Neuropsychopharmacology (2012) 37, 2057–2066

www.neuropsychopharmacology.org

Open

Norepinephrine and Dopamine Modulate Impulsivity on the Five-Choice Serial Reaction Time Task Through Opponent Actions in the Shell and Core Sub-Regions of the Nucleus Accumbens

Daina Economidou[1], David EH Theobald[1], Trevor W Robbins[1], Barry J Everitt[1] and Jeffrey W Dalley*[,1,2]

[1]*Behavioral and Clinical Neuroscience Institute and Department of Experimental Psychology, University of Cambridge, Cambridge, UK;* [2]*Department of Psychiatry, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK*

Impulsive behavior is a hallmark of several neuropsychiatric disorders (eg, attention-deficit/hyperactivity disorder, ADHD). Although dopamine (DA) and norepinephrine (NE) have a significant role in the modulation of impulsivity their neural loci of action is not well understood. Here, we investigated the effects of the selective NE re-uptake inhibitor atomoxetine (ATO) and the mixed DA/NE re-uptake inhibitor methylphenidate (MPH), both with proven clinical efficacy in ADHD, on the number of premature responses on a five-choice serial reaction time task, an operational measure of impulsivity. Microinfusions of ATO into the shell, but not the core, sub-region of the nucleus accumbens (NAcb) significantly decreased premature responding whereas infusions of MPH in the core, but not the shell, sub-region significantly increased premature responding. However, neither ATO nor MPH significantly altered impulsive behavior when infused into the prelimbic or infralimbic cortices. The opposing effects of ATO and MPH in the NAcb core and shell on impulsivity were unlikely mediated by ancillary effects on behavioral activation as locomotor activity was either unaffected, as in the case of ATO infusions in the core and shell, or increased when MPH was infused into either the core and shell sub-region. These findings indicate an apparently 'opponent' modulation of premature responses by NE and DA in the NAcb shell or core, respectively, and suggest that the symptom clusters of hyperactive-impulsive type ADHD may have distinct neural and neurochemical substrates.
Neuropsychopharmacology (2012) **37,** 2057–2066; doi:10.1038/npp.2012.53; published online 18 April 2012

Keywords: atomoxetine; methylphenidate; nucleus accumbens; prefrontal cortex; impulsivity

INTRODUCTION

Impulsivity, the tendency to act prematurely without foresight, represents a core feature of several neuropsychiatric disorders (eg, attention-deficit/hyperactivity disorder (ADHD), mania and substance abuse) (Dalley *et al*, 2011; Moeller *et al*, 2001). The catecholamines dopamine (DA) and norepinephrine (NE) are widely implicated in the modulation of impulsivity through the clinical efficacy of drugs that increase brain DA and NE function (eg, methylphenidate (MPH) and atomoxetine (ATO)) and by evidence that DA-ergic and NE-ergic transmission are deficient in patients with impulse control disorders (Comings *et al*, 2003; Faraone *et al*, 2005).

*Correspondence: Dr JW Dalley, Behavioral and Clinical Neuroscience Institute and Department of Experimental Psychology, University of Cambridge, Downing Street, Cambridge, Cambridgeshire, CB2 3EB, UK, Tel: +44(1223) 765 291, Fax: +44(1223) 333 564,
E-mail: jwd20@cam.ac.uk
Received 16 February 2012; revised 19 March 2012; accepted 21 March 2012

Animal studies support a role for DA and NE in impulsivity. Thus, depletion of DA in the ventral striatum of rats, including the nucleus accumbens (NAcb), has the profound effect of reducing impulsivity on a five-choice serial reaction time task (5-CSRTT) (Cole and Robbins, 1989) similar to the effects of DA receptor antagonists infused locally into the NAcb core (NAcbC) (Pattij *et al*, 2007; Pezze *et al*, 2007). Drugs that increase brain DA neurotransmission (eg, amphetamine and MPH) produce divergent effects on impulsive behavior, generally acting to improve stopping performance on stop-signal reaction time tasks and reducing delay-discounting impulsivity while increasing 5-CSRTT impulsivity (Eagle *et al*, 2007; van Gaalen *et al*, 2006; Navarra *et al*, 2008). Enhancing extracellular NE levels by systemic treatment with the selective NE re-uptake transporter (NET) inhibitor ATO decreases several distinct forms of impulsivity in rats (Robinson *et al*, 2008). However, the precise neural mechanisms underlying the regulation of impulsivity by NE and DA are not yet clear and could involve both cortical (eg, prefrontal cortex, PFC) and subcortical regions,

especially the NAcbC and NAcb shell (NAcbS) (Dalley *et al*, 2008, 2011).

Data from both animals and humans converge to suggest that PFC-striatal circuitry is an important substrate for the manifestation of pathologically impulsive behavior (Dalley *et al*, 2008; Potenza *et al*, 2003). Indeed, optimal levels of DA and NE are critical to PFC function and suboptimal transmission can lead to PFC dysfunction and symptoms resembling ADHD (Arnsten and Li, 2005; Arnsten and Pliszka, 2011). Although the NAcb receives a dense dopaminergic innervation from the ventral tegmental area (Haber *et al*, 2000; Voorn *et al*, 1986), only the NAcbS receives a significant input from locus ceruleus and the medullary A2 group of NE neurons (Berridge *et al*, 1997; Delfs *et al*, 1998; McKittrick and Abercrombie, 2007). However, the functional importance of this afferent NE-ergic innervation of the NAcbS in impulsivity is unknown.

Here, we investigated the effects on impulsivity of the selective NET inhibitor ATO and the mixed DAT/NET inhibitor MPH (Gatley *et al*, 1996; Wong *et al*, 1982) infused directly into either the NAcbC or NAcbS, or the prelimbic (Prl) and infralimbic (IL) cortex. Impulsivity was defined as the number of premature responses on the 5-CSRTT, an attentional paradigm requiring action restraint during a waiting interval for a reward-predictive target stimulus (Robbins, 2002). To rule out nonspecific effects, we also investigated the effects of intra-PFC and intra-NAcb infusions of ATO and MPH on spontaneous locomotor activity.

MATERIALS AND METHODS

Animals

Outbred male Lister Hooded rats (Charles River, Margate, UK) weighing 280–300 g at the beginning of the experiments were used. Water was available *ad libitum* and food was given at the end of each day's testing. Rats were housed under temperature and humidity controlled conditions and a reversed 12-h light–dark cycle (lights off at 0700 hours). All procedures conformed to the UK (1986) Animal (Scientific Procedures) Act (Project license 80/2234).

