain 5HT levels. We included an extensive cognitive test battery with tasks stemming from animal models, human cognitive studies and computational modelling. The tasks expanded across the domains discussed above and each chapter represents a cognitive domain studied.

Study hypotheses

Overall, we hypothesized, based on prior literature on serotonergic interventions, that participants receiving an acute escitalopram dosage of 20mg will make more errors during both probabilistic learning and after reversal of stimulus-reward contingencies (perseverative errors) (Chamberlain et al., 2006). We hypothesised no effect or possible improvement (Ye et al., 2014) on tests of action restraint or cancellation. Deficits were expected in tests of cognitive flexibility (although predominantly in reversal). We also hypothesized that acute escitalopram would enhance affective interpretation bias in the three emotional processing tasks (i.e. more positive affective interpretation bias) as negative affective biases are well-documented in major depression (Murphy et al., 1999) and following ATD in healthy volunteers (Murphy et al., 2002) and acute administration of a clinically relevant dosage of citalopram in healthy volunteers was previously shown to increase recognition of happy faces and attention to socially relevant stimuli (Harmer et al., 2003b).

Chapter 2. Materials and methods

This study was approved by the NHS East of England - Cambridge Central Research Ethics Committee (REC reference: 15/EE/0004). All volunteers were given both verbal and written information and they gave written consent prior to screening and participation.

Participants and study design

Sixty-six healthy participants aged 18-45 years old were recruited through printed and electronic advertisements in local community and university settings. A priori power calculation was performed in order to define the sample size (Soper, D.S. (2014) A-priori Sample Size Calculator for Student t-Tests [Software]). Desired statistical power level was set to 0.8, probability level (p- value) was set to 0.05 and anticipated effect size (Cohen's d) was set to 0.66 based on findings from previous study exploring model-based and model-free learning following serotonergic manipulation (Worbe et al., 2016). Power calculation resulted in a total sample size of 60 people. An additional number of 3 participants per group (10% of the initial desired sample size) was added to account for potential side effects, as it was prepared as standard policy from the pharmacy for pharmacological research studies.

As soon as a potential participant expressed interest by contacting the research team, a detailed information sheet, which included the study eligibility criteria and explanation of the study procedure, and a pre-consent form for contact details and requesting permission to contact over the phone and enquire about medical history to ensure eligibility were sent. Thus all potential participants were pre-screened over the phone, prior to enrolment, with a screening form approved by the local ethics committee for active major medical problems and the MINI International Neuropsychiatric Interview for any psychiatric symtpoms. Screening was conducted by a qualified medical doctor and eligibility was confirmed with a senior psychiatrist. Exclusion criteria included current or past neurological and psychiatric symptoms, family history of neurological or psychiatric disorders, past history of gastrointestinal disorders, renal/hepatic impairment, head injury, impaired vision/hearing/movement, diabetes, asthma, epilepsy, glaucoma, bleeding disorders, thyroid dysfunction and personal or family history of cardiac (heart or circulation) problems. Participants were excluded if they were receiving any medication for the

aforementioned reasons, except for oral contraceptives for females, and they were requested to report any major illness during the last month prior to study day. Additional exclusion criteria included alcohol abuse (defined as more than 28 units per week for males and 21 units per week for females), nicotine consumption more than five cigarettes per day and significant history of drug abuse (including cannabis, amphetamines, cocaine, heroin, ecstasy, barbiturates, tranquilizers, opiates or psychedelics) defined, as per Chamberlain et al., 2006, as: 1) use of any listed substances within the last month, 2) more than occasional (which is defined as more than five times throughout lifetime) intake of all listed drugs except for cannabis, or, 3) regular (defined as more than once monthly) cannabis intake.

Upon confirmation of eligibility, appropriate dates were selected for testing day. The compound was prepared by the Cambridge University Hospital pharmacy and dispensed for collection by researchers on testing day.

Upon arrival to the research facilities located in Addenbrooke's Hospital, participants repeated the screening in order to ensure that nothing has changed since the phone session, for example medication or illness (es). Then the participants completed the Beck Depression Inventory (BDI) (Beck, 1972). The researchers explained the procedure, provided again the information sheet and answered any questions the participants had. All participants underwent an electrocardiogram (ECG) on study day to exclude cardiac abnormalities including any pre-existing QT- interval prolongation that could be affected by escitalopram administration (Castro et al., 2013). Female participants were requested to undertake a urine pregnancy test to exclude pregnancy. Participants were requested to refrain from alcohol 24 hours prior to study day. During testing day, participants were prompted to have the typical coffee or tea intake as per every day.

Study design rationale

A double-blind, parallel-groups design was employed in order to eliminate experimenter bias and practice effects (extensive training) on learning tasks. Escitalopram was applied so as to increase brain 5HT levels as per previous neurocognitive studies (Ye et al., 2014) and findings of increasing extracellular 5-HT concentration in the prefrontal cortex more than 4-fold without direct effect on norepinephrine and DA levels (Hyttel, 1995), compared to other serotonergic agents commonly used such as fluoxetine (Bymaster et al., 2002). Escitalopram 20mg is a clinically relevant based on guidelines for the treatment of major depression and anxiety disorders in adults (British National Formulary 2013) and shows high selectivity for SERT (Owens et al., 1997), the lowest potential among other

SSRIs for drug-drug interactions (Hiemke et al., 2000) and minimum potential side effects. Single dose citalopram was previously shown to successfully change brain serotonergic levels and performance in tasks measuring response inhibition (Ye et al. 2015; Chamberlain et al., 2006) and probabilistic learning (Chamberlain et al., 2006) in our lab and in other labs (Wienberg et al., 2010; Harmer et al., 2003). Escitalopram was selected, compared to the racemic mixture citalopram due to higher efficacy in comparable dosages (Burke, 2002). Peak plasma levels are reached within 3 to 4 hours after oral escitalopram intake (Rao, 2007).

Pharmacological properties of escitalopram

Escitalopram (S-citalopram) is a selective 5HT reuptake inhibitor (SSRI) used for the treatment of generalized anxiety disorder (Davidson et al., 2004), panic disorder (Stahl et al., 2002), obsessive-compulsive disorder (Stein et al., 2007) and social anxiety disorder (Kasper et al., 2002). It is the S-stereisomer of the antidepressant drug citalopram (Hyttel et al., 1992), a well-established therapeutic tool for the treatment of anxiety and mood disorders (Vaswani et al., 2003). Both R- and S- enantiomers occupy to some degree the SERT in the human brain in vivo (Lundberg et al., 2007) but in-vitro studies suggest that the affinity of the S-enantiomer for the SERT is 30-fold higher than the R-enantiomer (Burke, 2002). Thus, the activity of citalopram is supposed to lie primarily on the S-enantiomer (Owens et al., 2001), which is shown to inhibited by the R-enantiomer possibly via an allosteric interaction with the SERT (Sanchez et al., 2004), resulting to the lower efficacy of citalopram compared to escitalopram (Burke, 2002).

Escitalopram is quickly absorbed from the gastrointestinal tract after ingestion without any influence of the food intake (Sogaard et al., 2005) and reaches bioavailability rates of 80% (Bareggi et al., 2007). Peak serum concentrations are achieved 3-4 hours following oral administration (Sogaard et al., 2005) with a mean SERT occupancy of 87% (Lundberg et al., 2007). In vitro studies show that escitalopram exhibits negligible effects on cytochrome P450 enzymes (Burke, 2002) and thus, a low potential for interactions to other clinically relevant drugs (Malling et al., 2005). Mean escitalopram half-life is reported as approximately 27-33 hours (Areberg et al., 2006). It has been reported that in comparable doses, which refer to the S- enantiomer dosage, escitalopram has higher efficacy and quicker onset of action than citalopram (Sanchez et al., 2004) possibly attributed to the aforementioned inhibition of the S-enantiomer by the R-enantiomer in citalopram.

Most frequent side effects of escitalopram, as reported in double-blind studies, include headaches (18%), nausea (17%), insomnia (9%), fatigue (8%), diarrhoea (8%), dry mouth (6%), dizziness (6%) and tiredness (5%) (Baldwin et al., 2007). Because of highest selectivity amongst other antidepressants, including sertraline, paroxetine, fluoxetine and fluvoxamine (Owens et al., 2001), with almost no affinity to other tested receptors (Höschl et al., 2008; Sanchez et al., 2004), the side-effect profile of escitalopram is relatively mild (Brunton et al., 2010).

Escitalopram and serotonin transporter occupancy

Meyer et al. (2004) applied receptor-ligand imaging and showed that the serotonin transporter (5HTT) occupancy in striatum in minimum therapeutic dosages of citalopram (20-40mg) was 81.4%. Occupancy of 5HTT increased as the dose or plasma levels increased, reaching a plateau in plasma levels in high doses. The estimated citalopram dose required to obtain occupancy of striatal 5HTT was 3.4 mg/day as assessed by the SSRI administration to healthy and depressed volunteers for four weeks. In the same study, 5HTT occupancy was estimated in other brain regions; specifically, 5HTT occupancy was estimated as 72.3% in bilateral thalamus, 83.2% in anterior cingulate cortex, 79.9% in the prefrontal cortex, 87.5% in the midbrain and 69% in bilateral cuneus. The researchers tested other commonly used SSRIs and showed that minimum clinical dosages also produced occupancy of 80%. The researchers noted that they tested the 5HTT binding potential from 6 to 13 hours following last SSRI dose administration. Distinct 5HT receptor subtypes are found in different concentrations in distinct brain regions. Localization of SERT in the brain using auto-radiographic and positron emission tomographic methods showed higher density of 5-HT_{1A} and 5-HT_4 receptors in the superficial layers of the neocortex and higher 5-HT_{2A} receptor density in the middle layers. High 5-HT_{1A} receptor density was shown in limbic cortical regions including the (posterior) entorhinal cortex and the hippocampus and a trend of higher density of 5-HT_{1B} receptors was reported in the ventral striatum and the ventral pallidum (Varnäs et al., 2004).

Comparison of escitalopram and citalopram

Compared to citalopram, previously applied in human cognitive studies assessing learning (Chamberlain et al., 2006) and response inhibition (Ye e al., 2014), rodent studies show superior efficacy of escitalopram in the forced swimming test (Sanchez et al., 2004), the

chronic mild stress test (Sanzhez et al., 2003; Montgomery et al., 2001) and the assertive behaviour test (Mitchell, 2005). Clinical studies also confirm these findings as a 24-week clinical study comparing 10 mg escitalopram effect to 20mg citalopram in depressed patients showed superior efficacy of escitalopram treatment for global disease severity (Colonna et al., 2005). A 8-week clinical study showed higher response to treatment with 10-20 mg escitalopram compared to 20-40 mg citalopram, significant reduction of depressive symptomatology measured with the Montgomery–Asberg Depression Rating Scale following escitalopram treatment compared to placebo, and adverse event withdrawal rates similar to placebo (Lepola et al., 2003). Superior efficacy of 20mg escitalopram compared to 40mg citalopram in major depression patients was shown by Moore et al. (2005). Burke et al. (2002) showed at least similar efficacy in reducing depressive symptomatology 10 mg escitalopram to 40 mg citalopram in a 8-week double blind study with clinical response of escitalopram demonstrated within one week of treatment.

Possible limitation of pharmacological manipulation

Nord et al. (2013) found that the same dose of escitalopram as used here (20mg oral) produced changes in PET ligand binding suggestive of reduced 5-HT in cortical (rather than subcortical) regions in male humans. Higher acute i.v. doses in female rhesus monkeys produced apparently increased 5-HT levels, particularly in cortical and thalamic regions presumably because auto-receptor effects are outweighed by greater effects at the synaptic terminals.

Experimental procedure

On testing day and upon arrival to the NIHR Cambridge Biomedical Research Centre (Mental Health theme), participants were administered a pill containing either escitalopram or placebo accompanied with a glass of water. The researcher, the facilities' nurses (who administered the pill) and participants were blinded. Placebo medication consisted of cellulose, microcrystalline with 1% magnesium stearate capsules, manufactured to match the appearance of the escitalopram capsules. Both escitalopram and placebo capsules were prepared by the pharmacy manufacturing unit of the Royal Free London NHS Foundation Trust according to standard specifications. One participant did not complete the neuropsychological testing due to experiencing side effects. Appropriate procedure according to local research ethics guidelines was followed.

Neuropsychological testing started 3 hours following drug or placebo administration to achieve peak escitalopram plasma concentrations (Rao, 2007). In the interim, participants waited in a quiet room. Light lunch was offered as food does not interact with escitalopram absorption (Rao, 2007). Two blood samples were collected, the first at 2.5 hours post-drug administration and the second at 5.5 hours post-drug administration. Following the end of the testing session, participants were given verbal recommendations on the possible side effects detailed in the information sheet and advice on how to reduce any side effects within twelve hours from end of testing session.

Behavioural testing was performed in a quiet room on a Windows portable computer and the complete task battery lasted three and a half hours. Groups were matched for demographic characteristics.

Measures	**Placebo group**	**Escitalopram group**	**Group difference***
Participants	33	32	n.s.
Male:female	17:16	16:16	n.s.
Age	25 (5)	27 (7)	n.s.
NART	43.45 (4.93)	42.9 (5.27)	n.s.
IQ	121.98 (4.4)	122.39 (4.7)	n.s.
Years of education	16.6 (2.7)	16.9 (2.5)	n.s.
State-Trait Anxiety Inventory			n.s.
State	31.2 (6.8)	33.3 (7.3)	n.s.
Trait	35.2 (8.6)	34.6 (9.5)	n.s.
Beck Depression Inventory	5.3 (5.1)	4.1 (4.4)	n.s.
Barratt Impulsiveness Scale			n.s.
Total score	66.67 (7.6)	64.6 (6.6)	n.s.
Second order factors			n.s.
Attentional	16.4 (3.6)	14.9 (2.6)	n.s.
Motor	27.7 (3.6)	27.1 (3.2)	n.s.
Non-planning	22.6 (3.9)	22.7 (4.7)	n.s.

Table 1: Group demographics. Mean (SD) *** Group difference**: p-values of two-tailed t-tests, n.s.= not significant, $p>0.05$

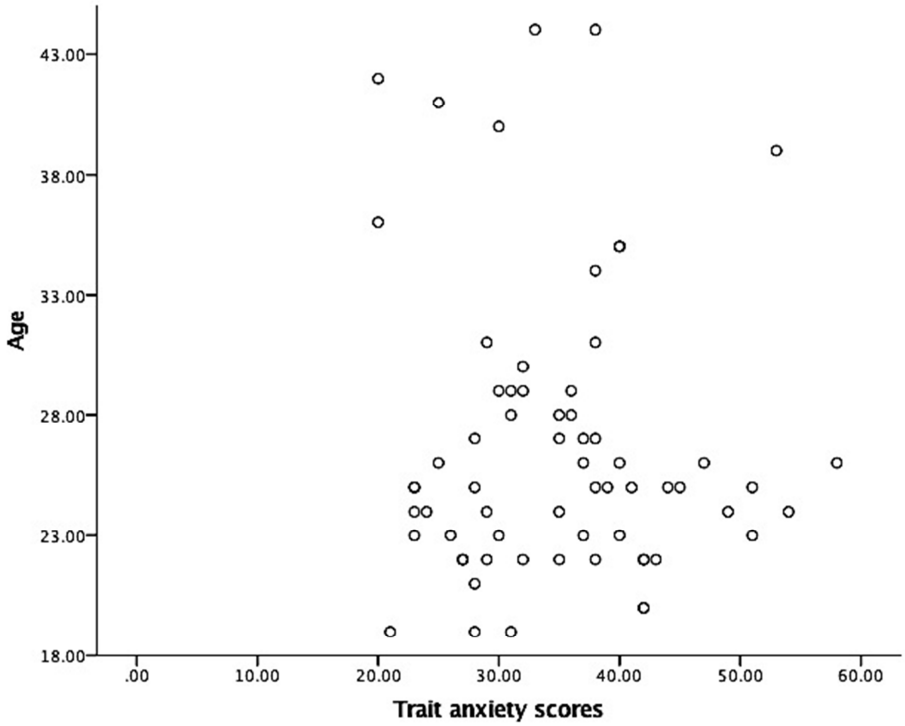


Figure 1: Scatterplot of participants' age and trait anxiety scores

Participants additionally completed a 27-item monetary choice questionnaire (Kirby et al, 1999) where they chose between small, immediate and large, delayed monetary rewards. The discounting rate (k) was separately calculated for small, medium, and large reward magnitudes. The formula applied was: V=U/(1+kD), where V is the subjective value of a reward of amount U after a delay D. The questionnaire Impulsive choice is characterized by the greater values of k, which corresponds to a steeper discounting rate. The questionnaire was administered as a hypothetical measure; participants were prompted to answer every question as if they would receive the reward stated (Grant and Potenza, 2011). The questionnaire assessed impulsive choice indicated by larger values of k and thus steeper discounting rate.

	Escitalopram	**Placebo**	**Group difference***
Small k	0.03 (0.06)	0.02 (0.04)	n.s.
Medium k	0.02 (0.05)	0.01 (0.02)	n.s.
Large k	0.02 (0.05)	0.01 (0.02)	n.s.

Table 2: Discounting rate (k) results in the 27-item monetary choice questionnaire. Mean (SD)
*** Group difference**: p-values of two-tailed t-tests, n.s.= not significant, p>0.05

Neuropsychological testing

Participants performed a computerized neuropsychological test battery assessing learning, emotional processing, decision-making and response inhibition. We applied a probabilistic learning task previously shown to be sensitive to serotonergic pharmacological manipulations (Chamberlain et al., 2006; Park et al., 1994). Participants also completed the (simplified) fabulous food game (de Wit et al., 2007), an appetitive instrumental task designed to evaluate the balance between goal-directed and habitual behaviour which has been previously used in a number of pharmacological studies (de Wit et al., 2012a) in healthy volunteers (de Wit et al., 2012b) and clinical populations (Ersche et al., 2016; Gillan et al., 2011). Sixty-one of the participants, thirty-one in placebo and thirty in escitalopram groups, completed the Intra-extra dimensional set shift task from the CANTAB battery measuring cognitive flexibility (http://www.cambridgecognition.com). Participants completed additional tests assessing cognition in a counterbalanced order.

Participants were reimbursed with a fixed amount for the time spent in the research facilities plus a flat added amount for travel expenses. Participants were told that they should try to perform as best as possible in each task, as they would receive additional monetary reward for task performance. Due to the size of the test battery, participants were told that researchers would add the points from all the tasks and according to how well they did, they would gain an extra amount ranging from 0 to 5 pounds which they would receive in the end of the experimental session in order to maximise motivation for optimal task performance throughout testing day. In reality, after the end of testing procedure all participants received the maximum additional amount despite task performance. The tasks are described in detail in each chapter.

Mood self-reports

Results

Alertness significantly decreased during testing day in both groups, $p=0.048$, $F(2,102)=3.139$, partial $\eta^2=0.058$, but no interaction of time and treatment was reported, $p=0.260$, $F(2,102)=1.364$, partial $\eta^2=0.026$. There was a significant interaction of time of rating and treatment in calmness versus excitement reported by participants, $p=0.029$, $F(2,40)=3.881$, partial $\eta^2=0.162$. Tests of simple main effects revealed a significant increase in excitement in the escitalopram group following drug administration and prior to beginning of neuropsychological testing, $p=0.028$, $F(1,29)=5.368$, partial $\eta^2=0.156$, but this was

abolished by the end of testing session, p>0.05. Contentment and happiness did not significantly change during testing day and there was no interaction with treatment [contentment; time: F(2,100)=0.666, p=0.516, treatment x time: F(2,100)=0.821, p=0.443, happiness; time: F(2,100)=1.164, p=0.316, treatment x time: F(2,100)=0.193, p=0.825]. Interest significantly decreased during experimental day, F(2,100)=10.707, p<0.001, partial η^2=0.176, but no significant interaction of treatment and time was reported, F(2,100)=0.724, p=0.487. There was no change over time in willingness to engage in social interaction, p=0.22, F(1,59)=1.538 nor was it affected by treatment [time x treatment interaction, p=0.438, F(1,59)=0.609]. No treatment effect was reported during the experimental session for feeling tense [treatment: p=0.141, F(1,60)=2.226] and at the end of testing session [treatment: p=0.55, F(1,54)=0.363]. Results are summarized in Table 1 in the Appendix.

Biochemical analysis

Blood samples were collected for sixty-four participants and they were centrifuged within 20 minutes at 4°C for 10 minutes at 4000rpm. Plasma samples were extracted and stored in -20°C. The samples were transported in -80°C dry ice to a collaborator in Leipzig, Germany. Analysis showed a significant group difference of escitalopram concentration (measured in ng/ml) between the two groups in both time points, 2.5 hours, p< .001, t(54)= 18.835, and 5.5 hours after start of testing session p< .001, t(54)= 20.548.

Escitalopram (ng/ ml)	Placebo	Escitalopram	Group difference*
2.5 hrs	0	21.14	p< .001
5.5. hrs	0	17.24	p< .001

Table 3: Escitalopram levels in placebo and escitalopram groups. ***Group difference**: p-values of two-tailed t-tests or non-parametric tests, as appropriate

Chapter 3. Emotional processing and social behaviour

Background

Emotional processing and SSRIs

5HT is implicated in fear recognition and anxiety. SSRIs constitute well-established therapeutic tools for anxiety and mood disorders (Vaswani et al., 2003). Attention biases in information processing have been consistently reported in the literature in depression (Erickson et al., 2005; Murphy et al., 1999), mania (Murphy et al., 1999), substance dependence (Ersche and Sahakian, 2007) and anxiety disorders (Mogg et al., 1995). Emotional processing changes were shown to occur early in treatment with antidepressants leading to the suggestion that these effects on negative biases can act as biomarkers predicting overall antidepressant therapeutic response (Shiroma et al., 2014; Harmer et al., 2009).

The role of 5HT in emotional processing

Harmer et al. (2003a) found a selective decreased recognition of fearful facial expressions on a facial expression task following ATD in females but not male healthy volunteers with stimuli derived from the Pictures of Affect Series (Ekman and Friesen 1976). In another study applying the same stimuli, a single intravenous dose of citalopram 10 mg resulted in increased recognition of fearful facial expressions (Harmer et al., 2003b). The treatment group though showed reduced amygdala activity for the fearful facial expressions. Similar to Harmer et al. (2003b), Browning et al. (2007) showed increased recognition of fearful facial stimuli and increased baseline startle response following oral administration of citalopram 20 mg. It was suggested that this effect might mediate the anxiogenic effect seen with acute SSRI administration (Harmer et al., 2003b; Kent et al., 1998). Elevation of brain serotonergic levels through dietary tryptophan administration resulted in increased recognition of fearful and happy facial expressions (Attenburrow et al., 2003).

The observed mixed effect of increased recognition of both fearful (Browning et al., 2007; Harmer et al., 2003) and happy (Harmer et al., 2003) facial stimuli was attributed either to modulation of cognitive mechanisms mediating the significance of facial stimuli as social cues or decreased brain serotonergic levels, rather than increased ones, following acute citalopram administration (Labuschagne et al., 2010), possibly due to activation of auto-receptors located in the pre-synaptic neuron (El Mansari et al., 2005). It is worth noting that this mixed effect was not produced with the antidepressant mirtazapine, an antagonist

of a_2-adrenoreceptors and post-synaptic 5-$HT_{2A/2C}$ receptors. Acute mirtazapine administration in healthy volunteers reduced fear processing, an effect similar to chronic SSRI treatment (Armone et al., 2009).

Interestingly, two studies yielded dissimilar results. Del-Ben et al. (2005) showed that intravenous citalopram administration (7.5mg) reduced amygdala activity for fearful facial stimuli in healthy male volunteers although no effect on behavioural measures of face recognition was observed. Murphy et al. (2009) showed that single oral administration of citalopram 20mg reduced amygdala response to fearful facial stimuli (Ekman and Friesen 1976) in accordance with amygdala hyperactivity (Drevets, 2003) and increased emotional processing, specifically for threat-related and aversive stimuli (Bhagwagar et al., 2007), reported in depression and anxiety disorders. Additionally, it is suggested that the clinical effect of SSRIs is partially mediated by restoration of amygdala activity to sad and fearful facial stimuli (Fu et al., 2004; Sheline et al., 2001) and preclinical findings that 5HT shows an inhibitory effect on amygdala (Stutzmann and LeDoux, 1999). In the Murphy et al. (2009) study, citalopram administration altered amygdala activity by reducing it, but did not affect recognition of fearful stimuli, as it would be expected based on the Harmer et al. (2003b) and Browning et al. (2009) studies. Additionally, the clinical finding of anxiogenic effect following acute SSRI administration is not supported by this study. Murphy et al. (2009) addressed this paradox by noting that acute SSRI-mediated anxiogenic effects are only seen in a subgroup of patients thus additional factors such as sex and genotype might play a role and that the sample in their study might not be the most suitable to study this effect.

Previous rat studies showed that acute fluoxetine administration induced anxiogenic-like behavior in the elevated plus maze (Silva and Brandao, 2000) and acute escitalopram administration increased anxiety-like behavior measured by reduced open arm exploration in the elevated plus-maze (Bondi et al., 2008). In contrast to previous study (Harmer et al., 2006), citalopram administration did not modulate amygdala activity when presented with masked fearful facial stimuli (Murphy et al., 2009). Improved recognition of both fearful and happy facial expressions was accompanied by faster reaction times in the Harmer et al. (2003b) study, thus leading to the suggestion that participants receiving single dose of citalopram were both better and faster in recognising these emotional stimuli. This effect was not mediated by any change in self-reported subjective states. Improved recollection of positive memories was also reported following the administration of both citalopram

(Harmer et al., 2004) and the dual 5HT and norepinephrine reuptake inhibitor duloxetine (Harmer et al., 2008).

Perhaps counter intuitively, recognition of happy facial expressions was also increased (with significance level equal to p= .05) in both aforementioned studies (Murphy et al. 2009; Harmer et al., 2003b) thus leading to the suggestion that acute citalopram administration might increase the perception of affiliative signals consistent with a role of 5HT in affiliative behaviour (Knutson et al., 1998). Sub-chronic administration of citalopram for seven days led to reduced identification of fearful and angry facial stimuli and suppressed the already initiated startle response towards negative affective stimuli (Harmer et al., 2004).

Labuschagne et al. (2010) reported a temporal modulation of emotional processing following citalopram administration with an enhanced cortical response to moderately intense sad facial expressions in the treatment group. The researchers attributed this modulation to cognitive mechanisms pointing out the significance of these facial stimuli as social cues. Another potential explanation is that the dosage of citalopram applied may have resulted to lower rather than higher brain serotonergic levels.

Neuropsychological test battery

We administered three tasks, one from the CANTAB battery and two from the EMOTICOM battery, which measure information processing biases using words and facial expressions respectively as stimuli. We combined these two tasks in order to eliminate any cross-cultural and educational individual differences that could affect performance from using the well-established CANTAB affective Go/No-Go word version alone (Bland et al., 2016). The EMOTICOM Social information preference task ('Theory of mind') was included in our task battery in order to further explore the serotonergic modulation on “hot” cognition (Roiser and Sahakian, 2013). Theory of mind is termed as the “ability to infer the mental states of others” (Frith and frith, 2003) and several tasks are applied to study this domain, including the “faux pas” tests and the “Reading the mind in the eyes” test by Baron-Cohen et al. (2001). Impairments in theory of mind are identified in autism (Happe and Frith, 1996) and schizophrenia (Bora et al., 2009; Green et al., 2008). We further included the ultimatum game, which measures emotion regulation through social interaction (Crockett et al., 2008).

Hypotheses

We predicted that acute escitalopram would enhance positive affective interpretation bias in the three emotional processing tasks, the EMOTICOM affective Go/No-Go task, the CANTAB affective Go/No-Go task and the EMOTICOM Social Information Preference Task or 'Theory of mind' task. We also hypothesized, given prior literature on acute citalopram dosage increasing attention to socially relevant stimuli (Harmer et al., 2003b) and our sample is comprised by healthy volunteers, that the escitalopram-treated group would select faces as more informative during the EMOTICOM "Theory of mind task".

I. EMOTIONAL PROCESSING TASKS

1. EMOTICOM affective Go/No-Go task

Task description

During the face affective Go/No-Go task, participants are presented with target emotions (happy, sad, neutral) and are requested to press space bar on the keyboard only when the target emotion is present. There are six blocks consisting of the following combinations: (1) happy target/sad distractor, (2) happy target/neutral distractor, (3) neutral target/happy distractor, (4) neutral target/sad distractor, (5) sad target/happy distractor and (6) sad target/neutral distractor (Bland et al., 2016). Participants completed 120 trials across six conditions. The task lasted six minutes. Primary outcome measures included number of "hit" responses for each of the presented emotion target and reactions times (RTs) for all "hit" responses for each of six conditions. We calculated affective bias scores, as per Bland et al. (2016), by subtracting the RTs in the sad target/happy distractor condition from the RTs in the happy target/sad distractor condition.

Figure 1: Participants were presented with different target emotions; happy, sad and neutral respectively

Participants

Overall sixty-four participants completed the face affective Go/No-Go task; thirty-two in the escitalopram and thirty-two in the placebo group. Mean demographics are presented on Table 1. Participants completed this task along with the additional cognitive tasks in a counterbalanced order. We predicted that acute escitalopram administration would increase responses for happy facial stimuli (targets).

Demographics	Placebo	Escitalopram	Group difference*
Male: female	16:16	16:16	n.s.
STAI trait	35.28 (8.6)	34.6 (9.54)	n.s.
BDI	5.31 (5.21)	4.1 (4.33)	n.s.

Table 1: Group demographics. Mean + SD. ***Group difference**: p-values of two-tailed t-tests n.s.= non-significant, p>0.05

Results

The mean number of 'hit' responses participants made throughout the six different conditions are shown in Table 2. We performed repeated measures ANOVA with type of condition as within-subjects factor. We found no significant main treatment effect, p= .821.

Conditions	Placebo	Escitalopram	Group difference*
happy/ sad	8.81	9.25	p= .407
sad/ happy	8.81	8.88	p= .906
happy/ neutral	9.19	9.38	p= .716
neutral/ happy	8.22	8.00	p= .754
neutral/ sad	8.75	9.00	p= .578
sad/ neutral	8.38	8.03	p= .590

Table 2: Mean 'hit' responses across the six conditions. *Statistical significance level set as p< .05

We further compared the RTs in the six conditions with repeated measures ANOVA with condition valence as within-subjects factor. We found a significant main valence effect, p< .038, but no interaction between treatment and valence, p= .268.

Condition	Placebo	Escitalopram	Group difference*
happy/ sad	0.50 (0.06)	0.48 (0.9)	0.203
sad/ happy	0.5 (0.07)	0.5 (0.1)	0.837
happy/ neutral	0.48 (0.07)	0.46 (0.08)	0.126
neutral/ happy	0.52 (0.8)	0.52 (0.09)	0.956
sad/ neutral	0.52 (0.07)	0.5 (0.08)	0.256
neutral/ sad	0.55 (0.08)	0.52 (0.07)	0.133

Table 3: The two groups did not differ in RTs across the six conditions, $p < 0.05$. Mean (SD) in milliseconds

We compared affective bias between treatment groups and we found no significant treatment effect, $p = .896$, $t(62) = -.131$.

Trait anxiety and depressive symptomatology

Subjects were divided into low and high-anxious groups based on a median split on trait anxiety scores from the State-Trait anxiety inventory (Spielberger et al., 1983); demographics for the subgroups are presented in Table 4. We performed repeated measures ANOVA as above with valence and distractor type as within-subjects factors and trait anxiety and treatment as between-subjects factors. Dependent variables were mean reaction times in the four conditions; happy/sad, happy/neutral, sad/happy and sad/neutral. We found a significant interaction between valence and trait anxiety, $p = .026$, $F(1,60) = 5.195$, with high trait anxiety group responding significantly slower for sad targets compared to happy ones. We found no significant interaction between valence, trait anxiety and treatment, $p = .640$, $F(1,60) = .222$, and no interaction between treatment and trait anxiety on the reaction times of the four target/distraction conditions, $p = 0.162$, $F(1,60) = 2.008$.

Trait anxiety group		Low Trait Anxiety	High Trait Anxiety	Group difference*
Number of volunteers	Placebo	13.00	19.00	
	Escitalopram	19.00	13.00	
Gender	Females	16.00	16.00	
	Males	16.00	16.00	
Age		26.81 (6.26)	25.03 (4.9)	n.s.
BDI		3.06 (3.71)	5.34 (2.28)	n.s.
NART		42.94 (4.8)	42.19 (6)	n.s.
Years of education		16.5 (2)	16.14 (2.15)	n.s.

Table 4: Demographics of low- and high-trait anxiety groups, n.s.=not significant

Taking into account the extensive literature on affective information biases in depression (Erickson et al., 2005; Murphy et al., 1999), we repeated the ANOVA with two within-subjects factors (valence and distractor type) adding Beck Depression Inventory scores as covariates. We report no significant treatment effect, p= .592, F(1,61)= .291, no valence effect, p= .677, F(1,61)= .175, and no interaction between treatment and valence, p= .603, F(1,61)= .273.

Previous studies reported sex-dependent effects of ATD on emotional processing (Ellenbogen, et al., 1996; Booij et al., 2003; Harmer et al., 2003) therefore, we added sex as between-subjects factor in our initial analysis on number of "hits" and RTs. We found no significant main interaction between treatment and sex on number of "hits", p= .133 and no significant main interaction between treatment and sex on RTs, p= .116.

2. CANTAB affective Go/No-Go task

Task description

Participants were rapidly presented with a series of words on the screen consisting of three distinct valence types: positive (for example warmth), negative (for example mistake) and neutral (for example item). Words from two valence categories were presented and participants are instructed to press a button every time they see a word matching a specific valence target given. The task consisted of twenty 18-word blocks and it lasted ten minutes. Primary outcome measures included correct latency, commission errors (incorrect response towards a distractor), omission errors (omission of response towards a target stimulus) and affective response bias (the difference between correct latency for positive blocks minus correct latency for negative blocks). Commission and omission errors were calculated based on whether they were performed during a shift or a non-shift block. In shift blocks participants had to change the valence of their response orientation between blocks, for example from positive to negative target, in contrast to non-shift blocks where participants did not have to change their response orientation, for example in positive to positive target presentation.

Figure 2: Participants are presented with words of difference valence type such as positive ones

Participants

Overall sixty participants completed the affective Go/No-Go task; twenty-nine in the escitalopram and thirty-one in the placebo group. Demographics are presented on Table 5. Participants completed this task along with the additional cognitive tasks in a counterbalanced order. We predicted that acute escitalopram administration would increase responses for happy facial stimuli (targets).

Demographics	Placebo	Escitalopram	Group difference*
Male: female	16:15	15:14	n.s.
STAI trait	34.46 (8.01)	34.99 (9.59)	n.s.
BDI	5.1 (5.19)	4.24 (4.52)	n.s.

Table 5: Group demographics. Mean + SD. ***Group difference**: p-values of two-tailed t-tests
n.s.= non-significant, p>0.05

Results

We found no main treatment effect on mean correct latencies for positive and negative target stimuli, p= .651, F(1,55)= .206, and no interaction between treatment and valence, p= .799, F(1,55)= .065. Commission error data was square root transformed to reduce skew and normalize variance and outliers removed as appropriate. We found no significant treatment effect on commission errors made in the shift, p= .438, t(57)= .781, and the non-shift blocks, p= .191, t(55)= 1.324. We further compared the two treatment groups in

commission errors in the shift blocks for positive and negative target stimuli. Repeated measures ANOVA showed no valence effect, p= .146, F(1,55)= 2.179 and no interaction between valence and treatment, p= .444, F(1,55)= .595. Omission error data were log transformed to normalize variance and reduce skewness. The two groups showed no difference in omission errors performed in shift, p= .843, t= .20, and non-shift blocks, p= .712, t= .109. Treatment had no effect on the affective response bias, U= 497, z= .703, p= .482.

Measure	Placebo	Escitalopram	Group difference*
Correct latencies			
Positive	538 (65.17)	524.59 (61.77)	n.s.
Negative	509.87 (63.77)	502 (56.81)	n.s.
Omission errors			
Shift blocks	2.9 (3.89)	2.03 (2.4)	n.s.
Non-shift blocks	2.62 (3.8)	1.87 (2.29)	n.s.
Commission errors			
Shift blocks	4.1 (3.13)	4.7 (3.96)	n.s.
Non-shift blocks	2.9 (2.4)	4.04 (3.83)	n.s.
Affective bias	-9.22	-1.15	n.s.

Table 6: The two treatment groups did not differ in any task measure, RTs measured in milliseconds, mean (SD), n.s.= non-significant

We further separated participants into low and high trait anxiety groups using a median split on the STAI trait anxiety scores (Table 7), as per previous analyses on the face affective Go/No-Go task. We compared again the correct latencies for positive and negative target stimuli. We found no main treatment effect, p= .686, F(1,56)= .165, no trait anxiety effect, p= .212, F(1,56)= .212, and no interaction between treatment and trait anxiety, p= .590, F(1,56)= .294. We added BDI scores in our analysis and we found no interaction between BDI scores and treatment on response latencies, p= .119, and affective response bias, p= .382.

Trait anxiety group		Low Trait Anxiety	High Trait Anxiety	Group difference*
Number of volunteers	Placebo	16.00	14.00	
	Escitalopram	12.00	15.00	
Gender	Females	14.00	15.00	
	Males	18.00	13.00	
Age		26.49 (6.32)	25.64 (5.37)	n.s.
BDI		3.06 (3.94)	4.44 (3.71)	n.s.
NART		42.85 (4.51)	43.04 (4.54)	n.s.
Years of education		16.05 (1.2)	16.36 (3.1)	n.s.

