Title

From impulses to maladaptive actions: the insula is a neurobiological gate for the development of compulsive behavior

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Abstract

Impulsivity is an endophenotype of vulnerability for compulsive behaviors. However, the neural mechanisms whereby impulsivity facilitates the development of compulsive disorders, such as addiction or obsessive compulsive disorder, remain unknown. We first investigated, in rats, anatomical and functional correlates of impulsivity in the anterior insular cortex (AI) by measuring both the thickness of, and cellular plasticity markers in, the AI with magnetic resonance imaging and situ hybridization of the immediate early gene Zif268, respectively. We then investigated the influence of bilateral anterior insular cortex lesions on the high impulsivity trait, as measured in the 5-choice serial reaction time task (5-CSRTT), and the associated propensity to develop compulsivity as measured by high drinking levels in the schedule-induced polydipsia procedure. We demonstrate that the anterior insular cortex (AI) causally contributes to individual vulnerability to impulsive-compulsive behavior in rats. Motor impulsivity, as measured by premature responses in the 5-CSRTT was shown to correlate with the thinness of the anterior region of the insular cortex, of which highly impulsive rats (HI) expressed lower zif268 mRNA levels. Lesions of AI reduced impulsive behavior in HI rats, which were also highly susceptible to develop compulsive behavior as measured in a schedule-induced polydipsia (SIP) procedure. AI lesions also attenuated both the development and the expression of SIP. This study thus identifies the AI as a novel neural substrate of maladaptive impulse control mechanisms that may facilitate the development of compulsive disorders.

Key Words: Impulsivity, Compulsivity, Insular cortex, 5-Choice serial reaction time task, Schedule induced polydipsia

Introduction

The neurobiological mechanisms subserving individual vulnerability to obsessive compulsive spectrum disorders (OCSDs), such as addiction or Obsessive/Compulsive Disorder (OCD), remain poorly understood. Impulse-control deficits commonly observed in patients suffering from OCSDs[1, 2], are increasingly recognized as endophenotypes underlying the vulnerability to, and severity of, these disorders[1-5]. Thus, a high impulsivity trait[6] and impulse control deficits such as those observed in attention-deficit/ hyperactivity disorder[7] predict the emergence of compulsive drug use[8], while impulsivity may also predict the severity of OCD symptomatology[9].

Similarly, in preclinical models, trait-like impulsive behavior in rats, as measured by the inability to suppress premature, anticipatory responses in the five-choice serial reaction time task (5-CSRTT)[10] predates and causally contributes to the development of various forms of compulsive behavior[11, 12], thus revealing a serial transition from impulsivity to compulsivity in the development of compulsive disorders. Indeed, highly impulsive (HI) rats are not only predisposed to develop compulsive cocaine self-administration[12], but they have also been shown to be highly vulnerable to develop schedule-induced polydipsia (SIP)[11], a compulsive adjunctive drinking behavior[13, 14] that recapitulates key hallmarks of compulsive behavior in OCD[15]. Thus, the compulsive drinking of SIP is not related to homeostatic thirst regulation[13, 14], but is instead an excessive, repetitive, and maladaptive behavior that decreases markers of anxiety[16].

Although the behavioral and psychological relationship between impulsivity and various forms of compulsivity is well characterized, the neural mechanisms whereby impulse control deficits facilitate the development, and contribute to the severity, of compulsive disorders remain poorly understood.

Increasing evidence suggests that the insular cortex, which is involved in interoception and associated emotional processes[17], may be a neural locus that influences impulsive-compulsive behaviors[18-23]. Specifically, it has been suggested that somatic states associated with reward or anxiety may be processed by this region and thus provide a substrate for anticipatory responses contributing to the induction of impulsive behavior[24] as well as compulsive avoidance behavior[25]. Anatomical and functional imaging studies in humans demonstrate that patients suffering from compulsive disorders, and their siblings[26], show reduced grey matter density[26-29], and altered function[20, 30, 31] of the insular cortex compared with controls. Moreover, neuroimaging studies have implicated the anterior insular cortex (AI) in impulse control in both healthy individuals[20, 32] and those addicted to drugs[33]. In preclinical studies, the AI is associated with reward processing[34, 35] and impulsive decision making[36].

These findings suggest that altered processing of interoceptive information in the AI may be involved in the facilitation of compulsive behaviors[37] by underlying impulsivity traits. We therefore investigated whether the AI is causally involved in the expression of impulsivity in rats and the increased vulnerability this trait confers on the development of compulsive behavior, as measured by SIP[15].

Materials & Methods

The timeline of the five different experiments of this study is presented **figure 1**.
A detailed description of the materials and methods is provided in **SOM**.

Subjects

One hundred and forty male rats were used in this study. All experiments were conducted between 8:00 am and 6:00 pm in agreement with the European Community Council Directives (86/609/EEC).

Apparatus

5-choice serial reaction time task

The 5-choice serial reaction time task was performed in 5-hole boxes that have already been described[11].

Schedule-induced polydipsia

The schedule induced poludipsia task (SIP) was performed in operant chambers that have already been described[11].

Behavioral procedures

5-choice serial reaction time task

The 5-CSRTT procedure has been extensively described [38, 39]. Detailed methods are provided in **SOM**.

Schedule-induced polydipsia

SIP is a maladaptive, excessive intake of freely available water resulting in a decrease in corticosterone levels[16] in the face of predictable intermittent food delivery that has been suggested to generate distress in animals[40]. Being excessive, maladaptive, somehow divorced from its immediate consequences and anxiolytic, performance under a SIP schedule captures the hallmark features of compulsive disorders as defined in the DSM[15]. Detailed methods for SIP experiments (experiments 4 & 5) 1 are provided in **SOM**.