5-CSRTT

A detailed description of the apparatus and procedures employed has been described previously (Bari *et al*, 2008). A PC using WhiskerServer software and FiveChoice client controlled the apparatus (Cardinal and Aitken, 2010). Briefly, subjects were trained on the 5-CSRTT to detect the location of a brief visual stimulus (0.5 s) presented pseudo-randomly in one of five spatially distinct apertures on the front wall of the chamber. Each session consisted of 100 discrete trials and lasted approximately 30 min. Trials were initiated by animals entering the food magazine on the opposite wall, and after an intertrial interval (ITI) of 5 s had elapsed, the visual stimulus was presented in a single location that varied on a trial-by-trial basis. Rats were rewarded with a food pellet (Noyes dustless pellets, Research Diets, UK) if they correctly located the position of the target stimulus with a nose-poke response; deemed a 'correct' response. A failure to respond within 5 s of the target presentation resulted in a time-out (TO) period of 5 s and was deemed an 'omission'. Responses made before the target stimulus or in an adjacent hole were deemed 'premature' or 'incorrect' responses, respectively, and also resulted in TO. Responses during TO were not further punished. Responses made in any hole after a correct or incorrect response but before collecting the food pellet were deemed 'perseverative' responses and were never punished. Performance was assessed using seven variables: premature responses; choice accuracy (% correct/correct + incorrect responses); omissions (% omissions/correct + incorrect + omissions); perseverative responses; latency to make a correct or an incorrect response after the onset of the target stimulus (ms); and, latency to collect food from the magazine after a correct trial (ms).

Following stable baseline performance on the 5-CSRTT (ITI = 5 s), drug testing begun. As the main dependent variable in our studies was the number of premature responses, on drug treatment days we increased the inter-trial interval to 7 s to avoid potential floor effects. We have found that imposing a fixed long ITI (LoITI) significantly increases the frequency of premature responses (Dalley *et al*, 2007) and thus widens the window for detecting decreases as well as increases in impulsive behavior. Before drug testing began, animals experienced three LoITI sessions spaced at weekly intervals to generate a stable baseline level of heightened impulsivity.

Locomotor Activity

Locomotor activity was evaluate in 12 chambers (Med Associates; 29.5 × 32.5 × 23.5 cm) equipped with infrared photocell beams and controlled by a PC. The day before initiation of drug testing, animals were habituated to the locomotor chambers for 2 h. On drug treatment days, animals were initially placed into the locomotor chambers for 30 min (habituation). Animals were then removed from the chambers, given the respective drug/vehicle infusion and returned to the chambers 5 min later. Locomotor activity was then recorded for a further 30 min and was measured as photocell beam interruptions. Drug testing was conducted every third day.

Drugs

ATO hydrochloride (a gift from Eli Lilly, Basingstoke, UK) was dissolved in 0.01 mol/l phosphate-buffered saline (PBS). MPH hydrochloride (Sigma, Cambridge, UK) was dissolved in 0.9% sterile saline. Both drugs were given by intracranial infusions (0.5 μl per infusion).

Intracranial Surgery

Rats were anesthetized with ketamine hydrochloride (100 mg/kg; Ketaset, Fort Dodge Animal Health, Southampton, UK) and xylazine (9 mg/kg; Rompun, Bayer, Newbury, Germany), and secured in a stereotaxic frame with the incisor bar set at −3.3 mm relative to the interaural line in flat skull position. Bilateral 22-gauge double guide cannulae (Plastics One, Sevenoaks, UK) were implanted bilaterally in the cortex overlying the medial (m)PFC (PrL/IL), NAcbC, or the NacbS, according to published stereotaxic coordinates (Table 1; Paxinos and Watson, 2004).

Table 1 Brain Stereotaxic Coordinates (Relative to Bregma; mm) for NAcb and mPFC Guide Cannula

Region	Antero–posterior	Lateral	Ventral
mPFC (PrL/IL)	+3.0	± 0.75	1.6 (from dura)
NAcbS	+1.7	± 0.75	2.0 (from skull)
NAcbC	+1.7	± 1.9	2.2 (from skull)

Drugs were injected through a plastic injector (28-gauge) protruding beyond the cannula tip: 1.5 mm for the PrL, 3.0 mm for the IL, 5.25 mm for the NacbS, and 5.0 mm for the NAcbC.

Cannulae were secured to the skull with dental acrylic and stainless steel screws and occluded by a stylet. After surgery animals were allowed to recover for 1 week.

Intracranial Microinfusions

Drug infusions were given 5 min before behavioral testing. Animals were habituated to the infusion procedure over two daily sessions and received on both occasions a single vehicle infusion over 1 min (ATO; PBS, MPH; saline, 0.5 μl). During this procedure, rats were gently restrained by the experimenter while the obturators were removed from the cannulae and the respective bilateral injectors lowered into the intended brain region. Following each infusion, the injector remained in the brain for 2 min. The injector was then removed and the obturator replaced before placing the animal into the respective test apparatus.

Histological Assessment

At completion of the experiments, rats were killed by an overdose of sodium pentobarbital (1.5 ml per rat, Dolethal 200 mg/ml, Rhone-Merieux, Athens), and perfused transcardially with 0.01 M PBS followed by 4% paraformaldehyde. The brains were removed, post-fixed in 4% paraformaldehyde, and transferred to 20% sucrose solution in 0.01 M PBS overnight before being sectioned into 60 μm coronal sections. Every third section was mounted and stained with Cresyl Violet. Cannulae placements were verified under light microscope and mapped onto standardized coronal sections of the rat brain (Paxinos and Watson, 2004).

Experiment 1: Effects of Intra-NAcbS Infusions of ATO or MPH on 5-CSRTT Performance

Two groups of rats with bilateral cannulae aimed at the NAcbS ($n = 7$–8 per group) were infused with either ATO or MPH (0.5–5.0 μg), or their respective vehicle (see above) before 5-CSRTT testing. Infusions were given according to a Latin square design and were delivered at 0.5 μl per site over 1 min. Before the drug treatment day, rats were run daily on the 5-CSRTT with a reduced ITI of 5 s. One week after the last drug infusion animals received a vehicle infusion (ATO or MPH vehicle, respectively) and were re-tested with a LoITI session. Following histological assessment, three animals from the ATO group and two animals from the MPH group were excluded from further analysis.

Experiment 2: Effects of Intra-NAcbC Infusions of ATO or MPH on 5-CSRTT Performance

Two groups of rats ($n = 9$ per group), were implanted with guide cannulae above the NAcbC. One group of rats received intra-NAcbC infusions of ATO (0.0, 1.5, or 5.0 μg); the second group received intra-NAcbC MPH (0.0–5.0 μg). Following termination of drug treatments, animals received a vehicle NAcbC infusion and were tested on a control LoITI session (see, experiment 1). Following histological assessment, cannulae were misplaced in one animal from the MPH group and therefore was excluded from further analysis.