Table 7: Demographics of the low- and high-trait anxiety groups, n.s.=not significant

Sex was added as a between-subjects factor and yielded no significant results on affective response bias [treatment by sex interaction, p= .408] and correct latencies [treatment by sex interaction, p= .564].

3. EMOTICOM Social Information Preference Task or 'Theory of mind' task

Background

Serotonin has been implicated in social processing with sub chronic SSRI administration shown to promote affiliative behaviour in both primate and human studies (Raleigh et al., 1991; Knutson et al., 1998). In autism, characterised by deficits in social cognition, SSRIs are shown to reduce OCD behaviour and anxiety (Hollander et al., 2005) mediated by SERT binding in medial frontal cortex and temporal lobe.

The task applied was developed by Bland et al. (2016). The majority of tasks widely used in the literature are not sensitive to the (optimal) performance variation observed in healthy adults. This task assesses information sampling in socially ambiguous situations; there is no right or wrong answer therefore greater response variation can be studied. Unlike existing tasks, mainly used in clinical populations, this task assessed the 'extent to which people choose to use theory of mind information' (Bland et al., 2016) rather than whether participants are capable or not of applying theory of mind information.

Task description

Participants are presented with a scene with pieces hidden from view including three faces revealing feelings, three revealing thoughts and three revealing facts. Participants are prompted to select up to only four out of nine pieces of information to help resolve ambiguity. Subsequently, they are requested to choose between three possible outcomes of the situation (negative, positive or neutral valence). This choice provides a measure of interpretational bias.

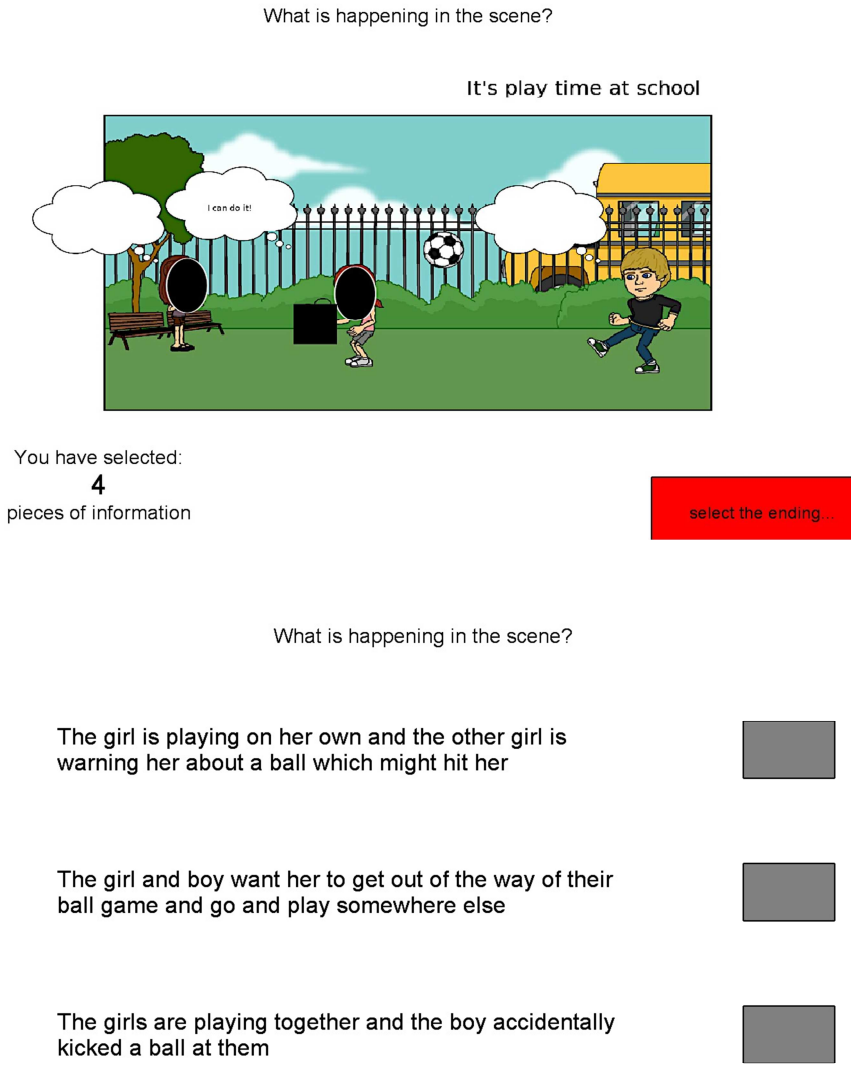


Figure 3: Participants are presented with various scenarios where they can select pieces of information

Following selection of the outcome scenario, participants were requested to rate their confidence on a computerised scale on the choice made.

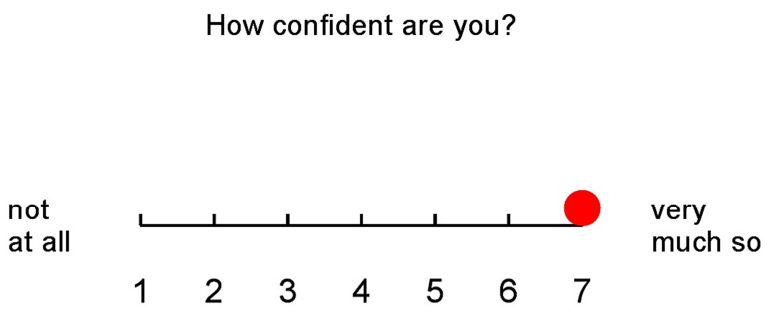


Figure 4: Confidence ratings following outcome selection

The task lasted ten minutes and eighteen scenarios were presented. The affective bias in interpretation was calculated by subtracting the proportion of negative outcomes chosen from the proportion of positive outcomes chosen (Bland et al., 2016).

Participants

Fifty-eight participants completed the task; twenty-seven in the escitalopram and thirty-two in the placebo group. Demographics are presented in Table 9.

Trait anxiety group		Low Trait Anxiety	High Trait Anxiety	Group difference*
Number of volunteers	Placebo	14.00	16.00	
	Escitalopram	12.00	11.00	
Gender	Females	16.00	13.00	
	Males	17.00	13.00	
Age		26.49 (6.32)	25.64 (5.37)	n.s.
BDI		3.33 (4.75)	4.16 (3.17)	n.s.
NART		42.7 (4.77)	43.2 (4.52)	n.s.
Years of education		15.6 (2.5)	16.24 (1.5)	n.s.

Table 9: Demographics in low and high trait anxiety groups, n.s.= not significant

Results

We performed repeated measures ANOVA with the information type (faces, thoughts, facts) as within-subjects factor and dependent variable the proportion of information chosen. We found a significant main effect of information type, $p < .001$, $F(2,56) = 221.302$, partial $\eta^2 = .795$, and an interaction of information type and treatment close to significance, $p = .06$, $F(2,56) = 2.956$, partial $\eta^2 = .095$. Follow-up, independent samples t-tests showed that the escitalopram group chose significantly higher proportion of facts, $p = .017$, $t = 2.451$, partial $\eta^2 = .095$, without any significant difference in proportion of faces, $p = .346$, $t = -.950$, partial $\eta^2 = .016$, and thoughts chosen, $p = .330$, $t = -.983$, partial $\eta^2 = .017$.

Measure	Placebo	Escitalopram	Group difference*
Information chosen			
Facts	0.15 (0.1)	0.1 (0.07)	p= 0.017, t= 2.451
Faces	0.27 (0.11)	0.29 (0.1)	n.s.
Thoughts	0.58 (0.12)	0.61 (0.1)	n.s.
Affective bias in outcome interpretation	2.78	1.91	n.s.
Information deliberation RTs	19.47 (7.01)	17.95 (5.53)	n.s.
Outcome deliberation RTs	8.5 (3.35)	8.89 (2.81)	n.s.

Table 10: Escitalopram-treated participants chose a higher proportion of facts, p= .017, with no difference in other task measures. Information type presented as proportions

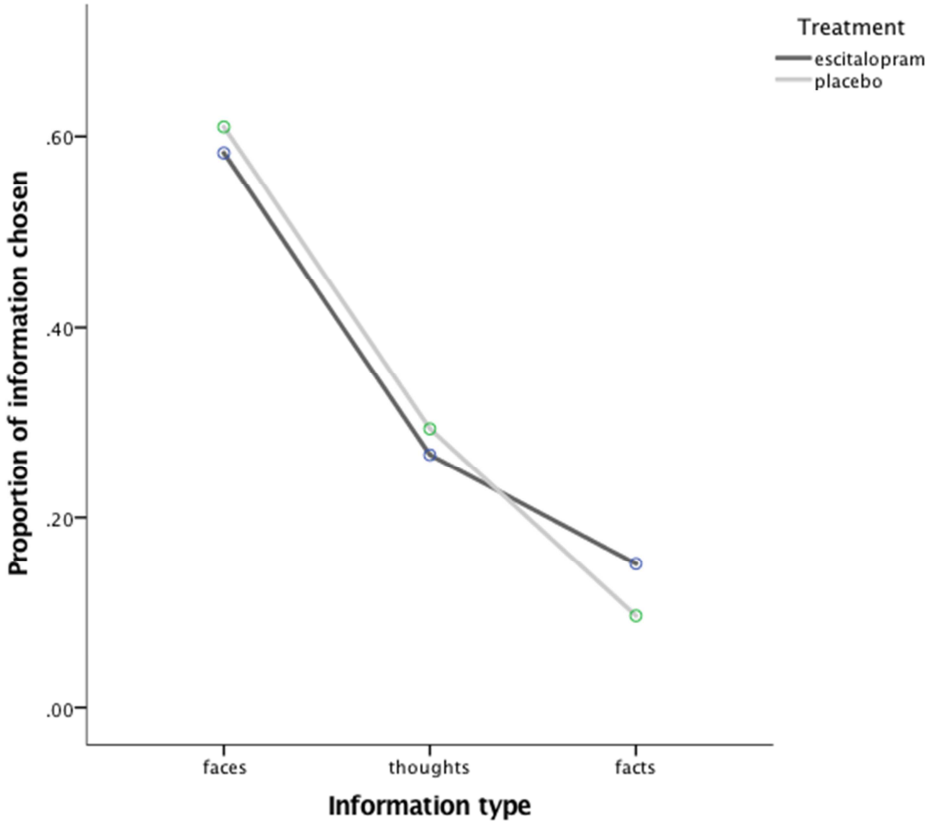


Figure 5: The escitalopram-treated group chose significantly higher proportion of facts, p= .017

We performed repeated measures ANOVA with scenario outcome valence (positive, neutral and negative) as within subjects-factor and treatment as between-subjects factor. We found a significant valence effect, p< .001, F(2,51)= 16.745, partial η^2= .396, but no interaction between valence and treatment, p= .621, F(2,51)= .481. Participants in both treatment groups chose more positive scenario outcomes.

We found no difference between treatment groups in affective bias, p= .235, F(1,57)= 1.444. Previous studies showed a sex specificity effect of serotonergic manipulation on emotional processing (Harmer et al., 2003a) thus we further included sex in our analysis and found no interaction between sex and treatment in affective bias, p= .358.

We also found no interaction between BDI scores median split and treatment, p= .601, and no interaction between STAI scores median split and treatment, p= .863. Data on response latencies for info and outcome deliberation and for indicating level of confidence in choices made were compared between groups using non-parametric tests as per violation of tests of normality. The treatment groups did not differ in either of these measures [info

deliberation latencies, p= .456, U=383, z= -.746; outcome deliberation latencies, p= .743, U= 395, z= -.328; latencies for confidence ratings, p= .518, U= 389.5, z= -.647].

Both groups had similar confidence ratings, p= 0.931, F(57)= 1.672, thus showing that escitalopram did not affect decision confidence. There was no change over time in willingness to engage in social interaction as measured with a visual analogue scale, p=0.22, F(1,59)=1.538, between the two treatment groups [time x treatment, p=0.438, F(1,59)=0.609].

Conclusion

Contrary to our initial hypotheses, escitalopram administration had no effect on affective bias in either of the three emotional processing tasks. Escitalopram administration increased the proportion of chosen facts in the EMOTICOM Social Information Preference Task or 'Theory of mind' task.

II. EMOTICOM Ultimatum game

Background

Social behaviour requires emotional regulation and behavioural control. A suggested role for serotonergic transmission in being implicated in social behaviour is through modulation of emotional regulation. To this end, we included in our study the Ultimatum Game, which has been used to study emotion regulation through social interaction (Crockett et al., 2008; Fehr and Camerer, 2007). In this task, one player is the proposer, thus proposing a way to split money with another player, who is the responder. If the responder accepts the suggested offer, then they both gain respectively, whereas if the responder declines it, neither of the two earns anything. Guth et al. (1982) showed that people tend to reject offers that are less than 20-30% unfair. Responses to unfairness are mediated by 5HT as ATD was previously shown to increase rejection rates for unfair offers (Crockett et al., 2008) without this effect being attributed to changes in mood, fairness judgement or

response inhibition which remained unaffected by the manipulation replicating previous studies in primates (Clarke et al., 2005).

Along with other tasks assessing social cognition, the ultimatum game has been applied in autism (Andari et al., 2010), schizophrenia (Agay et al., 2008) and psychopathy (Rilling et al, 2015). In our study, we attempted to capture the effects of acute escitalopram administration on both emotional processing and emotional regulation.

Task description

During the task (Bland et al., 2016), participants were asked to select the avatar they wished to use during the task thus personalising the game. The opponent's avatar was randomly selected by the computer from a pool of both male and female pictures. In each single shot, the opponent avatar changed.

In the beginning of the trial, participants were requested to select 3 balls from a choice of 9 overall. Depending on the colours revealed behind the balls, they won money, which was then combined with the opponent's total (full design explanation in Bland et al., 2016). We manipulated contribution to the total amount with three different contribution levels; level 1, where the participant contributed more, level 2, the opponent contributes more and level 3, equal contribution. Participants were informed of the amount contributed by both themselves and the opponents. This design allowed us to observe independent effects of escitalopram administration on responding to different levels of fairness accounting for different amounts of personal contribution.

During each session, participants were informed whether they would decide on how to split the money earned or whether the opponent would make the decision. There were 15 games where the participants acted as the proposers choosing from four different fairness levels; 50:50, 40:60, 30:70 and 20:80. There were 36 trials where the participants acted as responders with seven offer levels ranging from fair (50:50) to increasingly unfair (10:90). If the participant accepted the split proposed by the proposer, the money was correspondingly divided. If the participant rejected, neither of the two earned anything.

Participants were provided with written instructions on the computer screen and a practice trial. Subsequently, the experimenter asked for verbal confirmation they understood the task. Participants were told that the money they would gain would be converted to points. All trials were counterbalanced. Overall the task lasted 12 minutes.

Participants

Overall sixty-two volunteers completed this task; thirty- two in escitalopram and thirty in placebo group. Participants completed this task along with others in counterbalanced order.

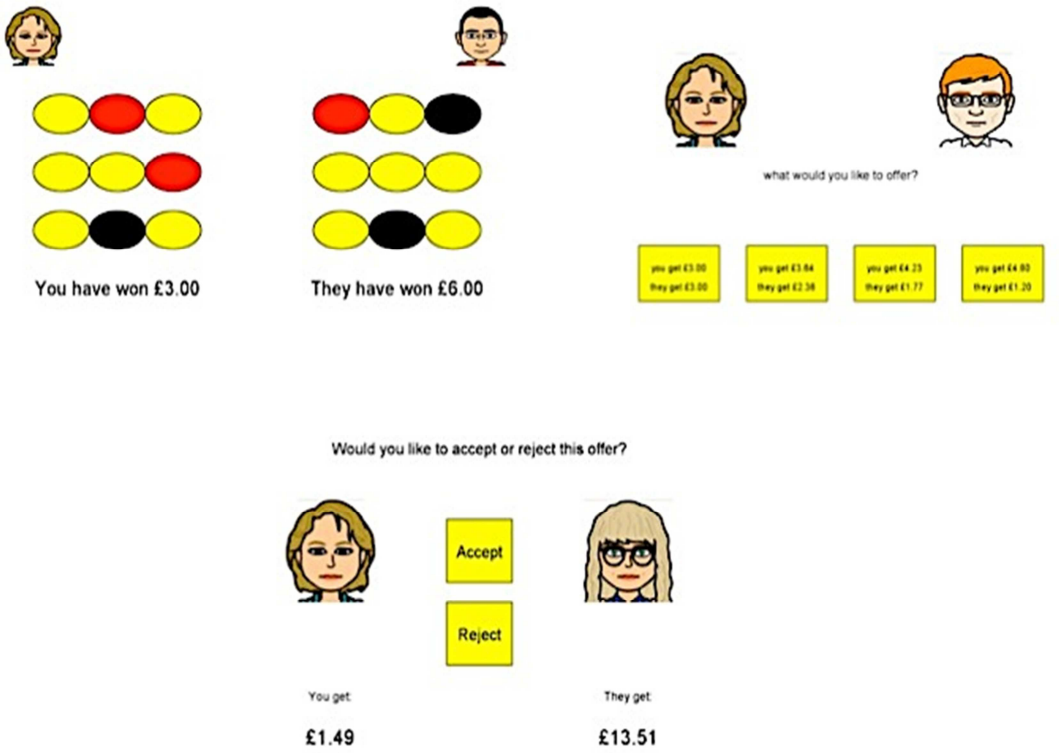


Figure 6: Participants selected 3 balls (a). Depending on the colours revealed behind the balls, participants could win money**.** Participants acted as proposers (b) choosing from 4 different levels of fairness to offer and as receivers (c) where they could accept or reject the offer made the proposer

Outcome measures included risk adjustment and value of offers chosen.

Risk adjustment = (2*acceptance at 50% offer) + (1* acceptance at 40% offer) + (0* acceptance at 30% offer) − (1* acceptance at 20% offer) − (2* acceptance at 10% offer)/Average offer

Hypothesis

We predicted that escitalopram will affect rejection rates, based on fairness level in the EMOTICOM Ultimatum Game. We hypothesized escitalopram-treated participants will display a higher tolerance towards unfair offers (mirroring the opposite effects of lowering brain 5HT levels with ATD; Crockett et al., 2009).

Results

We found no difference between groups for risk adjustment, p= .548, F(1,61)=.366.

We grouped the different fairness levels, where the participants were the responders, into fair (50:50), moderate (40:60, 35:65, 30:70, 25:75) and unfair (20:80, 10:90). We performed repeated measures ANOVA with level of fairness (fair, moderate, unfair) as within-subjects factor and treatment as between-subjects factor. We found a significant main effect of fairness level, p< .001, F(2,59)= 164.773, partial η^2= .848, but no interaction between fairness level and treatment, p= .682.

Measure	Placebo	Escitalopram	Group difference*
Risk adjustment	0.22 (0.22)	0.18 (0.29)	n.s.
Participant as responder Fairness levels	**Rejections rates**		
Unfair	72.22 (27.95)	69.8 (34.12)	n.s.
Moderate	40.37 (28.66)	38.2 (26.54)	n.s.
Fair	5.19 (7.57)	7.99 (9.03)	n.s.

Table 11: The two treatment groups did not differ in the main task measures, n.s.= not significant, p>0.05

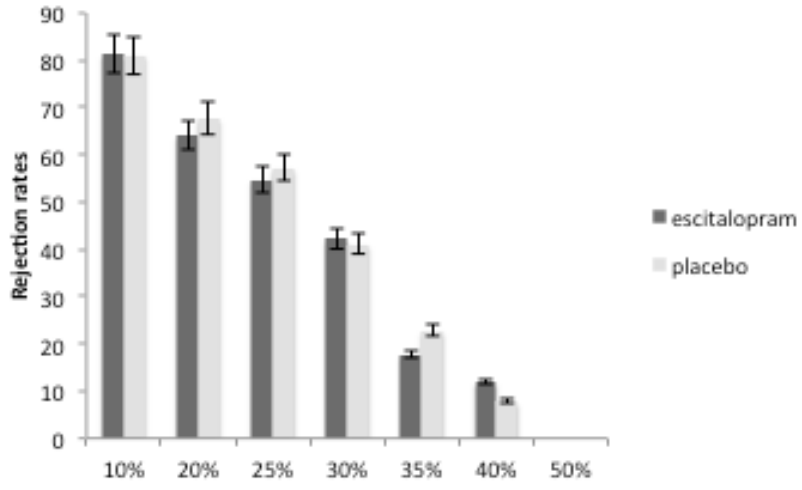


Figure 7: Rejection rates for the different levels of fairness

We included Kirby discount rate as covariate; we found no effect of treatment on rejection rates across the different fairness levels, p= .245.

Conclusion

Escitalopram administration produced no effect in social behaviour measured with the Ultimatum game from the newly developed EMOTICOM battery (Bland et al., 2016). Participants treated with escitalopram showed no difference from placebo in rejection rates which was the key measure.

Discussion

Contrary to our initial predictions, escitalopram administration did not produce any effect in the emotional processing domains even taking into account trait anxiety, BDI and gender influences. This apparently contrasts with Harmer et al. (2003a) and Browning et al. (2006), who reported enhanced recognition of both fear and happiness in faces by (mainly) healthy female volunteers following acute citalopram (oral 20mg or iv. 10mg) - and decreased recognition of fear in faces following ATD in female volunteers (Harmer et al., 2003b). However, Murphy et al. (2009) found that oral citalopram reduced the BOLD response to fearful faces in the amygdala, with no behavioural effect on recognition *per se*. Moreover, Del-Ben et al. (2005) found no behavioural effects on face recognition of i.v. citalopram (7.5mg) treatment.

The lack of observed effects in the affective faces go/no-go task, compared to previous studies using acute citalopram administration and facial stimuli, might be driven by the differences in the tasks applied; in our face go/no-go task we only used three valence types of happy, sad and neutral stimuli whereas in the Harmer et al. (2004; 2003b) fearful stimuli was also applied to which the significant response following acute citalopram administration was observed. It is important to note that Harmer et al. (2003b) also showed an increased recognition of happy facial stimuli, which we did not observe in our task. Additionally, although we employed different tests i.e. face recognition tests in Harmer et al. (2003b; 2004) versus Affective Go/No-Go tasks in our study, face recognition could be measured in the EMOTICOM faces affective Go/No-Go task as participants had to respond to a target face ignoring the distractor and escitalopram produced no effects on accurate 'hit' responses. The groups also did not differ in omission errors for distinct target valence words in the CANTAB affective Go/No-Go task – a task previously shown to be sensitive to ATD (enhancing negative affective bias) and depression (Murphy et al., 2002; 1999). Nevertheless, the effects of acute citalopram administration and ATD have overall yielded contrasting results in previous studies thus pointing out the complexity of serotonergic

modulation (and the techniques applied, as previous studies have used both oral and intravenous citalopram and other SSRI administration) in mediating emotional processing.
We did observe an effect on social cognition with the application of a novel 'theory of mind' task, where participants receiving escitalopram chose a larger proportion of facts compared to other types of information during the initial, in order to interpret socially ambiguous scenarios.
An alternative account is the final net concentration in brain 5HT levels produced by acute escitalopram administration in distinct brain regions mediating distinct aspects of cognitive mechanisms ("cold" versus "hot" cognitive mechanisms). But again, in this case we would expect effects similar or close to the ones observed with ATD in previous studies, which was not observed in our study.

Chapter 4. Instrumental and probabilistic learning

Background

Cognitive flexibility entails two distinct –both behaviourally and anatomically- functions of reversal learning and attention set shifting; these are differentially affected by distinct neurotransmitter systems innervating the prefrontal cortex (PFC) (Clarke et al., 2005). 5HT is traditionally related to behavioural inhibition in light of punishments or negative events (Crockett et al., 2009; Deakin and Fraeff, 1991). Global brain tryptophan depletion is shown to improve outcome prediction for punishments but not for rewards (Cools et al., 2008a) and affect subsequent choices (Evers et al., 2005). The role though of 5HT in aversive processing is not clear; a contrasting effect is shown for 5HT on behavioural inhibition (in terms of response suppression) related to punishment (Cools et al., 2008b; Tye et al., 1977; Graeff et al., 1970). A possible account for this is the differential effect of serotonergic transmission in cortical and subcortical areas thus affecting processing of punishments and inhibitory control respectively (Cools et al., 2008). Alternatively, it may relate to different tasks applied in these studies, measuring distinct aspects of aversive processing and response inhibition. Reversal learning is shown to be mediated both by dopaminergic and serotonergic systems. Reversal deficits have been observed in patient groups, including schizophrenia (Pantelis et al., 1999) and frontal lesion patients (Rolls et al., 1994). Izquierdo et al. (2017) illustrate that rule learning and subsequent use combined with reward learning is the optimal strategy in a probabilistic learning task, rather than motor response control. Thus suggestions of reversal learning paradigms measuring response inhibition (Jones and Mishkin, 1972) are not supported. The task has been applied across species thus proving to be of translational significance.

Brain areas implicated in reversal learning

The prefrontal cortex is associated with modulation of attention with distinct parts accounting for distinct functions. Nagahama et al. (2001) reported activation of antero-dorsal PFC during set shifting between different perceptual dimensions, where increased response time was also recorded, and activation of postero-ventral PFC during both set shifting and reversal of stimulus-response contingencies. The mPFC (Cools et al., 2002) and orbitofrontal cortex are implicated in reversal learning in several studies across species (humans, Rolls et al., 1994; Kringelbach and Rolls, 2003; non human primates, Dias et al.,

1996; rat, Boulougouris et al., 2007). Patients with lesions in ventral areas of the frontal lobe show deficits during reversal learning (Rolls et al. 1994).

Animal studies suggest that initial stimulus discrimination is successfully acquired but reversal of S-R contingencies is impaired in OFC lesions. Suggested accounts include the selective reversal of neuronal firing for neurons encoding specific cues (Rolls et al., 1996) meaning that the reversal of cues is first learnt in OFC and then propagated to other brain areas which though is not support by findings showing that other brain areas, for example, the amygdala reverses the contingencies prior to the OFC function (Schoenbaum et al., 1999). Alternative explanation refers to the OFC encoding predictions for expected outcomes; thus during a reversal the OFC will facilitate the new representation of S-R contingencies in other brain areas which is supported by findings of rat basolateral amygdala lesions abolishing OFC-induced deficits in reversal learning (Stalnaker et al., 2007). The same study showed that basolateral amygdala lesions alone did not affect reversal-learning performance (Stalnaker et al., 2007). Thus amygdala features as playing a supportive role possibly for tracking prior outcomes and using them as a comparison to expected ones (Izquierdo et al., 2017); this is supported by evidence that outcome representation in amygdala incorporates both the stimuli predictive of these outcomes and prior reward history (Paton et al., 2006). Rugyla et al. (2015) showed that localised 5HT depletion in the marmoset OFC impaired discrimination learning and reduced response suppression in a novel probabilistic visual discrimination task with reward and punishment conditions. In the same study, localised 5HT depletion in the marmoset amygdala impaired choices mediated by increased sensitivity to misleading feedback (fro both reward and punishment) and reduced response suppression in a variable interval test of punishment sensitivity (Rygula et al., 2015). This pattern of effects is suggested to reflect encoding of anticipatory signals – for both rewards and punishments- by the DRN 5HT neurons (Miyazaki et al., 2014).

Amygdala receives cortical input and projects to dorsal (Rogers et al., 2000) and ventral (Cools et al., 2002) striatal areas, which in turn are implicated in reversal learning leading to suggestions of a “functional circuit supporting reversal learning” (Izquierdo et al., 2017). The striatum in turn receives input from the OFC and projects to nucleus accumbens and dorsomedial striatum as shown in the rodent (Izquierdo et al., 2017). Lesions in the dorsomedial striatum produce reversal-learning deficits in both rats (Castañé et al., 2010) and marmosets (Clarke et al., 2008). The contribution of serotonergic neurotransmission in OFC function to behavioural flexibility is suggested to include a role

for “preventing competing, irrelevant, salient stimuli from biasing responding” (Walker et al., 2009) as the researchers showed that 5-HT OFC depletion produced “stimulus-bound” responding in both a conditioned reinforcement and a discrimination extinction test. Additionally, it produced biased responding towards previously rewarded stimulus.

Neurotransmitter systems implicated in reversal learning

Three major neurotransmitter systems are widely studied in relation to reversal learning paradigms; 5HT, DA and glutamate. Each neurotransmitter system exerts effects on distinct brain processes employed during reversal learning paradigms. Studies have also manipulated brain cholinergic levels, as will be discussed below.

The serotonergic system: Selective serotonergic depletion in the marmoset prefrontal cortex with the neurotoxin 5,7-DHT resulted in impaired performance in a serial reversal-learning task (Clarke et al., 2004). This effect was produced during the reversal stage and it was attributed to perseverative responding to the previously rewarded stimulus. The aforementioned effect on reversal was manifested with 5-HT depletion but not with DA depletion (Clarke et al., 2007). Boulougouris et al. (2008) showed a differential effect of antagonism of 5HT receptors on rat performance on a serial spatial reversal learning task; $5HT_{2A}$ receptor antagonists increased errors to attaining learning criterion following stimulus contingencies’ reversal attributed to increased perseverative errors to previously maximally rewarded stimulus. Antagonism of $5HT_{2C}$ receptors improved performance, which was mediated by the OFC (Boulougouris and Robbins, 2010). Reversal learning deficits have been observed in an attention set shifting task in rats following two weeks of chronic intermittent cold stress (CIC) which was subsequently overturn by two week treatment with citalopram. The task included both intra- and extra-dimensional shifts and reversals of stimulus discrimination (Lapiz and Morilak, 2006). Both acute and chronic systemic intravenous citalopram administration reversed this effect (Danet et al., 2010) replicating a previous study by this group (Lapiz- Bluhm et al. 2009). Both CIC and control groups had comparable baseline 5-HT levels in the OFC measured with micro dialysis but the CIC group showed significantly reduced OFC 5-HT levels as per samples collected during testing. Tryptophan depletion in naïve rats showed a selective impairment in reversal in the attention-switching task similar to the one observed with CIC (Lapiz-Bluhm et al. 2009). Finger et al. (2007) showed no effect of tryptophan depletion in reversal errors in healthy volunteers where they showed reduced responding to rewarding stimuli in a passive avoidance task.

The dopaminergic system: Prefrontal DA depletion in marmosets produced no effect in reversal learning (Roberts et al., 1994). Administration of oral methylphenidate to healthy individuals produced differential effects of D_2/D_3 receptor availability in distinct brain areas, which correlated with performance in cognitive tasks (Clatworthy et al., 2009). Performance in the reversal learning task was predicted by methylphenidate effect on D_2/D_3 receptor availability in post commissural caudate, as measured with PET scan, and baseline trait impulsivity differences measured with the Barratt Impulsivity scale; a positive association of trait impulsivity scores and improved performance was reported with high trait impulsivity participants gaining more from methylphenidate administration in terms of reversal learning ability. Impaired reversal learning was observed following bromocriptine (a D2 receptor agonist) administration to healthy volunteers (Mehta et al., 2001) and in medicated Parkinson's disease (PD) patients (Swainson et al., 2000) performing a probabilistic reversal learning task attributed to "overdosing of the DA system related to reversal learning" (Mehta et al. 2001) which is the dopaminergic "innervation to the ventromedial head of the caudate, which has anatomical connections to the lateral orbitofrontal cortex" (Alexander et al. 1986). This was suggested for both the PD patients, where dopaminergic drugs restore the DA loss and thus interact with "different baseline levels of DA function modulating cognitive processes that rely on separate regions of the frontal cortex" and the healthy volunteers, where areas implicated in reversal learning are "overdosed", most prominently in novel and unexpected situations (Schultz et al., 1997).

Interactions between serotonergic and dopaminergic systems on reversal learning: The dissociative effects of distinct neurotransmitter systems to reversal learning show that deficits of reversal produced by reduced serotonergic transmission in the OFC are neuro-chemically specific (Clarke et al., 2007; Walker et al., 2006) and not dependent on effects through the dopaminergic system.

Other neurotransmitter systems: Adminitration of a clinically relevant dosage (60mg) of atomoxetine, a selective noradrenaline reuptake inhibitor, to healthy volunteers did not produce any effect on probabilistic and reversal learning (Chamberlain et al., 2006). It is worth noting that prefrontal cholinergic depletion in marmosets resulting from excitotoxic lesions impaired performance on discrimination reversals (Roberts et al., 1990) attributed to an extent to increased perseveration (Roberts et al., 1992), similar to OFC lesion produced perseveration and amygdala lesion mediated impairments in serial reversal

learning. Systemic scopolamine (a muscarinic receptor antagonist) administration in rats produced deficits in reversal learning (Chen et al., 2004).

5HT transmission and reversal learning in human volunteers

Citalopram administration (20mg) to healthy volunteers (Chamberlain et al., 2006) impaired performance both during probabilistic learning, indicated by increased number of errors prior to reaching learning criterion, and after reversal of stimulus-outcome contingencies during the second stage of the task, identified as perseverative errors to the previously maximally rewarding stimulus. The citalopram-treated group showed higher sensitivity to misleading feedback, reminiscent of behaviour in depression (Tavares et al., 2008) and longer response latencies during the first stage of the task. Elliott et al. (1996) previously reported that depressed patients –among several neuropsychological deficits including recognition working memory and planning- showed higher probability of failing in a task following failure in a previous task, thus demonstrating the detrimental role of negative feedback on depressed patients' performance. This was demonstrated in matching to samples and the new Tower of London tasks, which employ distinct neural substrates, but both involve explicit and immediate feedback provided. In these two tasks, depressed patients showed higher tendency towards incorrect responses following negative feedback. This effect was localised to accuracy and not speed of responding, as participants did not show any difference in response latencies, and it was correlated with future hospitalization rates (Elliott et al., 1996).

This 'catastrophic response to failure' has been previously described (Beats et al., 1996) and Elliott et al. (1996) suggested that depressed patients might be less able than controls to use negative feedback in order to update their performance. Indeed, Murphy et al. (2003) demonstrated depressed patients were more "sensitive" to misleading negative feedback than controls, as they were more likely to switch to the incorrect pattern in subsequent trials following misleading feedback. On the contrary, the same study showed that depressed patients were able to use accurate negative feedback to adjust their performance in a spatial working memory task applied. This was not attributed to impaired acquisition of visual discrimination (as patients and controls did not differ in number passing learning criterion and errors made), instead did differ in ability to maintain the visual discrimination acquired and applying the probabilistic rule to adjust performance – when misleading feedback was provided. Increased sensitivity to misleading feedback was also shown following 5HT depletion in the marmoset amygdala (Rugyla et al., 2015).

In this study, we applied a probabilistic and reversal learning task (Chamberlain et al., 2006) and the CANTAB Intra-Extra Dimensional (ID/ED) Set Shift task (Rogers et al., 1999) which included reversal stages and was previously shown to be sensitive to serotonergic manipulations (Park et al., 2004).

I. Probabilistic reversal learning task description

Participants were presented with two patterns (one red, one green) and required to select which one is the correct over a series of trials. After each choice, they received feedback on whether their choice was correct or incorrect, presented on the screen as '**CORRECT**' and '**INCORRECT**' respectively accompanied with auditory (high versus low pitch sound). The pattern that participants chose in the first trial was the correct one and participants received a ratio of 80:20 of positive: negative feedback for this one. The opposite ratio was given for the "incorrect" one (Chamberlain et al., 2014). Probabilistic learning serves the purpose of slowing down the rate of learning (Izquierdo et al. 2017) and excludes a simple stay-win/lose-shift strategy for completing the task. The task consisted of two stages each of which had 40 consecutive trials; during the second the stimulus – outcome contingencies reverse, meaning if the green pattern was correct in the first stage, now red became the correct one. The ratio of positive: negative feedback are now reversed, meaning that 80% positive feedback and 20% negative feedback are now given for the red one. The same two patterns were presented throughout the task. Participants were instructed that on some trials they will receive feedback they made the wrong choice, although having made the correct one, and to start choosing the other pattern only when certain that the rule changed. Administered instructions detailed in Appendix. Participants made their choice by touching the respective pattern on the screen.

Participants' ability to encode the stimulus-outcome contingency was assessed by the number of errors until reaching the learning criterion for the first stage, set as eight correct consecutive responses (Chamberlain et al., 2006). The same learning criterion was applied in the second stage of the task following reversal of stimulus outcome contingencies.

Participants' ability to reverse their choice during the second stage was measured by the number of errors made labelled as 'perseverative' errors to the previously most rewarding stimulus (as per Chamberlain et al., 2006). We assessed feedback sensitivity indicated by the number of trials participants stayed or switched following accurate and misleading

feedback in the first stage. We further assessed 'stickiness', termed as making the same choice as per previous trial regardless of feedback.

Hypothesis

We hypothesized that the escitalopram-treated group will perform more errors during the probabilistic learning and after reversal (Chamberlain et al., 2006) and will show higher sensitivity to misleading feedback.