Anxiety

All rats exposed to SIP have been tested for their anxiety level, either before (experiment 4), or after SIP exposure (experiment 5) on an elevated plus maze (EPM, View point Life science, France) as

described in[41, 42]. A detailed description of the procedure for experiments 4 and 5 is provided in **SOM**.

Imaging

Magnetic resonance imaging was performed on anaesthetised rats using a 4.7T Bruker BioSpec 47/40 system at 150µm isotropic resolution with a RARE sequence (imaging parameters: TR/TE 3500/45ms, ETL 16, BW 40kHz).

Voxel-based cortical thickness was used to assess cortical thickness through the entire brain[43]. The thickness maps were assessed using SPM8 (Wellcome Trust Centre for Neuroimaging, UCL, London) with the SPMMouse toolbox[44] using a general linear model to find correlations with premature responding with an exploratory threshold of $p < 0.005$ (uncorrected). See **SOM** for more details.

Surgery

Stereotaxic neurosurgery was conducted under isoflurane anaesthesia as previously described. Lesions of the anterior insular cortex (n = 20) were performed by bilateral infusions of 0.8 µL of 0.09 M quinolinic acid through a 26-gauge cannula (Phymep, Paris, France) at the following coordinates: AP + 1.44 mm and ML ± 5.2 mm from bregma, DV - 6.8 mm from skull surface[45], incisor bar at - 3.3 mm. For sham-operated rats, the injector was lowered bilaterally into the targeted area but no infusion was performed. Histological assessment of the lesions was performed as described in **SOM**.

In situ hybridization

The in situ hybridization experiments were performed on the same drug naïve animals as initially used in a previous study[39], with similar methodology as described in[46]. Briefly, 12µm thick coronal sections of the anterior insular cortex (3 to 5 / rat) were subjected to pre-hybridisation treatment and hybridised overnight with a validated Zif 268 oligoprobe[39]. Sections were then subjected to post-hybridization and exposed 10 days to Kodak MR X-ray films. OD was quantified from digitized films using ImageJ software on regions of interest illustrated in **Figure 3**.

Data and statistical analyses

HI and LI rats were selected based upon their level of premature responses during the two long inter-trial interval (LITI) sessions performed prior to surgery. The percentage of premature responses of each LITI session were averaged and ranked. Depending on the spread of the distribution of the population (example from experiment 3 provided **Figure S1**), rats were designated as HI or LI on the criteria of premature responses greater than or equal to 40 or 50 for HI or premature responses less than or equal to 20 or 30 for LI (adapted from[47]), whichever is closest to the upper and lower quartiles.

High drinkers (HD) and low drinkers (LD) were selected in the upper and lower quartile of the population (**Figure S1**), respectively, according to their average water intake during the last three SIP sessions as described previously[11]. Figures show group means ± SEM. Data were analysed using Statistica 10® and were subjected to analysis of variance (ANOVA) [with time (bins or pre vs post AIC lesion blocks, or insular structures as within-subject factors; and impulsivity, i.e. HI vs LI or lesion or compulsivity, i.e., HC vs LC, as between subject factors].

The normality of the different distributions was assessed with the Kolmogorov-Smirnov test. When assumptions for homogeneity of variance, i.e., for zif268 mRNA levels, tested with the Cochran C test, were violated, data were subjected to a log-transformation.

The relationships between impulsivity and insular thickness and between the level of SIP and the magnitude of the deficits in compulsive behaviour observed after AI lesions in experiment 5 were investigated using a parametric Pearson correlation. For this the deficit in compulsive behaviour was calculated as: $\frac{(Water\ intake\ day\ 20\ posts{-}surgery){-}(Water\ intake\ day\ 20\ pre{-}surgery)}{Water\ intake\ day\ 20\ pre{-}surgery}\ X\ 100.$

For all analyses, upon confirmation of significant main effects, differences among individual means were analysed using the Duncan's post hoc test. For all analyses, significance was accepted at $\alpha = 0.05$. To further support the observed effect of post-training SIP AI lesions carried on relatively small samples (experiment 5) partial Eta-squared values (partial η^2) are reported as a measure of the effect size.

Results

We investigated whether impulsivity in rats measured in the 5-CSRTT, was associated with structural abnormalities in the insular cortex, as observed in impulsive-compulsive human syndromes. Rats characterized as high or low impulsive[12, 38] on the basis of their level of premature responses during long inter-trial interval challenges[47] in the 5-CSRTT were subjected to MRI scanning under general anesthesia. We found that impulsivity was inversely related to, and highly predicted by, the thickness of the insular cortex in rats (**Figure 2A-B**) [R^2=0.62, p<.0001]. These results demonstrate that the relationship between insular cortex thickness and impulsivity is observed across species and that the former may be a neurobiological marker of impulse control deficits.

According to the posterior-to-anterior organization of information integration within the insular cortex[48], the AI, where interoceptive sensory mechanisms overlap with emotional evaluative processes to produce subjective 'feelings'[49], is the primary output domain of the insula and projects to the prefrontal