Experiment 3: Effects of Intra-PrL and Intra-IL Infusions of ATO or MPH on 5-CSRTT Performance

Two groups of rats ($n = 10$ per group) were implanted with guide cannulae overlying the mPFC. The first group received intra-PrL infusions of ATO (0.0–5.0 μg) and following a 1-week washout period, the same rats received infusions of ATO into the IL. A second group of rats received infusions of MPH (0.0–5.0 μg) into first the PrL and latter into the IL after a 1-week washout. Both sets of rats (ATO and MPH) received the respective vehicle infusion 1 week after the last drug infusion and were tested under a LoITI. Following histological assessment, two animals from each group (ATO and PMH) were excluded from further analysis.

Experiment 4: Effects of Intra-NAcbS and NAcbC Infusions of ATO and MPH on Locomotor Activity

The effects of ATO and MPH (0.0 and 5.0 μg) on locomotor activity were investigated in two groups of rats with bilateral cannulae above the NAcbS ($n = 14$) or the NAcbC ($n = 10$). Animals destined for NAcbS infusions were divided in two subgroups ($n = 7$ per group) with similar levels of baseline locomotor activity during habituation. The first subgroup of rats received, in a counterbalanced manner, intra-NAcbS infusions of ATO (0.0 or 5.0 μg), whereas the second subgroup received MPH (0.0 or 5.0 μg). Subsequently, the drug treatment was reversed. Thus, the first subgroup now received MPH (0.0 or 5.0 μg), whereas the second subgroup now received ATO (0.0 or 5.0 μg). Animals destined for NAcbC infusions were divided in two subgroups ($n = 5$ per group), and were tested for locomotor activity following ATO and/or MPH treatment as described above. Following histological assessment, misplaced cannulae were found in five NAcbS animals and in two NAcbC operated animals. These animals were excluded from further analysis.

Experiment 5: Effects of Intra-PrL and Intra-IL Infusions of ATO and MPH on Locomotor Activity

Rats ($n = 12$) with bilateral cannulae aimed at the PrL received, in a counterbalanced order, 5.0 μg ATO or its vehicle. Following a 1-week washout, the same rats received ATO or vehicle infusions into the IL in a counterbalanced order. A second group of rats ($n = 10$) received MPH (0.0 or 5.0 μg), into both the PrL and IL, as described above. The histological assessment identified four animals from the ATO group and two animals from the MPH group with

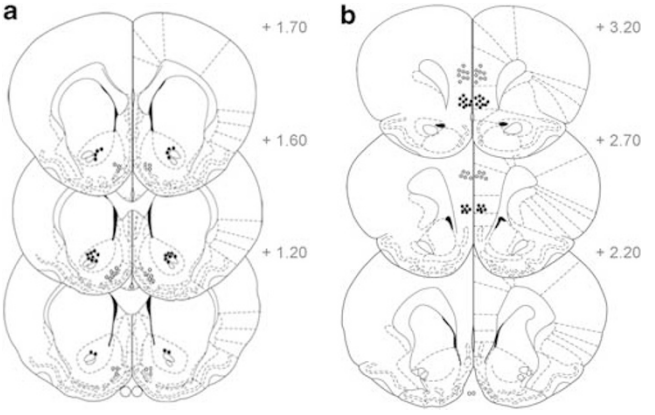

Figure 1 Schematic representations of injector tips in the (a) NAcbS (open circles), NAcbC (closed circles) and (b) PrI (open circles) and IL cortex (closed circles). Co-ordinates are expressed in mm. Drawings adapted from Paxinos and Watson (2004).

Table 2 Final Group Sizes with Cannula Placements Histologically Verified

	Experiment 1	Experiment 2	Experiment 3	Experiment 4		Experiment 5
	NAcbS	NAcbC	PrL/IL	NAcbS	NAcbC	PrL/IL
ATO	5	9	8	9	8	8
MPH	5	8	8	9	8	8

misplaced cannulae. These animals were excluded from further analysis.

Statistical Analysis

The results are presented as means (±SEM) for each behavioral variable (5-CSRTT: premature responses, choice accuracy, omissions, perseverative responses, latency to make a correct or an incorrect response and latency to collect food reward; locomotor activity: photocell beam interruptions). Behavioral data were analyzed using repeated-measures analysis of variance (ANOVA) with dose as a within-subjects factor (SPSS, Chicago, IL). *Post-hoc* Newman–Keuls comparisons were used where appropriate. Statistical significance was set at $p<0.05$.

RESULTS

Histology

Figure 1 shows the most central location of the infusion cannulae tips in the NAcbS and NAcbC and in the PrL and IL. Following the histological assessment, animals in which the cannulae were positioned outside the target areas were excluded from the study. The final group sizes for each experiment are shown in Table 2.

Experiment 1: Effects of Intra-NAcbS Infusions of ATO or MPH on 5-CSRTT Performance

Intra-NAcbS infusions of ATO resulted in a significant decrease in the number of premature responses ($F_{(3,12)}=7.2$, $p<0.01$) (Figure 2a). *Post-hoc* tests revealed a significant decrease in premature responding after treatment with all doses of ATO. ATO did not significantly affect attentional accuracy, omissions, the latency to make a correct response, or the latency to collect food reward (all p-values = NS) (Table 3). During the final control LoITI session, there was a significant increase in the number of premature responses compared with the baseline response (ie, measured during a 5 s ITI) but not compared with the vehicle-treated groups, suggesting that there was no habituation in premature responding during the experiment (Figure 2a).

In contrast, intra-NAcbS MPH infusions had no significant effect on premature responding ($F_{(3,12)}=0.8$, $p=$NS) (Figure 2c), attentional accuracy, omissions, or the latency to collect food from the magazine (all p-values = NS) (Table 3). However, MPH (1.5 μg) did reduce the latency to make a correct response ($p<0.05$) (Table 3). A similar pattern of effects was observed during the final control LoITI session to that described for ATO above (Figure 2c).

Experiment 2: Effects of Intra-NAcbC Infusions of ATO or MPH on 5-CSRTT Performance

Intra-NAcbC ATO infusions had no effect on premature responding ($F_{(2,16)}=0.5$, $p=$NS) (Figure 2b), or any other behavioral variables (all p-values = NS) (Table 3). By contrast, intra-NAcbC infusions of MPH resulted in a marked increase in premature responding ($F_{(3,21)}=6.7$, $p<0.01$), which was significant at the 5.0 μg dose ($p<0.01$) (Figure 2d). This response was highly selective with no additional effects on any other behavioral variable (all p-values = NS) (Table 3).

Experiment 3: Effects of Intra-PrL and Intra- IL Infusions of ATO or MPH on 5-CSRTT Performance

Intra-PrL infusions of ATO had no significant effect on premature responding ($F_{(2,14)}=1.9$, $p=$NS) (Figure 3a), or any other variables on the 5-CSRTT (all p-values = NS) (Table 4). Intra-IL ATO also had no significant effect on premature responding ($F_{(2,10)}=0.1$, $p=$NS) (Figure 3b), or any other behavioral variables (all p-values = NS) (Table 4). A similar profile of effects was observed following infusions of MPH in the PrL and IL with no significant effects ($p=$NS) on any of the behavioral measures except a significant decrease in omissions following intra-IL treatment ($p<0.05$) at the dose of 5.0 μg (Figures 3c–d, Table 4).