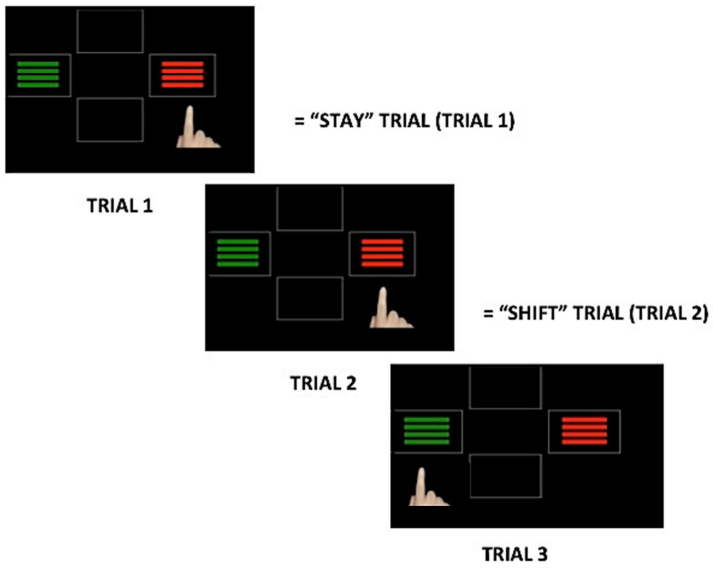


Figure 1: Participants needed to make a two-alternative forced choice during the probabilistic learning task (a) and received either accurate (80%) or misleading feedback (20%) (b). Contingencies were reversed during the second stage

Results

Overall, sixty-five participants completed the task, thirty-three in the placebo and thirty-two in the escitalopram group. Data from one participant in escitalopram group were not used in the analyses as per incomplete file produced despite completing the task during testing session. Data was analysed with t-tests and non-parametric Mann Whitney tests, when data transformation applied did not normalise variance. The following data refers to 31 participants in the escitalopram group and 33 in the placebo one.

Stage 1 Acquisition Phase: The learning criterion was achieved by 26/31 participants in the escitalopram and 30/33 participants in the placebo group, χ^2=1.393, p=0.268. However, the escitalopram group made significantly more errors, U=314, z=-2.850, p=0.004 (Table 2).

Stage 2 Reversal Phase: The learning criterion was achieved by 25/31 participants in the escitalopram and 27/33 participants in the placebo group, χ^2=0.014, p=0.904. Errors and RTs were both similar between groups (p> 0.05).

Various probabilistic and reversal learning tasks have variable task structures with series reversals (Boulougouris et al., 2007), concurrent reversals including learning many stimuli pairs of which some reverse and some not (Wilson and Gaffan, 2008). The task we applied, based on previous literature (Chamberlain et al., 2006), had one reversal, which occurred after 40 trials.

Win-stay/lose-shift: In stage 1, *a priori* planned analyses showed that the escitalopram group showed a significantly lower probability of win-stay, p=0.043, F(1,62)= 4.272, partial η^2=0.064, and a significantly higher probability of lose-shift, p=0.013, F(1,62)= 6.575, partial η^2=0.096. We further assessed sensitivity to valid (80% of trials) or misleading (20% of trials) feedback by separating the above probabilities based on feedback type. Table 2 shows that the escitalopram group had a significantly higher probability of lose-shift after both misleading, p=0.003, F(1,62)= 9.724, partial η^2=0.136, and accurate (valid) feedback, p=0.032, F(1,62)= 4.834, partial η^2=0.72.

We applied the Benjamini–Hochberg procedure to control for multiple comparisons in the task (Benjamini and Hochberg, 1995) with a false discovery rate set at q<0.15; as Table 1 shows that increased lose-shifting behaviour after misleading feedback survived significance.

The two groups did not differ in win-shift behaviour after accurate (valid) feedback, p>0.05. There was no difference in either of these measures during stage 2.

	Measure		Placebo	Escitalopram	Group difference*
Stage 1	Errors		1.76 (3.73)	4.55 (6.66)	**p=0.004**
	RTs (ms)		2016.06 (937.32)	2165.79 (968.95)	p=0.532
	Feedback type 80% accurate 20% misleading				
Probabilities	Reward-stay	80%	86.74	75.6	p=0.043
		20%	1.14	4.03	p =0.104
	Reward-shift	80%	5.97	9.48	p =0.125
		20%	4.92	8.47	p =0.279
	Lose-stay	80%	1.42	4.13	p =0.125
		20%	88.64	72.18	**p =0.009**
	Lose-shift	80%	5.87	10.79	p =0.032
		20%	5.3	15.32	**p=0.003**
Stage 2	Errors		6.58 (2.57)	7.58 (4.46)	p=0.689
	RTs (ms)		2251.86 (6285.86)	1280.32 (865.23)	p=0.237
	Feedback type 80% accurate 20% misleading				
Probabilities	Reward-stay	80%	68.75	63.71	p=0.376
		20%	11.74	15.32	p=0.359
	Reward-shift	80%	6.06	8.57	p=0.238
		20%	4.92	6.58	p=0.442
	Lose-stay	80%	16.1	16.32	p=0.237
		20%	70.08	62.9	p=0.971
	Lose-shift	80%	10.07	12.6	p=0.684
		20%	13.26	14.92	p=0.355

Table 1: Mean (SD) errors and response latencies and probabilities of win-stay/lose-shift behaviour based on feedback type (80% valid, 20% misleading) in the two treatment groups ***Group difference:** p-values of one-way ANOVAs. Significant p-values following control of false discovery rate at q <0.15 with the Benjamini-Hochberg procedure are shown in bold

Additional exploratory analyses

We additionally calculated the number of trials during the two stages that participants spontaneously shifted their choice. We found that during stage 1, escitalopram group made significantly more spontaneous shifts compared to placebo, U=330, z=-2.487, p= .013,

Cohen's d equal to 0.31. We found no significant difference for spontaneous shift in stage 2.

We further explored if spontaneous shift might explain (partially) the increased errors in stage 1. We performed a Pearson correlation analysis in the escitalopram group, which showed a strong correlation between stage 1 errors and number of trials performing a spontaneous shift, r= .778, n= 31, which was statistically significant, p< .001.

Given the role of stress in impairing decision-making (Eysenck et al., 2007), we performed a median split on trait anxiety scores of the State-Trait Anxiety Inventory (Spielberger, 1983) thus dividing participants to high and low trait anxiety groups (Table II). We separately compared treatment effect on errors made during stage 1 for low and high trait anxiety groups using non-parametric tests. Participants in the high trait anxiety group made significantly more errors when receiving escitalopram compared to placebo group, U=58, z=-2.424, p=.015, Cohen's d equal to 0.98. We found no significant treatment effect in the low trait-anxiety group; p=.08.

STAI trait score	Placebo group	Escitalopram group
Low	14	19
High	19	12

Table 2: Number of participants in treatment groups based on high and low trait anxiety scores

STAI trait score	Placebo group	Escitalopram group	Group difference*
Low	26.31	28.00	n.s.
High	41.42	43.83	n.s.

Table 3: Mean STAI trait scores in the treatment groups ***Group difference:** p-values of two-tailed t-tests or non-parametric tests, as appropriate. n.s.= not significant, p>0.05

Trait anxiety group		Low Trait Anxiety	High Trait Anxiety	Group difference
Number of volunteers	Placebo	14.00	19.00	
	Escitalopram	19.00	12.00	
Gender	Females	16.00	17.00	
	Males	17.00	15.00	
Age		27.5 (5.61)	23.25 (4.67)	n.s.
BDI		3.21 (2.4)	4.5 (2.4)	n.s.
NART		42.7 (4.77)	43.2 (4.52)	n.s.
Years of education		15.6 (2.5)	16.24 (1.5)	n.s.

Table 4: Demographics in low- and hig-trait anxiety groups, n.s.= not significant

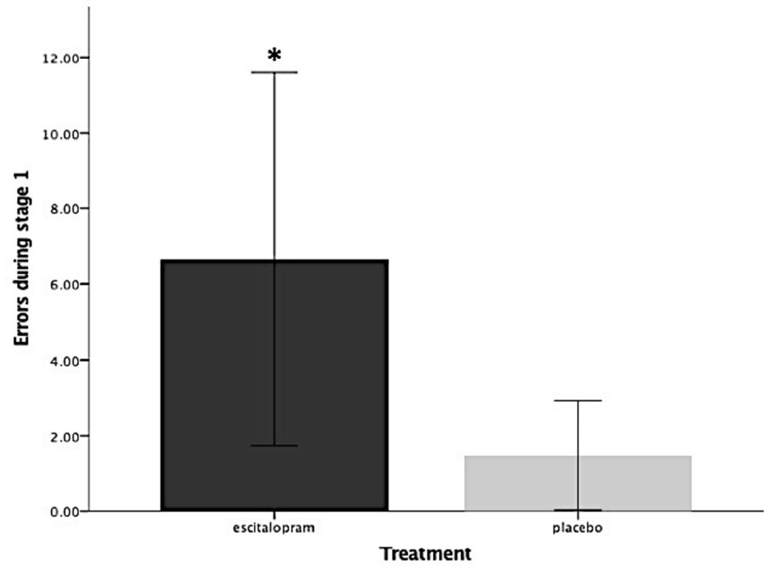


Figure 2: Treatment effect on number of errors made during stage 1 in high trait anxiety group

We further added sex as between-subjects factor in our initial analysis. We found no significant main sex and treatment by sex effect on number of errors made and win-stay/lose-shift behaviour, p> .05.

II. CANTAB Intra-Extra Dimensional Set Shift task

Task description

Attention set shifting, another aspect of cognitive flexibility, has been widely studied using the CANTAB Intra-Extra Dimensional Set Shift task (Rogers et al., 1999). Shift and reversal learning are suggested to be distinct operations (Krushke, 1996) and animal

studies show that deficits in these two domains are anatomically dissociable (Lawrence et al., 1998).

The task, based on the Wisconsin card sort test (Grant and Berg, 1948), measures cognitive flexibility (Rogers et al. 1999; Lawrence et al., 1998a). It consists of nine stages of visual discrimination, attention set formation and rule acquisition, maintenance of attention, shifting and flexibility of attention and rule reversal (Table IV). In the beginning of the task, participants need to make a visual discrimination of two simple colour-filled shapes and learn which is the correct one. When learning criterion is reached (six correct responses), the stimuli and/or rules change. The shift is initially intra-dimensional (the only relevant dimension is the colour-filled shapes) and then extra-dimensional, as another dimension is introduced consisting of white lines (Figure 7). These subsequently become the only relevant stimuli. If at any stage participants fail to reach learning criterion, the task terminates after 50 trials. The task lasted for 7 minutes. The order of stages is shown in Table IV. Outcome measures include errors and number of trials and stages completed.

Blocks	Stages	
1	Simple discrimination	One dimension; two simple, colour-filled shapes
2	Simple reversal	
3	Compound discrimination 1	Second dimension added; white lines
4	Compound discrimination 2	
5	Compound reversal	
6	Intra-dimensional shift (IDS)	
7	Intra-dimensional shift reversal (IDR)	
8	Extra-dimensional shift (EDS)	
9	Extra-dimensional shift reversal (EDR)	

Table 5: CANTAB Intra-Extra Dimensional Set Shift task block summary

Background

Frontal lobe lesion patients show impairments in extra-dimensional shifts but not in intra-dimensional shifts (Owen et al., 1991), which in subsequent study was correlated with increased perseveration (Owen et al., 1993). Parkinson's disease patients also show deficits in extra-dimensional shifts with distinct differences based on medication status; thus medicated patients exhibit higher 'learned irrelevance' and un-medicated patients equal significant impairment in both perseveration and 'learned irrelevance'. The latter is a term introduced by associative learning studies in animals and refers to the inability to learn

about stimuli that were previously irrelevant ones (Mackintosh, 1983). Both perseveration of an attention set and 'learned irrelevance' may contribute to deficits in switching between distinct perceptual dimensions of stimuli (Owen et al., 1993).

The task was developed initially for monkeys and subsequently translated in humans based on the intra- and extra-dimensional shifts from the Wisconsin card sorting test (WCST), a classical test of 'cognitive set' (Lawrence et al., 1998a). 'Attention set' encompasses attention to be preferentially directed towards a relevant dimension of a stimulus resulting in increased processing of information relevant to this stimulus and suppression/reduction of processing of irrelevant information (Brown and Tait, 2016). The WCST was extensively applied in frontal cortex damage patients (Rogers et al., 2000; Milner, 1963), cognitive dysfunction in older adults (Ridderinkhof et al., 2002), attention deficit/hyperactivity disorder (Loge et al., 1990), Parkinson's disease (Lees et al., 1983), Alzheimer's disease (Nagahama et al., 2005) and schizophrenia (Gold et al., 1997). The WCST used stimuli that varied in three dimensions. Brain areas implicated in attention set shifting versus reversal of S-R contingencies have been extensively studied across species (Clarke et al., 2004; Cools et al., 2008). Healthy volunteers with the 5-HTTLPR s-allele show superior performance in the Wisconsin card-sorting test (Borg et al., 2009).

Brain areas implicated in attention set shifting

Lesions in medial prefrontal cortex in rats impair switching of attention set between dimensions of complex stimuli (Birrell and Brown, 2000). Lesions in orbitofrontal cortex in rats produced selective impairment in reversals but no effect on formation, maintenance or shifting of attention (McAlonan and Brown, 2003). These effects were reported in an attention-switching task of the same structure as the one used in primates. Lesions in the lateral prefrontal cortex (Brodmann's area 9) in marmoset monkeys produce attention shift deficits (Dias et al., 1996a). Lesions in dorsolateral and ventrolateral human brain areas (Rogers et al., 2000; Milner et al., 1963). Impairments in set shifting have been reported in clinical populations including depression (assessed with the WCST, Merriam et al., 1999)

'Cognitive set' refers to a 'set to respond according to abstract aspects of input, that is not tied to a particular motor response' and 'response set' to the 'prior assignment of probability of selection from the repertoire of available responses' (Robbins and Brown, 1990). Previous research suggested people with localized frontal lobe excisions show poor performance in extra-dimensional set shifting but not to intra-dimensional set shifting (Owen et al., 1991). Huntington's disease patient studies show impaired performance in

the extra-dimensional set shift stage (Lawrence et al., 1998b) and increased perseverative responding during the reversal learning phases of the task in advanced stages of the disease (Lange et al., 1995). Extra-dimensional shifts (EDS) require more trials to be succeeded than intra-dimensional shifts (IDS) (Rogers et al., 2000; Roberts et al., 1988) as they incur a 'switch cost' arising from shifting attention to one dimension to another (Brown and Tait, 2016). Compared to the Wisconsin sort card test, the ID/ED task captures the succession through sort cards within one dimension (intra-dimensional), which induces an attention bias towards this dimension (Rogers et al., 2000). This needs to be subsequently adjusted, as participants need to redirect their attention to another dimension (extra-dimensional). In both tasks, an attention set has developed through accumulation of prior reinforcement and this is now challenged through either changing sorting rule (WCST) or re-direction of the visual discrimination (intra- or extra-dimensional). Both frontal areas mediate ED shift learning, as frontal lesions in both patients and nonhuman primates are associated with deficits in this stage. The ID/ED task has been used in animal studies (Clarke et al., 2005) and pharmacological manipulations in healthy (Rogers et al., 1999; Park et al., 1994) and clinical populations (Rock et al., 2014).

Neurotransmitter systems implicated in attention set-shifting

Prefrontal, 6-hydroxyDA (6-OHDA) induced DA depletion in primates improved EDS shifting (Roberts et al., 1994) leaving unaffected the acquisiting of visual discrimination during the IDS or during reversals. The lesioned group had comparable IDS and EDS performance, thus indicating that possibly this group had not formed an attention set during the IDS stages and thus did not need to reverse this during the EDS one. Thus, the lesioned group might have employed a strategy throughout the task based on figures such as combining shapes and lines into one figure, as described by the researchers. Crofts et al. (2001) showed that 6-OHDA induced DA depletion produced differential effects on caudate nucleus and frontal areas in monkeys; frontal lesioned monkeys showed an impairment of forming an attention set but better ability of attention shifting whereas the caudate lesioned monkeys showed relatively preserved performance in the attention shifting task and lower distractibility in a distractor test. In terms of neurotransmitter changes, the prefrontal DA depletion was accompanied by a long-term increase of extra-cellular DA in the caudate nucleus. The authors attributed the effects on attention set shifting in the balance between prefrontal and striatal DA availability (Roberts et al.,

1994). Interestingly, 6-OHDA produced DA depletion in the marmoset caudate nucleus did not affect the first shift in the attention set shifting task but impaired the re-engagement of a previously relevant perceptual dimension (Collins et al., 2001).

Administration of sulpride (a D2/D3 receptor antagonist) to healthy participants produced deficits during the EDS stage (Mehta et al., 1999). Impairments during the EDS stage are reported in Parkinson's disease patients (Downes et al., 1989), where prefrontal DA loss is prominent, and these are accompanied by impairments in acquiring and maintaining an attention set (Owen et al., 1992). Administration of tolcapone, a cathechol-O-methyltranferase inhibitor used in the treatment of Parkinson's disease, ameliorated performance during the EDS stage in a rat study (Tunbridge et al., 2004).

5HT and attention set-shifting

Selective prefrontal 5HT depletion in marmosets produced no effect on attention set shifting either in the intra-dimensional or the extra-dimensional set shifts (Clarke et al., 2005). A difference was observed in reversals included in the attention set shift task with the lesioned group making more errors than the control group (Clarke et al., 2005) indicative of perseverative errors as previously reported (Clarke et al., 2004). A small significant increase in errors to learning criterion was observed on a distractor test, where monkeys had to learn that novel exemplars replaced the previous irrelevant exemplars, thus indicating a role for 5-HT in encoding stimulus salience (Clarke et al., 2005).

Walker et al. (2006) showed that 5,7-dihydroxytryptamine-induced 5-HT depletion of the OFC also impaired performance on a detour-reaching task which is dependent on intact OFC function; during the task, animals need to inhibit a pre-potent tendency to reach immediately for a reward placed inside a Perspex box and instead make a detour around it in order to reach the reward (food). The lesioned monkeys made more attempts to reach directly the reward, along the line of their sight, compared to the sham group. This is consistent with previous study showing that excitotoxic lesions of the OFC produced increase in attempts for reaching directly the reward (Wallis et al., 2001). The results of these lesions are attributed to direct failure of inhibitory control and not to any interference of other processes involved such as action-outcome learning or applying a specific strategy as the direct reaching for the reward was through seeing the later behind a transparent barrier as in previous study having used an opaque barrier this did not affect learning the detour reaching task (Wallis et al., 2001). These effects of OFC 5-HT depletion in the

detour-reaching task, which involves inhibitory processes, shows that the impairments reported by prefrontal 5-HT depletion in serial reversal learning tasks by Clarke et al. (2004) can be extended to other tasks which evaluate distinct behavioural processes. A potential explanation for this perseverative responding, as clearly illustrated in Walker et al. (2006), is that monkeys' behaviour is controlled by Pavlovian conditioning; thus the monkeys may be unable to inhibit a conditioned stimulus elicited response, which in this case would involve food as an appetitive stimulus. This could be in accordance to previous suggestions that impairments in reversal learning tasks following OFC lesions (Rolls et al., 1994) might be guided by an inability to "un-learn" the stimulus-reward association (O'Doherty et al., 2003). This though is suggested by Walker et al. (2006) not to explain their findings as the reward properties do not change throughout the task (that is, food remains as still valuable in the inside of the box without any changes during the detour-reaching task). Elliott et al (1996) showed that depressed patients did not differ from healthy volunteers in terms of intra- and extra-dimensional set shift errors they made in the CANTAB ID/ED task.

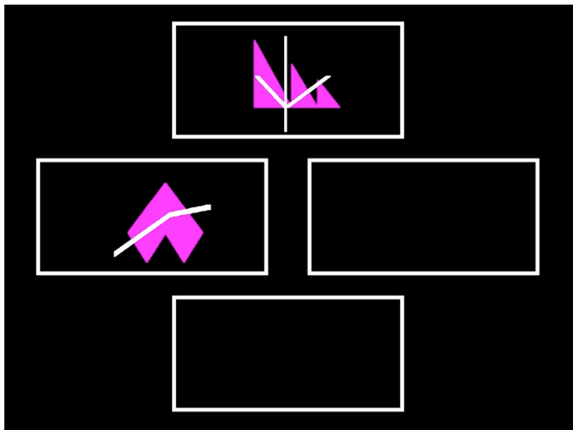


Figure 3: Example of the CANTAB Intra-Extra Dimensional Set Shift task, with both dimensions – shapes and white lines- shown

Hypothesis

We predicted that acute escitalopram administration would produce suboptimal performance in the CANTAB ID/ED task, predominantly in reversals with anticipated increased perseverative errors (Clarke et al., 2006; 2005; 2004).

Results

Data were analysed with repeated measures ANOVA. Outliers were removed as appropriate when attempts to reduce skewness and normalise variability with data transformation was unsuccessful. Data are presented in Table 6.

Stages	Description	Escitalopram group	Placebo group	Group difference*
1 (SD)	Simple discrimination	1.13 (1.61)	0.79 (0.69)	0.296
2 (SR)	Simple reversal	2.17 (2.56)	1.42 (0.58)	0.197
3 (C/D)	Compound discrimination 1	0.47(0.9)	0.64 (0.83)	0.441
4 (CD)	Compound discrimination 2	0.3(0.6)	0.07 (0.26)	0.067
5 (CDR)	Compound reversal	1.33 (0.61)	1.25 (.52)	0.577
6 (IDS)	Intra-dimensional set shift	0.37 (0.56)	0.32 (0.48)	0.741
7 (IDR)	Intra-dimensional set shift reversal	1.23 (0.68)	1.11 (0.31)	0.373
8 (EDS)	Extra-dimensional set shift	11.4 (10.49)	5.43 (6.93)	**0.014**
9 (EDR)	Extra-dimensional set shift reversal	4.33 (8.57)	1.11 (0.57)	0.052

Table 6: The escitalopram-treated group made significantly more EDS errors, p= 0.014. *Group difference defined as the p-values of one-way ANOVAs

We found a significant treatment main effect, p= .001, $F(1,41)=12.658$, partial $\eta^2=.236$, a significant block effect, p< .001, $F(8,34)= 58.589$, partial $\eta^2=.932$, and a marginally significant interaction between block and treatment, p= .049, $F(8,34)= 2.233$, partial $\eta^2= .344$. Follow up one-way ANOVAs showed that treatment did not affect ID shift errors, p= .629. The escitalopram group made significantly more EDS errors, p=0.014, $F(1,56)= 6.449$, partial $\eta^2=0.103$. We further performed a repeated measures ANOVA for ID and ED shift stages; there was a significant stage effect, p< .001, $F(1,52)=41.447$, partial $\eta^2=.444$, with both groups making less errors in ID shift compared to ED shift. This validates the treatment effect in EDS errors as it should be easier to perform the ID than the ED shift in either group, as previously reported (Brown and Tait, 2016).

The escitalopram group overall made more total errors (adjusted), M=8.25, $t(59)=2.563$, p=.013, and more errors on the completed stages, $t(59)=2.264$, p=.027.

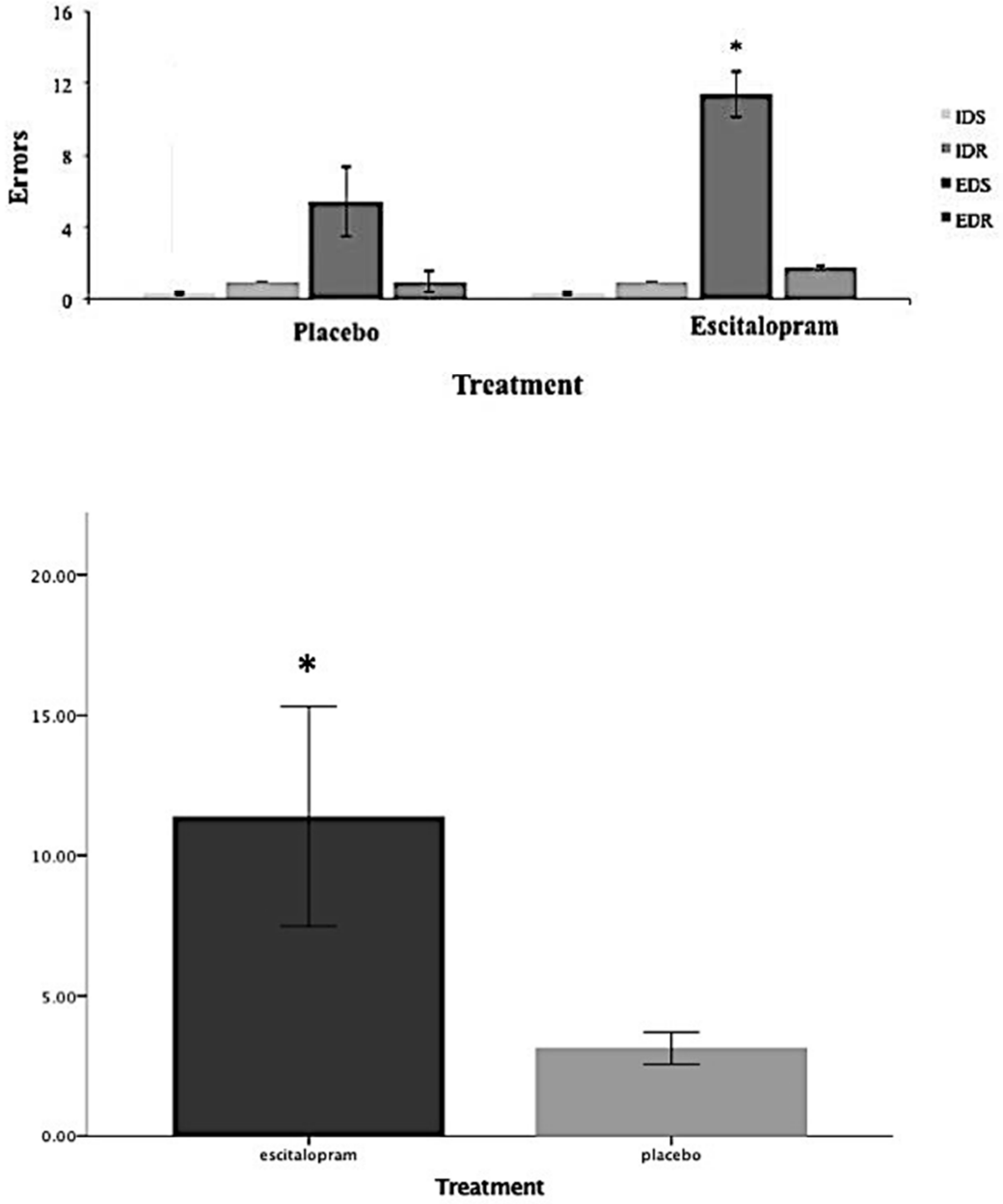


Figure 9: The two treatment groups did not differ in the IDS, IDR and EDR stages (a) but the escitalopram-treated group made more errors during the EDS stage, p= .014 (b)

The escitalopram group required more trials in the completed stages, U=308.5, z=-2.260, p=.024, Cohen's d equal to 0.6046. We ran repeated measures ANOVA with response latencies in each of the nine stages. We found a significant block, p< .001, F(8,40)= 57.848, partial η^2=.920, and interaction of block and treatment effect, p=.013, F(8,40)=2.85, partial η^2=.363. Following up with one way ANOVAs, we show that participants in escitalopram group showed higher response latency in stage 8, p= .026,

t(59)=-2.307, partial η^2=.102 [following data transformation to reduce skewness and normalise variance].

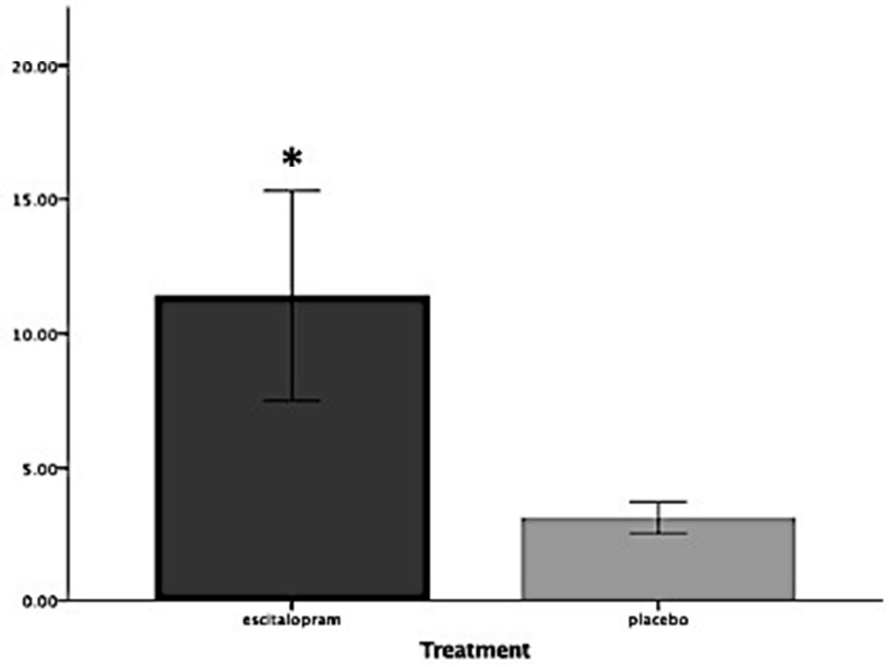


Figure 10: The escitalopram-treated group showed significantly longer EDS response latencies, p= .026

We further split participants based on trait anxiety scores into two groups. Repeated measures ANOVA with treatment and trait anxiety group as between-subjects factor showed a significant treatment main effect, p= .015, F(1,31)= 6.634, but no interaction between treatment and trait anxiety group, p= .446. We examined the effect of sex in the errors made and we found no interaction between treatment and sex, p> .05.

Discussion

In the probabilistic learning task, we found that the escitalopram group made significantly more errors prior to reaching the learning criterion during stage 1, similar to Chamberlain et al. (2006) study, which used 30 mg citalopram. This could not be attributed to choice stickiness. We also showed that escitalopram group was affected by misleading feedback and employed a less optimal task strategy by shifting rather than staying following misleading feedback. This is in accordance to previous literature on 5HT affecting feedback sensitivity shown in ATD studies (Evers et al., 2005; Clarke et al., 2004) and

depressed patient studies (Murphy et al., 2003). Unlike Chamberlain et al. (2006) we found no effect of escitalopram on the second stage.

This effect of misleading feedback on stay and shift could resemble a model-free RL strategy where rewarded behaviour is reinforced. Worbe et al. (2016) did indeed show that ATD tipped the balance towards model-free RL in the reward version of a two-step decision task. Nevertheless, we applied the same task in our study and did not see this effect (please see Chapter 4 for further details).

Izquierdo et al. (2017) nicely summarise in their review the processes employed in the brain when performing the reversal learning task and consist of; (1) reward learning after choosing different patterns/stimuli, (2) computation and representation of probability, based on prior ones, of upcoming reversal and/or (3) representation of "task space" which incorporates understanding of "hidden variables" relevant to task structure. These processes are/may be computed by distinct neural substrates and delineating these in future experiments will aid towards a better understanding of the exact brain processes involved when performing a reversal learning task in both human and animal work.

An alternative explanation could be the net effect of escitalopram administration on brain 5HT levels. We applied a clinically relevant dosage of escitalopram to acutely increase brain serotonergic levels. Nevertheless, the high dosage we applied might have resulted in the opposite direction (Chamberlain et al., 2006) through activation of 5HT auto-receptors in the pre-synaptic neurons. Previous study had shown that acute escitalopram administration in rats produced a deficit in an attentional set-shifting task as rats took more trials to criterion similar to a group that was subjected to chronic stress (Bondi et al. 2008). In humans, acute citalopram administration in healthy males impaired contextual processing, measured with the delayed non-matching to sample task, and this effect was abolished in "chronic" treatment for 28 days (Almeida et al., 2010).

We additionally tested participants on a set-shifting task as studies have shown distinct effects of 5HT on reversal learning and set shifting (Clarke et al., 2004). Participants receiving escitalopram made significantly more errors during the EDS with associated higher response latency. Studies on rats (e.g. Danet et al., 2010, Barlow et al., 2015) and marmoset monkeys (e.g. Clarke et al., 2005) implicate 5-HT in deterministic reversal learning deficits, rather than ED-shifting (Dias et al., 1996), with ATD impairing reversal learning (errors) and lengthened response latencies at certain stages of reversal and ID-shifting (Rogers et al., 1999). However, the common deficits produced on probabilistic learning and ED-shifting here suggest effects of the drug on neural circuits including the

right ventro-lateral prefrontal cortex (BA47), as both of these tasks are associated with activations of this region (Cools et al., 2002). Moreover, the pharmacological fMRI study of Del-Ben et al. (2005) emphasised strong interactions between i.v. administration of the SSRI citalopram and different task-related activations of this region

Previous study suggested inter-individual differences in both stress exposure and cognitive capacities to account for distinct employment of learning strategies (Friedel et al., 2017). Escitalopram administration in our study increased errors during stage 1 in the probabilistic learning task only in the high trait anxiety group but we found no interaction between treatment and trait anxiety scores in the CANTAB ID/ED task. Cools et al. (2005) showed that the effect of tryptophan depletion on goal-directed learning was highly correlated with individual differences in a subcomponent of the Barratt impulsiveness scale. We provide further evidence that individual baseline differences, such as trait anxiety, may potentially modulate the effect of serotonergic manipulations in healthy volunteers but this appears to be task-dependent and needs further exploration.

Chapter 5. Goal-directed and habitual learning

Introduction

Decision-making constitutes a complex process that involves choice selection and action performance that aim to maximize reward and minimize punishment. Decisions are mediated through signals that are created in anticipation of future events. These signals are based on past experience and therefore are dependent on previous rewards. In order to make decisions, people have to make initially reliable predictions (Schultz and Dickinson, 2000). The concept of reward is used to describe the positive value that is attributed to behaviours or actions, and the candidate underlying neural substrates include the midbrain dopaminergic neurons as well as their projections to the striatum and the frontal cortex.

Goal-directed versus habitual behaviour

The dual-control theory, the foundations of which come from the philosopher and psychologist William James (1842-1910), was formulated by Evans (1984), who suggested that reasoning arises from heuristic processes, which entail the selection of the information relevant to a given situation, and analytic processes, with the application of relevant information to make judgements on the situation; these two systems can be defined as the habitual and goal-directed systems respectively (Dickinson and Balleine, 1993).

When behaviour is controlled by the goal-directed system, actions are performed in light of either succeeding desirable goals or avoiding undesirable situations; thus values are represented as action-outcome associations followed by a subsequent evaluation of the outcomes. Shopping the ingredients needed to cook a specific meal is one example of goal-directed behaviour. Goal-directed behaviour is thus characterized by response representation and the representation of respective outcomes and it is characterized as "reflective" behaviour. In contrast, habitual behaviour is mediated through reliance on previously experienced stimulus-response associations without any associative interference of the action outcomes (Balleine and O'Doherty, 2010) thus it is "reflexive" in nature.

These two mechanisms have been described to rely on distinct neuronal substrates. Goal-directed behaviour is mediated by the medial prefrontal and the medial orbitofrontal cortex and the homologous prelimbic cortex in rats. The aforementioned prefrontal regions project to areas of dorsal striatum; thus anterior caudate (Tanaka et al., 2008) in humans and dorsomedial striatum in rats account for goal-directed behaviour. The orbitofrontal and

ventrolateral prefrontal cortex along with the amygdala and the medio-dorsal thalamus form the 'associative cortico-basal-ganglia loop' (Balleine, 2005). It is suggested that the orchestration of contextual information dependent on hippocampal function and affective information derived from amygdala function combined with the prefrontal cortex guide appropriate goal-directed action selection (Goto and Grace, 2005; Grace, 2000; Mogenson et al., 1980). The orbitofrontal cortex and dorsal striatum are shown to compute the value of goal-directed actions (de Martino et al., 2009) similar to previously shown activation of dorsal striatum in light of expected rewards (Delgado et al., 2003). Brain areas implicated in habitual learning include the posterior lateral putamen in humans (Tricomi et al., 2009) and the dorsolateral striatum in rats (Balleine and O'Doherty, 2010; Balleine, 2005), the cortico-thalamic loop (Yin et al., 2006) and the infra-limbic cortex (Coutureau and Killcross, 2003). Individual differences in cortico-striatal connectivity are suggested to underlie manifested slips-of-action; de Wit et al. (2012) showed that caudate-ventromedial PFC connectivity predicted goal-directed choice behaviour in an instrumental learning task whereas posterior putamen-premotor cortex connectivity predicted habitual responding.

Differences between the two types of instrumental choice

The two mechanisms have two major differences: first, they differ in their sensitivity to any changes in the outcomes associated with an action and second, in the sensitivity to changes in the causal relationship between these actions and the outcomes. Both types of decision-making mechanisms have advantages and disadvantages; goal-directed behaviour can be more accurate as deliberate thinking takes place, but comes with an energy cost. On the contrary, habitual behaviour control is less cognitively demanding and it is more efficient. This can be advantageous during stress or when cognitive resources are limited. The disadvantage is that it comes at the cost of behavioural inflexibility. This in turn can lead to inappropriate behaviour or 'slips-of-action' (Dickinson, 1985), which are characterised by the intrusion of well-established habits into goal-directed behaviour, either when the environmental contingencies or our desires change. An example of changes in the environment would be moving houses; in this case, a 'slip-of-action' would be to mistakenly drive from work back to the old house after the end of the day. Several daily behaviours thus are automated and mediated by the habitual system.

When initially starting to perform an action, this happens in light of a goal; with extensive repetitions, habitual action control takes over and behaviour is guided by antecedent stimuli (Adams, 1982; Adams and Dickinson, 1981). Thus, cues elicit the relevant

response regardless of outcome value (Dickinson and Balleine, 2002). Adams (1981) demonstrated this early on in rats, showing that following extensive training, lever pressing was no longer sensitive to devaluing the outcome. Dual-system theories suggest that the balance between these two types of behaviour determines the final behavioural output (de Wit and Dickinson, 2009).