cortex, the amygdala and the ventral striatal nodes of the corticostriatal circuitry that is involved both in impulse control[47, 50-52] and compulsive behavior such as SIP[53, 54]. We therefore investigated whether HI and LI rats differentially recruit the AI, as indexed by the activation of the cellular plasticity marker zif268[55, 56], under conditions in which impulse control was challenged (**Figure 3A**). For this, we used *in situ* hybridization to quantify zif268 mRNA levels[39] in the granular, agranular and dysgranular AI (**Figure 3B**) of brains harvested from HI and LI rats sacrificed after a 5-CSRTT session[39]. HI rats (n=6), which displayed higher levels of premature responses than LI rats both during LITIs [$F_{1,9}$ =17.84, $p < .001$] and baseline sessions [$F_{1,9}$ = 21.79, $p < .001$], including the one that preceded sacrifice [$F_{1,9}$ = 13.28, $p < .01$] exhibited a reduced level of zif268 mRNA in the AI as compared with LI rats (n=5) (**Figure 3C**) [main effect of impulsivity: $F_{1,9}$ = 5.54, $p < .05$ and impulsivity x structure interaction: $F_{2,18}$ = 5.23, $p < .017$]. This difference was primarily attributable to significantly lower zif268 mRNA levels in the agranular and dysgranular AI [ps<.05], both of which have major reciprocal connections with the prefrontal cortex and the basolateral amygdala and project to the ventral striatum, as opposed to the granular cortex, which projects to the amygdala and more dorsal territories of the striatum[48, 57]. The difference in Zif268 mRNA expression could not be attributable to other differences in task performance nor in rewards obtained since HI and LI rats did not differ in accuracy [$F_{1,9}$ = 3.75, $p > 0.85$], omissions [$F_{1,9} < 1$] or perseveration responses [$F_{1,9} < 1$] (**Figure 3D-E**). Thus altered cellular plasticity mechanisms in the AI may underlie high impulsivity.

To demonstrate a causal involvement of the AI in impulse control deficits we assessed the effects of bilateral excitotoxic lesions of the AI (**Figure 4**) on the level of impulsivity of HI (n=14) and LI rats (n=15) (**Figure S1A-B**). As shown **figure 4**, selective bilateral AI lesions resulted in a significant decrease in premature responding in HI rats only (**Figure 4B & C**) [main effect of period (pre- vs post-surgery assessment): $F_{1,36}$ = 7.3, $p < .05$; impulsivity group x period x surgery interaction, $F_{2,36}$ = 3.39, $p < .05$]. With an average reduction of 48% in premature responses as compared to pre-lesion performance, lesioned HI rats no longer differed from LI rats (either sham or lesioned) in their level of impulsivity (**Figure 4C & D**) [all ps > .05]. The effect of bilateral AI lesions was specific to impulse control because they had no effect on other psychological processes assessed in the 5-CSRTT, including attention and motivation (**Figure S2**) [all Fs < 2, all ps > .05]. Overall these results show that the AI contributes to impulse control deficits displayed by HI rats.

To understand further the common contribution of AI to impulsivity and the associated increased propensity to develop compulsivity, we capitalised on the recently reported propensity of HI rats to

develop excessive levels of water intake in a SIP procedure[11]. We thus investigated the effect of bilateral AI lesions (**Figure 5A**) on the development of SIP (**Figure 5B**) and the long-term expression of this compulsive behavior in high-drinking rats (**Figure 5C-D**). Thirty-four rats received bilateral excitotoxic or sham AI lesions prior to exposure to the SIP procedure. Bilateral AI lesions prevented the development of SIP (**Figure 5B)** [time x surgery interaction: $F_{25,800}$=1.61, p<.05]. Thus, despite a small trend towards increasing their water intake over time as compared to baseline conditions the schedule-induced drinking behavior of lesioned rats never differed from baseline [all ps > .05] as opposed to sham rats in which water intake progressively increased over time, differing from baseline by session 18 [all ps < .05] (**Figure 5B**). This decreased level of compulsive drinking observed in lesioned rats was specific to drinking as they did not differ from shams in either magazine entries or in food intake, thus exhibiting normal feeding motivation (**Figure S3A**) [all Fs < 1].

We next investigated whether the AI is part of the neuroendophenotype that underlies the individual propensity to express high levels of compulsive behaviour, as operationalized in rats selected for their high level of SIP. An independent cohort of rats was exposed to the SIP procedure so that, after 20 daily sessions high compulsive (HC) intermediate (IC) or low compulsive (LC) rats were identified as previously described[11] (**Figure S1C-D**). Rats then received either bilateral excitotoxic or sham AI lesions, and following a post-surgery recovery period of 10 days they were re-exposed to the SIP procedure for 20 post-surgery daily sessions. As illustrated in **Figure 5**, bilateral AI lesions (**Figure 5A**) resulted in a marked decrease in SIP, specifically in compulsive rats (**Figure 5C-D**) [surgery x group x period interaction: $F_{2,34}$ = 4.68, p < .05, partial $\eta^2 = .216$]. Thus whereas HC sham operated rats rapidly recovered their pre-surgery levels of compulsive behavior, displaying daily levels of water intake much higher than their physiological need (as measured in d1 post surgery, **Figure 5C**) [p < .001], lesioned HC rats no longer displayed such compulsive behavior as revealed by their low level of water intake which never differed from baseline. *Post-hoc* analysis revealed that bilateral AI lesions resulted in a ~30% decrease in SIP in HC rats (**Figure 5D**) [p < .01] which differed from sham HC animals throughout the post-surgery training period [p < .01]. Thus HC rats were similar to both sham and lesioned LC animals [all ps > .05] that, akin to IC rats (**Figure S4A-B**), were unaffected by AI lesions (**Figure S4C-D**) [all ps > .05]. This between subject analysis was further strengthened by population-based dimensional analyses (**Figure 5E-F)**. Thus, the magnitude of the deficit in compulsivity following bilateral AI lesions (n = 20) was predicted by the level of compulsive behaviour displayed during the final stages of SIP training [session 19: R = .55, R^2 = .31, p < .002, partial

$\eta^2 = .3$] (**Figure 5E, table S1)**. This correlation was not observed in sham rats (n = 20) (**Figure 5F, table S1)** Thus, bilateral AI lesions dramatically influenced SIP performance only in animals prone to develop high levels of compulsivity.