Experiment 4: Effects of Intra-NAcbS and NAcbC Infusions of ATO and MPH on Locomotor Activity

Locomotor activity was not significantly affected by either intra-NAcbS or intra-NAcbC ATO infusions. By contrast, locomotor activity was significantly increased following both intra-NAcbS and intra-NAcbC infusions of MPH ($p<0.001$) (Figures 4a–b).

Experiment 5: Effects of Intra-PrL and Intra-IL Infusions of ATO and MPH on Locomotor Activity

Intra-PrL ATO infusions did not significantly affect locomotor activity. However, when infused in the IL, ATO

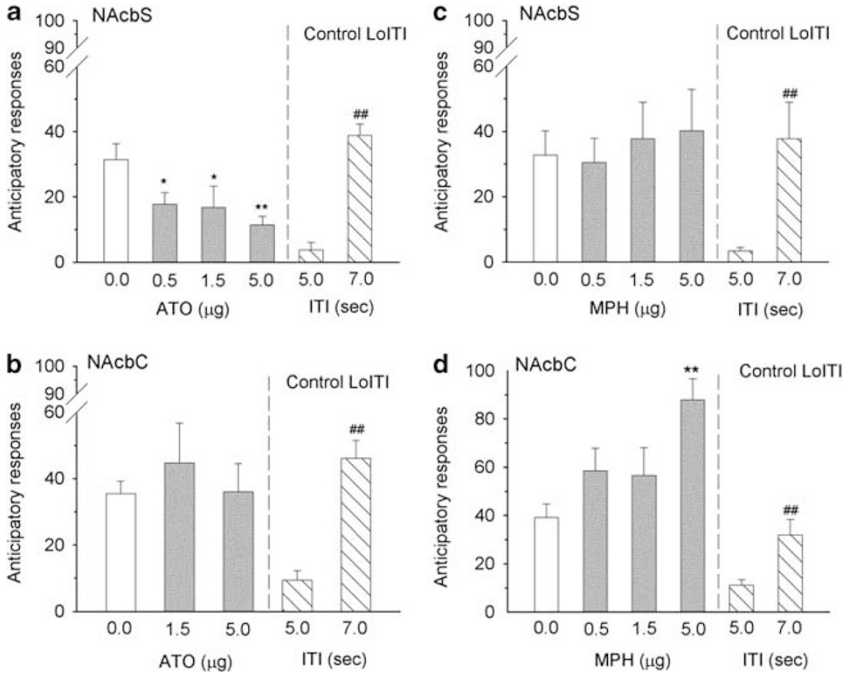

Figure 2 Mean (± SEM) number of premature responses following intra-NAcbS (a) or intra-NAcbC (b) infusions of ATO and following intra-NAcbS (c) or intra-NAcbC (d) infusions of MPH in rats during performance in the 5-CSRTT. Intra-NAcbS treatment with the selective NET inhibitor ATO significantly decreased premature responding at all doses tested (a) whereas infusions of the mixed DAT/NET inhibitor MPH into the NAcbC produced the opposite effect (d). *$p<0.05$ and **$p<0.01$, compared with vehicle-treated animals; $^{\#\#}p<0.01$, compared with baseline responding (ITI = 5.0 s).

Table 3 Summary of the Effects of Intra-NAcbS and Intra-NAcbC Infusions of ATO and MPH on 5-CSRTT Performance

	ATO				MPH			
	0.0	**0.5**	**1.5**	**5.0**	**0.0**	**0.5**	**1.5**	**5.0**
NAcbS								
Accuracy (%)	87.3 ± 0.9	89.2 ± 1.1	87.6 ± 2.9	89.6 ± 1.1	87.5 ± 2.5	90.7 ± 1.0	88.3 ± 2.0	84.4 ± 1.5
Omissions (%)	5.2 ± 1.1	4.6 ± 2.0	5.0 ± 2.3	8.2 ± 2.9	8.2 ± 3.4	5.4 ± 2.0	6.0 ± 2.3	11.0 ± 1.9
Perseverative NPs	35.4 ± 4.2	46.4 ± 8.5	45.8 ± 11.1	47.8 ± 13.3	43.6 ± 11.8	52.0 ± 16.5	53.4 ± 16.5	81.0 ± 14.5
Correct latency (ms)	513.1 ± 29.0	519.7 ± 32.3	497.5 ± 32.9	550.1 ± 46.2	480.3 ± 19.2	444.3 ± 19.5	432.7 ± 17.4*	462.7 ± 13.2
Incorrect latency (ms)	1297.5 ± 174.3	1118.2 ± 209.3	1350.1 ± 329.7	1665.3 ± 262.1	1221.0 ± 179.4	995.6 ± 197.3	1046.3 ± 120.1	1192.2 ± 187.9
Collect latency (ms)	1728.2 ± 229.0	2134.2 ± 494.9	2022.9 ± 487.0	1968.9 ± 342.5	1642.7 ± 226.3	1576.6 ± 118.1	2028.3 ± 330.9	1675.2 ± 135.4
NAcbC								
Accuracy (%)	79.1 ± 1.6	—	80.2 ± 2.2	76.7 ± 3.9	79.2 ± 3.8	78.2 ± 3.7	69.9 ± 8.2	74.7 ± 5.2
Omissions (%)	9.8 ± 2.1	—	9.2 ± 1.7	11.3 ± 1.7	7.6 ± 1.4	13.3 ± 5.9	20.7 ± 7.1	20.3 ± 3.3
Perseverative NPs	76.9 ± 10.8	—	63.7 ± 6.1	85.7 ± 17.7	55.3 ± 6.9	61.3 ± 8.9	58.4 ± 7.7	67.6 ± 9.0
Correct latency (ms)	576.5 ± 35.2	—	633.1 ± 60.4	672.3 ± 78.3	575.9 ± 37.5	647.6 ± 43.8	609.1 ± 43.7	608.0 ± 60.4
Incorrect latency (ms)	1174.3 ± 94.3	—	1337.0 ± 130.2	1503.2 ± 177.4	1430.1 ± 175.9	1458.0 ± 137.5	1558.0 ± 148.5	1937.4 ± 208.2
Collect latency (ms)	1552.7 ± 88.2	—	1545.2 ± 108.3	1612.8 ± 104.6	1701.0 ± 69.7	1770.5 ± 97.4	1639.5 ± 54.3	1591.7 ± 63.7

*$p<0.05$, compared with vehicle-treated animals.
Data are shown as mean (± SEM).

resulted in a significant decrease in locomotor activity ($p<0.01$) (Figure 4a). MPH did not alter locomotion when infused in the PrL or IL cortex (Figure 4b).