Experimental demonstration of goal-directed versus habitual behaviour

In terms of distinct demonstration of these two types of behaviour in experimental settings there are so far two established ways; outcome devaluation and contingency degradation. In order to elicit habitual responding, outcome devaluation can be employed after extensive training either through taste aversion learning (Colwill and Rescorla, 1985) or by inducing satiety to a specific food reward (Balleine and Dickinson, 1998). Adams and Dickinson (1981) employed outcome devaluation to illustrate goal-directed behaviour in rats. The researchers trained the rats to press a lever to receive sucrose, which was subsequently associated with illness by injecting lithium chloride, thus devaluing the sucrose. When rats were allowed to press levers during an extinction test, where they received no feedback regarding the devalued outcome, their performance decreased, thus indicating that 1. rats were able to encode the association of response (lever press) and outcome (receipt of sucrose) and 2. they were able to use this to change their subsequent performance. Adams (1981) also showed that the amount of training affects the influence of devaluation on rat lever pressing thus supporting that extensive training mediates the shift from goal-directed to habitual behaviour.

Tricomi et al. (2009) showed similar findings in humans; after being trained to press buttons, which were eventually associated with two different snacks, the volunteers ate a snack until reaching a state of satiety (thus devaluing the snack). In an extinction task, volunteers having received little training showed reduced actions associated with receipt of the devalued snack whereas over-trained volunteers were insensitive to snack devaluation as their responses were comparable for both valuable and devalued snacks.

Neurotransmitter systems encoding the arbitration between goal-directed and habitual responding

The role of DA transmission in the arbitration between these two types of behaviour is well studied, in contrast to 5HT transmission that few studies have examined in regards to goal-directed and habitual learning. It is shown that ascending dopaminergic projections affect

both types of action control. Goal-directed action control is regulated by both prefrontal and striatal areas with mesolimbic dopaminergic neurotransmission to nucleus accumbens playing a central role (Berridge and Robinson, 1998). Goto and Grace (2005) showed that within the nucleus accumbens distinct dopaminergic receptor activation (D_1 versus D_2 receptors) affect distinct aspects of goal-directed action selection (strategy learning versus set shifting) corresponding to distinct afferents. de Wit et al. (2012a) applied an instrumental discrimination learning task, specifically designed to discriminate between goal-directed and habitual learning, to healthy volunteers and reduced global brain DA levels through acute dietary phenylalanine and tyrosine depletion. The researchers showed a shift towards habitual learning only in female participants after global depletion of DA levels (de Wit et al., 2012a). Amphetamine, a DA releaser (Calipari and Ferris, 2013) administration to rats accelerated the shift from goal-directed to habitual action control after extensive training, without directly impairing goal-directed responding (Nelson and Killcross, 2006), an effect reversed by a D_1 receptor antagonist (Nelson and Killcross, 2013).

The role of the 5HT system

5HT is proposed to be in an opponent (Lorrain et al., 1999) or, in a functional, valence-dependent (aversive or appetitive), synergistic relationship with DA (Boureau and Dayan, 2011; Deakin, 1983) and its role in the balance between these two distinct aspects of choice selection is poorly understood. Worbe et al. (2015) showed that ATD, which causes central global 5HT depletion (Crockett et al., 2012), increased habitual responding in healthy volunteers as they performed more 'slips-of-action' for devalued stimuli. The dorsolateral striatum, which mediates habitual responding, receives serotonergic afferents (Di Matteo et al., 2008) and is consisted by several 5HT receptor subtypes, mostly $5HT_{1A/1B}$ and $5HT_{2A/2C}$. Over-expression of $5HT_6$ receptors in the dorsolateral striatum decreased habitual lever pressing in operant tasks in rats without affecting acquisition of stimulus-outcome contingencies (Eskenazi and Neumaier, 2001).

Insights into the two types of action control can offer insights into functional and dysfunctional behaviours (de Wit et al., 2012a) and thus aid understanding psychiatric and neurological disorders. A disruption of the balance between these two types of behavioural control is suggested to underlie the manifestation of compulsions in obsessive-compulsive disorder (OCD) (Gaybriel and Rauch, 2000), which is characterized by the presence of obsessions (intrusive, unwanted and repetitive thoughts) and compulsions (persistent,

ritualistic and stereotypical actions). Typically, compulsions occur in response to/ are associated with a specific obsession; such as excessive hand-washing (compulsion) in view of possible contamination (obsession). Gillan et al. (2011) used an appetitive instrumental learning task to experimentally study this hypothesis. They showed that OCD patients had intact ability to learn from feedback but were less sensitive to outcome devaluation compared to controls. The patient group made more 'slips-of-action' errors in a stage of the task specifically designed for goal-directed and habitual action competing over behavioural control. This was correlated with symptom severity as measured with the Yale-Brown Obsessive Compulsive scale. The patient group also showed impaired knowledge of outcomes, which was correlated with 'slips-of-action' errors, but intact knowledge of responses compared to controls.

Over-reliance in habitual responding in patient groups

The Gillan et al. (2011) results suggest that OCD patients show a deficit in goal-directed learning and a subsequent over-reliance on habitual system. One possible limitation of the study is that the majority of the OCD patients were receiving chronic SSRI treatment, thus complicating the interpretation of the results due to possible confounding medication effect. Considering that compulsions are portrayed as responses to irrational beliefs (obsession) and are related to avoidance, rather than approach, the researchers in a subsequent study (Gillan et al., 2014) used a shock-avoidance paradigm during which participants needed to respond using foot pedals to avoid electric shocks after being warned by task stimuli. To test for habits, the researchers subsequently disconnected one of participants' wrist from the stimulator which delivered the electric shock and measured the number of responses participants continued making to the task stimuli that were now labelled as 'safe' as they were not associated with shock delivery. Following overtraining, OCD patients performed more habitual responses to the devalued stimuli; interestingly, prior to overtraining, when habits were not yet formed, the patient group did not differ in number of responses to the devalued stimuli. Along with no manifested difference between the two groups of task contingencies and shock expectancy, the researchers suggested that the inappropriate responses the patient group made after overtraining were not in light of a goal but rather the result of habitual control of behaviour.

An over-reliance on habitual system has been shown in other patient groups including obesity (Volkow and Wice, 2005) and drug abuse (Everitt et al., 2001). Everitt and Robbins (2015) suggested that drug addiction can be portrayed as a transition from

instrumental initial use of drugs (with associated hedonic effects) to a maladaptive habitual and compulsive act mirrored in the neural level with transition from prefrontal cortical to striatal regions and from ventral to dorsal striatal areas. Experimentally induced stress in healthy volunteers, using the socially evaluated cold pressor test (Schwabe et al., 2008a), produced a shift towards habitual responding in an instrumental learning task (Schwabe et al., 2009a). This was shown by persistent responding towards devalued outcomes and reduced explicit knowledge of R-O contingencies. Increased S-R learning was previously shown in both humans (Schwabe et al., 2007) and rodents (Kim et al., 2001) following exposure to stress and after pharmacological manipulation of cortisol levels in humans (Schwabe et al., 2009b). Interestingly, high levels of chronic stress, as measured in a chronic stress questionnaire, increased inflexible S-R learning (Schwabe et al., 2008b).

I. Instrumental discrimination learning task

Task description

The task, developed originally by de Wit et al. (2007), provides a measurement of the balance between goal-directed and habitual choice behaviour deriving from experimental psychology literature. The theoretical background of the task involves an integrative model of ideomotor (O-R) theory and R-O theory, proposing that in the majority of daily actions both types are employed. The proposed associative-cybernetic model (AC model, de Wit and Dickinson, 2008; Dickinson and Balleine, 1994) suggests that behaviour starts from habit memory, which contains stimulus units responsive to environmental stimuli and response units related to corresponding behaviour. The relationship between the stimulus and response units can be strengthened through the reinforcement process described by Thorndike (1911) facilitated by a reward unit in the incentive system. Within the AC framework, goal-directed behaviour is conceptualised as dependent on engagement of essential sensory events, including the sensory feedback (F) related to performing the target response and the outcome (O) of the response.

For actions to be characterised as goal-directed two criteria need to be met: the belief criterion and the desire one. In the AC model, formation of F->0 associations in the associative memory represents the belief criterion, indicating knowledge between selected response and manifested outcome, and engagement of the incentive system representing

the desire criterion, encoding the desirability of the outcome/reward (de Wit and Dickinson, 2009). The integrative model accounts for behavioural autonomy as well, namely the transition from flexible, but computationally costly, goal-directed to inflexible, but efficient in stable environment, habitual behaviour (de Wit and Dickinson, 2009). Adams (1982) was the first to show that extensive training of lever pressing renders goal-directed behaviour in rats impervious to devaluation of the food outcome. Behavioural autonomy has been exhibited in humans as well through response conflict mechanism due to stimulus-outcome associative interference in an incongruent version of the fruit game (de Wit et al., 2007).

Taking into consideration the tryptophan depletion results of Worbe et al. (2015) and the study by Gillan et al. (2011), where over-reliance of OCD patients on habitual responding might also have reflected a chronic SSRI treatment effect, we investigated the effect of the attempted increase in central 5HT transmission through acute escitalopram administration. The insights could further help understand underlying mechanisms in disorders such as depression where perseverance and maladaptive behaviour represent hallmark symptoms and cognitive dysfunction is suggested as an important target for treatment (National Academies of Sciences, Engineering, and Medicine, 2015).

Stages

1. Instrumental discrimination training stage

Participants initially performed an instrumental discrimination training stage, during which they learnt how to make responses to stimuli in order to gain rewards. They were presented with closed boxes with pictures of fruits on the outside and they were requested to learn by trial and error the correct response that was either left or right key press. An accurate response revealed another fruit on the inside of the box and they earned points. When the incorrect key was pressed, the box did not open and no points were earned **(Figure 1).** Total points were shown on the screen following the end of each trial.

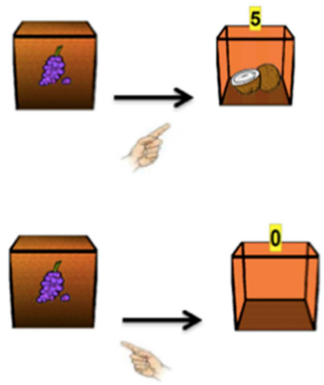


Figure 1: Instrumental discrimination training phase. Participants were presented with a closed box with a fruit picture on the outside. They were requested to press either the right or left key to open the box

Participants were prompted to respond as fast as possible to gain extra points. The points awarded for correct responses within the following latencies ranges were: 0-1 s, 5; >1- 1.5 s, 4; >1.5 – 2 s, 3; >2 – 2.5 s, 2; >2.5 s, 1. Participants received 8 blocks of 12 trials each in this stage. Verbal instructions were administered as following:

"In this game, you will get the chance to earn points by collecting fruit from inside a box on the screen by opening the box by pressing either the right 'm' or left 'z' key. If you press the correct key, the box will open to reveal a fruit inside and points will be added to you total score. However, if you press the incorrect key, the box will be empty and no points will be added to your total. Your task is to learn which is the correct key to press. Sometimes it will be the left-hand one and sometimes it will be the right-hand one. The picture on the front of the door should give you a clue about which is the correct response. The quicker you make the correct response the more points will be added to your total. Your accumulated points will appear at the top of the screen. You should try to learn the types of fruits that are found inside the boxes following left-hand and right-hand responses because later on you will be asked to gather some types of fruit but not others."

Before the beginning of the task, participants were shown a demo to ensure instructions were clear and understood.

2. Outcome-devaluation test

This stage was designed to assess knowledge of response-outcome contingencies. Participants were presented with two open boxes containing fruits; the one had been

previously earned by a right-key press and the other one by a left-key press. One of the fruits was now devalued, indicated by a red cross superimposed on it **(Figure 2)**. Participants were instructed to press the key that had previously earned the still valuable fruit. One block of 36 trials was completed. Verbal instructions were administered as following.

"Now two open boxes will appear on the screen with different types of fruit inside them. One fruit was earned by a left response in the previous stage and the other by a right response. Although both types of fruit were valuable previously, one of them is now devalued and earns no points, whereas the other is still valuable and gains points. The devalued fruit will have a cross on it".

Participants completed one block of 36 trials.

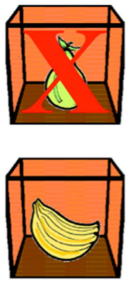


Figure 2: Outcome-devaluation test

3. Slips-of-action test

The aim of this task was to assess more directly relative goal-directed versus habitual behaviour. Participants were presented with a screen showing six open boxes with the same fruit images as used in the instrumental discrimination stage; a cross superimposed on two out of the six fruits indicated that if these fruits were collected, points would be subtracted from the total score. Verbal instructions were administered as following:

"In the next part of the game, you'll see a series of boxes with pictures of fruit on the outside and once again you can press left or right to open these and win points. The pictures on the box will be the same as the ones in the first game, and the correct response, left or right, will also be the same as what you learnt in the first half of the last game. However, unlike before, some of the fruits inside the box are no longer valuable, meaning

you can no longer earn points for them. In fact, if you try and open a box, which contains a non-valuable fruit inside, you will have points subtracted from your total"

Following ten seconds, closed boxes were presented in quick succession. Participants were instructed to press the key that earned the still valuable fruits as per the initial instrumental discrimination stage and withhold from pressing any key when a box appeared with a fruit picture on the outside that signalled a no longer valuable fruit on the inside **(Figure 3)**. Participants completed 108 trials.

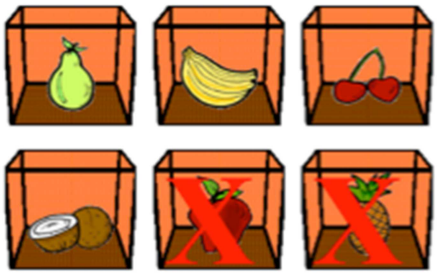


Figure 3: Slips-of-action test.

4. Baseline test

This stage was included to control for general task characteristics of the slips-of-action test and assess inhibitory response control. The same rules as in the latter task applied except that now, stimuli are devalued instead of outcomes (cross superimposed on the fruit picture of the outside of the box). Again, participants need to withhold their response when a fruit currently devalued appears (Figure 4). Participants completed 108 trials. No feedback was provided in any of these stages.

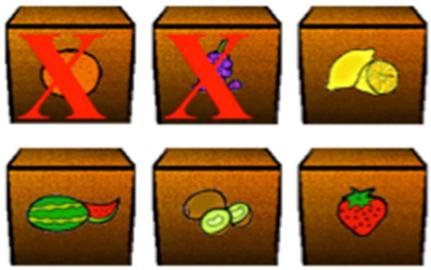


Figure 4: Baseline test

5. Questionnaires

We assessed participants' instrumental contingency knowledge by asking them to indicate on a printed questionnaire whether a right or left response had earned each fruit that had acted as a discriminative stimulus ('stimulus-response' knowledge) and which was the correct fruit found inside the box following correct response ('response-outcome' knowledge). Participants were requested to indicate which fruit was found inside a box when separately shown each fruit stimulus ('stimulus-outcome' knowledge). We subsequently asked participants to indicate their confidence from 0 to 100 for the choices they made by moving the cursor across a line on the screen.

Participants

Overall sixty-five participants completed the instrumental learning task; thirty-three in the placebo group and thirty-two in the escitalopram group. Participants completed this task along with the additional cognitive tasks in a counterbalanced order.

Hypothesis

We predicted that participants receiving escitalopram will rely less on habitual responding and thus exhibit fewer slips-of-action during the task.

Results

Instrumental learning stage

Repeated measures Analysis of Variance (ANOVA) was performed on accuracy and reaction time during the instrumental learning stage. Treatment did not have a statistically significant effect on accuracy over the blocks but a trend towards significance is reported, $p= .079$, $F(1,63)= 3.197$, with pre-planned, pair-wise comparisons using Bonferroni corrections showing that overall participants on escitalopram had lower accuracy (85,52%) than on placebo (89,11 %). We found no statistically significant difference in reaction times between the two groups, $p= .671$, $F(7,52)= .702$.

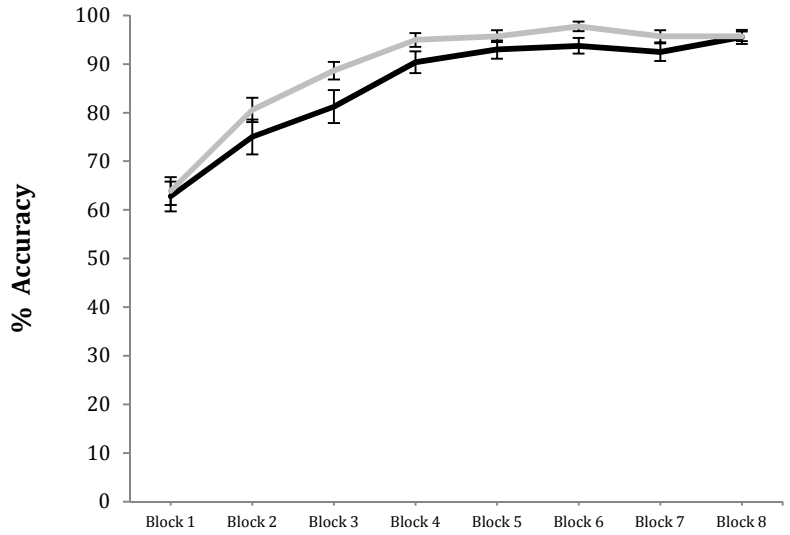


Figure 5: The escitalopram-treated group showed lower accuracy during the instrumental discrimination learning stage, p= .079. Error bars represent standard error of the mean (S.E.M.)

Blocks	Placebo group	Escitalopram group	Group difference*
1	63.89 (16.36)	62.76 (17.32)	0.788
2	80.56 (14.38)	75 (20.3)	0.207
3	88.64 (10.37)	81.25 (19.17)	0.057
4	94.95 (8.05)	90.37 (12.54)	0.083
5	95.71 (6.95)	92.97 (10.61)	0.221
6	97.73 (5.62)	93.75 (8.98)	0.036
7	1.23 (0.68)	1.11 (0.31)	0.142
8	11.4 (10.49)	5.43 (6.93)	0.938

Table 1: Performance during the eight blocks of the instrumental learning stage

Anxiety has been linked to cognitive impairments, including diminished attention control and processing efficiency (Eysenck et al., 2007) and altered decision-making (de Visser et al., 2010). We performed a median split on trait anxiety scores of the State-Trait Anxiety Inventory (Spielberger, 1983) thus dividing participants to high and low trait anxiety groups **(Table 1).**

STAI trait score	Placebo group	Escitalopram group	Group difference*
Low	26.31	28.00	n.s.
High	41.42	43.23	n.s.

Table 2: Mean STAI trait scores in the treatment groups. ***Group difference:** p-values of two-tailed t-tests, n.s.= not significant, p>0.05

We performed a two-way repeated measures ANOVA with treatment and trait anxiety group as between subjects factors; we found a statistically significant treatment main effect, $F(1,61) = 5.228$, $p = .026$, partial $\eta^2 = .079$, trait anxiety effect, $F(1,61) = 5.098$, $p = .028$, partial $\eta^2 = .079$, and interaction of treatment and trait anxiety group effect, $F(1,61) = 4.694$, $p = .034$, partial $\eta^2 = .071$, on overall accuracy. A significant block effect is reported, $p < .001$, $F(7,427) = 88.869$, partial $\eta^2 = .593$ and an interaction between block and trait anxiety close to significance, $p = .060$, $F(7,247) = 1.952$, partial $\eta^2 = .031$. No interaction between block, trait anxiety and treatment is reported, $p = .226$.

Follow-up, one-way ANOVAs on overall accuracy across blocks during the instrumental discrimination learning stage showed that participants with high trait anxiety receiving escitalopram had lower accuracy compared to high trait anxiety participants receiving placebo, $M=8.59$, $t(30) = -2.814$, $p = .009$. No treatment effect on accuracy was found in the low trait anxiety group, $p = .924$.

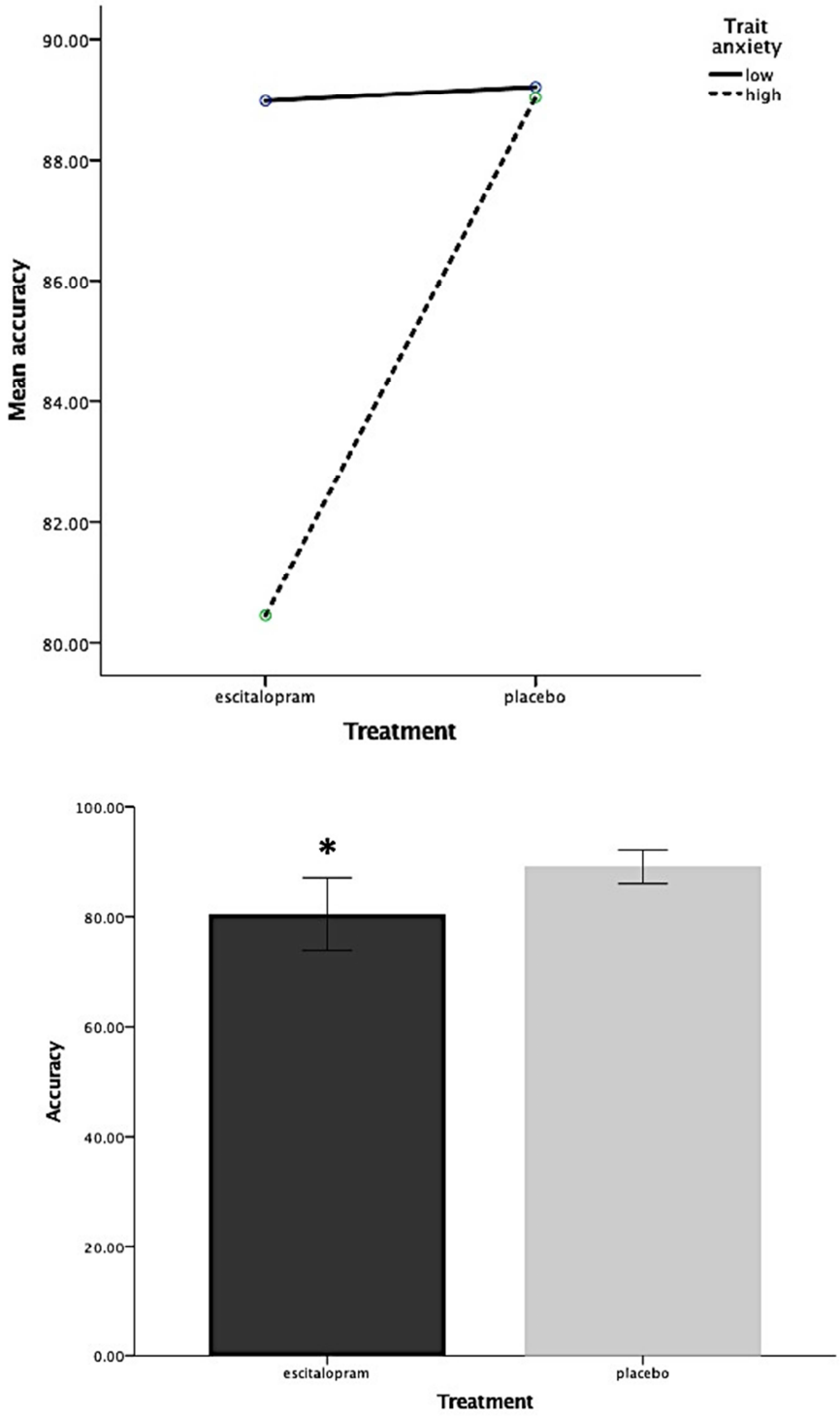


Figure 6: Treatment effect on mean accuracy in high and low trait anxiety groups (a) and total accuracy across blocks in high trait anxiety groups (b)

We found no difference in reaction times between the two treatment groups, p= .914, and no interaction between treatment and block, p= .841. We further compared reaction times after splitting participants in high and low anxiety groups, as above, and we found that

high trait anxiety participants showed longer response latencies across blocks compared to the low trait anxiety group, p= .018, F(1,61)= 5.878, partial η^2 = .088, but this was not affected by treatment, p= .877.

Outcome devaluation stage

A t-test revealed no statistically significant difference between the two groups on accuracy, p= .957, and reaction times, p= .256, during this stage. We further compared reaction times after splitting participants in high and low anxiety groups and we found no significant interaction between treatment and trait anxiety, p= .235.

Slips of action and baseline test

We performed repeated measures ANOVA with type of test (baseline or slips-of-action) and outcome value (valuable or devalued) as within-subjects factors and treatment as between subjects factor. Bonferroni corrections were applied as appropriate. There was a significant interaction of outcome value and test, p= .001, F(1,61)= 13.155, partial η^2= .177. We found no difference in percentage of responses made between the two treatment groups, p= .703.

Stress (Schwabe and Wolf, 2009; 2010) and anxiety (Eysenck et al., 2007) are shown to increase reliance to habitual responding therefore we performed repeated measures ANOVA with treatment and trait anxiety as between-subjects factors and test type and outcome value as within subjects factors. We found a statistically significant interaction between outcome value, treatment and trait anxiety, p= .038, F(1,61)= 4.518, partial η^2 = .069 which was further investigated with tests of simple effects.

For the slips of action test, we found that in both treatment groups, high trait anxiety participants made significantly more responses for valuable outcomes compared to devalued one, p< .001, F(1,30)= 84.042, partial η^2= .737, but there was no interaction between outcome value and treatment group, p= .169. Participants with high trait anxiety receiving escitalopram made fewer responses for valuable outcomes compared to placebo controls, p= .007, F(1,30)= 8.360, partial η^2= .218. Treatment did not affect responses made for devalued outcomes in participants with high trait anxiety, p= .680. In the low trait anxiety group, participants in both treatment groups made significantly more responses for valuable outcomes compared to devalued ones, p< .001, F(1,31)= 73.472, partial η^2 = .793, but here was no interaction between outcome value and treatment group, p= .263.

Treatment did not affect responses made for devalued outcomes in participants with low trait anxiety, p= .329.

For the baseline test, we found that high trait anxiety participants made significantly more responses for valuable outcomes compared to devalued one, p< .001, F(1,30)= 391.051, partial η^2= .929, and a significant interaction between outcome value and treatment group was shown, p= .031, F(1,30)= 5.094, partial η^2= .145. Participants with high trait anxiety receiving escitalopram made fewer responses, close to significance, for valuable outcomes compared to placebo controls, p= .058, F(1,30)= 3.870, partial η^2= .114. Treatment did not affect responses made for devalued outcomes in participants with high trait anxiety, p= .125. In the low trait anxiety group, participants in both treatment groups made significant more responses for valuable outcomes compared to devalued ones, p< .001, F(1,31)= 263.239, partial η^2 = .793, but here was no interaction between outcome value and treatment group, p= .511. Treatment did not affect responses made for devalued outcomes in participants with low trait anxiety, p= .476.

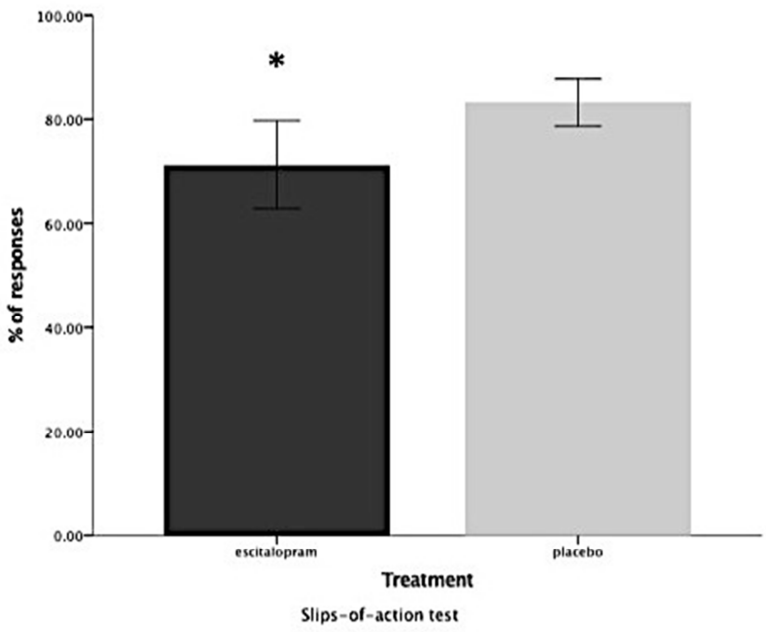


Figure 7: Percentage of responses made for valuable outcomes in the slips of action test in the high trait anxiety group. Participants receiving escitalopram showed fewer responses compared to placebo group

Questionnaires

Participants in both treatment groups showed a significant difference in explicit knowledge among the three types of S-R-O contingencies, p< .001, F(2,126)= 17.72, partial η^2= .213; participants were less able to report S-O compared to S-R contingencies, p< .001, and R-O

contingencies, p= .005. We found no significant difference in knowledge between treatment groups for the three types of instrumental contingencies, p= .902. We further split participants in high and low anxiety group and we found a significant interaction between treatment and trait anxiety for stimulus-response knowledge, p= .044, F(1,61)= 4.246, partial η^2= .065. In the high trait anxiety group, participants receiving escitalopram had impaired knowledge of stimulus-response contingencies compared to placebo, p= .045, F(1,30)=4.362, partial η^2 = .127. No treatment effect is reported in the low trait anxiety group, p= .810. Participants showed no statistically significant difference in confidence ratings between the treatment groups, p= .125, F(42)= 7.490.

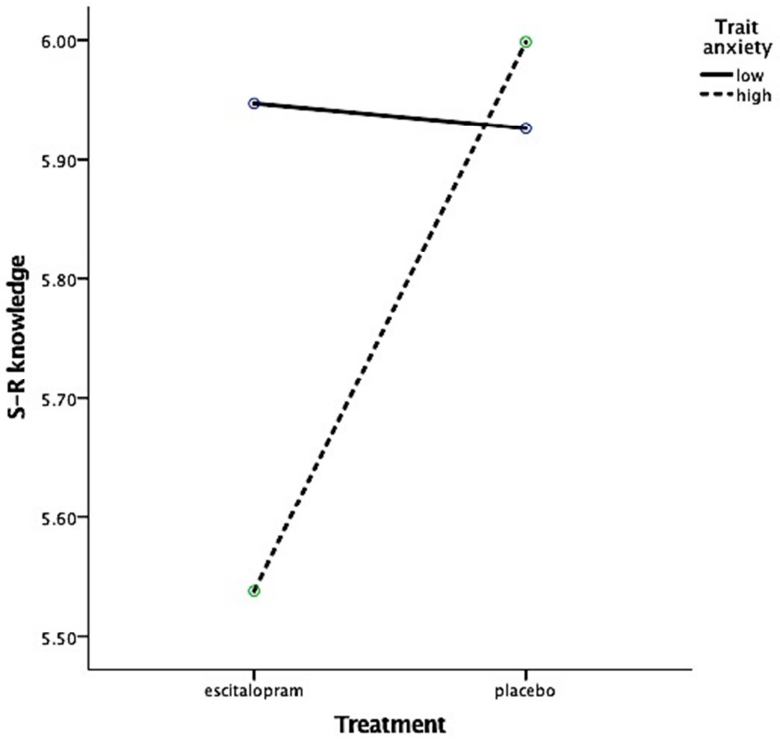


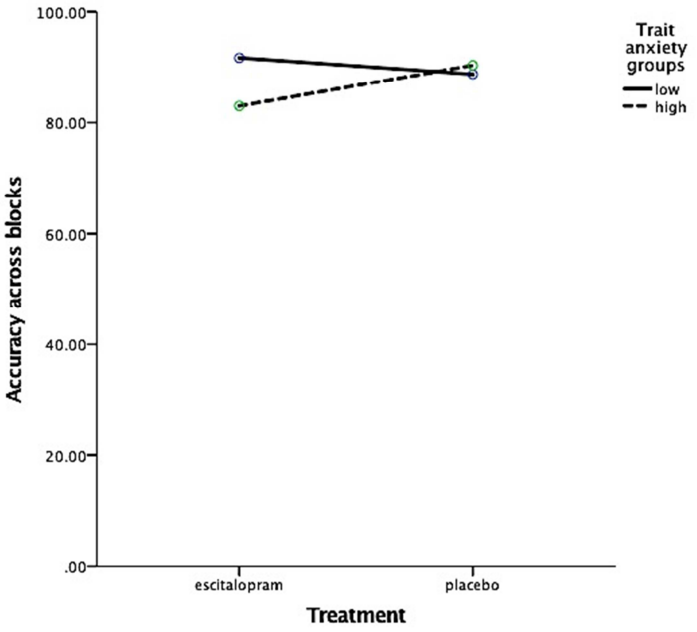
Figure 8: Knowledge on stimulus-response questionnaire among the treatment groups

Task stages	Measures	Escitalopram Mean (SD)	Placebo Mean (SD)	Group difference*
Outcome devaluation test	Accuracy	91.93 (13.65)	91. 75 (12.3)	n.s.
	RTs	1240.56 (430.04)	1298.26 (510.98)	n.s.
Slips-of-action test	% Responses to devalued stimuli	27.26 (24.31)	91.67 (28.79)	n.s.
	RTs for responses to devalued stimuli	718.48 (75.58)	716.66 (42.41)	n.s.
	% Responses to valuable stimuli	77.04 (14.91)	80.6 (11.81)	n.s.
	RTs for responses to valuable stimuli	704.07 (90.37)	683.95 (95.7)	n.s.
Baseline test	% Responses to devalued stimuli	19.88 (16.15)	18.77 (12.89)	n.s.
	RTs for responses to devalued stimuli	709.72 (50.15)	708.05 (41.75)	n.s.
	% Responses to valuable stimuli	80.51 (13.08)	84.01 (10.15)	n.s.
	RTs for responses to valuable stimuli	701.06 (78.41)	663.76 (119.95)	n.s.
Explicit knowledge of S-R-O contingencies	% Accuracy for S-R	78.13	80.11	n.s.
	% Accuracy for R-O	89.59	90.86	n.s.
	% Accuracy for S-O	96.35	99.46	n.s.

Table 3: Participants in the two groups did not differ in performance in stages 2-4 of the appetitive instrumental learning task including the outcome devaluation test and slips-of-action test which directly measures goal-directed versus habitual behaviour. For statistical analysis purposes, participants not reaching learning criterion in stage 1 were assigned the number of errors to criterion as the worst performing subjects in the respective group. Repeated measures ANOVA with type of test (baseline or slips-of-action) and outcome value (valuable or devalued) as within-subjects factors and treatment as between subjects factor and Bonferroni corrections showed a significant interaction of outcome value and test, $p= 0.001$, $F(1,61)= 13.155$, partial $\eta^2= 0.177$. Participants in both treatment groups showed a significant difference in explicit knowledge among the three types of S-R-O contingencies, $p< 0.001$, $F(2,126)= 17.72$, partial $\eta^2= 0.213$; participants were less able to report S-O compared to S-R contingencies, $p< 0.001$, and R-O contingencies, $p= 0.004$. ***Group difference:** p-values of one-way ANOVA. n.s.= not significant, $p>0.05$

De Visser et al. (2010) reported sex specificity in the effect of trait anxiety in decision-making applying the Iowa gambling task. Both male and females participants were assigned to low, medium and high trait anxiety groups. Male participants in the low and high trait anxiety groups showed impaired decision-making compared to participants in the medium one, whereas female participants only in the high trait anxiety group showed impaired performance in the task compared to both low and medium trait anxiety groups. Additionally, impairment was manifested during the exploration phase of the task in males and during the exploitation phase in females.

We therefore further tested for the effect of treatment on accuracy in the instrumental learning discrimination stage separately in male and female participants. We separated male participants in low and high trait anxiety groups; repeated measures ANOVA revealed no interaction between treatment and trait anxiety, p= .374. We separated female participants as well in low and high trait anxiety groups; we found a significant interaction between treatment and trait anxiety groups, p= .02, F(1,28)= 6.097, partial η^2= .179. Follow-up, one-way ANOVAs revealed that in the escitalopram group, female participants with high trait anxiety showed lower accuracy across blocks than female participants with low trait anxiety, p= .035, F(1,14)= 5.446, partial η^2= .280, whereas in the placebo group, female participants did not differ in accuracy depending on trait anxiety scores, p= .407.



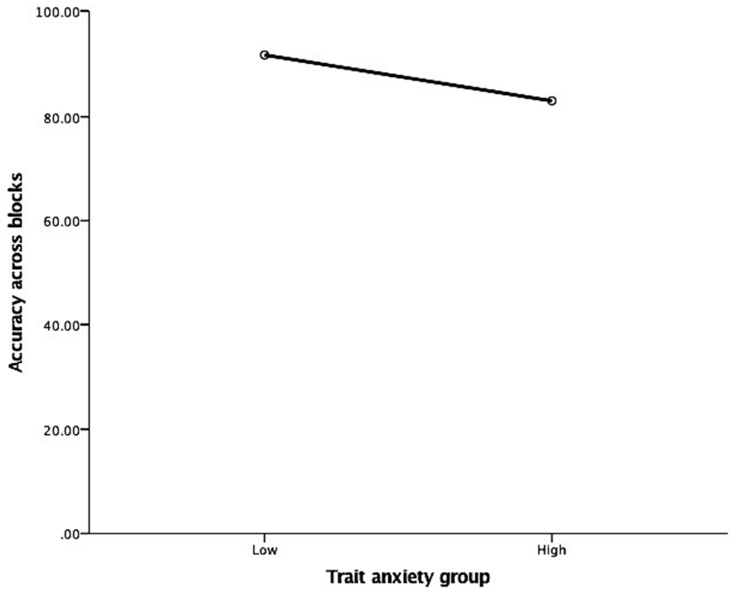


Figure 9: Distinct effect of treatment on mean accuracy across blocks dependent on trait anxiety group (a) and mean accuracy across blocks in low and trait anxiety groups for female participants receiving escitalopram (b)

We added sex as covariate in repeated measures ANOVA for the effect of treatment and trait anxiety scores on percentage of responses in the slips-of-action and baseline tests. We found no significant sex effect, p= .434.