The differences in water consumption observed in HC lesioned rats were specific to drinking behavior because these animals did not differ from the other groups in their magazine entries (**Figure S3B-C**) [Fs < 1). Thus the effects of bilateral AI lesions on the compulsivity displayed by HC rats were unlikely to be attributable to altered homeostatic regulation since (i) excessive drinking in the SIP procedure is unrelated to thirst[14]; (ii) homeostatic-related baseline levels of water intake in HC, IC and LC rats were not influenced by AI lesions; and (iii) water intake during the SIP procedure was not affected by AI lesions in LC and IC rats (**Figure S4**). Moreover the effect of bilateral AI lesions on SIP is unlikely to be mediated by an altered anxiety state since anxiety indexed on the elevated plus maze was no different between sham and AI lesioned rats (**Figure S5).**

Discussion

The definition of a neural circuit that contributes to impulsivity in its multifaceted forms and the associated vulnerability to develop compulsivity is one of the major objectives in the search for endophenotypes of neuropsychiatric disorders of the impulsive/compulsive spectrum. The present findings show that the AI may be a core component of such a neural circuit, being functionally involved in impulse control deficit and associated propensity to develop compulsive behavior. Indeed, the present results show: (i) that a high impulsivity trait was predicted by AI cortical thinness, a potential anatomical marker of impaired inhibitory control that has also been observed in clinical studies of compulsive stimulant drug users[33], (ii) HI rats showed reduced zif268 mRNA levels indicating blunted cellular plasticity processes in the AI following performance on the 5-CSRTT, an observation in line with the evidence from BOLD imaging that the AI is associated with motor impulsivity in humans[20].

The present results further demonstrate that the AI is causally involved in impulse control deficits and the associated propensity to develop a form of compulsive behavior as operationalized by high levels of drinking in a SIP procedure. Selective bilateral lesions of the AI resulted not only in a marked decrease in impulsivity in HI rats[11, 12], but also prevented the development of compulsive behavior and markedly attenuated its long-lasting expression in highly compulsive rats.

The effects of bilateral AI lesions on impulse control could not be attributed to attentional or motivational deficits, since accuracy and latency to magazine entry, as well as markers of attention

and motivation in the 5-CSRTT, were not affected. Additionally, histological assessment ruled out the possibility that the behavioral effects of AI lesions were due coincidental damage to the adjacent orbitofrontal cortex (OFC). Moreover, bilateral excitotoxic lesions of the OFC have no effect on premature responding, but instead increase perseverative responding in the 5-CSRTT[58]. This double dissociation suggests distinct contributions of the AI and OFC respectively to impulsive and perseverative behavior, as measured in the 5-CSRTT.

Intriguingly, despite suggestions that these OFC-dependent perseverative responses in the 5-CSRTT may represent a form of compulsive behavior linked to OCD[59], they do not correlate with compulsive cocaine self-administration[12], nor with nucleus accumbens dependent behavior such as impulsive responses in the 5CSRTT[10] and SIP[11 and present study], nor are they influenced by atomoxetine, a noradrenaline reuptake inhibitor that decreases both impulsivity and SIP[11]. This suggests that perseverative responding in the 5-CSRTT may depend on neural and psychological processes that are dissociable from those underlying the compulsive nature of SIP.

Bilateral AI lesions influenced neither baseline water intake nor the increased anxiety state that follows chronic exposure to the SIP procedure. Thus, the influence of AI lesions in the development of SIP on general populations or the long-term expression of high levels of SIP in high compulsive rats cannot be attributable to an effect on the basic mechanisms governing thirst or the expression of anxiety.

The lack of effect on anxiety of AI lesions despite their effect to reduce levels of SIP may reflect the selective involvement of the insular cortex in the development of compulsive habits. Indeed, while corticosterone levels have been shown to be reduced after as little as 4 ml of drinking in polydipsic rats[60], the excessive drinking displayed by the HC rats was not motivated solely by a homeostatic tendency to reduce stress. If this was the case, then the prevention of SIP would be associated with an increase in anxiety levels, but AI lesions had no effect on elevated plus maze measures of anxiety. The data are more compatible with the hypothesis that, after more than three weeks of training, SIP had become compulsive following over-training and therefore divorced from any stress-reducing functions it may have served during its acquisition.

The effects of bilateral AI lesions on both impulsive and compulsive behaviors were only partial, e.g. between 30 and 40% decrease in behaviour, and state-dependent in that they were only observed in vulnerable HI or HC rats. This suggests that the AI is a key neural node in a network inducing these behaviors, whilst not necessarily itself subserving impulse control and the associated propensity to

express compulsive behaviors (see [61]). The precise nature of the neural network through which the AI influences impulse control and the transition to compulsivity remains to be established but likely involves the prefrontal cortex and the amygdala with which the AI has extensive reciprocal connections[52, 62], as well as the nucleus accumbens[4].

The anatomical e.g.[63] as well as functional relationships[64] between the AI and the nucleus accumbens, long viewed as a functional interface between emotions and actions[65], may contribute to the modulation of impulse control and the associated transition to compulsivity[66, 67]. AI-accumbens functional interactions are involved in incentive anticipation[64], while the nucleus accumbens has been shown to regulate both impulsivity and the development of SIP[51, 54, 68]. Another potential neural network by which the AI influences impulse control is through its projections to the amygdala, since the reciprocal interactions between the AI and the amygdala have been shown to facilitate the conscious awareness of emotional states related to subjective feelings[69]. Neuroimaging studies have shown that the stronger the connectivity between insular cortex and amygdala, the lower was impulse control in a group of abstinent heroin addicted patients[70]. This observation is in line with our current findings demonstrating that AI lesions restore impulse control in highly impulsive rats and prevents the development of compulsivity.