DISCUSSION

The results of these experiments show that the NAcb is a key area for regulating the expression of impulsive behavior, assessed by premature responding on the 5-CSRTT, which is critically and oppositely modulated by NE-ergic and DA-ergic mechanisms in the shell and core sub-regions of the NAcb. There was a reduction in impulsivity following intra-NAcbS, but not intra-NAcbC infusion of the selective NET inhibitor ATO. In contrast, intra-NAcbC, but not intra-NAcbS infusions of the mixed NET/DAT inhibitor MPH resulted in the opposite effect and significantly

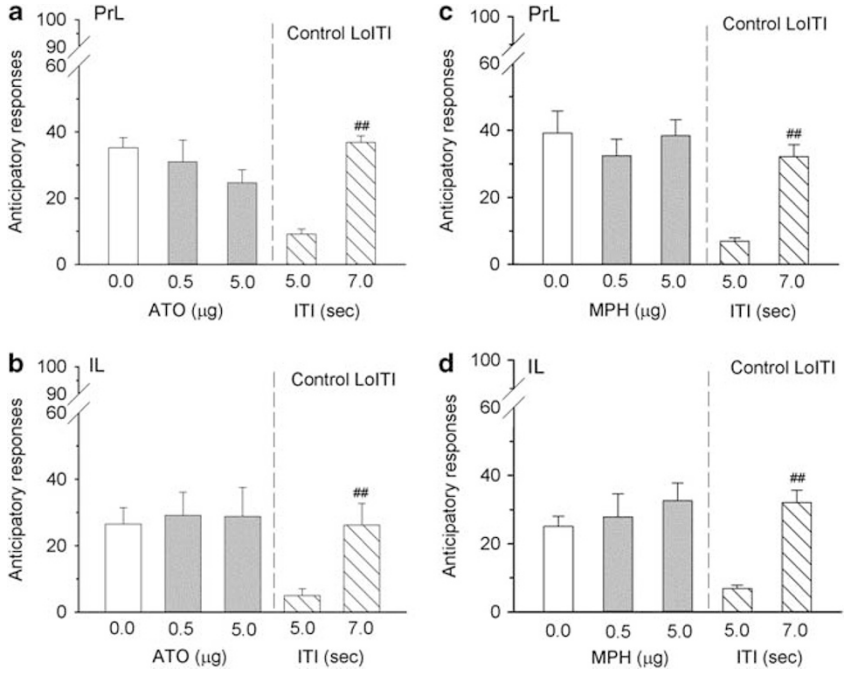

Figure 3 Effects of intra-PrL (a) and intra-IL (b) infusions of ATO and of intra-PrL (c) and intra-IL (d) infusions of MPH on premature responses in the 5-CSRTT. Neither compound produced significant effects on premature responding. Data are mean (±SEM) number of premature responses. $^{\#\#}p<0.01$, compared with baseline responding (ITI = 5.0 s).

Table 4 Summary of the Effects of Intra-PrL and Intra-IL Infusions of ATO and MPH on 5-CSRTT Performance

	ATO			MPH		
	0.0	**0.5**	**5.0**	**0.0**	**0.5**	**5.0**
PrL						
Accuracy (%)	87.0 ± 1.3	90.2 ± 2.2	87.8 ± 1.9	83.1 ± 3.8	82.1 ± 2.8	81.3 ± 3.2
Omissions (%)	3.3 ± 1.0	3.6 ± 1.1	3.9 ± 0.7	4.6 ± 0.9	4.9 ± 0.8	5.9 ± 2.3
Perseverative NPs	36.3 ± 8.1	37.6 ± 4.8	52.3 ± 9.2	54.6 ± 10.8	50.1 ± 9.0	61.3 ± 10.5
Correct latency (ms)	461.9 ± 17.2	496.7 ± 26.3	480.1 ± 17.8	468.7 ± 7.1	460.8 ± 12.7	467.3 ± 28.9
Incorrect latency (ms)	1039.1 ± 75.9	1130.3 ± 249.2	1258.6 ± 219.9	1151.5 ± 169.7	1083.7 ± 162.1	1363.7 ± 178.9
Collect latency (ms)	1397.4 ± 70.4	1525.8 ± 151.0	1558.6 ± 93.4	1454.9 ± 107.2	1427.1 ± 61.8	1807.3 ± 395.4
IL						
Accuracy (%)	88.0 ± 1.9	86.3 ± 2.2	88.3 ± 1.8	86.2 ± 2.0	87.0 ± 3.0	87.2 ± 2.7
Omissions (%)	5.8 ± 1.2	5.7 ± 1.8	4.3 ± 0.5	5.6 ± 1.0	4.1 ± 1.1	2.9 ± 0.9*
Perseverative NPs	41.0 ± 10.1	51.5 ± 13.9	46.2 ± 7.4	59.9 ± 9.3	56.3 ± 6.7	55.1 ± 8.2
Correct latency (ms)	429.0 ± 17.4	488.8 ± 39.1	456.7 ± 28.4	486.2 ± 12.1	488.9 ± 20.7	481.2 ± 18.6
Incorrect latency (ms)	1367.0 ± 233.2	1246.1 ± 154.5	1180.7 ± 207.4	1173.5 ± 206.1	1339.2 ± 145.5	1077.6 ± 199.2
Collect latency (ms)	1447.4 ± 40.1	1464.9 ± 117.5	1428.5 ± 82.9	1535.1 ± 82.1	1554.9 ± 65.6	1569.9 ± 97.1

*$p<0.05$, compared with vehicle-treated animals.
Data are shown as mean (± SEM).

increased premature responding. Neither of these drugs altered impulsivity on the 5-CSRTT following intra-PrL or intra-IL administration. In a separate experiment, intra-NAcbC MPH, while producing high levels of premature responding, also elicited locomotor hyperactivity. However, although to a lesser extent, increased locomotor activity was also found following intra-NAcbS infusions of MPH, which was an ineffective site for inducing premature or impulsive responding in the 5-CSRTT. Locomotor activity was not altered by MPH when infused into the PrL and IL cortex. In contrast, although ATO had no significant effect on locomotor behavior when infused into any of the NAcb sub-regions, it did result in a decrease when infused into the IL. The reported effects were unlikely to be mediated by sites distal to the microinjection location because (i) ATO produced strongly divergent effects on behavior when