Discussion

In our study, escitalopram administration produced a tendency towards lower discrimination learning ability, which was pronounced by baseline trait anxiety scores. Unlike the Worbe et al. (2015) study using tryptophan depletion and showing increased habitual responding, we did not observe any such effect following acute escitalopram administration.

We found that during both the baseline and slips-of-action tests, participants with high trait anxiety receiving escitalopram responded less for valuable outcomes, thus indicating a primary learning impairment. This was supported by impaired explicit knowledge of S-R knowledge. This could be in accordance to clinical findings of increased anxiogenic effect during initial treatment with SSRIs (Taylor et al., 2015). Thus this anxiogenic effect might not be the result of changes in emotional processing during treatment initiation (Browning et al., 2007), but through effect on “cold” cognitive mechanisms (Roiser and Sahakian, 2013). Indeed, for sustained improvement in mood to be observed with chronic SSRI

administration, Harmer and Cowen (2013) proposed that cognitive re-appraisal and re-evaluation of emotions is needed thus reflecting the role of learning. Previous studies suggested a role for baseline personality traits in mediating the effect of serotonergic manipulations; Di Simplicio et al. (2014) showed that citalopram administration for seven days in healthy individuals increased fear re-activity only in the high neuroticism-group. An alternative explanation is that our dosage produced a net decrease rather than increase of brain serotonergic levels (Labuschagne et al., 2010) possibly due to activation of auto-receptors located in the pre-synaptic neurons (El Mansari et al., 2005). Previous studies have also shown sex specificity in serotonergic manipulations (Ronbinson et al., 2009) but further research is needed for delineating the underlying mechanisms; hormonal interactions might play a key role.

II. Reinforcement learning accounts

Computational accounts of reinforcement learning (RL) theories (Sutton and Barto, 1998) are suggested in terms of underpinning neural areas of goal-directed and habitual behaviour, which derive from animal experimental psychology. Two distinct classes are identified; model-free RL, which is associated with dopaminergic neurons and their (mostly dorsolateral) striatal projections, and model-based RL, which is associated with the prefrontal cortex circuit (Daw et al., 2005). The terms do not entirely overlap with the constructs of goal-directed and habitual behaviour derived from animal literature but they do share many common features (Dolan and Dayan, 2015); suggestions though have been made that habitual learning is not restricted solely to a model-free RL model but instead might be better explained by a hierarchical model where automaticity is one aspect of action sequences (for detailed review, please refer to Dezfouli et al., 2014). Both model-based and model-free RL mechanisms entail both advantages and disadvantages which justifies the account of more than one action controllers and points out what controls arbitration.

Within the suggested framework, the two systems represent the opposite ends in regards to statistical efficiency and computational flexibility. A prominent model-free RL method is temporal-difference learning, referring to the activity of dopaminergic neurons in classical and instrumental learning tasks (Schultz et al., 1997). This RL account is based on “the association of an action with a scalar summary of its long-run future value” which is

termed as ‘”caching” (Daw et al., 2005). Under this account, dopaminergic neuronal firing encodes the reward predicted by related stimuli; this was demonstrated experimentally by Schultz et al. (1997) who recorded the activity of single DA neurons on monkeys performing a classical pavlovian task. During the task, monkeys were presented with various appetitive stimuli (such as juice). When the stimulus was presented in the absence of prediction, a positive error occurred in the prediction of reward and DA neurons were activated. The stimuli were associated with reward, thus learning occurred; DA neurons were activated by the conditioned stimulus and not reward delivery per se. This computational account of “caching” is simple and does not incur any computational cost (parallel to energy cost, previously described for habitual behaviour), but it does entail inflexibility, as re-evaluation of outcomes does not directly change the stimulus’ value (again, parallel to habitual behaviour where outcome devaluation is experimentally shown not to directly affect responses made).

On the contrary, model-based RL, represented in the neural level as mentioned by prefrontal circuit, does not entail any “cached” values; instead, short-term predictions about the immediate outcomes of each action are computed in a sequence. This is termed as “tree-search” as it involves the exploration of “a branching set of possible future situations” (Daw et al., 2005). This demands both time and working memory capacity and is prone to more errors during the process, thus rendering it inefficient. On the other hand, compared to model-free RL, re-evaluation of outcomes is directly encoded. This allows for behavioural flexibility and ‘goal-directed’ behaviour.

It has been shown, as previously discussed, that extensive training underlies the shift from goal-directed to habitual behaviour. Daw et al. (2005) described how this binds with the RL accounts proposed; after moderate training of lever-pressing, rats show a sensitivity to outcome devaluation, which is encoded by the model-based or “tree-search” system, whereas extensive level-pressing shows an insensitivity to outcome devaluation (Adams, 1982), thus indicating a predominance of the model-free or “caching” system. Dopaminergic input for dorsolateral striatal areas is suggested to underlie this “transfer”, as shown by lesions of the dorsolateral striatum (Yin et al., 2004) and the nigrostriatal pathway (Faure et al., 2005), which disrupt the development of S-R habits.

One of most widely applied tasks for dissociating between model-based and model-free choice behaviour is the two-stage Markov decision-making task (Daw et al., 2011). During the first stage, participants make an initial choice between two stimuli that lead with fixed probabilities (70% and 30% of the choices respectively) to one of two pairs of stimuli in

stage 2. Each of the four stimuli of stage 2 is associated with a probabilistic monetary reward, with the probability changing slowly and independently during the course of the task (ranging from 0.25 to 0.75). The stimuli applied are Tibetan characters (Daw et al., 2011). Healthy volunteers employ a mixed pattern of behaviours in this task using both model-based and model-free RL strategies (Daw et al., 2011).

It is suggested that competition of these two systems and reliance on the system with the most accurate predictions for a given situation determines the decision-making process (Daw et al., 2005). Nevertheless, the conditions that control the balance between these two systems in choice behaviour have not yet been fully clarified. Daw et al. (2011) suggested that both the "caching" and "prefrontal" controllers aim towards rational end points and, provided the appropriate settings, on the same operations might be mediated by the model-free RL controller more effectively than the model-based RL one thus pointing towards a "rationality" underlying both the plurality and the arbitration of these two systems. Numerous studies have focused on the role of DA (Schultz et al., 1997) as administration of drugs that increase brain DA levels enhances the representation of errors in the prediction of rewards and increases the possibility of choosing the most rewarding option during instrumental learning tasks (Pessiglione et al., 2006; Frank et al., 2004). L-DOPA administration to healthy volunteers, resulting in elevated brain DA levels, enhanced reliance on model-based versus model-free behavioral (Wunderlich et al., 2012) mirroring the opposite effect (predominance of habitual learning) following diminished global brain DA levels in female healthy participants using an instrumental learning discrimination task (described previously, de Wit et al., 2012a).

The traditional view is that 5HT opposes the role of DA in reward processing; reduced DA neuronal firing is shown to encode punishment in instrumental learning tasks through the representation of negative prediction errors (Schultz et al., 1997) but this cannot fully account for the large range of punishment values (Palminteri et al., 2012; Bayer and Glimcher, 2005); thus, from a physiological perspective, 5HT is suggested to have an opponent role to DA through punishment-related/aversive processing (Grossberg, 1988).

Nevertheless, this view has been contradicted (Boureau and Dayan, 2011), as experimental studies of tryptophan depletion produced mixed results (Worbe et al., 2016; Crockett et al., 2009; Tanaka et al., 2009; Cools et al., 2007; Evers et al., 2005), and alternative theoretical accounts are proposed (Dayan and Huys, 2008). McCabe et al. (2010) showed that sub-chronic citalopram administration in healthy volunteers reduce neural processing for both appetitive and aversive food stimuli. Palminteri et al. (2012) administered a subliminal

instrumental learning task to un-medicated OCD patients and OCD patients receiving 5HT-reuptake inhibitors and compared their performance with previous results from patients with Gilles de la Tourette syndrome (un-medicated and group receiving neuroleptics), which is characterised by basal ganglia dysfunction and manifested maladaptive behaviours. OCD patients showed impaired performance in the task, which was restored by the 5HT-reuptake inhibitors (which was not related to either motor or choice impulsivity, as assessed by the task design); this improvement in performance was not related to outcome valence, as previously shown in the Gilles de la Tourette patients (Palminteri et al., 2009).

5HT manipulations and model-based/model-free choice behaviour

Worbe et al. (2016) used the two-stage decision-making Markov task, adapted by Daw et al. (2011), to include a punishment condition. Behavioural analyses showed that participants used both choice strategies in order to perform the task. The researchers showed tryptophan depletion in healthy volunteers impaired model-based strategy in the reward component of the task whereas it enhanced model-based behaviour in the punishment condition, an effect though not supported by subsequent computational modelling (Worbe et al., 2016). Additionally, in the reward condition, the behavioural data showed a shift towards model-free learning in the tryptophan depletion group.

These findings support a dual role for 5HT in model-based and model-free learning dependent on outcome valence. Participants' performance on initial learning discrimination and the outcome devaluation stage did not differ from the tryptophan-balanced control group, suggesting that the effect on S-R responding was not driven by lack of knowledge of R-O contingencies or inability to use this knowledge for action selection.. In terms of possible explanations, it is suggested that reduction of 5HT transmission promotes responses to punishments (Dayan and Huys, 2009). Worbe et al. (2015) used an appetitive instrumental learning task with explicit rewards (points) but the lack of reward delivery can be conceptualised- and perceived- as "punishment" by participants. This could be in accordance to proposed reference dependency of value by Kahneman and Tversky (1979) in their influential prospect theory. Worbe et al. (2015) alternatively suggested that cognitive inflexibility produced by tryptophan depletion, as shown in primates by Clarke et al. (2004), and choice perseveration (Seymour et al., 2012) could drive increased habitual

responding. Overall response dis-inhibition cannot explain these findings, as tryptophan depletion did not alter performance in the baseline test.

An alternative explanation, for this dual role, would be that 5HT alters the average reward representation; this is based on suggestions from the computational modelling literature that 5HT contributes to average reward representation (Cools et al., 2011; Daw et al., 2002) which plays a role in the use of model-based choice strategy as it encodes opportunity cost of time spent deliberating for rewards or benefit for punishments respectively (Keramati et al., 2011; Niv et al., 2007). Indeed, Keramati et al. (2011) account of opportunity cost supports the shift towards model-free learning when average reward is high as the time spent deliberating will be contrasted with reward gained (Worbe et al., 2016).

Worbe et al. (2016) support that opportunity cost becomes a benefit (Boureau and Dayan, 2011) thus a reverse effect is observed. In support of this view, Worbe et al. (2016) suggested that different neural substrates might mediate the effects of diminished central 5HT neurotransmission in model-free and model-based learning in their experiment. This could be explained by the distinct anatomical correlates within the cortico-striatal circuitry for the two types of learning (Daw et al., 2011; Tricomi et al., 2009) and the independent effects of tryptophan depletion in enhancing model-based aversive and model-free appetitive learning (Worbe et al., 2016).

The effect of stress on model-based/model-free choice behaviour

Stress, which is associated with impaired decision-making (de Visser et al., 2010), affects these two distinct types of choice strategies. Otto et al. (2013a) showed that acute stress, induced with the cold pressor test, impairs model-based choice strategy use in healthy participants with low working memory; no effect was observed in the high working memory group thus pointing towards a distinct effect of stress based on availability of cognitive resources. This effect was suggested to be mediated by the dopaminergic system given the well-established role of DA in both types of choice strategy. The researchers had previously shown (Otto et al., 2013b) that depletion of working memory resources in healthy volunteers produced a detrimental effect on model-based learning contributions whereas model-free learning remained intact. A recent study (Friedel et al., 2017) showed a different effect of accumulated real-life stress versus acute stress, previously studied, on employment of the two strategies. The researchers applied the two-step task (Daw et al., 2011), the social readjustment rating scale to measure accumulated exposure to stress and a

digit symbol test to assess cognitive speed. Participants with high stress exposure and high cognitive speed showed increased reliance on model-based RL and reduced employment of model-free RL, whereas the opposite pattern was observed for people with low cognitive speed (i.e. increased model-free and reduced model-based behaviour) (Friedel et al., 2017).

Participants

Overall sixty-five participants completed the two-step decision-making task with reward and punishment conditions; thirty-three in the placebo group and thirty-two in the escitalopram group. Participants completed this task along with the additional cognitive tasks in a counterbalanced order.

Task description

The task, implemented by MATLAB 2010a and Cogent 2000, had identical design to Worbe et al. (2016) with both reward and punishment conditions. The reward identical is identical to the initial task (Daw et al., 2011). The punishment version used different colour code and stimuli set in the first and second task stages. Transition probabilities were the same in both conditions. Monetary reward in stage 2 was a pound coin and punishment was denoted by the same coin with a cross-superimposed on it. Participants received self-paced, computer-based instructions, which explained the task structure and performed practice examples. In order to incentivise participants for best performance, they were instructed to try to win as much as possible in the reward condition and try to avoid losing in the punishment condition. Participants were told that they would win additional points depending on how they perform in both conditions (the consensus across study day was that participants would receive a flat rate for the time spent in the facilities and additional amount dependent on task performance calculated through cumulative points, please see Chapter 2 for further details). Participants completed overall three blocks in reward condition and three blocks in punishment conditions with a total 201 trials in each version (mean session duration was 9 minutes). During each task stage, participants had 2 s to make a selection. Transition time between stages was 1.5 s. After 2 s, if no response was made, the trial was aborted – a red cross was super imposed on the stimuli. Omitted trials were not associated with any outcome and they were not used for data analysis.

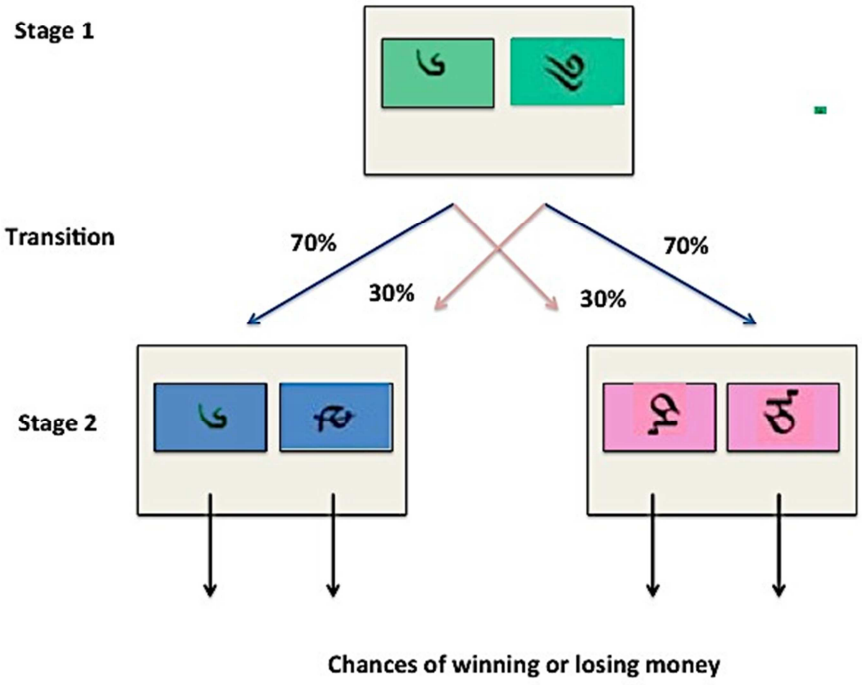


Figure 10: The two-step task

Hypothesis

We predicted that escitalopram administration would affect the arbitration between model-based and model-free strategies in a valence-dependent manner; specifically, we anticipated an increase in goal-directness in the reward condition and an increase in model-free behaviour in the punishment condition.

Results

As per previous studies (Worbe et al., 2016), we performed two types of analyses; a factorial analysis of staying and shifting and an analysis based on the application of computational modelling. In the factorial analysis, we calculated the probability at the first stage to choose the same stimulus as in the previous trial based on the type of transition (probability of transition on previous trial, which was either 70% for common transition or 30% for rare transition), the outcome (rewarded, no rewarded, punished, not punished) and valence (reward or punishment) as within-subjects factors and treatment group

(escitalopram or placebo) as between-subjects factor which were all fitted in a repeated measures mixed ANOVA.

Stay and shift of behaviour were considered to represent model-based and model-free learning respectively (Worbe et al., 2016). We found main effect of outcome, $p < .001$, $F(1,63) = 87.986$, partial $\eta^2 = .580$, main effect of outcome x transition, $p = .003$, $F(1,63) = 9.791$, partial $\eta^2 = .135$, and marginally significant outcome x transition effect, $p = .048$, $F(1,63) = 4.063$, partial $\eta^2 = .061$. We found no main effect of valence, $p = .111$. The finding of significant outcome and outcome x transition effect shows that participants in both groups used both model-based and model-free strategies throughout the task, as per previous studies (Worbe et al., 2016; Daw et al., 2011). The marginal but significant outcome x treatment effect shows that model-free learning is modulated by escitalopram treatment. Follow up analyses with tests of simple effects with outcome (rewarded, no rewarded, punished, not punished) and valence (reward or punishment) and treatment group (escitalopram or placebo) showed a significant outcome, $p < .001$, $F(1,128) = 100.952$, partial $\eta^2 = .441$, outcome x treatment effect, $p = .032$, $F(1,128) = 4.710$, partial $\eta^2 = .035$, and valence effect, $p = .032$, $F(1,128) = 4.690$, partial $\eta^2 = .035$. No valence x treatment effect is reported, $p = .291$. Follow-up, one-way t-tests revealed that escitalopram group shows lower probability to stay after being punished, $p = .018$, $t = -2.405$, partial $\eta^2 = .043$. No effect was observed in the other measures, $p > .05$.

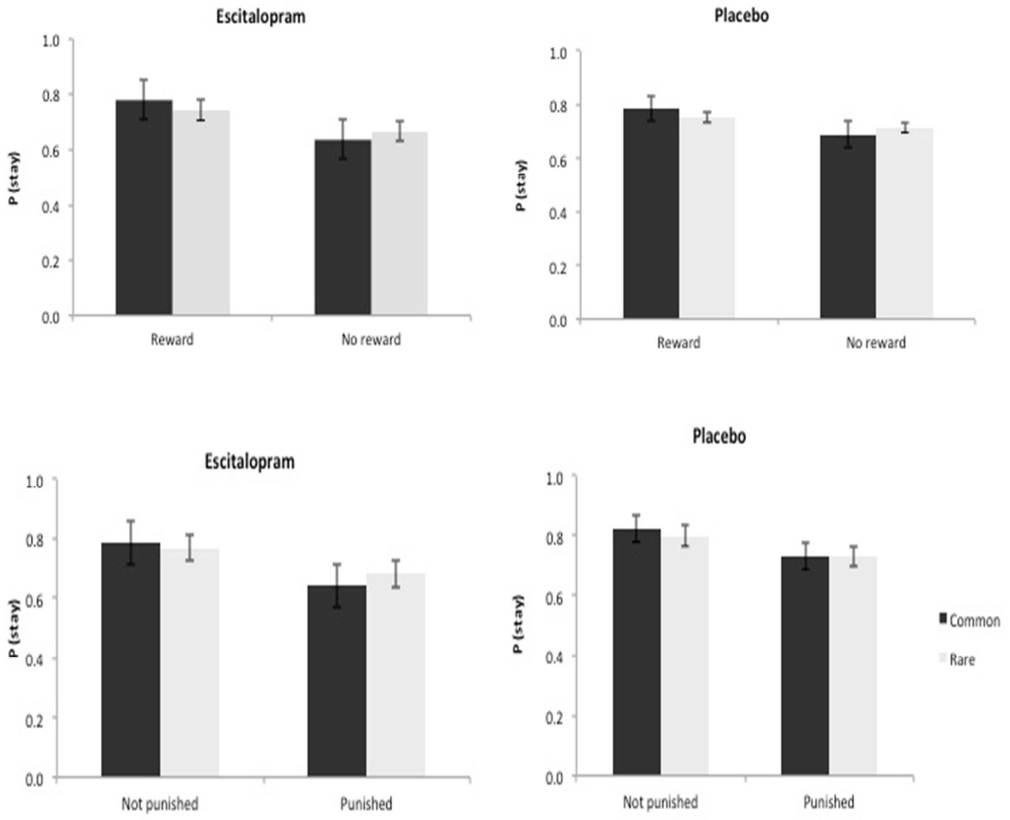


Figure 11: Factorial analysis results of behaviour for reward (a, b) and loss (c, d) conditions

Based on previous studies of stress effect on model-based/model-free RL, we split participants in high and low trait anxiety groups; we found a significant outcome, $p < .001$, and outcome x treatment effect, $p = .033$, as per previous analysis, but no interaction between trait anxiety and treatment, $p = .406$.

We further fitted a computational model previously described (Daw et al., 2011; Worbe et al., 2016) to the behavioural data. This is a hybrid model, detailed in Daw et al. (2011), with an algorithm including both model-based and model-free subcomponents allowed for mapping each state-action pair to its expected future value. The model-free strategy was computed using the SARSA (λ) temporal difference learning. At each stage *i* of each trial *t*, the value for the each state-action pair was calculated as follows:

$$Q_{TD}(s_{i,t}, a_{i,t}) = Q_{TD}(s_{i,t}, a_{i,t}) + \alpha_i \delta_{i,t}$$

where $\delta_{i,t} = r_{i,t} + Q_{TD}(s_{i+1,t}, a_{i+1,t}) - Q_{TD}(s_{i,t}, a_{i,t})$ and α_i is a free learning parameter.

The full model allows different learning rates α_1 and α_2 for the two task stages. The reinforcement eligibility parameter (λ) determines the update of the first-stage action by the second-stage prediction error as follows: $Q_{TD}(s_{1,t}, a_{i1,t}) = Q_{TD}(s_{1,t}, a_{1,t}) + a_1 \lambda \delta_{2,t}$.

The model-based reinforcement-learning algorithm was computed by mapping state-action pairs to a transition function and assuming that participants choose between two possibilities, as follows: $P(S_B / S_A, a_A) = 0.7$, $P(S_C / S_A, a_B) = 0.7$ for the common or $P(S_B / S_A, a_A) = 0.3$ $P(S_C / S_A, a_B) = 0.3$ for the rare transition, where S is a state (first stage: S_A; second stage: S_B and S_C), and a is an action (two actions - a_A and a_B).

The action value (Q_{MB}) was computed at each trial from the estimates of the transition probabilities and rewards and was defined for the first stage as follows:

$$Q_{MB}(s_A, a_i) = P(s_B | s_A, a_i) \max_a Q_{TD}(s_B, a) + P(s_C | s_A, a_i) \max_a Q_{TD}(s_C, a)$$

Finally, to connect the values to choices, the weighted sum of the model-free and model-based values was computed for the first stage as defined:

$$Q_{net}(s_A, a_j) = w\, Q_{MB}(s_A, a_j) + (1-w)\, Q_{TD}(s_A, a_j)$$

where *w* is the weighting parameter. Assuming that two approaches coincide at the second stage, and that $Q_{MB} = Q_{TD}$, at the second stage $Q_{net} = Q_{MB} = Q_{TD}$. Then, the probability of a choice is the softmax equation for Q_{net}:

$$P(a_{i,t} = a | s_{i,t}) = \frac{\exp(\beta_i [Q_{net}(s_{i,t}, a) + p * rep(a)])}{\sum_{a'} \exp(\beta_i [Q_{net}(s_{i,t}, a) + p * rep(a)])}$$

where the free inverse temperature parameters (β_i) control the choice randomness, and *p* captures perseveration ($p > 0$) or switching ($p < 0$) in the first-stage choices.

Overall, the fully parameterized model contains 7 free parameters (β1, β2, α1, α2, λ, π, ω); ω is an index of the relative engagement of model-free versus model-based choice, with low values indicating a shift to model-free and high values indicating a shift to model-

based choices. Special cases of pure model-based and model-free strategy use correspond to ω values of 1 and 0 respectively. Parameter results are summarised in **Table II**.

Parameter	Placebo	Escitalopram
	Reward condition	
β1	5.011	4.241
β2	3.545	3.158
α1	0.479	0.49
α2	0.487	0.407
λ	0.541	0.68
π	0.217	0.163
ω	0.362	0.348
	Punishment condition	
β1	4.532	3.695
β2	2.419	2.026
α1	0.499	0.515
α2	0.468	0.603
λ	0.559	0.628
π	0.366	0.253
ω	0.407	0.332

Table 1. Parameter estimates for reward and punishment conditions of the two-step task

The parameter estimates were fitted into a multivariate ANOVA with treatment as between-subjects factor separately for reward and punishment conditions. We found no difference between treatment groups for either of the seven parameters calculated.

Discussion

In the two-step task, the factorial analysis of the behavioural data showed that participants receiving escitalopram showed lower probability to make the same choice after being punished. This is in accordance to the literature of the role of 5HT in aversive processing and behavioural inhibition (Deakin and Graeff, 1991). We found no effect of acute escitalopram administration on the reward version of the task. We also found no effect of escitalopram administration in the balance between model-based and model-free choice behaviour; this is in accordance to the aforementioned findings with the instrumental discrimination learning task where escitalopram did not affect responses for devalued

outcomes in the slips-of-action test (indicative of habitual responding). Worbe et al. (2016) had previously reported an effect of tryptophan depletion in model-free and model-free learning in the two-step task dependent on the valence of the condition (reward vs. punishment); we did not observe such effect. The lack of valence effect in the two-step task task may point towards a non-serotonergic specific effect on model-based *versus* model-free behaviour; i.e. towards an effect on learning and/or working memory capacity. Indeed, in the instrumental learning fruit task we did find that the two groups substantially differed in learning rates- although this did not reach significant- thus a possible explanation for this would be that escitalopram might modulate confidence about reward delivery. Indeed uncertainty can modulate learning rates as previously shown (Behrens et al., 2007) and 5HT was previously shown to modulate risky decision-making (Long et al., 2009). Nevertheless, we did apply a gambling task to capture decision-making under uncertainty but we found no effect of the serotonergic manipulation. This though may be related to task differences compared to other studies.

Chapter 6. Additional cognitive functions

I. Executive functions

1. The CANTAB Spatial working memory task

Background

Working memory can be described as the temporary storage of information in a manner that makes this information available for engagement from other cognitive processes. The Baddeley and Hitch (1974) influential multi component model of working memory suggests that there are distinct components of working memory; the central executive, the visuo-spatial working memory, the phonological loop and the episodic buffer. Baddeley (1986) suggested a distinction in working memory for verbal and visuospatial information and a further distinction for spatial and visual information. An alternative account to the above theory proposes a unitary model of memory where visual, spatial and verbal information are based on different levels of representation (Jones et al., 1995) contrasting the multi component working memory model. The neural areas implicated in spatial working memory include a network consisting of the superior frontal, dorsal parietal and occipital cortex (Awh and Jonides, 2001).

Working memory deficits

Several studies show impairments of working memory in impulse control disorders (Willcut et al., 2005; Evenden, 1999). Frontal lobe lesion patients, shown to exhibit impulse control disturbances (Bechara and van den Linden, 2005), were impaired in the CANTAB spatial working memory task (SWM) (Owen et al., 1990). Specifically, patients with both frontal and temporal lobe damage made more ‘between’ and ‘within’ errors during the searches consisting of four, six and eight boxes. The frontal lobe damage patient group was less efficient in the strategy used thus indicating that the impaired performance might be partially attributed to a primary impairment in applying appropriate strategies (Owen et al., 1990) as per previous report (Petrides and Milner, 1982).

Neural substrates underlying working memory

The role of 5HT is not clear unlike the dopaminergic one, where distinct components of WM are differentially affected via both the striatum and frontal cortex (Cools et al., 2007). Ascending mesencephalic DA projections to striatum and PFC are implicated in several cognitive functions including cognitive flexibility, learning by reinforcement (Schultz et al., 1997) and working memory (Clatworthy et al., 2009). Levy et al. (1997) reported increased activity in the head of caudate nucleus in spatial delayed response visual working memory task in monkeys. Rat studies suggest the involvement of nucleus accumbens in tasks dependent on working memory such as the T-maze (Taghzouti et al., 1985) or the radial arm maze task (Floresco et al., 1997). D_1 and D_2 receptors are implicated in working memory as shown in monkey studies (Lee et al., 2007). Kimberg et al. (1997) showed a that an effect of bromocriptine (a D2 receptor agonist) on working memory in young volunteers dependent on baseline working memory capacity; participants with high capacity deteriorated in their performance whereas the opposite pattern was observed for low capacity volunteers. Mehta et al. (2001) showed improved working memory in healthy people following administration of bromocriptine mediated by effects on the dorsolateral prefrontal and posterior parietal cortex and dependent baseline working memory capacity; the improvement was more prominent in participants with lower baseline working memory capacity. Cools et al. (2007) showed differential effects of bromocriptine in a working memory task in healthy volunteers based on individual differences of trait impulsivity. High trait impulsivity participants showed improved switching -or flexible updating- of relevant information following bromocriptine administration. This was correlated with altered activity in the putamen as measured with neuroimaging. No effect was observed in low trait impulsivity volunteers. The researchers applied the delayed match to sample task that included both resistance to distraction and the need to flexible update representations. Bromocriptine administration modulated the lateral prefrontal cortex activity during distraction; again, this effect was significant only for the high-trait impulsivity group. No effect of bromocriptine on prefrontal cortex activity was observed during switching; this is interesting as a large body of literature points towards the frontal cortex as key brain area in task switching and set shifting (as explained in other chapters). The researchers suggested that the dopaminergic effect on striatal activity might “bias impulsivity” (Cools et al., 2007) by “mediating PFC-evoked input to the striatum”. Indeed, previous research showed that injection of D2 receptor agonist and antagonist in the rat striatum produced both impairment and facilitation of striatal activity invoked by frontal cortex stimulation

(Goto and Grace, 2005). Methylphenidate administration is shown to modulate working memory through altered DA receptor availability in the ventral striatum (Clatworthy et al., 2009).

Serotonergic modulation of working memory

The role of 5HT in working memory is less clearly understood. Findings from a limited number of animal studies suggest a generic distinction between high 5HT levels impairing SWM – along with additional cognitive functions- and low 5HT facilitating it (Luciana et al., 2001). Both systemic and topical (into dorsal hippocampus) injection of 8-hydroxy-2-(di-n-propulamino)tetralin (a 5-HT_{1A} receptor agonist) in rats impaired spatial learning in water maze (Carli and Samanin, 1992; Carli et al., 1992) and systemic administration of the same agent impaired performance in a radial maze task (Winter and Petti, 1987), an effect though suggested to be mediated through regulation of DA activity (Luciana et al., 1998). Acute administration of the 5-HT agonist fenfluramine impaired performance in a SWM task where participants had to indicate the location of appearance of visual cues after variable delays (Luciana et al., 1998). The drug effect was observed when delay exceeded a threshold of 4000 milliseconds; researchers attributed this effect not directly on disturbance of mnemonic processes- as these remained intact during the task- but rather on the use of strategies to perform a specific goal/task including inhibitory control, focused attention and motivation. Modulatory influence on the dopaminergic system is suggested (Luciana et al., 1998) by additional studies showing that 5-HT inhibits the facilitatory DA actions in several behaviours (Soubrie, 1986). In a subsequent study, Luciana et al. (2001) found that increased 5HT levels with tryptophan loading impaired performance in the digit span and the ability to maintain affective aversive information in working memory with no effect on SWM. Vortioxetine, a multimodal antidepressant acting on the serotonergic system, reversed memory deficits induced by ATD in rats in contrast to both escitalopram and duloxetine (Jensen et al., 2014) and these effects were possibly mediated through agonism of $5HT_{1A}$ receptors with no involvement of antagonising $5HT_3$ receptors (du Jardin et al., 2014).

Hypothesis

We added this task to our task battery to control for any effects of acute escitalopram administration on SWM that could mediate the anticipated in the balance between goal-directed and habitual choice behaviour. Previous studies showed an interaction between

sticking to a goal and manifested memory deficits with $5HT_{1A}$ receptor agonist (Luciana et al., 1998) and differential effects of acute stress on the arbitration between model-based and model-free choice strategies based on underlying working memory capacity (Otto et al., 2013a).

Task description

The task required participants to retain spatial information and store and appropriately use items in working memory. A number of coloured boxes were presented on the screen and participants had to search through these by touching each box and revealing what was in the inside. The aim was to find, by elimination, one blue token in each of a number of boxes and to use it to fill an empty column on the right hand side of the screen called the 'home'. Participants would then start a new search for the next blue token. This would end again when the next blue token was found and placed in the 'home' column. The important instruction was that once a blue token was found inside a box then this particular box could not be used to hide another token and therefore the participants should not go back searching in the same box again. Therefore the total numbers of blue tokens corresponded to the total number of boxes on the screen. There are two types of mistakes the participants could perform; a 'between-search error' which the participants would make every time they touched a box where a blue token was already found and a 'within-search error' when the participants would go back and touch a box that had already opened during this particular search and was found empty. Participants decide on their own search strategy for finding all the blue tokens. The computer determines how many empty boxes need to be visited. A strategy that could be applied to efficiently solve the task is to begin with a specific box and, after the blue token is found, start the new search by returning to the same box (Owen et al., 1990). For example, Joyce et al. (1996) showed impaired strategy use in patients with chronic fatigue syndrome.

Total task duration was approximately nine minutes. Participants were given clear instructions on how to perform the task (www.cambridgecognition.com, Manual version 5.0.0, 2012). Participants were initially shown a demonstration trial during which the experimenter touched the boxes while administering the instructions. This was followed by a practice trial of 3 boxes and it was not assessed. The assessed trials included three searches consisting of 4 boxes, three searchers consisting of 6 boxes, three searches consisting of 8 boxes, three searches consisting of 10 boxes and three searches consisting of 12 boxes. Detailed instructions, as administered to participants, are listed in Appendix.

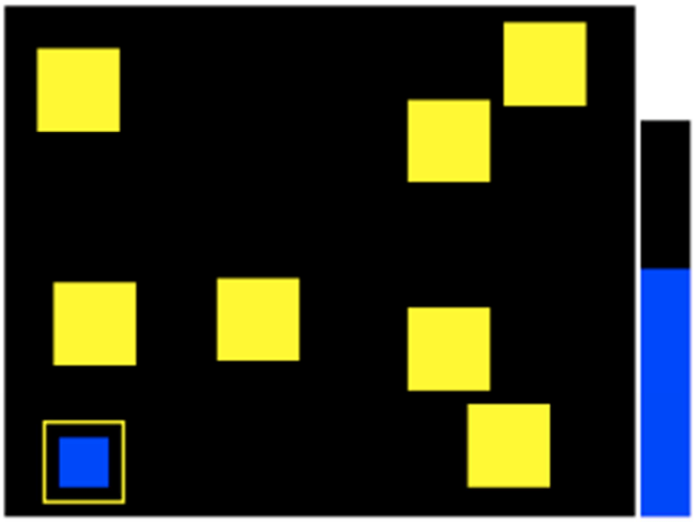


Figure 1: The spatial working memory task screen with eight boxes shown. The black column on the right hand side, called the 'home', is where the blue tokens need to be placed

Measures

The primary outcome measure was the number of 'between search' errors. We additionally compared treatment groups for 1. 'within search' errors, 2. time taken to search between tokens and 3. heuristic strategy at the hardest levels, an estimate of which is obtained by the number of times participants begin a new search with the original search box, instead of starting with different boxes at the 6 and 8 boxes levels. Therefore, low scores indicate better strategy use. Higher scores correspond to poorer use of strategy. Efficient strategy reduces the workload posed on working memory. Mean token-search preparation time was calculated as the time required since a new search problem was presented on the screen until participants first touched the screen to open a box, for the first touch of each search problem, and as the time taken since a token was placed in the 'home' area until the next box was touched, for subsequent box touches within the same search. Lower scores were indicative of better task performance.

Participants

Overall sixty-one participants completed the spatial working memory task; thirty-one in the placebo group and thirty in the escitalopram group. Participants completed this task along with the additional cognitive tasks in a counterbalanced order.

Results

All participants in both groups reached the maximum number of problems that equals to 17 overall (including one demonstration and one practice trial). Repeated measures ANOVA showed a significant effect of the total number of boxes on 'between search' errors made in both groups, $p< .001$, $F (4,54)= 38,849$, partial $\eta^2= .775$. Participants made more errors as the number of boxes presented on the screen increased. We found no interaction between number of boxes and treatment group on the number of 'between search' errors made, $p= .912$. The escitalopram group made more 'between-search' errors compared to placebo but this did not reach statistical significance, $p=.398$. Similarly, we found that participants in both groups made more 'within search' errors as the number of boxes increased, $p< .001$, $F (4,57)= 7.951$, partial $\eta^2= .358$. No interaction between number of boxes and treatment group was observed, $p= .689$. The escitalopram group made more 'within-search' errors in all searches but this did not reach statistical significance, $p= .583$. Mean scores of strategy did not differ between the two groups, $p= .182$.