The role of the AI in the modulation of impulse control and the associated propensity to develop compulsive behavior demonstrated in the present study is consistent with the theoretical framework of Bechara and Damasio[71, 72] suggesting that interoceptive mechanisms play a major role in regulating cognitive processes. Thus impaired AI-dependent emotionally evaluated interoceptive states[19] may tune corticostriatal processes governing control over internally-driven urges, thereby facilitating impulsivity and hence the transition to compulsivity under stress.

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"Supplementary information is available at Molecular Psychiatry's website"

References

1. Menzies L, Achard S, Chamberlain S, Fineberg N, Chen C, del Campo N *et al.* Neurocognitive endophenotypes of obsessive-compulsive disorder. *Brain* 2007; **130**(Pt 12)**:** 3223-3236.

2. Robbins TW, Gillan CM, Smith DG, de Wit S, Ersche KD. Neurocognitive endophenotypes of impulsivity and compulsivity: towards dimensional psychiatry. *Trends Cogn Sci* 2012; **16**(1)**:** 81-91.

3. Lejuez CW, Magidson JF, Mitchell SH, Sinha R, Stevens MC, de Wit H. Behavioral and biological indicators of impulsivity in the development of alcohol use, problems, and disorders. *Alcohol Clin Exp Res* 2010; **34**(8)**:** 1334-1345.

4. Dalley J, Everitt B, Robbins T. Impulsivity, compulsivity, and top-down cognitive control. *Neuron* 2011; **69**(4)**:** 680-694.

5. Ersche K, Turton A, Chamberlain S, Muller U, Bullmore E, Robbins T. Cognitive dysfunction and anxious-impulsive personality traits are endophenotypes for drug dependence. *Am J Psychiatry* 2012; **169**(9)**:** 926-936.

6. Fernandez-Serrano M, Perales J, Moreno-Lopez L, Perez-Garcia M, Verdejo-Garcia A. Neuropsychological profiling of impulsivity and compulsivity in cocaine dependent individuals. *Psychopharmacology (Berl)* 2012; **219**(2)**:** 673-683.

7. Wilens T. The nature of the relationship between attention-deficit/hyperactivity disorder and substance use. *J Clin Psychiatry* 2007; **68 Suppl 11:** 4-8.

8. Brook D, Brook J, Zhang C, Koppel J. Association between attention-deficit/hyperactivity disorder in adolescence and substance use disorders in adulthood. *Arch Pediatr Adolesc Med* 2010; **164**(10)**:** 930-934.

9. Lopez-Ibor JJ. Impulse control in obsessive-compulsive disorder: a biopsychopathological approach. *Prog Neuropsychopharmacol Biol Psychiatry* 1990; **14**(5)**:** 709-718.

10. Robbins TW. The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. *Psychopharmacology*, vol. 163(3-4)2002, pp 362-380.

11. Ansquer S, Belin-Rauscent A, Dugast E, Duran T, Benatru I, Mar AC *et al.* Atomoxetine decreases vulnerability to develop compulsivity in high impulsive rats. *Biol Psychiatry* 2014; **75**(10)**:** 825-832.

12. Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ. High impulsivity predicts the switch to compulsive cocaine-taking. *Science* 2008; **320**(5881)**:** 1352-1355.

13. Falk JL. The motivational properties of schedule-induced polydipsia. *J Exp Anal Behav* 1966; **9**(1)**:** 19-25.

14. Moreno M, Flores P. Schedule-induced polydipsia as a model of compulsive behavior: neuropharmacological and neuroendocrine bases. *Psychopharmacology (Berl)* 2012; **219**(2)**:** 647-659.

15. Association AP. *The Diagnostic and Statistical Manual of Mental Disorders: DSM 5*. bookpointUS2013.

16. Dantzer R, Terlouw C, Mormede P, Le Moal M. Schedule-induced polydipsia experience decreases plasma corticosterone levels but increases plasma prolactin levels. *Physiol Behav* 1988; **43**(3)**:** 275-279.

17. Critchley HD, Wiens S, Rotshtein P, Ohman A, Dolan RJ. Neural systems supporting interoceptive awareness. *Nat Neurosci* 2004; **7**(2)**:** 189-195.

18. Phillips ML, Marks IM, Senior C, Lythgoe D, O'Dwyer AM, Meehan O *et al.* A differential neural response in obsessive-compulsive disorder patients with washing compared with checking symptoms to disgust. *Psychol Med* 2000; **30**(5)**:** 1037-1050.

19. Fineberg N, Potenza M, Chamberlain S, Berlin H, Menzies L, Bechara A *et al.* Probing Compulsive and Impulsive Behaviors, from Animal Models to Endophenotypes: A Narrative Review. *Neuropsychopharmacology* 2009.

20. Dambacher F, Sack AT, Lobbestael J, Arntz A, Brugman S, Schuhmann T. Out of control Evidence for anterior insula involvement in motor impulsivity and reactive aggression. *Soc Cogn Affect Neurosci* 2014.

21. Strigo IA, Matthews SC, Simmons AN, Oberndorfer T, Klabunde M, Reinhardt LE *et al.* Altered insula activation during pain anticipation in individuals recovered from anorexia nervosa: evidence of interoceptive dysregulation. *Int J Eat Disord* 2013; **46**(1)**:** 23-33.

22. Hoptman MJ, Antonius D, Mauro CJ, Parker EM, Javitt DC. Cortical thinning, functional connectivity, and mood-related impulsivity in schizophrenia: relationship to aggressive attitudes and behavior. *Am J Psychiatry* 2014; **171**(9)**:** 939-948.