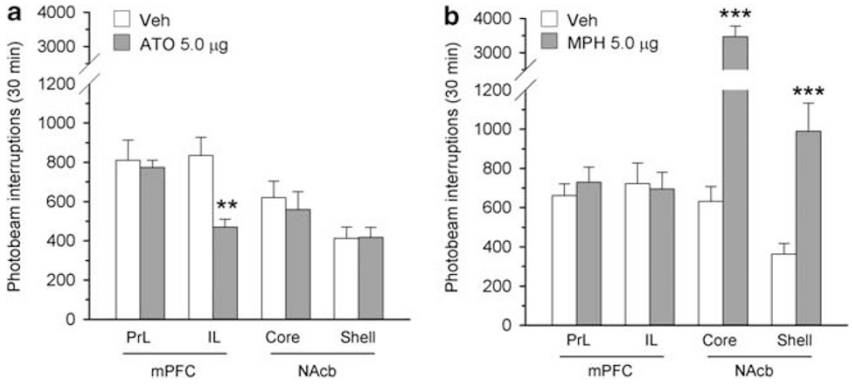

Figure 4 Effects of ATO (a) and MPH (b) infusions into the NAcbC and NAcbS and into the PrL/IL cortex on spontaneous locomotor activity. Data shown are mean (± SEM) photocell beam interruptions. $^{**}p<0.01$ and $^{***}p<0.001$, compared with vehicle-treated animals.

injected into the closely flanking core and shell sub-regions of the NAcb, and (ii) dissociable behavioral effects were observed after infusions of ATO into the PFC and NAcb.

The premature or anticipatory index of responding in the 5-CSRTT is considered a valid measure of a key construct of impulsivity, namely 'waiting impulsivity', which depends critically on the integrity of the NAcbC (Cardinal *et al*, 2001; Dalley *et al*, 2011). However, the neurochemical mechanisms that modulate this NAcb control over the ability to anticipate signals predictive of reward, wait for them, and respond on the onset of a stimulus have not been defined. The NAcb is a heterogeneous structure with two anatomically and functionally distinct compartments, the core and shell (Groenewegen *et al*, 1999). A major distinction between these sub-territories is related to their NE-ergic innervation, which predominantly targets the shell, with inputs primarily originating in the medullary A2 NE-ergic cell group of the nucleus tractus solitarius (Berridge *et al*, 1997; Delfs *et al*, 1998; McKittrick and Abercrombie, 2007). This is of particular interest in light of our results showing opponent modulation of impulsivity by ATO and MPH in the NAcbS and NAcbC. Thus, intra-NAcbS infusions of ATO resulted in a marked reduction in impulsivity of a magnitude similar to the effects of systemically administering ATO (Paterson *et al*, 2011; Robinson *et al*, 2008). This effect is likely attributable to NET blockade and increased NE transmission in the NAcbS. Conversely, the increased impulsivity seen following local intra-NAcbC infusions of the mixed DAT/NET inhibitor MPH can instead be attributed to DAT blockade and increased DA transmission in the NacbC given a scarce local NE innervation (Berridge *et al*, 1997; Delfs *et al*, 1998; McKittrick and Abercrombie, 2007) and the absence of any trend of effect following direct ATO infusion. By contrast, in the NAcbS, the lack of effect of MPH on impulsivity could be attributed to its dual inhibitory effect on DAT and NET (Gatley *et al*, 1996). Of particular interest in this context are anatomical data demonstrating reciprocal connections between the shell and the core (van Dongen *et al*, 2005; Groenewegen *et al*, 1999) and microdialysis data suggesting a functional interaction between these two areas indicating a prominent role of the NE system in modulating DA release within the NAcb (Mizoguchi *et al*, 2008; Verheij and Cools, 2008). Therefore, we hypothesize that the NAcbS, via its NE-ergic innervation, acts to regulate extracellular DA levels in the NAcbC and thereby determines the level of inhibitory control over a prepotent response (Dalley *et al*, 2011; Goto and Grace, 2008).

In support of this hypothesis, intra-NAcbC infusions of DA receptor agonists and antagonists respectively increase and decrease premature responding in the 5-CSRTT (Besson *et al*, 2010; Pattij *et al*, 2007; Pezze *et al*, 2007). Support for the notion of a balanced interaction between the NAcbS and the NAcbC in the mediation of impulsivity in the 5-CSRTT is the finding that excitotoxic lesions of the NAcbC increased, whereas NAcbS lesions decreased impulsivity produced by the mixed NET/DAT inhibitor D-amphetamine (Murphy *et al*, 2008).

In contrast to the dissociable effects of intra-NAcb core and shell infusions of ATO and MPH on 5-CSRTT impulsivity, there were no effects on impulsive behavior following local infusions of these drugs into the PrL and IL. This result was rather unexpected considering evidence of: (i) a dysfunctional PFC in ADHD patients (Castellanos *et al*, 2002; Rubia *et al*, 1999), (ii) top–down prefrontal control over cognitive and executive functions regulated by catecholamine transmission (Arnsten and Li, 2005; Arnsten and Pliszka, 2011), and (iii) significant increases in premature responding on the 5-CSRTT following selective lesions of the IL cortex (Chudasama *et al*, 2003). As NET is abundant within the mPFC, compared with DAT (Gehlert *et al*, 1993; Sesack *et al*, 1998), a beneficial effect on impulsivity of intra-mPFC (especially intra-IL) ATO treatment might have been expected. But the results of our experiments clearly indicated that this was not the case. However, other evidence indicates that serotonergic modulation of this region may be an important feature in the context of premature responding on the 5-CSRTT (Passetti *et al*, 2003; Winstanley *et al*, 2003). We have also recently reported evidence that the effect of ATO to speed stop-signal reaction time performance (a measure of 'stopping impulsivity', (Dalley *et al*, 2011)) is not mediated by actions within the IL or PL, but instead within the anterior cingulate and orbitofrontal cortices (Bari *et al*, 2011). One implication is that the multifaceted nature of impulsivity depends on different neural circuitries that are subject to different forms of modulation at both prefrontal cortical and striatal sites. Thus, the beneficial effects of systemically administered

ATO may be mediated by effects in multiple neural sites, the precise outcome depending on the specific behavioral demand of the task.

We also investigated whether shared or distinct neurochemical mechanisms modulate premature behavior and locomotor activity, as the latter could in some instances contribute significantly to determine the former. We found identical trends in behavior following MPH and ATO treatment, with MPH increasing, and ATO decreasing, spontaneous locomotor activity. However, for the locomotor-activating actions of MPH, similar but non-identical, intra-NAcb mechanisms were found to control this effect. In fact, both intra-NAcbC (similarly to impulsivity) and intra-NAcbS (differentially from impulsivity) infusions of MPH stimulated locomotor activity. This result is consistent with the well-established effect of increased NAcb DA to enhance behavioral output, producing a state of 'behavioral activation' (Robbins and Everitt, 1982; Swanson *et al*, 1997). By contrast, we demonstrated dissociable mechanisms by which ATO decreases impulsive behavior and locomotor activity. Unlike its effects in the 5-CSRTT, intra-NAcbS NET inhibition by ATO did not alter locomotion but, surprisingly, it was the intra-IL infusions of ATO that produced a significant decrease in locomotor activity, possibly reflecting the known role of the IL cortex in the behavioral suppression of fear and drug-seeking behavior (Peters *et al*, 2009). These results may indicate that NAcbS NE is specifically recruited in conditions of alertness, also potentially involving the adaptation of behavior over delays to reinforcement (eg, 5-CSRTT). In contrast, the functional importance of an accumbens NE-ergic control over DA transmission in this area seems to be less important when vigilance and attentional demands are low (ie, locomotor activity).