The escitalopram group showed significantly higher mean token-search preparation latencies compared to placebo across all stages [treatment main effect; $p= .002$, $F (1)= 1507.6$, partial $\eta^2= .970$]. Participants in both groups showed significantly different mean token-search preparation time based on the number of boxes they were presented with, $p= .009$, $F (1)= 7.43$, partial $\eta^2= .137$. The escitalopram group required significantly more time during the searches presented with four [$p= .006$, partial $\eta^2= .152$], six [$p= .002$, partial $\eta^2= .193$], eight [$p= .012$, partial $\eta^2= .126$] and twelve boxes [$p= .032$, partial $\eta^2= .094$]. When presented with ten boxes, the escitalopram group again needed more time although this did not reach statistical significance, $p= .053$, partial $\eta^2= .077$. We repeated our analysis adding sex as between-subjects factor; we found a significant main treatment effect, $p= .022$, $F (1,58)= 5.564$, partial $\eta^2= .088$, but no interaction between treatment and gender, $p> .05$.

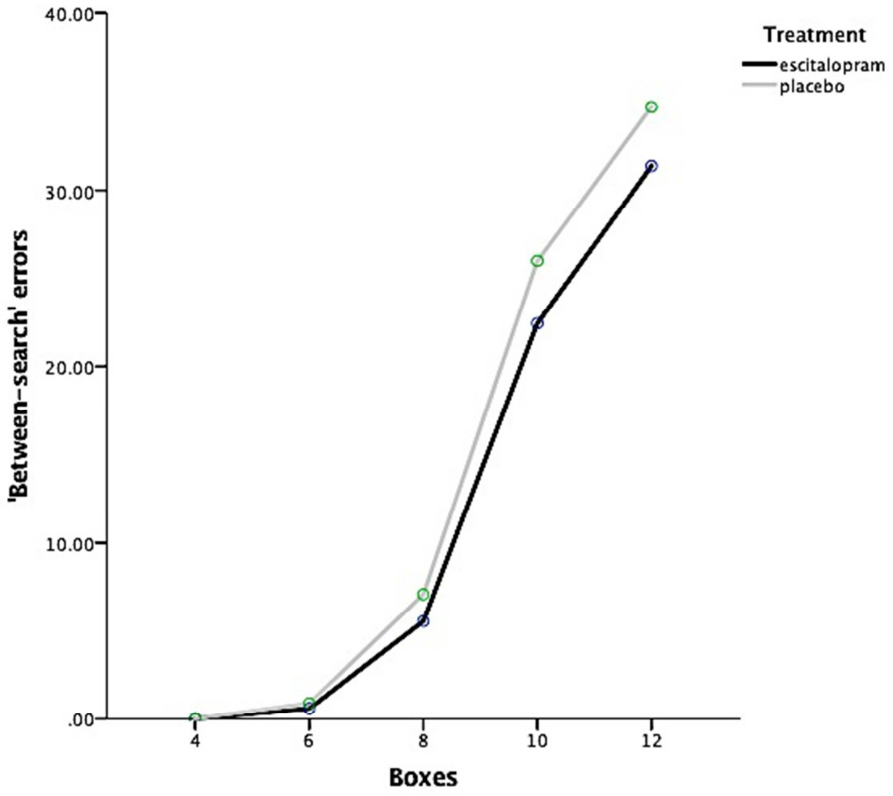


Figure 2: 'Between-search' errors in escitalopram and placebo groups

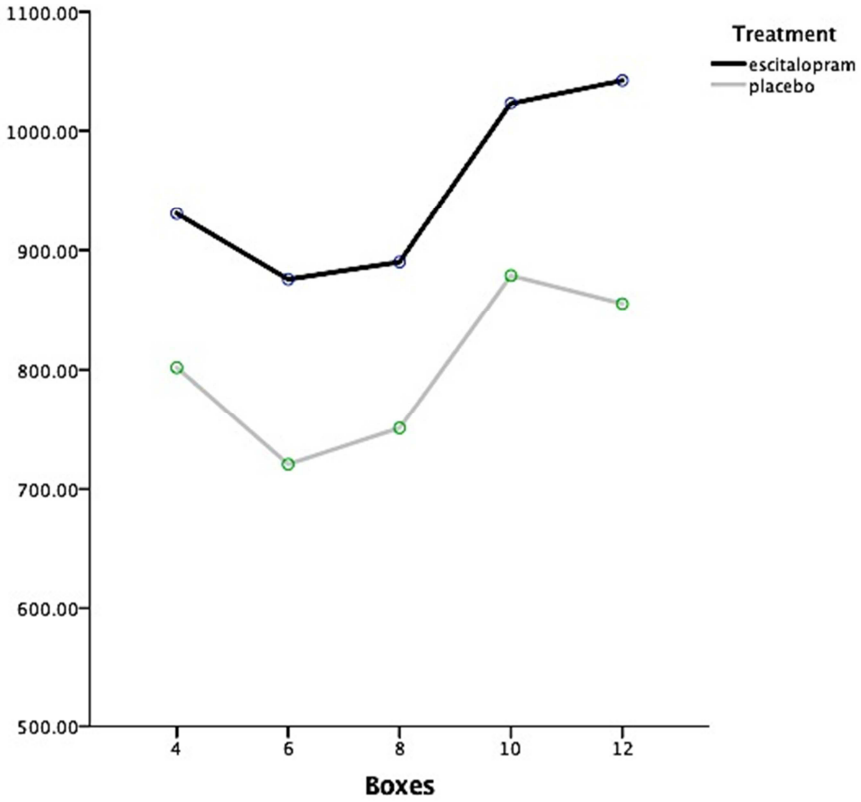


Figure 3: Mean overall token-search preparation time during the searches with 4, 6, 8, 10 and 12 boxes presented

2. The CANTAB Paired Associates Learning task

Background

Conditional associative learning is impaired in frontal lobe damage (Petrides, 1985b) and temporal lobe damage and patients having undertaken unilateral amygdalo-hippocampectomy (Owen et al., 1995) and in primates with frontal lesions (Petrides, 1982). Patients with idiopathic Parkinson's disease and patients with dementia of Alzheimer's disease type show severe impairment in a conditional associative learning task (Sahakian et al., 1988). Impairment in the CANTAB paired associates learning task (PAL) was reported in mild Alzheimer's disease and baseline performance in a subgroup of patients with mild cognitive impairment ('questionable dementia') was similar to Alzheimer's disease patients (Swainson et al., 2001) and correlated with subsequent global cognitive decline thus adding to the task's predictive value in early stages of dementia (Fowler et al., 1995). Impaired performance in the PAL task was found in bipolar patients, compared to controls, with an additional sex specificity as males performed worse than females (Sweeney et al., 2000). In the same study, a correlation of psychosis ratings, as measured with the scale for the assessment of positive symptoms, with number of errors performed was observed in unipolar depressed patients. Deficits in the PAL task were reported in depressed elderly individuals (Beats et al., 1996), which improved during patient recovery (Abas et al., 1990). Nevertheless, a study in outpatient depressed younger adults showed no difference between patient and control groups in performance in the PAL task although did show deficits in the Wisconsin card sort test, another test measuring executive function (Grant et al., 2001). Moderate significant impairment in the PAL and other tasks measuring executive functions are reported in a meta-analysis by Rock et al. (2013). Obsessive-compulsive disorder patients showed impaired performance in all the outcome measures of the task, which was more prominent in the most demanding stages (Morein-Zamir et al., 2010). The researchers attributed this deficit to non-spatial associative learning function (Murphy et al., 2004) rather than visuo-spatial or memory difficulties as no group difference was observed in the CANTAB spatial working memory task. Performance on the PAL task is mediated by frontal and medial temporal lobes (Owen et al., 1995) but parietal involvement may also be present (Morein-Zamir et al., 2010).

The involvement of dopaminergic neurotransmission in associative learning is well established (Robbins et al., 2010; Schultz et al., 1997). Acute administration of sulpiride (a

D2 receptor antagonist) though in healthy volunteers in variable dosages (200 and 400mg) did not affect performance on the CANTAB paired associates learning task (Mehta et al., 1999). A subsequent rat study showed impaired performance following administration of raclopride (a D2 receptor antagonist) (von Huben et al., 2006). Administration of a single dose of pramipexole (a D_2/D_3 agonist) in OCD and placebo volunteers produced deficits in both groups, whereas administration of amisulpride (a D_2/D_3 antagonist) produced no effect (Morein-Zamir et al., 2010). Impaired performance in the task is reported following scopolamine and dicyclomine administration in rats, whereas donepezil (a reversible cholinesterase inhibitor) facilitated rat performance (Bartko et al., 2010). Additionally, Morein-Zamir et al. (2010) found that OCD patients showed impaired performance in the PAL task compared to placebo further aggravated by administration of amisulpride as mentioned above and accompanied by deficient strategy in the SWM task.

Previous study showed the CANTAB paired associates learning task to be sensitive to serotonergic manipulations; ATD in healthy volunteers produced increased number of errors and significantly higher number of trials required to learn the visuo-spatial associations. No effect was observed on the "memory score" (Park et al., 1994) consistent with previously shown lack of effect on episodic and semantic memory after administration of the serotonergic agonist m-chlorophenylpiperazine (mCPP) in healthy older adults (Lawlor et al., 1989).

Task description

This task (Sahakian et al., 1988) involves visual pattern visuo-spatial memory and learning (Park et al., 1994) during which, participants learn the location of presentation of abstract patterns. White boxes are displayed on the screen and open one at a time in a randomised order. One or more boxes will contain a pattern. The patterns are then displayed in the middle of the screen, one at a time, and the subject must indicate in which box the pattern was originally located by touching the appropriate box. If the participant makes an error, the patterns are presented again as a reminder of their locations. The participant proceeds to the following stage once all the locations are correctly pointed. The task consists of a number of stages, which the participant must complete in order. If the participant cannot correctly complete one stage, then the test terminates. There are several modes of the task. We applied the high functioning mode as our sample involved healthy volunteers (www.cambridgecognition.com, Manual version 5.0.0, 2012). This mode consists of six stages with different number of patterns and boxes. The first stage is not assessed (practice

stage) and consists of 3 patterns and 6 boxes. The second stage consists of 3 patterns and 6 boxes, the third stage consists of 6 patterns and 6 boxes, the fourth stage consists of 8 patterns and 8 boxes, the fifth stage consists of 10 patterns and 10 boxes and the sixth stage consists of 12 patterns and 12 boxes. Stages 2-6 are assessed. Each stage can have up to 10 trials/ attempts overall which include the first presentation of all shapes and 9 repeat presentations. Participants are instructed when the start screen is displayed that they will initially see six white boxes which will open up in a random order and that a pattern will be shown in three of the boxes. They are instructed that they need to remember which pattern is located in which box and if they make any mistake the boxes will open up again to remind them of where the patterns were. Detailed instructions, as administered to participants, are listed in **Appendix**. Task duration was twelve minutes. During the task, an error was coded when participants touched a box that did not contain the pattern.

Primary outcome measure was the number of errors made across all assessed problems and all stages; this was adjusted for each stage not attempted due to a previous failure by summing the number of patterns not attempted and subtracting the number of patterns divided by the number of boxes from it. The result was then multiplied by the number of trials allowed for the stage. For the aborted runs, the adjustment was based on the stages, trials and responses not attempted due to the abort, with each missed response giving rise to an adjustment of 1 – 1 / number of boxes.

We also measured the total number of trials required to correctly locate all the patterns in all stages, the number of stages completed indicating the participants' overall success, and the number of stages completed on the first trial. We further calculated mean trials to success, which was the total number of trials required (maximum score = 10 trials per stage) to locate all the patterns correctly in all stages attempted divided by the number of successfully completed stages.

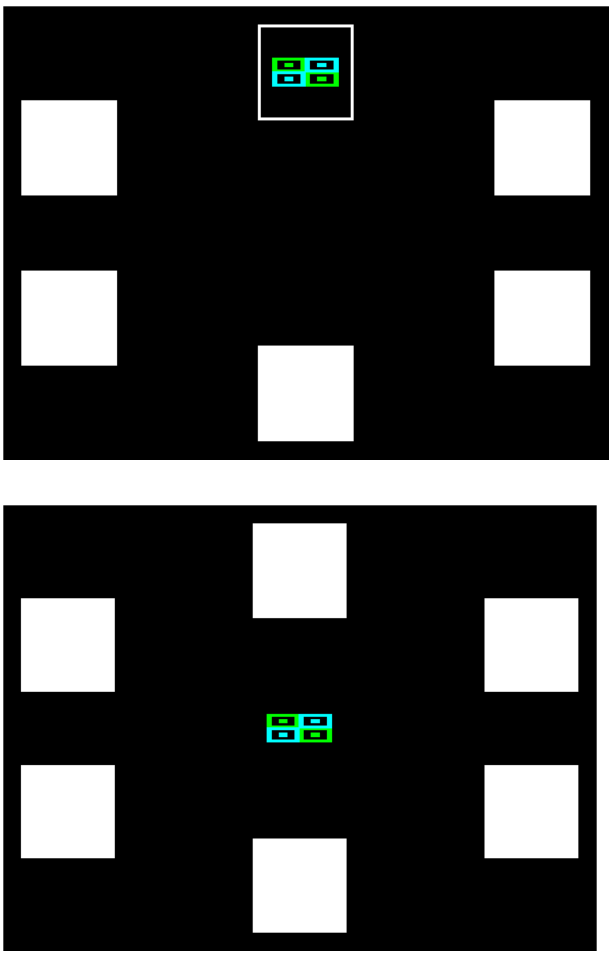


Figure 4: The test screen with one pattern shown in an open box (a) and the pattern shown in the middle of the screen (b)

Participants

Overall fifty-nine participants completed the paired associates learning task; thirty in the placebo group and twenty-nine in the escitalopram group. Participants completed this task along with the additional cognitive tasks in a counterbalanced order.

Hypothesis

We predicted that escitalopram-treated participants would exhibit superior performance in the PAL test.

Results

Repeated measures ANOVA showed a significant effect of number of patterns on total errors made, $p < .001$, $F(4,54) = 10.296$, partial $\eta^2 = .433$, but no difference between treatment groups between number of patterns and treatment groups, $p = .335$.

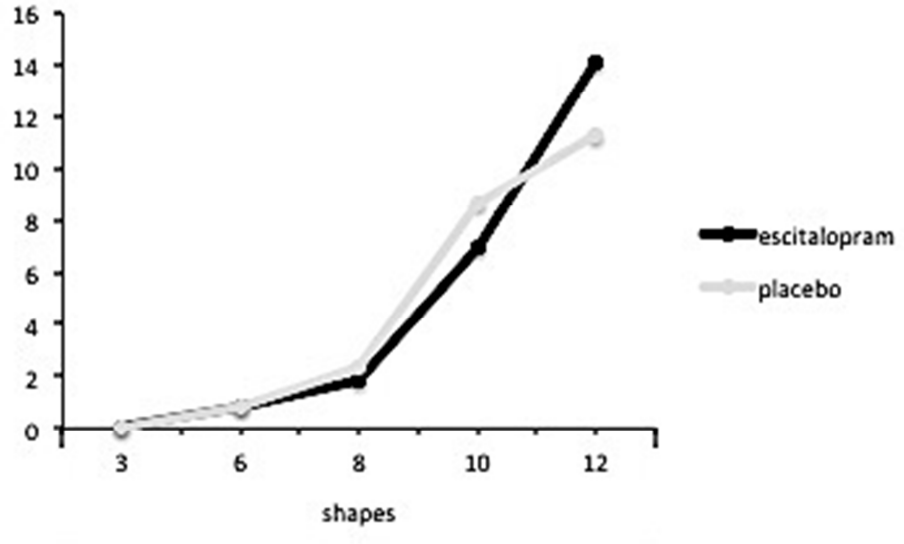


Figure 5: Total errors (adjusted) made in stages 2-6 with 3, 6, 8, 10 and 12 patterns respectively

Treatment groups did not significantly differ in the number of total trials required, U=415.5, z=-.299, p=.765, the number of stages completed, U=449.5, z=.577, p= .564 and the mean number of trials to success, U=415.5, z=-.299, p=.765. Participants in the two groups did not differ in the number of stages completed in the first trial, U= 475, z=.636, p=.525, and the first trial memory score, U=411, z=-.365, p=.715. We found no interaction between treatment and sex for the above measures, p> .05 for all.

3. The CANTAB Attention switching task

Task description

This task assesses cued attention set shifting. On each trial, an arrow appears either on the right or on the left hand side of the screen. A cue is presented on the top of the screen indicating whether participants need to indicate the direction or the side of the screen where the arrow appeared. For a number of trials, the side and the direction of the arrow are incongruent, for example, an arrow appearing on the right hand side of the screen is facing towards the left side (pictured below). Task duration is six minutes. Participants make their responses by touching two buttons, facing rightwards and leftwards, on the screen using their forefingers. Participants need to position their hands in order to move

their forefingers a short distance to touch the screen. Participants received progressive training (stages 1-4) to respond to the side and direction of the arrow. Only the last stage (stage 5) was assessed. Feedback was provided in practice stages but not in the assessed one. Participants are prompted by the experimenter to make a response based on the cue they receive, indicating the side or the direction of the arrow. Detailed instructions are listed in **Appendix**.

The primary outcome measure was the total number of commission errors, i.e. when participants responded too soon either prior to the end of the pre-empt window or before the stimulus appears. We additionally measured 1. the total number of omission errors where participants responded too late, i.e. after the end of the response window, 2. the percent commission trials, i.e. the percentage of trials where the outcome was a 'commission error', 3. the percent omission trials, i.e. the percentage of trials where the outcome was an 'omission error', 4. mean response latencies, measured from stimulus appearance until button press, 5. 'switch cost', i.e. calculated as the difference between the response latencies of non-switched versus switched assessed trials, and 6. 'congruency cost', i.e. calculated as the difference between the response latencies of congruent versus incongruent assessed trials.

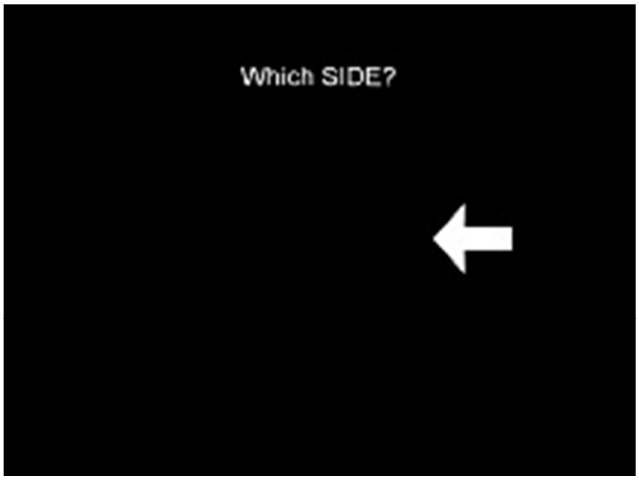


Figure 6: The task screen

Stage	Practice/ Assessed	Description
1	Practice	Arrow in centre of screen pointing to left or right. 8 trials. Subject must respond to DIRECTION of arrow. Feedback is provided.
2	Practice	Arrow on either side of screen pointing to left or right. 12 trials. Subject must respond to DIRECTION of arrow. Feedback is provided.
3	Practice	Arrow on either side of screen pointing to left or right. 16 trials. Subject must respond to which SIDE the arrow is on. Feedback is provided.
4	Practice	Arrow on either side of screen pointing to left or right. 8 trials. Subject must respond to SIDE or DIRECTION as cued. Feedback is provided.
5	Assessed	Arrow on either side of screen pointing to left or right. 160 trials. Subject must respond to SIDE or DIRECTION as cued. No feedback.

Table I: Structure of the Attention-switching task consisting of five stages

Participants

Overall fifty-nine participants completed the attention switching task, thirty in the placebo group and twenty-nine in the escitalopram group. Participants completed this task along with the additional cognitive tasks in a counterbalanced order.

Hypothesis

We predicted that escitalopram-treated participants would show better performance in the AST task.

Results

Data was square root transformed for normalising variance and reducing skewness as appropriate. Initially, we compared the groups for the total number of correct and incorrect trials and we found no significant difference [for correct trials, p= .359, t(58)= .924, and for incorrect trials, p=.515, t(58)=.655].

We found no significant difference between the treatment groups for our primary measure of commission errors, p= .527, t(58)=-.637. We also found comparable performance in terms of omission errors, p= .383, t(57)=-.880, and similar number of commission trials, p=.527, t(58)=-.637, and omission trials, p= .213, t(58)=-1.259.

Mean response latencies were comparable across the two groups, p= .915, t(58)=-.107. We further separately compared these for the congruent and incongruent trials. Repeated measures ANOVA showed that the type of trials (congruent or not) significantly affected latencies, as both groups showed higher response latencies in the incongruent trials and shorter latencies in the congruent trials, p< .001, F(1,58)=50.466, partial η^2= .465, but there was no interaction of trial congruency and treatment, p= .346, F(1,58)= .904. Participants did not differ in congruency cost, p= .256, and switch cost, p= .591.

We added sex as between-subjects factor in our primary analysis to examine if sex differences possibly mediate effects of escitalopram; we found no significant interaction between sex and treatment for commission errors, p> .05.

Conclusions

Contrary to our predictions, we found no effect of acute escitalopram administration on the three executive functions tasks from the well-established CANTAB battery. This lack of effect might be due to ceiling effects, as our sample consists of healthy, high-functioning volunteers thus it is possible that escitalopram administration would not produce any effect on these individuals. Interestingly, the only effect- in the opposite direction than expected- was higher mean token- search preparation times in the SWM task after escitalpram administration. The two groups did have comparable numbers of errors made thus it cannot be concluded that escitalopram administration impaired performance by increasing response latencies. It does point though towards a slight impairment – rather than improvement- of specific cognitive functions, but this needs to be interpreted along with the additional tasks.

II. Decision-making under uncertainty

The EMOTICOM Cambridge gambling task

Background

One of the most widely used tasks to study gambling behaviour is the Iowa Gambling Task (Bechara et al., 1994) where participants select among four separate decks of cards with various levels of rewards and punishments and gain or lose money. Optimal performance is selecting from the two "advantageous" decks (low reward and low punishment) compared to the two "disadvantageous" decks (high reward and high punishment) as this results in the long-term in a net gain, rather than a loss. Healthy volunteers initially choose from both decks but after a few trials, they select only from the advantageous one. Ventro-medial PFC lesion patients (Bechara et al., 2002; Fellows and Farah, 2005), pathological gamblers (Cavedini et al., 2002) and substance abuse patients (Bechara et al., 2001) are shown to persistently choose from the disadvantageous decks indicative of risky decision-making. Bechara et al. (2002) found that after a few trials, healthy subjects showed a stress response when choosing from the disadvantageous deck, as measured with skin conductance, in contrast to the patient group. This autonomous response was shown prior to cognitive appraisal thus leading to the development of the somatic marker hypothesis. Amygdala lesion patients showed similar performance and response in the gambling task (Bechara et al., 1999). It was suggested that both amygdala and ventro-medial PFC are implicated in decision-making process, with amygdala eliciting emotional/bodily responses to rewards and punishments whereas ventro-medial PFC mediating the representation of choices which influences subsequent choices in future options. Animal studies predominantly apply probability-discounting tasks, which measure the risk of lack of reward but not the risk of losing. Pathological gamblers show a hypoactive 5-HT system indicated by blunted prolactin response following intravenous administration of clomipramine (a tricyclic antidepressant) (Moreno et al., 1991) and oral administration of a serotonin $5HT_{2B/2C}$ agonist (Pallanti et al., 2006).

Serotonin and decision-making under uncertainty

Serotonergic and dopaminergic systems are implicated in gambling behaviour. Impaired performance in the Iowa gambling tasks is shown in healthy volunteers with the 5HTTLPR s-allele (Homberg et al., 2008). L-DOPA treatment in Parkinson's disease patients may

increase impulsive behaviour (Cools et al., 2003), possibly through modulating learning from appetitive but not aversive outcomes (Rutledge et al., 2009). SSRIs constitute a pharmacological management option for gambling disorders (Grant and Potenza, 2011; 2007). Additionally, we included the EMOTICOM cambridge gambling task in our test battery. The role of 5HT system in risky decision-making may be explained in terms of regulation of impulsive behaviours (Linnoila et al., 1983), enhanced stress (Meyer et al., 2004) and the role in aversive processing (Cools et al., 2008).

Rogers et al. (1999) showed that acute tryptophan depletion produced deficits in a probabilistic decision-making task; the effect was related to decision-making and not risk-taking. Depletion of central 5-HT levels following 5,7-dihydroxytryptamine injections in the rat DNR and MRN resulted in higher probability of choosing the immediate small reward more often than the delayed large one (Mobini et al., 2000). Critically, risky decision-making entails loss aversion, which is the tendency to avoid losses more than acquiring gains as it is suggested that losses provoke a psychological effect at least twice as great as equivalent gains (Kahneman and Tversky, 1979). Zeeb et al. (2009) addressed the lack of loss in rodent tasks by developing a rat gambling task with limited to time to select among four options associated with sugar pellets; reward is signalled by access to sugar pellet and loss by timeouts during which sugar pellets cannot be obtained. Zeeb et al. (2009) showed that administration of the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino)tetraline (8-OH-DPAT) increased choices of disadvantageous options; specifically, the rats chose showed impaired judgement of the probabilities and size of future rewards and punishments and subsequently the expected choice outcomes. Altered temporal judgements were not shown to underlie this effect, as options associated with shorter and longer punishing timeouts were chosen. Previous attempts were made to introduce loss to rat gambling tasks by adding quinine to reward pellets; this method though did not entail any risk of losing in the end of the task but rather a dissociation in the ratings of palatability among different rewards (van de Bos et al., 2006).

Task description

The task (Bland et al., 2016) included both reward and loss trials. During each trial, participants were presented with a roulette wheel; a proportion of the wheel was of purple colour and a proportion of orange colour. There were five different proportions ranging from very uncertain to very certain. Participants needed to place a bet on the expected outcome. A spinning point was displayed which stopped on one of the colours and

feedback was given to participants. The separate reward and loss conditions allowed for studying separately reward and punishment. Task duration was 10 minutes and the primary outcome measure was the risk adjustment score, calculated separately for reward and loss conditions as following:

Risk adjustment *= (2*bet at 90%) + (1*bet at 80%) + (0*bet at 70%) – (1*bet at 60%) – (2*bet at 50%)/ average bet*

Participants

Sixty-two participants completed the task, thirty in escitalopram and thirty-two in placebo group. Participants were instructed that the total amount displayed on the screen in the end of testing would be added to the points earned during the experimental day.

Results

We performed repeated measures ANOVA for reward and loss conditions on risk adjustment and we found a significant valence effect, $p < .001$, $F(1,60) = 7.115$; both groups showed lower risk adjustment for win compared to loss condition, but we found no treatment effect for either the win, $p = .585$, $F(1,61) = .302$, or the loss condition, $p = .352$, $F(1,61) = .881$. When comparing the mean response latencies for the win and loss conditions, we found a main effect of valence close to significance, with both groups showing higher response latencies for win compared to loss condition, $p = .098$, $F(1,60) = .626$, but no treatment effect in either condition, $p > .05$ for both.

Discussion

We applied well-established cognitive tasks on control for the effect of acute escitalopram administration on a large range of executive functions and how this effect might mediate the anticipated main effects of escitalopram on cognitive flexibility, probabilistic instrumental learning, response inhibition and emotional processing. We did not find any significant differences between the two treatment groups across the domains of working memory, risky decision-making and visual memory and new learning. Inconsistencies were previously reported in the literature across both learning and memory domains regarding the direction of effects of 5HT (Altman and Normille, 1988) attributed to the dosage and timing of agent used and type of memory and learning assessed (Harmer et al., 2002). Decreased serotonergic transmission was shown to lengthen thinking times in the Tower of

London planning task in subjects already familiar with the task- thus suggesting a retrieval deficit- but not affecting performance on the CANTAB SWM task (Park et al., 1994). ATD was also shown to affect long-term rather than short-term memory (Riedel et al., 1999) and delayed memory recall but not working memory performance (Harrison et al., 2004). Additionally, the effects of serotonergic manipulations show specificity for certain types of learning (Park et al., 1994) while leaving unaffected the mnemonic process involved in performing the PAL task, as per previous reports (Lawlor et al., 1989). These effects are reported following reduction of brain serotonin levels through ATD. Acute i.v. administration of 10mg of the SSRI citalopram enhanced delayed recall and recognition while leaving unaffected immediate recall on a verbal memory test (Harmer et al., 2002). Although in our study the escitalopram-treated group required more time to search for the token, their performance (in terms of between-search errors which is the primary outcome) was similar. There are two differences between our study and the Harmer et al. (2002) study; 1. different task applied i.e. our task was limited to spatial –not verbal- working memory with no component of long-term memory measured and 2. different manipulation applied as we used an oral dose vs the i.v. SSRI administration in the Harmer et al. (2002) study. Additionally we applied a higher dosage– as 20mg of escitalopram would be equivalent to citalopram 40mg (Höschl et al., 2008)- thus possibly our manipulation may have resulted in a net decrease, rather than increase, of brain 5HT levels through actions at auto-receptors on raphé 5-HT neurons (Blier et al., 1990). Additionally, sex-specificity of the effects of serotonergic manipulations were previously reported, as the Harmer et al. (2002) study showed effects on female participants; our sample was well-balanced in terms of sex (from overall number of 65 participants, 33 were males and 32 were females) and we found no interaction between treatment and gender replicated across several executive function tasks.

Chapter 7: Response inhibition

Background

Impulsive 'choice' (or 'waiting' impulsivity) and impulsive 'action' (or 'stopping' impulsivity) constitute two aspects of impulsive behaviour (Dalley et al., 2011). Among the tasks so far applied to study impulsive 'action', the continuous performance task (CPT) (Eagle et al., 2009) and the five-choice serial reaction time (5CSRT) task in rats are typically considered homologous with lack of correlation in performance reported between the continuous performance task and stop-signal task (Reynolds et al., 2008). Impulsive action is not a unitary construct. Schachar et al. (2007) described two distinct processes related to motor impulsivity, "action restraint" which refers to a response being triggered by a contextual cue and stopped before initiated and measured by Go/No-Go tasks (Casey et al., 1997), and "action cancellation" referring to inhibition of an already initiated action measured with Stop-signal paradigms (Logan et al., 1984). Eagle et al. (2009) showed tryptophan depletion differentially affected distinct forms of response inhibition by measuring stop-signal reaction time (SSRT) and a 'waiting' component using the Stop-signal task by placing the stop signal in the beginning of each stop trial. Rats had to withhold their response for an extended time period. Rats with forebrain 5HT depletion produced by intra-cerebroventricular 5,7-DHT administration showed a significantly reduced ability to wait, whereas no effect was produced by the lesion in the stop-signal reaction time thus pointing towards distinct roles of 5HT in various aspects of impulsive behaviour (Harrison et al., 1997; Eagle et al., 2008).

5HT and response inhibition

A crucial role for the serotoninergic system in behavioural inhibition has been well-documented (Soubrié, 1986) with low brain 5-HT levels being correlated with high levels of impulsivity (Dalley & Roiser, 2012; Walderhaug et al., 2002; Linnoila et al., 1983) as seen in several neuropsychiatric disorders, including depression and suicide ('impulsive aggression'), substance abuse and schizophrenia (Bourgeois, 1991; Millan, 2000; Mueck-Seler et al., 1996).

Several human studies have examined the effect of reduced central serotonergic transmission on response inhibition through tryptophan depletion on performance in Stop-signal and Go/No-Go tasks in healthy volunteers (Crockett et al., 2009; Cools et al., 2005;

Evers et al., 2006) and in animals applying the five-choice serial reaction time (5CSRT) task (Robbins et al., 1992). We will discuss these separately for the three tasks as they are not directly comparable in terms of underlying neural mechanisms (Eagle et al., 2009).

I. The human five-choice serial reaction time task

Background

The 5CSRT task was originally developed to investigate the deficits shown in children with attention-deficit/hyperactivity disorder (Carli et al., 1983) and it has been extensively used in rats to study attention and stimulus control through administration of several drugs and neurochemical lesions (Robbins et al., 2002). The task consists of five open apertures where a brief light stimulus is presented; rats need to make a nose-poke response in the aperture where the stimulus has just presented to obtain a primary reward (food pellet). If rats make a nose-poke response prior to stimulus presentation (within the inter-trial interval which lasts 5s), this constitutes a premature response. Correct responses are rewarded with food pellets, whereas premature responses, errors of commission (responding when stimulus has not presented) and errors of omission (not responding within a specific time limit when a stimulus has presented) are punished as a 'time-out' from positive reinforcement (e.g. with periods of darkness) and the initiation of a new trial. Correct responses assess integrity of spatial attention. Premature responses are indicative of 'waiting' impulsivity and they occur during the time period of reward anticipation and indicate failure to withhold a pre-potent action thus measuring impulsive action. Several behavioural manipulations are applied in the task (reviewed in detail in Robbins et al., 2002) including altering stimulus' brightness, introducing bursts of white noises at multiple time points during the inter-trial interval- in order to test selective attention to visual stimuli- and varying inter-trial intervals, including short and long sets (Robbins et al., 2002) thus altering stimulus predictability (Rogers et al., 2001). One of the rodent 5CSRT task features that enables its application in several pharmacological studies affecting distinct neurotransmitter systems and receptor types within each system is its stability in terms of baseline performance (Robbins et al., 2002) which reaches accuracy levels of at least 80%.

Brain areas implicated in the 5CSRT performance

Distinct cortico-striatal circuits and pharmacological manipulations, mainly targeting monoaminergic transmission, are shown in animal and human studies to differentially control distinct aspects of the task. Brain areas implicated in performance in the 5CSRT include primarily the anterior cortex and medial and lateral striatum (Robbins et al., 2002). Muir et al. (1996) showed that bilateral excite-toxic lesions of distinct rat PFC areas differentially affected task measures. Medial PFC (PFC) lesions negatively affected choice accuracy and resulted in increased response latencies for correct stimuli and perseverative responding (termed as repeated responding to the apertures after signalled food presentation). The effects were lasting as rats with mPFC lesions continued to show deficits in task performance when behavioural manipulations, including interpolated white noises prior to stimulus onset and change of stimulus duration (shorter), were introduced. In the same study, anterior cingulate cortex lesions increased premature responding. It is worth noting that similar but weaker effects to the ones produced by mPFC lesions by Muir et al. (1996) were previously reported by Zilles (1985) following excito-toxic lesions in the rat frontal area 2 (Fr2). Muir et al. (1996) also showed that lesions in the antero-dorsal frontal cortex affected task performance during behaviour manipulations (longer response latencies when stimulus duration was shorter and when interpolated white noises were present) but not in baseline. In the same study, parietal cortex lesions produced no effect. The mPFC expresses several 5HT receptor subtypes with a large density of $5HT_{1A}$ and $5HT_{2A}$ receptors (Barnes and Sharp, 1999).

Rogers et al. (2001) showed the involvement of distinct parts of the striatum in 5CSRT performance; lesions produced by quinolinic acid in medial striatum resulted in impaired choice accuracy, increased response latencies and increased premature and perseverative responding. These were distinct from effects following lateral striatum lesions where rat performance was “abolished” with a partial recovery during subsequent trials and rats were shown to “re-acquire” the task structure (Robbins et al., 2002). It is worth noting that the effects produced by medial striatal lesions appear of a broader range compared to corresponding cortical ones (Rogers et al. 2001) and projections from cortical areas contribute to control of these distinct aspects of task performance (Robbins et al., 2002). Also, these effects were not mediated by motivational deficits or motor impairments. Additional lesions in other brain areas are shown to impair performance in the 5CSRT task as rat medial dorsal thalamus lesions increase premature responding (Chudasama and Muir, 2001) during both baseline and unpredictable varying of inter-trial interval duration.

Lesions in anterior thalamic nuclei did not affect task performance, whereas pre-limbic cortex lesions increased perseverative responding (Chudasama and Muir, 2001). A study employing [^{14}C] deoxyglucose (DG) uptake showed lower neuronal activity in cingulate and ventro-lateral orbital cortices in low compared to high performing rats in the 5CSRT task (Barbelivien et al., 2001).

Neurotransmitter systems implicated in 'waiting' impulsivity

Systemic D-amphetamine administration increased premature responding in rats (Cole and Robbins, 1989; 1987) and impaired stimulus discrimination in rats with lesions in dorsal noradrenergic bundles (Cole and Robbins, 1987). These effects were mediated, to a large extent, by the dopaminergic system and partially, through an interaction of noraderenergic and dopaminergic systems (Robbins et al., 2002). D-amphetamine also affects latency for correct responses, as latency is increased after systemic administration and decreased after intra-cerebral (specifically intra-accumbens) administration (Robbins et al., 2002).

In humans, Morris et al. (2016) showed an association of premature responding in healthy volunteers with decreased connectivity of the sub thalamic nucleus with subgenual cingulate and ventral striatum. The researchers also showed distinct neural correlates for 'stopping' and 'waiting' impulsivity as measured with the stop-signal and the human 5CSRT task respectively in accordance to previous findings (Robinson et al., 2009). 'Stopping' impulsivity was associated with lower connectivity between hyper-direct projections of the right pre-supplementary motor area and the left sub-thalamic nucleus whereas 'waiting' impulsivity was associated with decreased connectivity between the sub thalamic nucleus and ventral striatum and subgenual cingulate cortex. Sub thalamic nucleus is a major area implicated in the indirect inhibitory pathway but also receives hyper-direct projections from cortical areas (Haynes and Haber, 2013).