23. Chung T, Clark DB. Insula white matter volume linked to binge drinking frequency through enhancement motives in treated adolescents. *Alcohol Clin Exp Res* 2014; **38**(7)**:** 1932-1940.

24. Verdejo-Garcia A, Bechara A. A somatic marker theory of addiction. *Neuropharmacology* 2009; **56 Suppl 1:** 48-62.

25. Paulus MP, Stein MB. An insular view of anxiety. *Biol Psychiatry* 2006; **60**(4)**:** 383-387.

26. Ersche K, Jones P, Williams G, Turton A, Robbins T, Bullmore E. Abnormal brain structure implicated in stimulant drug addiction. *Science* 2012; **335**(6068)**:** 601-604.

27. van den Heuvel OA, Remijnse PL, Mataix-Cols D, Vrenken H, Groenewegen HJ, Uylings HBM *et al. The major symptom dimensions of obsessive-compulsive disorder are mediated by partially distinct neural systems*, vol. 1322009, 853-868pp.

28. Moreno-Lopez L, Catena A, Fernandez-Serrano M, Delgado-Rico E, Stamatakis E, Perez-Garcia M *et al.* Trait impulsivity and prefrontal gray matter reductions in cocaine dependent individuals. *Drug Alcohol Depend* 2012; **125**(3)**:** 208-214.

29. Song A, Jung WH, Jang JH, Kim E, Shim G, Park HY *et al.* Disproportionate alterations in the anterior and posterior insular cortices in obsessive-compulsive disorder. *PLoS One* 2011; **6**(7)**:** e22361.

30. Velikova S, Locatelli M, Insacco C, Smeraldi E, Comi G, Leocani L. Dysfunctional brain circuitry in obsessive-compulsive disorder: source and coherence analysis of EEG rhythms. *Neuroimage* 2010; **49**(1)**:** 977-983.

31. Remijnse P, van den Heuvel O, Nielen M, Vriend C, Hendriks G, Hoogendijk W *et al.* Cognitive inflexibility in obsessive-compulsive disorder and major depression is associated with distinct neural correlates. *PLoS One* 2013; **8**(4)**:** e59600.

32. Churchwell JC, Yurgelun-Todd DA. Age-related changes in insula cortical thickness and impulsivity: significance for emotional development and decision-making. *Dev Cogn Neurosci* 2013; **6:** 80-86.

33. Ersche K, Barnes A, Simon Jones P, Morein-Zamir S, Robbins T, Bullmore E. Abnormal structure of frontostriatal brain systems is associated with aspects of impulsivity and compulsivity in cocaine dependence. *Brain* 2011; **134**(Pt 7)**:** 2013-2024.

34. Li C, Zhu N, Meng X, Li Y, Sui N. Effects of inactivating the agranular or granular insular cortex on the acquisition of the morphine-induced conditioned place preference and naloxone-precipitated conditioned place aversion in rats. *J Psychopharmacol* 2013; **27**(9)**:** 837-844.

35. Wu W, Li H, Liu Y, Huang X, Chen L, Zhai H. Involvement of insular muscarinic cholinergic receptors in morphine-induced conditioned place preference in rats. *Psychopharmacology (Berl)* 2014.

36. Pattij T, Schetters D, Schoffelmeer A. Dopaminergic modulation of impulsive decision making in the rat insular cortex. *Behav Brain Res* 2014; **270:** 118-124.

37. Naqvi N, Bechara A. The hidden island of addiction: the insula. *Trends Neurosci* 2009; **32**(1)**:** 56-67.

38. Murray JE, Dilleen R, Pelloux Y, Economidou D, Dalley JW, Belin D *et al.* Increased impulsivity retards the transition to dorsolateral striatal dopamine control of cocaine seeking. *Biol Psychiatry* 2014; **76**(1)**:** 15-22.

39. Besson M, Pelloux Y, Dilleen R, Theobald DE, Lyon A, Belin-Rauscent A *et al.* Cocaine modulation of frontostriatal expression of Zif268, D2, and 5-HT2c receptors in high and low impulsive rats. *Neuropsychopharmacology* 2013; **38**(10)**:** 1963-1973.

40. Platt B, Beyer C, Schechter L, Rosenzweig-Lipson S. Schedule-induced polydipsia: a rat model of obsessive-compulsive disorder. *Curr Protoc Neurosci* 2008; **Chapter 9:** Unit 9.27.

41. Dilleen R, Pelloux Y, Mar A, Molander A, Robbins T, Everitt B *et al.* High anxiety is a predisposing endophenotype for loss of control over cocaine, but not heroin, self-administration in rats. *Psychopharmacology (Berl)* 2012; (1)**:** 89-89.

42. Vanhille N, Belin-Rauscent A, Mar AC, Ducret E, Belin D. High Locomotor Reactivity to Novelty Is Associated with an Increased Propensity to Choose Saccharin Over Cocaine: New Insights into the Vulnerability to Addiction. *Neuropsychopharmacology* 2015; **40**(3)**:** 577-589.

43. Sawiak SJ, Wood NI, Carpenter TA, Morton AJ. Huntington's disease mouse models online: high-resolution MRI images with stereotaxic templates for computational neuroanatomy. *PLoS One* 2012; **7**(12)**:** e53361.

44. Sawiak SJ, Wood NI, Williams GB, Morton AJ, Carpenter TA. Voxel-based morphometry with templates and validation in a mouse model of Huntington's disease. *Magnetic resonance imaging* 2013; **31**(9)**:** 1522-1531.