Dysregulated NE and DA neurotransmission have been widely linked with the manifestation of maladaptive impulsive behavior and its related psychopathologies, such as ADHD (Arnsten, 2006; Biederman and Spencer, 1999). Human imaging data suggest altered activity in corticostriatal networks in such disorders, and that ATO and MPH exert their therapeutic effects through neuromodulation of these catecholaminergic systems within fronto-striatal circuits (Arnsten and Li, 2005; Arnsten and Pliszka, 2011). Here, we provide substantial evidence for a significant role of the ventral striatum in regulating the expression of impulsive behavior, but found no such evidence for the involvement of prefrontal areas. Thus, the altered prefrontal top–down control shown to be central in some aspects of impulse control disorders may be a consequence of a sub-optimal activity within the NAcb that may derive from a misbalance in NE and DA transmission within its sub-regions. However, as our experiments were carried out in rats not expressing trait-like impulsivity, our data do not exclude the possibility that the PFC is an important site for the beneficial effects of ATO or MPH treatment in patients with impulse control disorders, especially in view of data employing other behavioral test paradigms (Bari *et al*, 2011; Berridge *et al*, 2011). Rather, it appears that the beneficial actions of ATO are exerted at multiple sites, including striatum and PFC, depending on the precise dimension of impulsivity being measured (Bari *et al*, 2011; Robinson *et al*, 2008).

Finally, we demonstrated that distinct neural mechanisms underlie the modulation of impulsive and locomotor behavior, with the former more obviously depending on DA–NE mechanisms at the level of the ventral striatum. This is an important consideration for dissecting the symptom clusters of hyperactive-impulsive type ADHD, as it shows that these two elements, while often related, may have distinct neurochemical and neural substrates.

ACKNOWLEDGEMENTS

This work was funded by the Medical Research Council (G9536855, G0701500) and by a joint award from the Medical Research Council and Wellcome Trust in support of the Behavioral and Clinical Neuroscience Institute at Cambridge University. The authors thank Alan Lyon for technical assistance.

DISCLOSURE

TWR discloses his consultancy with Cambridge Cognition, Lilly, Lundbeck and GlaxoSmithKline, plus research grants with Lilly, Lundbeck and GSK. DE, DEHT, BJE and JWD declare no conflict of interest.

REFERENCES

Arnsten AFT (2006). Fundamentals of attention-deficit/hyperactivity disorder: circuits and pathways. *J Clin Psychiatry* **67**(Suppl 8): 7–12.

Arnsten AFT, Li B-M (2005). Neurobiology of executive functions: catecholamine influences on prefrontal cortical functions. *Biol Psychiatry* **57**: 1377–1384.

Arnsten AFT, Pliszka SR (2011). Catecholamine influences on prefrontal cortical function: relevance to treatment of attention deficit/hyperactivity disorder and related disorders. *Pharmacol Biochem Behav* **99**: 211–216.

Bari A, Dalley JW, Robbins TW (2008). The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. *Nat Protoc* **3**: 759–767.

Bari A, Mar AC, Theobald DE, Elands SA, Oganya KCNA, Eagle DM *et al* (2011). Prefrontal and monoaminergic contributions to stop-signal task performance in rats. *J Neurosci* **31**: 9254–9263.

Berridge CW, Shumsky JS, Andrzejewski ME, McGaughy JA, Spencer RC, Devilbiss DM *et al* (2011). Differential sensitivity to psychostimulants across prefrontal cognitive tasks: differential involvement of noradrenergic α(1)- and α(2)-receptors. *Biol Psychiatry* **71**: 467.

Berridge CW, Stratford TL, Foote SL, Kelley AE (1997). Distribution of dopamine beta-hydroxylase-like immunoreactive fibers within the shell subregion of the nucleus accumbens. *Synapse* **27**: 230–241.

Besson M, Belin D, McNamara R, Theobald DE, Castel A, Beckett VL *et al* (2010). Dissociable control of impulsivity in rats by dopamine d2/3 receptors in the core and shell subregions of the nucleus accumbens. *Neuropsychopharmacology* **35**: 560–569.

Biederman J, Spencer T (1999). Attention-deficit/hyperactivity disorder (ADHD) as a noradrenergic disorder. *Biol Psychiatry* **46**: 1234–1242.

Cardinal RN, Aitken MRF (2010). Whisker: a client-server high-performance multimedia research control system. *Behav Res Methods* **42**: 1059–1071.

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

Castellanos FX, Lee PP, Sharp W, Jeffries NO, Greenstein DK, Clasen LS *et al* (2002). Developmental trajectories of brain volume abnormalities in children and adolescents with attention-deficit/hyperactivity disorder. *JAMA* **288**: 1740–1748.

Chudasama Y, Passetti F, Rhodes SEV, Lopian D, Desai A, Robbins TW (2003). 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* **146**: 105–119.

Cole BJ, Robbins TW (1989). Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi on performance of a 5-choice serial reaction time task in rats: implications for theories of selective attention and arousal. *Behav Brain Res* **33**: 165–179.

Comings DE, Gonzalez NS, Cheng Li S-C, MacMurray J (2003). A 'line item' approach to the identification of genes involved in polygenic behavioral disorders: the adrenergic alpha2A (ADRA2A) gene. *Am J Med Genet B Neuropsychiatr Genet* **118B**: 110–114.

Dalley JW, Everitt BJ, Robbins TW (2011). Impulsivity, compulsivity, and top-down cognitive control. *Neuron* **69**: 680–694.

Dalley JW, Fryer TD, Brichard L, Robinson ESJ, Theobald DEH, Lääne K *et al* (2007). Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. *Science* **315**: 1267–1270.

Dalley JW, Mar AC, Economidou D, Robbins TW (2008). Neurobehavioral mechanisms of impulsivity: fronto-striatal systems and functional neurochemistry. *Pharmacol Biochem Behav* **90**: 250–260.

Delfs JM, Zhu Y, Druhan JP, Aston-Jones GS (1998). Origin of noradrenergic afferents to the shell subregion of the nucleus accumbens: anterograde and retrograde tract-tracing studies in the rat. *Brain Res* **806**: 127–140.

van Dongen YC, Deniau J-M, Pennartz CMA, Galis-de Graaf Y, Voorn P, Thierry A-M *et al* (2005). Anatomical evidence for direct connections between the shell and core subregions of the rat nucleus accumbens. *Neuroscience* **136**: 1049–1071.