The complex role of the serotonergic system in 'waiting' impulsivity

Manipulations of central 5HT levels show distinct effects on performance in the 5CSRT task depending on the method applied and area(s) affected. As an example, the neurotoxin para-chlorophenylalanine (which selectively and irreversibly inhibits tryptophan hyxroxylase involved in 5HT synthesis) decreased accuracy in the 5CSRT only when less intense stimuli were applied or during speeded rate of presentation (Jakala et al., 1992). Rat forebrain 5HT depletion increased premature responding in the 5CSRT task leaving unaffected accuracy of correct responses (Harrison et al., 1997a) and increased 'waiting'

impulsivity in the 'waiting' component of the Stop-signal task (Eagle et al., 2009), which resembled the behavioural manipulation of extended inter-trial interval previously applied in 5CSRT studies (Dalley et al., 2007; Robbins, 2002).

Administration of $5HT_{2A/2C}$ receptor agonists increased omissions in 5CSRT task (Carli and Samanin, 1992), which were reversed by ritanserin ($5HT_{2A/2C}$ receptor antagonist). Increased latencies for correct responses, without any effects on other measures, were observed after d-fenfluramine administration. Distinct serotonergic manipulations targeting distinct receptors show differential effects on 5CSRT performance. Carli and Samanin (2000) had previously reported that rats treated with systemic 8-OH-DPAT (a selective $5HT_{1A}$ agonist) showed reduced accuracy in the 5CSRT task, an effect mediated by activation of $5HT_{1A}$ auto-receptors specifically in the dorsal raphe nuclei. Additionally, rats treated with 8-OH-DPAT showed increased latencies for correct responses and more omission errors, an effect mediated by activation of post-synaptic $5HT_{1A}$ receptors. $5HT_1$ receptors are found predominantly post-synaptically in areas receiving innervation from the raphe nuclei (Barnes and Sharp, 1999) but are also located in pre-synaptic sites in the serotonergic neurons of the raphe nuclei (Winstanley et al., 2003).

Effects of direct mPFC administration of 5HT modulatory compounds

Modulation of the 5HT system within the mPFC through distinct receptor activation was also shown to differentially affect premature responding in the 5CSRT task (Winstanley et al., 2003). It is worth noting that direct mPFC administration of a selective $5HT\text{-}_{2A}$ antagonist improved accuracy and reduced high levels of premature responding, the latter possibly mediated by a reduction in the tonic activation of $5\text{-}HT_{2A}$ receptors in the mPFC. Indeed, previous studies had also shown distinct effects on the 5CSRT task depending on the type of 5HT receptor targeted as both systemic administration and direct infusion in the mPFC of the $5HT_{2A/2C}$ receptor antagonist ketanserin reduced premature responding (Passetti et al. 2003; Ruotsalainen et al. 1997) and in contrast, systemic administration of a $5HT_{2A/2C}$ receptor agonist increased premature responding (Koskinen and Sirvio, 2001; Koskinen et al., 2000). Carli et al. (2006) also reported distinct effects of direct mPFC infusions of the $5HT_{1A}$ receptor agonist 8-OH-DPAT and a selective $5HT_{2A}$ receptor antagonist on premature responding.

Distinct raphé nuclei lesions produce differential effects in the 5CSRT task

Infusion of the 5,7-DHT in both the MRN and DRN resulted in a cortical 5HT concentration decrease of 60% with the MRN lesions further depleting hippocampal 5HT and DRN lesions depleting 5HT in the nucleus accumbens and caudate-putamen (Harrison et al., 1997b). DRN lesion rats showed increased accuracy for correct trials, lower number of omitted trials and increased premature responding. Increased accuracy could be mediated either through effect on 5-HT receptors (Blue et al., 1988) or through interaction with the cholinergic system (Harrison et al., 1997b). MRN-lesioned rats showed increased latencies to collect the food rewards and increased premature responding without any effect on latencies for correct responses. Faster response latencies were indicative of increased motivation, similar to effect seen after d-fenfluramine administration (Carli and Samanin, 1992), but this was not supported by the duration of these effects in the two behavioural measures. When stimulus duration was shortened from 0.5s to 0.15s, DRN lesion rats showed reduced accuracy and increased latencies for correct responses with no effect on premature responding. In contrast, rats in the MRN group showed reduced accuracy and increased latencies for correct responses, increased number of omitted trials and increased premature responding (Harrison et al., 1997b). Prior to administration of the neurotoxin, rats were pre-treated with appropriate compounds (desipramine and nomifensine maleate) to block the neurotoxin uptake into noradrenergic and dopaminergic neurons respectively thus the effects could not be directly mediated by these two systems.

These results from selective DRN and MRN lesions along with the results from selective 5HT forebrain lesions point towards increased premature responding being mediated by the inhibitory effect of DRN projections to the striatum and nucleus accumbens (Harrison et al., 1997b). DRN serotonergic neurons are indeed implicated in impulsivity with activation of these neurons promoting patience for delayed rewards (Miyazaki et al., 2014; 2012) possibly by encoding an anticipatory signal for rewards in a larger time scale (Doya, 2002).

Does serotonergic system directly affect 'waiting' impulsivity?

The observed increased premature responding after forebrain 5HT depletion was suggested to be possibly attributed to the removal of an inhibitory control on dopaminergic neurotransmission. Thus increased premature responding in lesioned rats may reflect a lack of inhibiting motor responses (Soubrie, 1986) supported by the findings of increased premature responding following D-amphetamine administration (Cole and Robbins, 1987).

Nevertheless, D-amphetamine administered in rats post-operatively in the Harrison et al. (1997a) study did not show a synergistic action with 5HT depletion as in higher d-amphetamine doses, lesioned rats made less premature responses compared to the sham-operated group. Lesioned rats also omitted fewer trials in baseline and showed lower accuracy when interpolated bursts of white noise presented the presentation of the visual target; the latter can be attributed to inability to ignore distracting, irrelevant stimuli in lesioned rats. Harrison et al. (1997a) also showed increased omissions and longer trials following separate administration of D_1 and D_2 receptor antagonists, in accordance to previously reported effects after a-flupenthixol (combined D_1 and D_2 receptor antagonist) administration (Cole and Robbins, 1987). These results point towards differential effect of distinct DA receptors on striatal systems that in turn interact with distinct ascending 5-HT projections and thus possibly affecting distinct aspects of manifested impulsivity (Harrison et al., 1997a).

Another possible explanation could relate to the inverted U-shaped relationship of arousal and efficiency of performance (Hebb, 1955), which has been well-described for the case of dopaminergic transmission (Cools and D'Esposito, 2008). In support of this, in the Harrison et al. (1997a) study, no effect was observed on accuracy when the stimulus' intensity was reduced.

The human analogue of the five-choice serial reaction time task

Voon et al. (2014) developed the four-choice serial reaction time task, a human version of the rodent 5CSRT task and showed that alcohol-and methamphetamine-dependent volunteers and cannabis users made more premature responses compared to healthy controls supporting previous findings of high impulsive rats that subsequently compulsively self-administered cocaine (Belin et al., 2008). Voon et al. (2014) also assessed obese individuals with and without binge eating who did not show any difference in premature responding compared to controls. Acute methylphenidate administration in healthy participants also increased premature responding in the human analogue of the 5CSRT task (Voon et al., 2015). Young binge drinkers showed increased premature responding in the human 5CSRT task compared to healthy controls (Morris et al., 2016) possibly mediated by decreased connectivity between the sub thalamic nucleus and the subgenual cingulate and inferior parietal cortex.

As the findings from the aforementioned animal studies illustrate, the role of serotonergic transmission in mediating distinct aspects of impulsivity is far from clear. Worbe et al.

(2014) showed that ATD increases premature responding in healthy volunteers using the human version of the 5CSRT task. This effect was accompanied by increased accuracy and higher motivation index. These effects are similar to selective raphe nucleus lesions in rats but in contrast with the findings from global central 5HT depletion which can be attributed to task differences and wider extent of 5HT depletion produced in rats using the neurotoxin 5,7-DHT compared to the extent of depletion using ATD in humans (Worbe et al., 2014). The researchers also found no effect on delayed reward discounting measured with the 27-item monetary choice questionnaire (Kirby et al., 1999) prior and after the dietary procedure, thus providing further evidence of distinct underlying neural mechanisms of impulsive 'action' and 'choice'. Nevertheless, a clear distinction on how these two constructs were measured in the study is noted by the researchers (behavioural task for impulsive 'action' and self-administered questionnaire for impulsive 'choice', Worbe et al. 2014). A study in rats though had assessed the effect of citalopram (Baarendse and Vanderschuren, 2012) on the 5CSRT task and the delayed reward task (measuring impulsive 'action' and 'choice' respectively) showing reduced premature responding with an intermediate citalopram dosage (1.0 mg/kg) with no effect on accuracy, omission errors and perseverative responses. The researchers also applied longer inter-trial intervals in the 5CSRT and citalopram reduced premature responses in this condition.

Task description

The task (Voon et al., 2014) was programmed in Visual Basic using Visual Studio 2005 and Microsoft.NET Framework 2.0. Participants were seated in front of a touch screen computer (which was used to perform the additional cognitive tasks in a counter-balanced order) and a keyboard was located in an appropriate distance between them and the computer (approximately 20 cm), which was held constant during performing the task. Participants were asked to press and hold down the space bar using the index finger of their dominant hand. Pressing the space bar indicated the 'cue onset' and four boxes were shown on the screen. Following a random cue-target interval (2-10s), a green circle target appeared for a brief time period (32-64ms) inside one of the boxes. Participants released the space bar and touched the box where the target had appeared. They were requested to respond as quickly as possible and were trained to do so by receiving points as feedback which prompted them to respond more quickly, rather than accurately; this design aided towards creating 'false' alarms when participants released the space bar prematurely as previously described by Worbe et al. (2014; supplementary information). The task differs

from the previous CANTAB 5CSRT task (Sahakian et al., 1993) in two features; 1. response spaces were smaller (3x3 cm) and arranged in an arc in one section of the screen with each response space being 3cm apart from the other one, similar to the rodent task, and visual stimuli were presented more rapidly thus making the task more demanding, and 2. responses were prompted to be quick rather than accurate, thus specifically measuring premature responses.

Task structure

The task consisted of two baseline blocks of 20 trials each, the first in the beginning of the task and the second one after the first test block, where participants received no monetary feedback, and four test blocks of 40 trials each, where they received monetary feedback. As per previous study (Worbe et al., 2014), blocks with reduced target time, variable target intervals and distractor were applied to probe premature responding (Robbins, 2002). The final 10 trials from the baseline blocks were used to calculate respective mean reaction time (RT) and standard deviation (SD) for individualized feedback and incentivize quicker responses in the subsequent test blocks. During the test blocks, participants were prompted to respond as fast as possible. Experimenters prompted participants that they would not lose points for being inaccurate and they would win points for responses and more points for responding fast thus to try and be fast rather than accurate. When participants did not respond, they received feedback of losing points. When participants responded prematurely, they continued with touching the screen to complete the trial and they did not gain or lose any points. Total points were displayed when feedback was provided. Total task duration was twenty minutes. Participants were reimbursed with a fixed amount of money for their overall participation during testing day and they were incentivized in each individual task by the researchers to try and perform as best as possible as an additional amount would be added based on task performance.

Contrary to previous study (Worbe et al., 2014), we applied two additional test blocks thus extending the overall number of test blocks from four to six. The test blocks differed in stimulus duration, cue-target interval and the presence of distractors with the last two blocks being more challenging in performance.

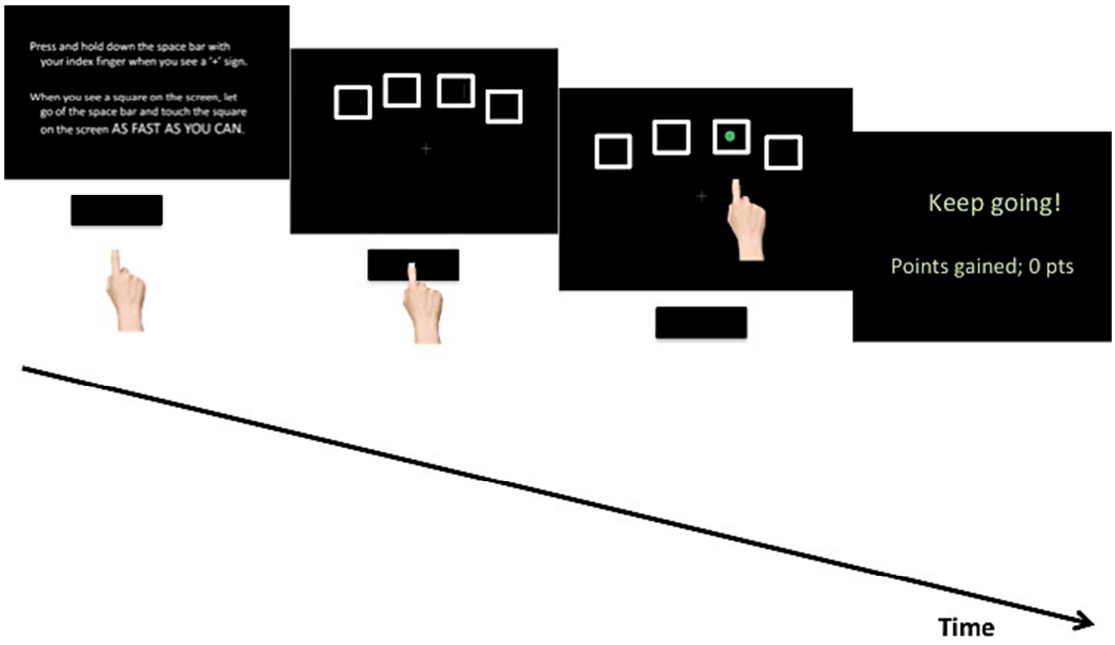


Figure 1: Structure of the human four choice serial reaction time task

Participants

Overall sixty-three participants completed the task; thirty-two in the placebo group and thirty-one in the escitalopram group. Participants completed this task along with the additional cognitive tasks in a counterbalanced order.

Task measures

As per previous study (Worbe et al., 2014), the primary outcome measure was premature responding, termed as the premature release of space bar prior to target onset, during the first four test blocks. We further calculated total points during the first four blocks, mean reaction times in baseline and first four testing blocks and accuracy and late responses (response time more than 1.5 SD) during the first four blocks. Accuracy was measured as the ratio of correct responses divided by the sum of correct and incorrect responses, where incorrect responses consisted of trials where participants touched the incorrect box after target onset. Motivation index measured response to reward feedback (points gained) and it was calculated as the ration of (mean RT during baseline block 1 – mean RT during baseline block 2) to (mean RT during baseline block 1 + mean RT during baseline block 2). Baseline blocks did not include any feedback on total points, but had otherwise similar

structure to test blocks. We additionally calculated premature responding, mean reaction times, accuracy and late responses for the two additional test blocks.

Hypothesis

We predicted that acute escitalopram administration would reduce premature responding in the 4CSRT task. We hypothesized this effect would not be accompanied by any changes in accuracy or motivational index.

Results

Data was analysed with independent samples t-test and repeated measures ANOVA, as appropriate. Four subjects, two in placebo and two in escitalopram group, produced incomplete data files containing the two baseline blocks and test block 1. Therefore, data from these four subjects were included solely in the analysis of motivational index. The following results refer to thirty (30) participants in the placebo group and twenty-nine (29) participants in the escitalopram group.

1. Premature responding: participants did not differ in premature responding, the primary task outcome, across the four test blocks, p= .905 [following square root transformation for reducing variance and normalising skewness]. We further compared premature responding across all six blocks and we found no treatment effect, p= .711.

2. Response latencies and motivational index: participants in both treatment groups showed significantly higher response latencies during baseline block 1 compared to baseline block 2, p<.001, F(1,61)= 19.381, partial η^2= .241, but there was no interaction between block order and treatment, p= .244. Treatment did not affect motivational index, p= .262, t(61)= -1.133. Response latencies for correct trials during test block 1 did not differ between treatment groups, p= .360, t(61)= .923.

3. Accuracy: across all six blocks, mean accuracy reached 89% for the escitalopram group and 92% for the placebo group, but the difference was close to significance, p= .077, t(57)= 1.801. We further compared accuracy in the first four test blocks and we found no significant treatment effect, p= .672, t(57)= -.425. We separately compared accuracy in blocks 5 and 6 as these were novel stages added in this study to test for the effect of additional task behavioural manipulations but we found no difference between treatment groups [for block 5, p= .11, and for block 6, p= .171].

4. Other measures: no group difference was shown for late responses, p= .613, or total points gained, p= .80, t(57)= -.254. In the last two blocks, participants did not differ in

premature responding [block 5; p= .298, t(57)= -1.051, block 6; p= .174, t(57)= 1.378] or accuracy [block 5; p= .11, t(57)= -1.625, block 6; p= .171, t(57)= -1.385].

Measures	Placebo	Escitalopram	Group difference*
RT Baseline block 1	554.99 (363.39)	507.26 (191.08)	n.s.
RT Baseline block 2	383.48 (125.39)	408.14 (150.67)	n.s.
Motivational index	0.15 (0.16)	0.10 (0.13)	n.s.
RT test block 1	469 (45)	508.67 (40.63)	n.s.
Accuracy			
Blocks 1-4	100%	90%	n.s.
Blocks 1-6	92%	89%	n.s.
Premature responding			
Blocks 1-4	11 (2)	10.1 (1.24)	n.s.
Block 5	3 (1)	2.27 (0.28)	n.s.
Block 6	1	2.13 (0.41)	
Late responses	0.09	0.07	n.s.
Total points	1986.7	1910.83	n.s.

Table 1: The two treatment groups did not differ in any task measure, Mean (S.E.M), n.s.= not significant, p> .05

Discounting rate k values for small, medium and large rewards representing impulsive choice (Table I) were added as covariates. After adjusting for k values, we found no treatment effect on motivational index (p> .05 for all three k values) or premature responding (p> .05).

k value	Escitalopram	Placebo	Group difference*
Small	0.03 (0.06)	0.02 (0.04)	n.s.
Medium	0.02 (0.05)	0.01 (0.02)	n.s.
Large	0.02 (0.05)	0.01 (0.02)	n.s.

Table 2: Discounting rate (k) results in the 27-item monetary choice questionnaire. Mean (SD)**,** n.s.= not significant, p>0.05

We found no treatment effect in motivational index, after adjusting for BIS 2nd motor factor, p= .290, F(1,60)= 1.138 and trait anxiety scores, p= .278, F(1,60)= 1.199.

Measure	Escitalopram	Placebo	Group difference*
Barratt Impulsiveness scale Second-order motor factor	26.9 (3.5)	27.5 (3.5)	n.s.
STAI trait	34.4 (9)	35 (8.7)	n.s.

Table 3: STAI trait anxiety and BIS scores, Mean (SD), n.s.= not significant, p>0.05

II. The interleaved Stop-signal, No-go trials task

Background

Stop-signal and Go/No-Go paradigms have been extensively applied to measure the ability to withhold a pre-potent action (Bari and Robbins, 2013). No-Go requires action selection and withhold mechanisms in order to inhibit a pre-potent action and Stop-signal indicates the cancellation of a response that has already been initiated by a previous cue (followed by a short delay period of approximately 200-300 milliseconds).

In both tasks, "Go" response, either as a key/lever press or touch-screen response to a visual stimulus, is common. Stop-signal reaction time (SSRT), which is the time taken to cancel an already initiated action and measured with the 'race' model initially developed by Logan (1994), is the key measurement in Stop-signal tasks. According to this model, response inhibition depends on a competition between Go and Stop processes resulting in response happening when Go wins and response being suppressed when Stop wins. Depending on the time-point when the stop signal is placed during the go process (the Stop-signal delay), the competition can be biased in favour of either of the two processes; if placed early enough, then the response will be inhibited every time whereas if placed towards completion of the Go process then the response will never be inhibited. As explained in depth in Eagle and Robbins (2003), an inhibition function can be plotted using the stop-signal delay and the probability of inhibition. SSRT can then be calculated based on this inhibition function. It is worth noting that the Stop signal task would resemble the Go/No-Go task for a Stop-signal delay of 0s, thus if a Stop trial was equivalent to a No-Go trial.

Cross-species application of the Go/No-Go and Stop-signal tasks

The tasks carry a translational significance as they have been used in both human and animal studies (Schall et al., 2002) with fairly similar format thus allowing for a cross-species comparability (Eagle and Robbins, 2003). The tasks are used, often interchangeably, to study deficient action inhibition exhibited in several neuropsychiatric disorders, including ADHD (Rubia, 2002; Oosterlaan et al., 1998), schizophrenia (Rubia, 2002), obsessive-compulsive disorder (Verbruggen and Logan, 2008), Parkinson's disease (Rae et al., 2016; Ye et al., 2014) and damage to the right inferior frontal gyrus (Aron et al., 2004). The tasks were previously shown sensitive to pharmacological manipulations with methylphenidate (Stop-signal task; Tannock et al., 1989), amphetamine (Stop-signal task; de Wit et al., 2000, lesions of medial striatum in rats combined with subsequent treatment with d-amphetamine; Eagle and Robbins 2003, Stop-change version of the task in rats; Feola et al., 2000), DA (Go/No-Go task, Guitart-Masip et al., 2014), ATD (Go/No-Go task, Crockett et al., 2009) and citalopram (Interleaved Stop-signal and No-Go trials task, Ye et al., 2014; Stop-signal task, Chamberlain et al., 2006; Go/No-Go task, Guitart-Masip et al., 2014).

Distinct brain areas are implicated in Stop-signal and No-Go processes

The two forms of response inhibition discussed have distinct underlying neural mechanisms (Eagle and Robbins, 2003) while both involving abnormalities in fronto-striatal loops (Rae et al., 2014; Robbins, 2007; Rubia et al., 2005). Traditionally, impulsivity has been linked to dopaminergic loading (Rowe et al., 2008). People characterized as impulsive show higher SSRT in Stop-signal paradigms (Logan et al., 1997).

Stop-signal process is mediated by the right inferior frontal cortex and associated with subcortical connections (Rubia et al., 2003; Eagle et al. 2008). Chamberlain et al. (2006) showed that administration of the selective norepinephrine inhibitor atomoxetine in healthy volunteers reduced SSRT. No effect was reported on response time for Go trials, thus this effect was not mediated by "speeding" reaction times overall. During a Go/No-Go task, distinct brain areas were activated during error processing (rostal anterior cingulate and posterior cingulate, left and right anterior insular cortex) and during response selection and inhibition in No-Go trials (dorsolateral PFC and inferior frontal cortex) (Menon et al., 2000) and the anterior part of the supplementary motor area (Simmonds et al., 2008).

5HT transmission in action restraint

Serotonergic transmission is shown to exert effect on action restraint measured with Go/No-Go tasks. The orbitofrontal cortex in rats and inferior frontal cortex in humans (Aron et al., 2004) are the main brain areas implicated in the effects of serotonergic manipulations on action restraint with diminished right inferior prefrontal activation during the No-Go condition following ATD (Rubia et al., 2005) and enhanced activation of the lateral orbitofrontal cortex during the No-Go condition following citalopram administration (Del-Ben et al., 2005). Animals showed inability to withhold responding during No-Go trials (Harrison et al., 1999), following selective forebrain depletion of 5HT levels with intracerebroventricular 5,7-DHT administration with reported affected areas including the neo-cortex and striatum (as DRN projection areas) and hippocampus (as MRN projection area). The lesioned rats were unimpaired in responding towards go trials compared to sham controls. Lack of reward delivery after commission errors (inappropriate responding to No-Go stimuli) viewed as 'aversive state' could possibly account for this effect (Gray and McNaughton, 2000). Impaired acquisition of a Go/No-Go task following regional 5HT depletion with the administration of parachloroamphetamine was also separately observed (Masaki et al., 2006).

Crockett et al. (2009) showed that ATD abolished punishment-induced inhibition in an orthogonal Go/No-Go task design shown by speeded responding on punished Go trials. This was not attributed to general motor inhibition as no effect was observed on commission errors (inappropriate responding to No-Go stimuli). Lack of effect of altered 5HT transmission on commission errors was also shown in other studies (Evers et al., 2006; Rubia et al., 2005; Del-Ben et al., 2005) although one study found increased commission errors in a Go/No-Go task after ATD in males with a history of alcoholism (LeMarquand et al., 1999). Interestingly, several of these studies showed effects on BOLD response during No-Go responding but no effect on the behavioural measures. Del-Ben et al. (2005) showed enhanced activation of the lateral orbitofrontal cortex following citalopram intravenous (i.v.) 7.5mg administration but no behavioural effect. Macoveanu et al. (2013) showed change in BOLD activation of right inferior frontal gyrus during No-Go responding following ATD but no significant effects on behavioural measures (errors and reaction times) of i.v. citalopram (20mg/h) on Go/No-Go or on a variant where an alternative response had to be made.

Crockett et al. (2009) also showed that the ATD effect on punishment-induced inhibition was not mediated by reduced sensitivity to aversive events, shown by lack of effect on difference in response bias between the Punishment-Go and Punishment/No-Go

conditions. There results were suggested to account for discrepancies in the relevant literature of 1. behavioural suppression shown in light of aversive stimuli (Eagle et al., 2009) and 2. lack of effect of altered serotonergic transmission on general motor inhibition (Eagle et al., 2009; Clark et al. 2005).

5HT role in action cancellation

Human studies showed no effect of ATD on SSRT in healthy volunteers (Clark et al., 2005 with forty-two healthy volunteers, Evers et al., 2005 with twelve male volunteers). Chamberlain et al. (2006) also showed no effect of 30 mg citalopram administration on SSRT in healthy volunteers. Blockade of 5-HT reuptake with citalopram in rats showed no effect on SSRT over a range of doses (Bari et al., 2009; Eagle et al., 2008). However a recent study by Ye et al. (2014) in Parkinson's disease patients showed a beneficial effect of 30mg citalopram on Stop-signal performance and increased inferior frontal activation on patients with more severe disease status as exhibited by higher scores in a disease rating scale. The interaction between the citalopram effect and disease severity suggests that the observed effects might be mediated through restoration of pre-existing changes in forebrain serotonergic projections in the patient group (Politis et al., 2012). Additionally a healthy volunteer study with and without history of alcoholism provided evidence for possible serotonergic effect on SSRT (Crean et al., 2002). The researchers suggested an interaction between genetic and serotonergic transmission and potentially a role for baseline impulsivity. Nevertheless, Eagle et al. (2009) reported that in rats baseline impulsivity did not mediate ATD effect on SSRT. It is worth noting that the effects seen in these studies refer to the inhibitory process itself as the Go process was unaffected (Eagle et al., 2009). Longer SSRT was though observed in patients with OCD and trichotillomania versus healthy controls (Chamberlain et al., 2006) with no effect on Go reaction times. OCD patients showed shorter SSRT compared to patients suffering from trichotillomania. Longer SSRT was also shown in unaffected, first-degree relatives of OCD patients (Chamberlain et al., 2007a) where SSRIs constitute the main pharmacological treatment.

Task description

We applied the No-Go and Stop-signal interleaved trials task (Ye et al., 2014), designed to measure both action restraint and action cancellation, thus attempting to capture the effect of serotonergic manipulation on both aspects of response inhibition in a single experimental design.

Participants were presented with three types of trials; 360 Go trials where they are requested to press either the right or left button depending on the direction of the black arrow on the screen (75%), 80 stop-signal trials where they should cancel a cued button press when the arrow turns red accompanied by a tone (17%) and 40 No-Go trials where they withhold themselves from pressing any button, as the arrow appears red accompanied by a tone (8%) (Figure 3).

According to the initial race model proposed by Logan and Cowen (1984) explained above, the probability of inhibiting the response can change by altering the time of appearance of the stop-signal during response execution. In our task design, the stop-signal delay (the time between initiation of Go response until the presentation of the stop-signal) varied from trial to trial following a step-up/down algorithm with initial estimate of 250 ms aiming towards maintaining 50% successful inhibition, similar to previous studies (Ye et al., 2014; Chamberlain et al., 2006).

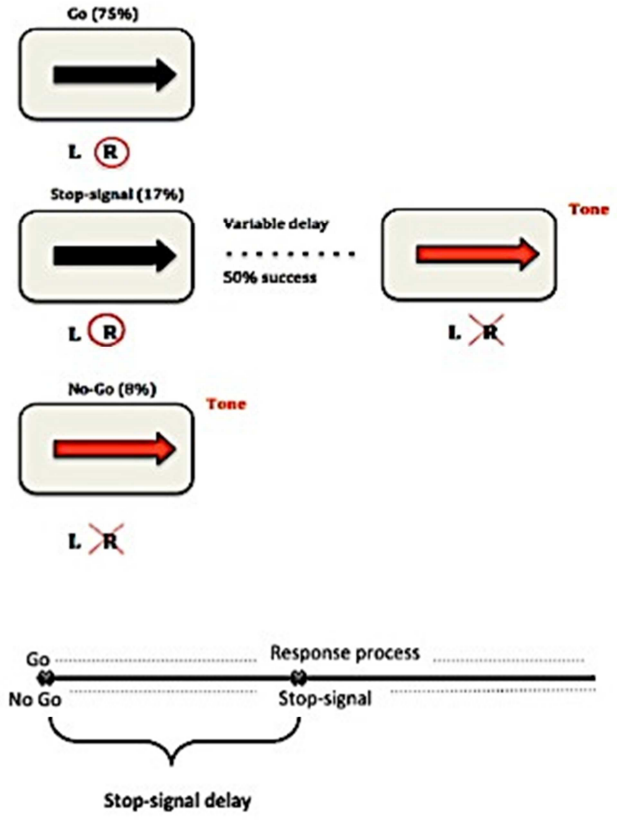


Figure 2: (a) Task structure; types and percentage of trials presented to participants. (b) During No-Go trials, the signal red arrow and tone are presented in the beginning of the Go response process. Thus participants need to make a pre-response or No-Go selection. Instead, in stop-signal trials the Go response is initiated and following a variable delay a stop-signal combined with a tone is presented. Participants need to appropriately alter their response

Participants

Sixty-five participants completed the task, thirty-three in the placebo group and thirty -two in the escitalopram group. Participants completed this task along with the additional cognitive tasks in a counterbalanced order.

Primary outcome measures was the Stop-signal reaction time (SSRT), i.e. the time required to abort an initiated action in the presence of a stop signal (measured in milliseconds). Additional measures included 1. mean reaction time in correct Go trials (measured in milliseconds), 2. rate of Go omission errors, 3. rate of Go commission errors which refer to wrong button press during Go trials and 4. rate of No-Go commission errors which refer to inappropriate button press during No-Go trials.

Hypothesis

Based on mixed results of prior literature on Stop-signal paradigms, we predicted that acute escitalopram administration would produce no effect on SSRT (although we note the study by Ye et al., 2014 reporting SSRT speeding). We predicted no effect of the drug on any other task measures including No-Go responding.

Results

Data from one participant was excluded as outliers identified using the generalized extreme studentized deviate test as appropriate. A one-way ANOVA determined whether SSRT differed between treatment groups. Mean SSRT was significantly lower in escitalopram (175.77 ms) compared to placebo (198.82 ms) group, $p=0.013$, $F(1,63)=6.501$. The observed group differences in SSRT cannot be attributed to strategic changes in responding speed as Go reaction time distributions between groups were similar.

Measures (Mean + SD)	Placebo	Escitalopram	Group difference*
SSRT (ms)	198.82 (40.02)	175.77 (31.46)	p=.013, F(1,63)=6.501
Go reaction time (ms)	463.52 (127.21)	492.19 (123.65)	n.s.
Go omission error rate	0.005 (0.01)	0.01 (0.04)	n.s.
Go commission error rate	0.11 (0.07)	0.1 (0.07)	n.s.
No Go error rate	0.11 (0.14)	0.08 (0.14)	n.s.

Table 4: Task performance and group differences. *** Drug effect**: p-values of two-tailed t-tests, n.s.= not significant, p>0.05

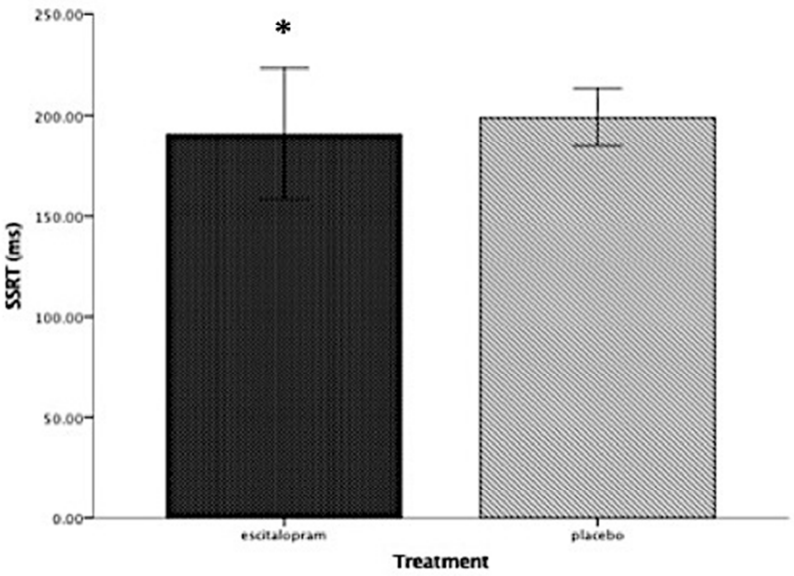


Figure 3: The escitaloparm-treated group showed a singificaly more rapid SSRT compared to placebo

Escitalopram treatment produced no significant effect on mean correct Go reaction times, p=364, F(1,62)= .835, omission error rate, p= .179, F(1,62)= 1.851, and Go commission error rate, p= .621, F(1,62)= .248. The treatment groups showed no difference in No-Go commission error rate, p= .459, F(1,62)= .555.

We performed Analysis of Covariance with Barratt Impulsiveness scale scores (Table III) as covariates. After adjusting for 1st order motor and self-control scores, escitalopram group showed significantly lower SSRT compared to placebo, p= .012, F(1,64)= 6.781, partial η^2= .102, and higher Go omission error rate close to significance, p= .055, F(1,60)= 3.840, partial η^2= .06.

Measures		Placebo	Escitalopram	Group difference*
BIS total score		66.67 (7.6)	64.6 (6.6)	n.s.
BIS 1st order factors	Motor	15.85 (3.1)	13.87 (4.3)	n.s.
	Self-control	12.63 (2.9)	11.03 (3.7)	n.s.
BIS 2nd order factors	Motor	27.72 (3.6)	26.94 (3)	n.s.
STAI trait		35.27 (8.5)	34.1 (8.9)	n.s.

Table 5: Barratt Impulsiveness scale and trait anxiety scores in the two groups

We repeated our analysis by adding trait anxiety median split scores as a between-subjects factor and we found no significant interaction between treatment and trait anxiety, p> .05,

on SSRT. We further added gender in our analysis but again found no interaction between treatment and gender for SSRT, p> .05.

Discussion

We administered a single clinically relevant dosage of escitalopram in healthy volunteers to examine distinct aspects of response inhibition through the application of a human analogue of the 5CSRT task measuring 'waiting' impulsivity and an interleaved Stop-signal and No-Go trials task measuring both action restraint and cancellation in the same volunteer sample. Escitalopram administration showed a significant beneficial effect in SSRT (speeded SSRT) in accordance with previous study reporting faster SSRT following citalopram administration in Parkinson's disease patients with more severe disease status (Ye et al., 2014) but unlike previous studies in human healthy volunteers. Similar to previous studies, we found no drug effect on median Go reaction times (Ye et al., 2014; Chamberlain et al., 2006; Clark et al., 2005). We found no effect of escitalopram on the 'waiting' aspect of impulsivity as measured with the human analogue of the 5CSRT task and no effect on behavioural measures of No-Go responding as per previous studies (Macoveanu et al., 2013; Del-Ben et al., 2005).

The effects of serotonin on behavioural inhibition depend on the paradigm employed, and action restraint differs from action cancellation (Dalley and Robbins, 2017; Del-Ben et al., 2005). Whilst sharing many neurobehavioral processes of inhibitory response control, including the engagement of fronto-striatal 'loops' involving the right inferior frontal gyrus (BA 44 and 45) (Rae et al., 2014; Rubia et al., 2005), the Stop-signal and Go/No-Go paradigms appear to have some distinct underlying mechanisms (Eagle et al., 2008). The SSRT might reflect a more sensitive (behavioural) measure than Go/No-Go performance – indeed the study by Macoveanu et al. (2013) showed no effect of i.v. citalopram (20mg/h) on a Go/No-Go task. Additionally, although Del-Ben et al. (2005) showed enhanced BOLD activation following citalopram intravenous (i.v.) 7.5mg administration, there was again no behavioural effect. Thus the present study is the first to show improvements in inhibitory response control performance by an acute high dosage SSRI in healthy volunteers.

The effects of serotonin manipulations may also vary according to baseline characteristics. For example, the change in activation of right inferior frontal gyrus during No-Go responding following ATD depended on neocortical 5-HT_{2A} receptor binding (Macoveanu

et al., 2013). However, none of the effects of escitalopram on inhibition in the current study were affected by trait anxiety, impulsiveness or gender.