45. Paxinos G, Watson C. *The rat brain in stereotaxic coordinates*. 5 edn. Elsevier Academic Press: Amsterdam, 2005.

46. Belin D, Deroche-Gamonet V, Jaber M. Cocaine-induced sensitization is associated with altered dynamics of transcriptional responses of the dopamine transporter, tyrosine hydroxylase, and dopamine D2 receptors in C57Bl/6J mice. *Psychopharmacology (Berl)* 2007; **193**(4)**:** 567-578.

47. Dalley JW, Fryer T, Brichard L, Robinson E, Theobald D, Laane K *et al.* Nucleus Accumbens D2/3 Receptors Predict Trait Impulsivity and Cocaine Reinforcement. *Science* 2007; **315**(5816)**:** 1267-1270.

48. Allen GV, Saper CB, Hurley KM, Cechetto DF. Organization of visceral and limbic connections in the insular cortex of the rat. *J Comp Neurol* 1991; **311**(1)**:** 1-16.

49. Zaki J, Davis J, Ochsner K. Overlapping activity in anterior insula during interoception and emotional experience. *Neuroimage* 2012; **62**(1)**:** 493-499.

50. Cardinal R, Pennicott DR, Lakmali C, Robbins TW. Impulsive Choice Induced in Rats by Lesions of the Nucleus Accumbens Core. *Science* 1252; (5526)**:** 2499-2501.

51. Besson M, Belin D, McNamara R, Theobald DE, Castel A, Beckett V *et al.* Dissociable Control of Impulsivity in Rats by Dopamine D2/3 Receptors in the Core and Shell Subregions of the Nucleus Accumbens. *Neuropsychopharmacology* 2009; (2)**:** 560-569.

52. Reynolds SM, Zahm DS. Specificity in the projections of prefrontal and insular cortex to

ventral striatopallidum and the extended amygdala. *J Neurosci* 2005; **25**(50)**:** 11757-11767.

53. Weissenborn R, Blaha CD, Winn P, Phillips AG. Schedule-induced polydipsia and the nucleus accumbens: electrochemical measurements of dopamine efflux and effects of excitotoxic lesions in the core. *Behav Brain Res* 1996; **75**(1-2)**:** 147-158.

54. Robbins TW, Koob GF. Selective disruption of displacement behaviour by lesions of the mesolimbic dopamine system. *Nature* 1980; **285**(5764)**:** 409-412.

55. Thomas K, Arroyo M, Everitt B. Induction of the learning and plasticity-associated gene Zif268 following exposure to a discrete cocaine-associated stimulus. *Eur J Neurosci* 2003; **17**(9)**:** 1964-1972.

56. Valjent E, Aubier B, Corbille AG, Brami-Cherrier K, Caboche J, Topilko P *et al.* Plasticity-associated gene Krox24/Zif268 is required for long-lasting behavioral effects of cocaine. *J Neurosci* 2006; **26**(18)**:** 4956-4960.

57. Shi CJ, Cassell MD. Cortical, thalamic, and amygdaloid connections of the anterior and posterior insular cortices. *J Comp Neurol* 1998; **399**(4)**:** 440-468.

58. Chudasama Y, Passetti F, Rhodes SE, Lopian D, Desai A, Robbins TW. Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic and orbitofrontal cortex in the rat: differential effects on selectivity, impulsivity and compulsivity. *Behav Brain Res* 2003; **146**(1-2)**:** 105-119.

59. Fineberg N, Chamberlain S, Hollander E, Boulougouris V, Robbins T. Translational approaches to obsessive-compulsive disorder: from animal models to clinical treatment. *Br J Pharmacol* 2011; **164**(4)**:** 1044-1061.

60. Brett LP, Levine S. The pituitary-adrenal response to "minimized" schedule-induced drinking. *Physiol Behav* 1981; **26**(2)**:** 153-158.

61. Cai W, Ryali S, Chen T, Li CS, Menon V. Dissociable roles of right inferior frontal cortex and anterior insula in inhibitory control: evidence from intrinsic and task-related functional parcellation, connectivity, and response profile analyses across multiple datasets. *J Neurosci* 2014; **34**(44)**:** 14652-14667.

62. Augustine JR. Circuitry and functional aspects of the insular lobe in primates including humans. *Brain Res Brain Res Rev* 1996; **22**(3)**:** 229-244.

63. Schilman EA, Uylings HB, Galis-de Graaf Y, Joel D, Groenewegen HJ. The orbital cortex in rats topographically projects to central parts of the caudate-putamen complex. *Neurosci Lett* 2008; **432**(1)**:** 40-45.

64. Cho YT, Fromm S, Guyer AE, Detloff A, Pine DS, Fudge JL *et al.* Nucleus accumbens, thalamus and insula connectivity during incentive anticipation in typical adults and adolescents. *Neuroimage* 2013; **66:** 508-521.

65. Mogenson GJ, Jones DL, Yim CY. From motivation to action: functional interface between the

limbic system and the motor system. *Prog Neurobiol* 1980; **14**(2-3)**:** 69-97.

66. Weller J, Levin I, Shiv B, Bechara A. The effects of insula damage on decision-making for risky gains and losses. *Soc Neurosci* 2009; **4**(4)**:** 347-358.

67. He Q, Xiao L, Xue G, Wong S, Ames S, Schembre S *et al.* Poor ability to resist tempting calorie rich food is linked to altered balance between neural systems involved in urge and self-control. *Nutr J* 2014; **13**(1)**:** 92.

68. Cardinal RN, Pennicott DR, Sugathapala CL, Robbins TW, Everitt BJ. Impulsive choice induced in rats by lesions of the nucleus accumbens core. *Science* 2001; **292**(5526)**:** 2499-2501.

69. Craig A. Emotional moments across time: a possible neural basis for time perception in the anterior insula. *Philos Trans R Soc Lond B Biol Sci* 2009; **364**(1525)**:** 1933-1942.