Eagle DM, Tufft MRA, Goodchild HL, Robbins TW (2007). Differential effects of modafinil and methylphenidate on stop-signal reaction time task performance in the rat, and interactions with the dopamine receptor antagonist cis-flupenthixol. *Psychopharmacology (Berl)* **192**: 193–206.

Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA *et al* (2005). Molecular genetics of attention-deficit/hyperactivity disorder. *Biol Psychiatry* **57**: 1313–1323.

van Gaalen MM, van Koten R, Schoffelmeer ANM, Vanderschuren LJMJ (2006). Critical involvement of dopaminergic neurotransmission in impulsive decision making. *Biol Psychiatry* **60**: 66–73.

Gatley SJ, Pan D, Chen R, Chaturvedi G, Ding YS (1996). Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. *Life Sci* **58**: 231–239.

Gehlert DR, Gackenheimer SL, Robertson DW (1993). Localization of rat brain binding sites for [3H]tomoxetine, an enantiomerically pure ligand for norepinephrine reuptake sites. *Neurosci Lett* **157**: 203–206.

Goto Y, Grace AA (2008). Limbic and cortical information processing in the nucleus accumbens. *Trends Neurosci* **31**: 552–558.

Groenewegen HJ, Wright CI, Beijer AV, Voorn P (1999). Convergence and segregation of ventral striatal inputs and outputs. *Ann NY Acad Sci* **877**: 49–63.

Haber SN, Fudge JL, McFarland NR (2000). Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *J Neurosci* **20**: 2369–2382.

McKittrick CR, Abercrombie ED (2007). Catecholamine mapping within nucleus accumbens: differences in basal and amphetamine-stimulated efflux of norepinephrine and dopamine in shell and core. *J Neurochem* **100**: 1247–1256.

Mizoguchi N, Saigusa T, Aono Y, Sekino R, Takada K, Oi Y *et al* (2008). The reboxetine-induced increase of accumbal dopamine efflux is inhibited by l-propranolol: a microdialysis study with freely moving rats. *Eur J Pharmacol* **601**: 94–98.

Moeller FG, Barratt ES, Dougherty DM, Schmitz JM, Swann AC (2001). Psychiatric aspects of impulsivity. *Am J Psychiatry* **158**: 1783–1793.

Murphy ER, Robinson ESJ, Theobald DEH, Dalley JW, Robbins TW (2008). Contrasting effects of selective lesions of nucleus accumbens core or shell on inhibitory control and amphetamine-induced impulsive behaviour. *Eur J Neurosci* **28**: 353–363.

Navarra R, Graf R, Huang Y, Logue S, Comery T, Hughes Z *et al* (2008). Effects of atomoxetine and methylphenidate on attention and impulsivity in the 5-choice serial reaction time test. *Prog Neuropsychopharmacol Biol Psychiatry* **32**: 34–41.

Passetti F, Dalley JW, Robbins TW (2003). Double dissociation of serotonergic and dopaminergic mechanisms on attentional performance using a rodent five-choice reaction time task. *Psychopharmacology (Berl)* **165**: 136–145.

Paterson NE, Ricciardi J, Wetzler C, Hanania T (2011). Suboptimal performance in the 5-choice serial reaction time task in rats was sensitive to methylphenidate, atomoxetine and d-amphetamine, but unaffected by the COMT inhibitor tolcapone. *Neurosci Res* **69**: 41–50.

Pattij T, Janssen MCW, Vanderschuren LJMJ, Schoffelmeer ANM, van Gaalen MM (2007). Involvement of dopamine D1 and D2 receptors in the nucleus accumbens core and shell in inhibitory response control. *Psychopharmacology (Berl)* **191**: 587–598.

Paxinos G, Watson C (2004). *The Rat Brain in Stereotaxic Coordinates - The New Coronal Set*. Academic Press: Boston.

Peters J, Kalivas PW, Quirk GJ (2009). Extinction circuits for fear and addiction overlap in prefrontal cortex. *Learn Mem* **16**: 279–288.

Pezze M-A, Dalley JW, Robbins TW (2007). Differential roles of dopamine D1 and D2 receptors in the nucleus accumbens in attentional performance on the five-choice serial reaction time task. *Neuropsychopharmacology* **32**: 273–283.

Potenza MN, Steinberg MA, Skudlarski P, Fulbright RK, Lacadie CM, Wilber MK *et al* (2003). Gambling urges in pathological gambling: a functional magnetic resonance imaging study. *Arch Gen Psychiatry* **60**: 828–836.

Robbins TW (2002). The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. *Psychopharmacology (Berl)* **163**: 362–380.

Robbins TW, Everitt BJ (1982). Functional studies of the central catecholamines. *Int Rev Neurobiol* **23**: 303–365.

Robinson ESJ, Eagle DM, Mar AC, Bari A, Banerjee G, Jiang X *et al* (2008). Similar effects of the selective noradrenaline reuptake inhibitor atomoxetine on three distinct forms of impulsivity in the rat. *Neuropsychopharmacology* **33**: 1028–1037.

Rubia K, Overmeyer S, Taylor E, Brammer M, Williams SC, Simmons A *et al* (1999). Hypofrontality in attention deficit hyperactivity disorder during higher-order motor control: a study with functional MRI. *Am J Psychiatry* **156**: 891–896.

Sesack SR, Hawrylak VA, Matus C, Guido MA, Levey AI (1998). Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. *J Neurosci* **18**: 2697–2708.

Swanson CJ, Heath S, Stratford TR, Kelley AE (1997). Differential behavioral responses to dopaminergic stimulation of nucleus accumbens subregions in the rat. *Pharmacol Biochem Behav* **58**: 933–945.

Verheij MMM, Cools AR (2008). Twenty years of dopamine research: individual differences in the response of accumbal

dopamine to environmental and pharmacological challenges. *Eur J Pharmacol* **585**: 228–244.

Voorn P, Jorritsma-Byham B, Van Dijk C, Buijs RM (1986). The dopaminergic innervation of the ventral striatum in the rat: a light- and electron-microscopical study with antibodies against dopamine. *J Comp Neurol* **251**: 84–99.

Winstanley CA, Chudasama Y, Dalley JW, Theobald DEH, Glennon JC, Robbins TW (2003). Intra-prefrontal 8-OH-DPAT and M100907 improve visuospatial attention and decrease impulsivity on the five-choice serial reaction time task in rats. *Psychopharmacology (Berl)* **167**: 304–314.

Wong DT, Threlkeld PG, Best KL, Bymaster FP (1982). A new inhibitor of norepinephrine uptake devoid of affinity for receptors in rat brain. *J Pharmacol Exp Ther* **222**: 61–65.