General discussion

Summary

We administered the SSRI escitalopram to healthy volunteers in a double-blind, randomised, parallel-groups design study. We applied a large cognitive task battery testing for a wide range of cognitive functions, including response inhibition, emotional processing, probabilistic and instrumental learning and cognitive flexibility. We further applied tasks from the newly developed EMOTICOM battery thus attempting to capture aspects of affective processing ('hot' cognition) (Roiser and Sahakian, 2013). Our volunteers had no current or prior history of psychiatric symptoms and additionally, no family history of psychiatric disorders. This is important in the context of cognitive function deficits shown in the same tasks as the ones used in our study (CANTAB task battery) in healthy first-degree relatives of patients with psychiatric disorders (Bora et al., 2009; Chamberlain et al., 2007). We selected escitalopram as it is highly selective for the 5HT system (Baldwin et al., 1995) and is previously shown to produce effects in cognitive and emotional processing tasks. Out study provides measurements of distinct cognitive domains directly comparable as per same sample of healthy volunteers and no practice effects possibly mediating discrepancies.

Study	Task	Population	Intervention	Findings	Current study
Response inhibition					
Action cancellation					**Escitalopram effects**
Ye et al., 2014	Interleaved Stop-signal/No-Go trials	PD patients (21)	Citalopram 30mg	More rapid SSRT and reduced No-Go errors in patients with relatively more severe stage of the disease*	**Speeded SSRT**
Chamberlain et al., 2006	Stop-signal	HVs (60)	Citalopram 20mg	No effect on SSRT	
Chamberlain et al., 2007	Stop-signal	HVs (60)	Buspirone (partial 5HT-1A agonist) 20mg and 30mg	No effect on SSRT	
Cools et al., 2005	Stop-signal	HVs (23)	ATD	No effect on SSRT	
Clark et al., 2005	Stop-signal	HVs (42)	ATD	No effect on SSRT	
Eagle et al., 2009	Stop-signal	Rats (24)	Central 5,7-DHT induced 5-HT depletion	No effect on SSRT	
Bari et al., 2009	Stop-signal	Rats (26)	Citalopram- various dosages	No effect	
Response inhibition					
Action restraint					
Macoveanu et al., 2013	Go/No-Go	HVs (22)	Citalopram, ATD	No effect on CEs Increased BOLD response during No-Go in left IFG with citalopram	**No effect on No-Go**
Del-Ben et al., 2005	Go/No-Go	HVs (12)	i.v. 7.5mg citalopram	No effects on CEs Increased right OFC activation Decreased mOFC activation	
Crockett et al., 2009	Go/No-Go	HVs (22)	ATD	No effect on CEs Abolishment of punishment induced slowing of RTs	
Rubia et al., 2005	Go/No-Go	HVs (9)	ATD	No effect on CEs Reduced BOLD response in right orbito-inferior prefrontal, superior and medial temporal cortex during No-Go	
Evers et al., 2006	Go/No-Go	HVs (13)	ATD	No effect on CEs No effect on BOLD response during response inhibition	
Harrison et al., 1999	Go/No-Go	Rats (19)	Global 5,7-DHT induced 5HT depletion	Increased responding during NoGo condition	
Probabilistic instrumental learning					
Murphy et al., 2002	Reversal learning	HVs (11)	ATD	No effect on errors Slower RTs after ATD	**Increased Stage 1 errors** **Increased sensitivity to misleading feedback**
Evers et al., 2005	Reversal learning	HVs (12)	ATD	No effect on errors Increased BOLD response in dmPFC during reversal switch errors	
den Ouden et al., 2013	Reversal learning	HVs (685)	Genotyping for SERT polymorphisms	Increased sensitivity to punishment in the l/l genotype	
Clarke et al., 2004	Reversal learning	Marmosets (3)	Selective 5,7-DHT induced prefrontal 5HT depletion	Increased perseverative errors after stimulus reversal	
Clarke et al., 2007	Reversal learning	Marmosets (28)	Selective 5,7-DHT induced OFC 5HT depletion	Increased perseverative errors	

Study	Task	Population	Intervention	Findings	Current study
Boulougouris et al., 2008	Reversal learning	Rats (34)	Systemic 5HT-2A antagonist- various dosages	Increased perseverative errors after stimulus reversal	
				Increased trials to criterion in highest dosage	
		Rats (33)	Systemic 5HT-2C antagonist- various dosages	Reduced perseverative errors	
				Reduced trials to criterion	
Cognitive flexibility					
Rogers et al., 1999	CANTAB ID/ED	HVs (15)	ATD	More errors and fewer subjects competing CD stage and more errors in IDS	**Increased EDS errors**
Talbot et al., 2006	CANTAB ID/ED	HVs (32)	ATD	No effect	**Increased EDS RTs**
Clarke et al., 2005	CANTAB ID/ED	Marmosets (16)	Selective 5,7-DHT induced prefrontal 5-HT depletion	No effect on EDS	

Table 1: Effects of serotonergic manipulations on the cognitive domains of response inhibition, probabilistic reversal learning and cognitive flexibility. *Abbreviations* **PD:** Parkinson's disease, **CEs:** commission errors, **5,7-DHT:** 5,7-dihydroxytryptamine, **5-HT:** serotonin, **CD:** Compound discrimination, **IDS:** Intra-dimensional set shift, **EDS:** Extra-dimensional set shift**, RTs:** Reaction times**, OFC:** Orbitofrontal cortex, **mCPP:** m-chlorophenylpiperazine**, dmPFC:** dorso-medial prefrontal cortex, **IFG:** inferior frontal gyrus, **SERT:** serotonin transporter, **SSRT:** Stop-signal reaction time**.** A reversal switch error in the probabilistic reversal-learning task denotes an incorrect response where participants reversed on the subsequent trial (Evers et al., 2005). *Quantified with the higher Unified Parkinson's Disease Rating Scale motor score

Effects of acute escitalopram on probabilistic learning and cognitive flexibility

We found that acute escitalopram administration increased errors during the first stage of the probabilistic learning task and increased sensitivity to misleading feedback as participants receiving escitalopram were more likely to shift in the next trial. This is in accordance to Chamberlain et al. (2006) study showing more errors made and increased feedback sensitivity using a similar probabilistic reversal-learning task. We found no effect of escitalopram on the ability to reverse task contingencies.

At the early stages of learning in the CANTAB ID/ED shifting paradigm, there were no effects of escitalopram, contrasting with effects of ATD (e.g. Park et al., 1994; Rogers et al., 1999). The relative lack of sensitivity of the latter visual discrimination-learning task may relate to its deterministic nature, whereas the probabilistic learning task entails greater uncertainty.

Effects of escitalopram on response inhibition

Acute escitalopram speeded the SSRT. This cannot simply be attributed to strategic changes in responding as Go RT and other measures were unchanged. The involvement of 5HT in response inhibition is extensively studied. Manipulating serotonergic levels shows distinct effects on aspects of response inhibition in both human and animal studies. Whereas the majority of human and rodent studies suggest no effect of altered 5HT transmission mainly through ATD on SSRT, a few studies did (Ye et al., 2014; Crean et al., 2002). One possible explanation is that they both involved patient groups where 5HT transmission in brain areas mediating response inhibition is altered by the disease and thus the effect of serotonergic manipulations might reflect a "restoration" effect of brain 5HT levels (Ye et al., 2016; 2014). Nevertheless, in our study we observed a beneficial effect of a high escitalopram dosage on action cancellation in healthy volunteers with intact baseline 5HT levels (no prior psychiatric disorder, no previous or current depressive symptomatology, and no possible forebrain neurodegeneration based on age cut-off limit of 45 years old). This effect was accompanied by similar Go reaction times in the two groups, which supports no effect of escitalopram on responding strategy (Eagle et al., 2009). Perhaps counter-intuitively, we observed no effect on action restraint. Crockett et al. (2009) showed abolishment of punishment-induced inhibition with ATD but this effect was derived from RTs for Go stimuli during punishment condition and not by differences in commission errors. Indeed, previous studies in humans (Rubia et al., 2006; Del-Ben et al., 2005) showed no effect of serotonergic manipulations on behavioural measures of No-Go.

Unlike the Crockett et al. (2009) study with orthogonal design and added punishment condition, we did not have any punishing outcome. Additionally, in our study, we applied a task with interleaved stop-signal and No-Go trials. This differs from previous studies in healthy volunteers where tasks included either Go and Stop-signal trials (Chamberlain et al., 2006) or Go and No-Go trials (Clark et al., 2005). From this perspective, the alternation of two types of response inhibition mechanisms that participants needed to employ for performing the task (action restraint versus cancellation) and the uncertainty of the type of trial that will follow might have produced a different behavioural effect and a possible conflict on participants' control over their response inhibitory ability.

Escitalopram increased response latencies in the SWM task thus a possible additional cognitive load (anticipation of stop-signal versus No-Go trial) might contribute to altered performance. To this end, we note that although there was no difference between treatment

groups on Go reaction times, our results show higher Go reaction times compared to the healthy volunteers in Clark et al. (2005) study but similar to controls and patients in the Ye et al. (2014) study which used the same task design as in ours. Thus, the task design per se might have additionally induced an effect on Go responding strategy. Thus, a possible explanation could be the higher number of stop-signal (17%) versus No-Go trials (8%) in our task design that could have re-directed attention towards stop-signal trials as "aversive" ones due to a temporal element entailed. Manipulations of task structure were previously shown to affect response inhibition in Go/No-Go tasks leading Simmonds et al. (2008) to define the process as "task-dependent". In simple tasks the same No-Go stimuli are applied whereas in more complex tasks No-Go stimuli require frequent updating of the S-R associations in working memory. This points towards response inhibition being mediated by other cognitive functions, thus any results need to be appropriately interpreted.

Effects on emotional processing

In contrast to its effects on learning and 'cold' executive performance, acute escitalopram had relatively little effect on emotional processing even taking into account trait anxiety, BDI and gender influences. This is in contrast to previous studies (Harmer et al., 2003a; Browning et al., 2006) where increased recognition of both fear and happiness in facial expression was reported after acute citalopram administration (oral 20mg or iv. 10mg) – as well as reduced recognition of fear in facial expressions after ATD in female volunteers (Harmer et al., 2003b). Murphy et al. (2009) though did find that oral citalopram reduced the BOLD response to fearful faces in the amygdala, but no behavioural effect on recognition per se was noted in accordance to Del-Ben et al. (2005) who again found no behavioural effects on face recognition of i.v. citalopram (7.5mg) treatment. Thus, it appears that different tasks applied as well as different method used (oral versus i.v. SSRI versus ATD) might produce differential changes in brain 5HT levels in distinct brain areas and to a different degree. These discrepancies in the literature highlight the complexity of delineating the role of 5HT system in cognition and emotional processing in humans (healthy and patient volunteers). We did find an effect on an information-sampling aspect of the novel EMOTICOM 'Theory of mind' task as escitalopram-treated participants selected a higher proportion of facts, rather than faces or thoughts, in order to solve socially ambiguous situations. This result suggests that although the emotional response to faces may possibly be enhanced by acute SSRIs, their informational value for decision-

making may thus paradoxically be diminished. But this finding needs to be further replicated in additional studies applying both acute and sub-chronic dosages of SSRIs.

Baseline 5HT levels possibly moderating the effects of serotonergic manipulations?

Another issue that needs to be raised is the possibility of differential effects on cognitive function based on baseline 5HT levels. This is nicely illustrated in the case of DA, where large variability is again seen in response to tasks measuring distinct cognitive functions and distinct experimental methods applied in both animal and human studies. The differential effect of drugs dependent on baseline levels is not new as it originates back to Wilder (1957). Cools and D'Esposito (2008) discuss the –mostly derived from animal studies- findings of dopaminergic drug effects being mediated by baseline DA levels in the PFC, the key area for executive functions and top down control on ascending neurochemical systems. Specifically, Cools and D' Esposito (2008) extend the empirical observation of an inverted U-shape function of DA transmission where both too little and too much brain DA levels show detrimental effects on cognitive functions (Cools and Robbins, 2004) to suggest that manipulations of brain baseline DA levels in the PFC and the striatum will produce distinct cognitive effects. High levels of activation of DA receptors in the PFC will improve cognitive stability, one aspect of cognitive control, whereas increased activation of DA receptors in the striatum will be beneficial for cognitive flexibility, another important aspect. Based on this suggestion, and the more widespread anatomical basis of 5HT system compared to DA along with the interaction between these two systems well shown in experimental studies, we assume that a similar pattern of distinct effects of altered 5HT transmission dependent on baseline 5HT brain levels, type of method applied (receptor agonism/antagonism versus tryptophan depletion in humans and neurotoxins in animals and rarely, tryptophan loading) and relevant target (receptors, differentially expressed in distinct brain regions, and appropriate dosage) and tasks used may produce different effects on the cognitive function studied. Indeed, Ye et al. (2016) who previously reported that the behavioural effect of citalopram depended on individual differences in baseline state of 5HT systems (Ye et al., 2014; 2015).

Regional neuroanatomical, morphological and pharmacological variations in the expression and subsequent activation of auto receptors by 5HT reuptake blocking agents (Blier et al., 1990) might also contribute to differences in enhancing or diminishing serotonergic transmission in distinct brain areas following administration of serotonergic agents (Hjorth et al., 2000). This can lead to differentially affecting distinct parallel

cortico-strital loops mediating information processing as described by Alexander et al. (1986). Indeed, this has been suggested for the neurocognitive deficits seen in Huntington's disease patients (Lawrence et al., 1998). It was also suggested that 5HT transmission in subcortical areas might encode a motivational aspect opposing DA transmission whereas direct modulation of OFC mediates top down inhibitory control over subcortical areas affecting distinct functions such as emotional processing (Cools et al., 2008).

Possible interactions with the dopaminergic system?

Although escitalopram does not directly affect dopaminergic transmission, an indirect effect could contribute to the effects of serotonergic manipulations on the inhibitory circuit. Another potential mechanism could include interactions between monoaminergic receptors and subsequent second-message cascades at the postsynaptic level (Albizu et al., 2011). Fluoxetine, another selective serotonin uptake inhibitor, is shown to increase extracellular serotonergic (along with dopaminergic) levels in the prefrontal cortex (Pozzi et al., 1999) and the addition of citalopram to treatment with methylphenidate produces a notable increase in prefrontal dopaminergic levels (Weikop et al., 2007). This was not though supported in a rat micro dialysis with citalopram administration (Bymaster et al., 2002).

The role of individual baseline traits

An issue that warrants attention is that pharmacological manipulations exert changes in task performance, which can vary across tasks and can be dependent on inter-individual differences (Cools and Robbins, 2004; Mattay et al., 2003). An example is the case of dopaminergic manipulations; high-impulsive healthy volunteers with low baseline working memory receiving bromocriptine (a DA D2 receptor agonist) showed improved flexible update of information related to changes in putamen activity compared to low-impulsive healthy volunteers that (Cools et al., 2007).

Indeed, serotonergic transmission in the OFC is shown to account for cognitive flexibility (Barlow et al., 2015) whereas amygdala is largely involved in aversive processing (Macoveanu et al., 2013). Additionally, depletion of OFC 5HT impairs reversal learning (Clarke et al., 2007) and impairs performance in Go/No-Go tasks by affecting the ability to learn the No-Go response rule (Harrison et al., 1999; Homberg, 2012). 5HT depletion is also shown to produce a shift to habitual, "stimulus-bound" responding be mediated by

reduced inhibitory control mediated by the OFC (Walker et al., 2009) thus showing a key role for this region in more than one aspects of cognitive control. Additionally, differential expression of 5HT receptors in distinct brain areas and in different dosages might account for the observed distinct effects. As previously discussed, rat studies show different effects on anticipatory (premature) and perseverative responding for 5-HT_{1A} and 5-HT_{2A} receptors expressed in the mPFC (Carli et al., 2006). Availability of $5HT_{1A}$ receptors in the DRN is related to amygdala connectivity with cortico-striatal regions for processing of aversive facial stimuli (Selvaraj et al., 2015).

In our study we found no effects of escitalopram on the strategy used or the between search errors made in the CANTAB SWM task. This is partially in accordance with a rat study showing no effect of escitalopram on ATD induced deficits in SWM (Jensen et al., 2014); the difference of course lies in the baseline SWM capacity. In our study participants had intact SWM capacity (healthy individuals, with no frontal degeneration suspicion or other disorder that could impair memory) whereas in the Jensen et al. (2014) study escitalopram possibly restored baseline 5HT levels but again produced no effect on reversing the SWM deficits unlike vortioxetine. This points towards a selective effect of 5HT receptors (in this case, possibly $5H_{T1A}$ as the researchers noted) in mediating SWM and possibly other cognitive functions. This could also account for the large discrepancy in the 5HT literature on how it affects cognitive functions spanning from inhibitory control to learning and affective processing. We note that we did observe significantly higher response latencies in the escitalopram group for searching for the token hidden inside the boxes thus indicating a possible impairment which in other conditions (for example, cognitive load) could show impairments in successfully completing the task.

Study limitations

Our study design carries specific limitations that need to be addressed. We used a single escitalopram dosage to increase brain serotonergic levels. This was the maximum clinically allowed dosage but given the mixed effects observed in the neuropsychological tests employed in the study, we note that this dosage might have resulted in diminished rather than enhanced brain serotonergic transmission. This might have been mediated through activation of the somato-dendritic, inhibitory $5HT_{1A}$ auto-receptors (Cools et al., 2008). Indeed, administration of 5-HT reuptake blocking agents has been shown to inhibit serotonergic neuronal discharge, synthesis and release (Hjorth et al., 2000; Adell and Artigas, 1991) attributed to somato-dendritic 5-HT_{1A} auto receptor activation (LePoul et

al., 1995) following increased extracellular 5-HT levels in the dorsal raphe nucleus induced by the 5-HT reuptake blocking agents (Bel and Artigas, 1992). A rat micro dialysis study showed increased nerve terminal 5-HT output level produced by citalopram treatment when agents blocking 5-HT_{1A} auto receptor-mediated negative feedback loops were co-administered (Hjorth, 1993). Suggestions of accelerating the action onset and improving the efficacy of serotonin reuptake blocking agents by co-administering a 5-HT_{1A} auto-receptor antagonist further support this view (Artigas, 1993).

Additionally, we applied a large cognitive test battery to capture several distinct cognitive functions. It is possible that the tasks applied did not measure the cognitive domain initially intended to, i.e. goal-directed and habitual learning might not be captured parallel to their computational forms of model-based/model-free learning by the respective tasks (instrumental discrimination learning and two-step tasks). Additionally, we did not use face recognition tasks as per Harmer et al. (2003b) and Browning et al. (2006) studies thus several of the discrepancies might arise from different task structure and lack of capturing the intended cognitive or emotional processing domain. Nevertheless, the majority of the tasks applied in this study are well-validated (CANTAB task battery) in both healthy and clinical group, but ceiling effects might be another factor to be taken into account as our sample consists of healthy volunteers, relatively young (mean age ranging from 25 to 27 years old in both groups and NART scores ranging from 43.45 to 42.9 in the two groups). An additional factor to take into consideration is the large number of cognitive tests administered which may have resulted in diminished attention and fatigue during the testing day; nevertheless, our tasks were administered in a counter-balanced order and we applied visual analogue scales measuring changes in mood state and drug side effects. We did find that alertness and interest significantly decreased throughout the experimental day but no effects on other measures including coordination, willingness to engage in social interaction and mental speed.

Acute versus chronic treatment with SSRIs

Cools et al. (2008) drew attention to the different acute *versus* chronic effects of SSRI administration and the need for this to be considered when interpreting the findings from studies using these compounds. Acute *versus* chronic serotonin-reuptake inhibition can have opposite functional effects on emotional processing and facets of information processing (Wienberg et al., 2010; Bari et al., 2010; Chamberlain et al., 2006; Harmer, 2006; Burghardt et al., 2004). Whether these opposite actions are respectively due to

globally diminished or enhanced 5-HT activity, is still unclear. However, the same acute dose can also produce a mixture of apparently opposite functional effects in different domains, e.g. improved SSRT and worsened ED-shifting, and enhanced processing of both 'fearful' and 'happy' emotions (Browning et al., 2006). This might arise because of differential effects of the SSRI in different brain regions sub-serving these functions, perhaps because of regional marked variations in SERT density (Majuri et al., 2017) or in differences in inhibitory auto-receptors in the dorsal and median raphé nuclei (e.g. Blier et al., 1990), as well as their projections to forebrain regions (to striatum, amygdala and neo-cortex and to hippocampus and limbic regions respectively; Hornung, 2010). The PET studies by Nord et al. (2013) also suggest that they result from diminished 5-HT post-synaptic actions in certain regions, mainly in the neo-cortex.

Comparison with ATD and other 5HT manipulations applied

We note that several of the findings on the role of 5HT in cognitive functions stemming from both the animal and human literature were produced by different manipulations of serotonergic brain levels. Compared with previous healthy volunteer studies using citalopram (Chamberlain et al., 2006), we note that differential effects on auto receptor activation may account for the discrepancy in the results. El Mansari et al. (2005) reported a four fold more potent suppression of firing activity of serotonergic neurons following acute challenge with escitalopram compared to citalopram and a faster recovery. This could be due to the R-citalopram enantiomer, which attenuates the effects of S-citalopram (as discussed in the introduction) on neuronal firing supporting the more rapid antidepressant onset of action of escitalopram (Sanchez et al., 2004).

The largest body of available previous findings come from ATD studies in human and 5HT depletion using neurotoxins in animals. These two are not directly comparable as they produce distinct patterns of depletion of brain 5HT levels, i.e the neurotoxin 5.7-DHT results in almost complete forebrain 5HT depletion (reaching 90%) whereas ATD is considered more mild (Cools et al., 2008). Additionally, contrasting escitalopram with ATD, the latter treatment may have very different effects on regional 5-HT function, distinct from those produced by acute escitalopram (Faulkner and Deakin, 2014). Although there have been relatively few direct comparisons of these treatments using brain-imaging methodology (e.g. Macoveanu et al., 2013), evidently acute escitalopram and ATD do not necessarily reflect opposite effects on 5-HT functioning.

Future directions and clinical implications

5HT is implicated in cognitive flexibility and behavioural inhibition. Serotonergic agents can support cognitive flexibility, which can of trans-diagnostic importance in disorders of compulsivity such as OCD. To this end, specific cognitive domains may be more useful as treatment targets than others. Further studies in healthy volunteers are needed to address the effects of altered serotonergic transmission on distinct cognitive functions. Action restraint and action cancellation need to be separately addressed in light of both rewarding and aversive outcomes will provide further insights into the role of 5HT in response inhibition similar to the reversal learning paradigm which, in its essential form, is an appetitive instrumental learning task with no punishment entailed. Nevertheless, aversive outcomes (Morris and Dolan, 2004) and reversal of Pavlovian contingencies have been applied (Burke et al., 2009). This will prove of clinical significance, as cognitive deficits are found in psychiatric patients and often persist during after symptom remission (National Academies of Sciences, Engineering, and Medicine, 2015).

Inhibitory control emerges as a candidate area of endophenotype where deficits can be viewed as trait of mood disorders including both unipolar and bipolar depression (Bora et al., 2013; Rock et al., 2012) and OCD (Chamberlain et al., 2007). Additionally, the effect of SSRI on reversing negative biases during early stages of treatment in major depression is suggested to act as a predictor of overall treatment response to this class of antidepressants (Harmer et al., 2013; 2008b). Attention set shifting and cognitive flexibility measured with intra- and extra-dimensional set shift tasks and reversal-learning paradigms can provide insights into "cold" cognitive processing and how it is affected across disorders. In accordance with the Research Domain Criteria, a proposed research classification of mental health disorders based on behavioural dimensions and neurobiological findings (Insel et al., 2010), cognitive deficits can be of trans-diagnostic importance thus helping to move away from symptom-based clustering of patients to mechanistic inferences of underlying pathophysiology (Stephan and Mathys, 2014). Indeed, as an example findings suggest a predominance of habitual versus goal-directed behaviour accounting for the compulsivity characterising obsessive-compulsive disorder (Gillan et al., 2011). Given mixed results in clinical studies regarding the effect of SSRIs used widely for management of depression and anxiety disorders it is imperative that the underlying effects of these compounds in healthy volunteers are defined. To this end, studies employing both acute and (sub) chronic SSRI administration will be useful.

The interaction of escitalopram effects on specific cognitive domains with baseline trait anxiety in our study also supports the need for patient stratification in psychiatry; clinically initial worsening of reported anxiety is observed in a few anxiety disorder patients (Taylor et al., 2015). The comorbidity of anxiety and depressive disorders and the large heterogeneity of patient populations pose both a necessity and a difficulty in characterising the patient sub-groups and the mechanisms that account for this differential response to serotonergic challenges. Baseline brain 5HT levels cannot fully account for this, as presumably these will be altered in a relatively uniform way across patients suffering from specific disorders. Pre-morbid personality traits might play a role. Distinct baseline trait anxiety, the measurements of which are not traditionally used as a tool in clinical practice, might provide some insights. This is only speculative based on our findings and a limited number of studies pointing towards neuroticism mediating effects in cognitive tasks (di Simplicion et al., 2014) and thus, it warrants further research studies validating the potential usefulness of this measurement in relation to serotonergic challenges. Additionally, although peripheral markers of the efficiency of these compounds in altering brain 5HT levels are useful (blood prolactin and cortisol measurements, plasma drug and metabolites' concentration) direct measurements of 5HT activity and temporal changes with interventions are needed. Combination of behavioural tasks with pharmacological challenges and brain imaging techniques (fMRI, PET scan, pharmaco fMRI) can further advance our understanding of the serotonergic mechanisms mediating cognitive functions and possibly provide useful insight in the long-term for clinical practice (Wandschneider et al., 2016; Arce et al., 2008).

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Appendix

I. Task administration detailed scripts

Instrumental learning task detailed description

Instructions provided verbally to participants during initial stage; *"In this game, you will get the chance to earn points by collecting fruit from inside a box on the screen by opening the box by pressing either the right 'm' or left 'z' key. If you press the correct key, the box will open to reveal a fruit inside and points will be added to you total score. However, if you press the incorrect key, the box will be empty and no points will be added to your total. Your task is to learn which is the correct key to press. Sometimes it will be the left-hand one and sometimes it will be the right-hand one. The picture on the front of the door should give you a clue about which is the correct response. The quicker you make the correct response the more points will be added to your total. Your accumulated points will appear at the top of the screen. You should try to learn the types of fruits that are found inside the boxes following left-hand and right-hand responses because later on you will be asked to gather some types of fruit but not others."* Before the beginning of the task, participants were shown a demo to ensure instructions were clear and understood. During this stage, the points awarded for correct responses within the following latencies ranges were: 0-1 s, 5; >1- 1.5 s, 4; >1.5 – 2 s, 3; >2 – 2.5 s, 2; >2.5 s, 1.

Verbal instructions during outcome devaluation stage were administered as following; *"Now two open boxes will appear on the screen with different types of fruit inside them. One fruit was earned by a left response in the previous stage and the other by a right response. Although both types of fruit were valuable previously, one of them is now devalued and earns no points, whereas the other is still valuable and gains points. The devalued fruit will have a cross on it"*.

Verbal instructions during slips-of-action test were administered as following;

"In the next part of the game, you'll see a series of boxes with pictures of fruit on the outside and once again you can press left or right to open these and win points. The pictures on the box will be the same as the ones in the first game, and the correct response, left or right, will also be the same as what you learnt in the first half of the last game. However, unlike before, some of the fruits inside the box are no longer valuable, meaning you can no longer earn points for them. In fact, if you try and open a box, which contains a non-valuable fruit inside, you will have points subtracted from your total"

Probabilistic reversal learning task

Instructions provided verbally to participants;

"On each go, the same two patterns will be presented. One of the patterns is correct and the other pattern is wrong and you have to choose the correct pattern on each go. However on some goes, the computer will tell you that you were wrong even if you chose the correct pattern. Your task is to stick to the pattern that is usually correct. So in other words always choose the pattern that is correct more often than the other pattern.

At some point during the task, the rule may change so that the other pattern is now usually correct. You then have to follow this new rule and choose the new pattern. It is important that you only start choosing the other pattern when you are sure that the rule has changed."

CANTAB Spatial working memory task

Instructions provided verbally to participants;

When the test screen initially appeared: *"For this test you will see some coloured boxes on the screen. What you have to do on each go is to look for a blue token that the computer has hidden inside one of the boxes. Only one token will be hidden at a time. You have to collect enough blue tokens to fill the black hole ('home') on the right of the screen. To look inside a box, all you have to do is touch it like this"*

Experimenter touches one box. *"This box does not have a blue token in it, so I shall try a different box"*. The experimenter touches another box. *"This box has a blue token inside it and now I am going to move it over here to fill the black hole"*, the experimenter touches the black hole on the right hand side of the screen and proceeds with the following instruction which is strongly emphasized *"Now I have found a blue token in this box..."* pointing towards the box where the token was found and adding *"...there will never be one in there again, so I must not go back to it. There are two more blue tokens to find, but the computer never uses the same box twice for the blue token, so I must touch another one"*. The experimenter touches another one; *"There is no token inside this box, so I will try another"* then touches the box that no token is yet found inside it and then touches the right hand side of the screen saying *"Now I have found two tokens and the last one must be in here"*. The experimenter proceeds with touching the only box not yet found a token inside and then touches the right hand side of the screen saying *"Now I have found all three token and I have finished because I have filled up the black hole completely with blue*

tokens". The computer displays COMPLETE playing a short tune and following a pause, a NEW SET (as per screen's wording) appears.

CANTAB Paired Associates Learning task

Instructions provided verbally to participants;

With the task start screen displayed: "In this test you will see six white boxes and they will open up in a random order. There will be a pattern in three of the boxes. You have to remember which pattern is in which box. If you make a mistake the boxes will open up again to remind you where the patterns were. Are you ready?". The experimenter presses the space bar to begin the first stage. When the patterns appear, the experimenter points to them and say: "There is one pattern… there is another pattern, and there is the third pattern". When all the boxes have been opened, the patterns appear in the middle of the screen, one by one. As the three patterns come up in the centre of the screen the experimenter prompts the participants with the instruction: "So which box was that pattern in? And which box was that pattern in? And which box was that pattern in?" and if needed the experimenter says: "Just touch the box if you think the pattern goes in there". If the subject touches the correct boxes, the screen will display '**All correct**'. The experimenter then says: "Now we are going to do the same thing again, but this time with different patterns. Are you ready?" and when the participant is ready the experimenter moves on to the next three-pattern stage by pressing space bar on the keyboard. If the participant has made a mistake during a trial, the screen will not display '**All correct'** immediately afterwards; instead the computer will pause and then re-present the patterns. During the pause, the experimenter says: "That was not quite right. The computer will open the boxes up again to remind you where the patterns were". To help the tester keep track of the number of trials the subject has completed for the current stage, a 2-digit number is shown in grey in the lower right-hand corner of the screen when the pattern is shown in the middle of the screen. The difference between the 2 digits is the number of the current trial as shown below.

Number displayed	Trial number
No number	1
53	2
96	3
84	4
72	5
93	6
70	7
91	8
90	9
0	10

When six patterns appear on the screen, the experimenter says: “This time there will be six patterns. You may not get this correct first time, but you can have more attempts until you do. Just try to do the best you can. Are you ready?”. When eight patterns appear on the screen, the experimenter says: “Now there will be eight boxes. All eight boxes will have a pattern inside, and you have to remember which pattern goes in which box. You may not get this correct first time, but you can have more attempts until you do. Just try to do the best you can. Are you ready?”. When ten patterns appear on the screen, “Now there will be ten boxes. Each box will have a pattern inside and you have to remember which pattern goes in which box. You may not get this all correct first time but you can have more attempts until you do. Just do the best you can. Are you ready?” and when twelve patterns appear on the screen “Now there will be twelve boxes. Each box will have a pattern inside and you have to remember which pattern goes in which box. You may not get this all correct first time but you can have more attempts until you do. Just do the best you can. Are you ready?”.

CANTAB Attention switching task

Instructions provided verbally to participants;

When the start screen is displayed, the experimenter prompts the participants; “In this task an arrow will appear on the screen pointing either to the left or to the right. Before each problem you will see the instruction “*Which DIRECTION?” When you see the arrow pointing to the left, press the left button. When you see the arrow pointing to the right,*

press the right button. Press the buttons as quickly as you can while trying to avoid making mistakes. Are you ready?". The experimenter pressed space bar to begin the stage.

In the next stage, the experimenter says: *"This time the arrows will appear in different places on the screen. Ignore where the arrow appears and continue to press the left button when the arrow points left, and the right button when the arrow points right. Are you ready?"* and presses the space button.

In the third stage, the experimenter prompts the participants by saying: *"This time you will see that the screen now says "Which SIDE?". You now need to ignore which way the arrow is pointing. Instead press the button on the side of the screen that the arrow appears on. Press the left button when the arrow is on the left side of the screen, and the right button when the arrow is on the right side of the screen. Are you ready?".*

In the next stage, the experimenter says *"For this next part, you should either press the button on the same SIDE of the screen as the arrow, or press according to the DIRECTION the arrow is pointing. You will see the words 'Which SIDE' or 'Which DIRECTION' before each arrow, to tell you which rule to follow"*.

In the final stage, which is assessed, the experimenter says, *"Now we are going to do the same thing again. This time there will be no feedback on the screen. Remember to keep going as quickly as you can while trying to avoid making mistakes"*.

Response inhibition; interleaved stop-signal and No-Go trials task

I. INTRODUCTION

"The next task looks at how we make movements and how we stop them. On the screen, you will see arrows pointing left and right, telling you whether the press the left or right button. We want you to be fast and accurate, so it is important that you try as **hard as possible to respond correctly and as fast as you can**."

II. TRAINING PHASE:

"Now let's practice the task. First you will get used one of two different buttons, until you are familiar with it.

Before we get started, there are a few tips for doing really well in this experiment:

- Use only index and middle finger of your right hand
- Respond as accurately as you can
- Respond as quickly as you can

You will see a black arrow either pointing to the left or to the right

When it points to the left, press the left button (index finger)

When it points to the right, press the right button (middle finger)

Occasionally the arrow goes red and you will hear a loud beep. When this happens, you must try to not press the button. So, don't press when the arrow is red, or if the arrow suddenly turns red.

Sometimes it will be hard to stop yourself from pressing the button, when it goes red This is normal, but please try to do your best.

Even though we are asking you to stop when you see a red arrow and a tone, do try to respond as quickly as you can.

The computer is keeping track of how long you take to respond and using that to tailor the task to your performance. Hesitating to respond (for example, to see if a red arrow and tone will come) will actually make the task more difficult."

II. Visual analogue scales

Subjective mood ratings	**Placebo**		**Escitalopram**	
	Baseline	**Post drug Pre testing**	**Baseline**	**Post drug Pre testing**
Alert- drowsy	35.17 (3.66)	36.59 (3.63)	29.72 (4.11)	43.7 (3.72)
Calm- excited	34.28 (3.63)	29.07 (3.02)	30.02 (4.02)	38.25 (4.11)
Strong- feeble	30.97 (2.17)	31.78 (3.14)	27.97 (3.41)	40.57 (4.42)
Muzzy- clear headed	71.42 (3.13)	68.57 (2.61)	72.4 (3.79)	59.06 (3.7)
Well-coordinated- clumsy	23.6 (2.41)	29.76 (3.09)	22.57 (3.48)	39.5 (4.3)
Lethargic- energetic	64.84 (2.98)	58.99 (3.58)	64.82 (4.7)	54.6 (4.2)
Contented- discontented	23.38 (2.58)	31.40 (3.86)	25.26 (3.2)	28.03 (3.1)
Troubled- tranquil	74.64 (2.2)	70.68 (2.73)	75.04 (2.81)	70.01 (3.24)
Mentally slow- quick witted	65.42 (2.32)	65.30 (3.04)	67.22 (3.23)	59.26 (3.89)
Tense- relaxed	76.19 (2.19)	71.46 (2.69)	67.59 (3.05)	66.85 (3.46)
Attentive- dreamy	29.11(2.87)	36.62 (3.18)	29.22 (3.22)	37.68 (3.79)
Incompetent- proficient	73.24 (2.27)	70.21 (2.39)	72.84 (2.99)	62.8 (3.88)
Happy- sad	24.12 (2.23)	28.51 (3.07)	25.79 (3.05)	28.8 (3.73)
Antagonistic- friendly	80.76 (2.16)	77.10 (2.44)	79.83 (2.48)	77.55 (2.7)
Interested- bored	24.76 (2.64)	32.83 (3.61)	23.86 (3.22)	32.76 (3.64)
Withdrawn- sociable	72.58 (2.77)	65.83 (3.51)	69.51 (0.6)	68.6 (4.26)

Table 1: Subjective ratings in the visual analogue scales. Mean + SEM. Subjects completed the scales in three time points during the testing session

Questionnaires and scales applied

VISUAL ANALOGUE SCALES

For each line below, put a vertical mark at the point which represents how you feel at this moment. The ends of each scale are to represent the "most" that you have ever felt in your life.

ALERT	_____	DROWSY
CALM	_____	EXCITED
STRONG	_____	FEEBLE
MUZZY	_____	CLEAR HEADED
WELL COORDINATED	_____	CLUMSY
LETHARGIC	_____	ENERGETIC
CONTENTED	_____	DISCONTENTED
TROUBLED	_____	TRANQUIL
MENTALLY SLOW	_____	QUICK WITTED
TENSE	_____	RELAXED
ATTENTIVE	_____	DREAMY
INCOMPETENT	_____	PROFICIENT
HAPPY	_____	SAD
ANTAGONISTIC	_____	FRIENDLY
INTERESTED	_____	BORED
WITHDRAWN	_____	SOCIABLE