70. Xie C, Li S, Shao Y, Fu L, Goveas J, Ye E *et al.* Identification of hyperactive intrinsic amygdala network connectivity associated with impulsivity in abstinent heroin addicts. *Behav Brain Res* 2011; **216**(2)**:** 639-646.

71. Bechara A, Damasio AR. The somatic marker hypothesis: A neural theory of economic decision. *Game Econ Behav* 2005; **52**(2)**:** 336-372.

72. Bechara A, Van der Linden M. Decision-making and impulse control after frontal lobe injuries. *Current Opinion in Neurology* 2005; **18**(6)**:** 734-739.

Figure legends

Figure 1: Timeline of the 5 experiments

Five experiments were carried out on independent cohorts of rats. Experiments 1 and 2 aimed at identifying anatomical and functional markers of impulsivity in the insular cortex of rats selected as high or low impulsive based on their level of premature responses in the 5-CSRTT. Experiments 2-5 aimed at investigating the influence of bilateral anterior insula lesions on impulsivity trait, the development of compulsive behavior as measured in the development of high levels of drinking in a SIP procedure and the expression of compulsivity in rats identified as high drinkers in the SIP procedure. 5-CSRTT: Five choice serial reaction time task. SIP: schedule-induced polydipsia. AI: anterior insula. EPM: elevated plus maze. HI: Highly impulsive, LI: low impulsive. HC: highly compulsive, LC: low compulsive.

Figure 2: Impulsivity is predicted by the thinness of the insular cortex in rats.

A three-dimensional reconstruction displaying typical cortical thickness in HI rats as compared with LI rats (**A**) suggests that the higher impulsivity the thinner the insula. A correlational analysis (**B**) between the thickness result from significant voxels in the insula cortex and impulsivity (premature responding rate (%) in the 5-CSRTT) revealed that those rats having higher rates of premature responding exhibit thinner insular cortices [R^2=0.62, p<.0001].

Figure 3: High impulsivity in the 5-CSRTT is associated with reduced Zif268 mRNA levels in the anterior insular cortex.

(**A**) As compared to LI rats, HI rats displayed higher levels of premature responses (an index of impulsivity) both during lITI and baseline sessions, including the one that preceded the assessment of zif258 mRNA. (**B**) *In situ* hybridization for the zif268 mRNA yielded excellent signal in the granular, agranular and dysgranular AI of HI and LI rats. (**C**) As compared to LI rats, HI rats displayed a lower level of zif268 mRNA in both the agranular and dysgranuar AI, but not in the granular AI. (D-E) HI and LI rats only differed in their impulse control since they displayed no differences in accuracy, omissions, and perseverative responses in the 5-CSRTT. HI, high impulsive rat; LI, low impulsive rat; II, intermediate-impulsive rat; Bl, baseline; lITI, long inter trial interval; AGI, agranular insula; DGI, dysgranular insula; GI, granular insula. Blue background represents post-lesion performance.

Figure 4: Bilateral anterior insular cortex lesion restores impulse control in highly

impulsive rats

(**A**) Schematic representation of the extent of the bilateral excitotoxic lesions of the AI from rats identified as high (HI) or low impulsive (LI). Areas shaded in grey represent the extent of neuronal damage. Coronal sections are +4.4 mm anterior through 0.00 mm anterior to Bregma. (**B**) After bilateral lesion of the anterior insular cortex (AI), the level of premature responses of HI rats during the LITI sessions was significantly reduced and did not differ from those expressed by intermediate and low impulsive rats. (**C**) Overall levels of impulsivity of the lesioned HI rats were significantly reduced post-surgery in marked contrast sham operated HI rats, which did not display a decrease in premature responses during the LITI sessions post-surgery (**D-E**). HI, high impulsive rat; LI, low impulsive rat; II, intermediate-impulsive rat; BI, baseline; LITI, long inter trial interval. *: $p<0.05$ different from pre-surgery levels.

Figure 5: Bilateral anterior insular cortex lesion prevents the development of SIP and its expression in high compulsive rats.

(**A**) Schematic representation of the extent of the bilateral excitotoxic lesions of the AI from rats identified as high (HC) or low compulsive (LC). Areas shaded in violet represent the extent of neuronal damage. Coronal sections are +4.4 mm anterior through 0.00 mm anterior to Bregma. (**B**). A pre-training bilateral lesion of the AI prevented the development of compulsive behaviour. Whereas Sham operated rats displayed an increase in water intake over time which reached levels significantly higher than baseline by session 18 (all ps<.05), lesioned rats never displayed levels of water intake that differed from baseline. (**C**). Bilateral AI lesions dramatically reduce compulsive behaviour in high compulsive rats. Following a bilateral AI lesion, only HC rats failed to re-developed SIP since their daily water intake over 20 SIP sessions no longer differed from baseline, contrary to sham animals for which water intake increased significantly relative to baseline from the first post-surgery SIP session (al ps<.05). A comparison of the overall average daily water intake displayed by sham and lesioned HC rats pre and post lesion (**D**) revealed that bilateral AI lesions decreased by ≈ 30% compulsive behaviour in HC rats ($p<.001$). (**E-F**) Regression analyses on cohorts of sham (n=20) or AI-lesioned (n=20) rats revealed that initial levels of performance under the SIP procedure predicted the magnitude of the deficit in compulsive behaviour following bilateral AI lesions.

Violet background represents post-lesion assessment; dotted line represents the post surgery water intake baseline; HC, high compulsive rat; *, different from baseline $p<.001$, $^{@,}$ different from HC

lesioned pre-surgery p<.001; [£], different from HC lesioned pre-surgery p<.01; [$], different from HC sham post-surgery p<.01. AP: antero-posterior, Bl: baseline.