ORIGINAL INVESTIGATION

Modulation of high impulsivity and attentional performance in rats by selective direct and indirect dopaminergic and noradrenergic receptor agonists

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Abstract

Rationale Impulsivity is associated with a number of psychiatric disorders, most notably attention deficit/hyperactivity disorder (ADHD). Drugs that augment catecholamine function (e.g. methylphenidate and the selective noradrenaline reuptake inhibitor atomoxetine) have clinical efficacy in ADHD, but their precise mechanism of action is unclear.

Objective The objective of this study is to investigate the relative contribution of dopamine (DA) and noradrenaline (NA) to the therapeutic effects of clinically effective drugs in ADHD using rats selected for high impulsivity on the five-choice serial reaction time task (5CSRTT).

Methods We examined the effects of direct and indirect DA and NA receptor agonists and selective DA and NA reuptake inhibitors in rats showing high and low levels of impulsivity on the 5CSRTT (designated high impulsive

'HI' and low impulsive 'LI', respectively). Drugs were administered by systemic injection in a randomized, counterbalanced manner.

Results Low doses of quinpirole (a D2/D3 agonist) and sumanirole (a D2 agonist) selectively reduced impulsivity on the 5CSRTT, whilst higher doses resulted in increased omissions and slower response latencies. The NA reuptake inhibitor, atomoxetine, and the alpha-2 adrenoreceptor agonist, guanfacine, dose dependently decreased premature responding. The dopaminergic reuptake inhibitor GBR-12909 increased impulsivity, whereas the nonselective DA and NA reuptake inhibitor methylphenidate had no significant effect on impulsive responses in HI and LI rats.

Conclusions These findings indicate that high impulsivity can be ameliorated in rats by drugs that mimic the effects of DA and NA, just as in ADHD, and that activation of D2/3 receptors selectively decreases high impulsivity on the 5CSRTT.

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Introduction

Impulsivity encompasses a wide variety of behavioural
phenomena, often described as a predisposition towards
rapid, unplanned responses to internal or external stimuli
with a diminished regard for their negative consequences
(Moeller et al. 2001; Chamberlain and Sahakian 2007;
Potenza 2007). In its pathological form, impulsivity
represents one of three main symptoms of attention deficit/
hyperactivity disorder [ADHD; DSM-IV-TR, American Psy-
chiatric Association (Newcorn et al. 2001; Solanto 2002)].
Treatment of ADHD relies on typical psychostimulant drugs,
notably amphetamine and methylphenidate, which act to
increase both noradrenergic and dopaminergic activity in the
brain by blocking the noradrenaline (NA) and dopamine
(DA) transporters (Elia et al. 1999; Kutcher et al. 2004;
Solanto 2002; McKittrick and Abercrombie 2007). However,
despite their clinical efficacy, the precise mechanism of
action of these treatments is poorly understood.

Clues to the aetiology of ADHD have mainly emerged
from brain imaging studies in affected individuals, which
strongly implicate brain DA dysfunction. For example, PET
studies demonstrate impaired presynaptic DA function in
the prefrontal cortex (PFC, Ernst et al. 1998), dysfunction
of the dopamine transporter (DAT, Dougherty et al. 1999;
Krause et al. 2000), and decreased DA D2/ D3 receptor
availability (Volkow et al. 2009). Further insights have
emerged from animal models of impulsivity (Fernando and
Robbins 2011), for example in rats exhibiting high
impulsive behaviour assessed using the five-choice serial
reaction time task (5CSRTT), which show a reduced
density of DA D2/D3 receptors in the ventral striatum
(Dalley et al. 2007). However, the relative involvement of
DA D2 and DA D3 receptors in modulating behavioural
impulsivity is largely unknown. In a recent study, infusions
of the DA D3 receptor preferring antagonist nafadotride in
the shell subregion of the nucleus accumbens (NAcb)
further exacerbated impulsive behaviour in high impulsive
(HI) rats. In contrast, infusions of the same compound in
the core subregion of the NAcb reduced impulsivity
(Besson et al. 2010). Since DA D3 receptors are present
in low abundance in the NAcb core relative to the shell
(Booze and Wallace 1995; Curran and Watson 1995), these
findings suggest separable roles of DA D2 and D3
receptors in the control of impulsivity. To test this
possibility, we investigated the effects of the selective DA
D2 receptor agonist sumanirole (McCall et al. 2005) on

impulsive responding in rats selected on the 5CSRTT. We
hypothesized that HI rats would respond differentially to
sumanirole if DA D2 receptors were selectively down-
regulated in these animals. For comparison, we also tested
the effects of the DA D2/3 receptor agonist quinpirole in
rats selected for extreme impulsivity phenotypes (HI and
low impulsive 'LI') on the 5CSRTT, as well as methylphe-
nidate and the selective DAT inhibitor GBR 12909
(Heikkila and Manzino 1984).

Interest has also focused on the brain noradrenergic system
as a potential target for the treatment of ADHD (Biederman
and Spencer 1999). Atomoxetine is the first non-stimulant-
based medication with proven efficacy in ADHD (Caballero
and Nahata 2003; Kratochvil et al. 2002), which acts by
blocking the uptake of NA in the brain (Bymaster et al.
2002), thereby facilitating noradrenergic modulation of
limbic cortico-striatal circuitry (Chamberlain et al. 2007).
Atomoxetine has been shown to be effective in reducing
impulsivity in rats on the 5CSRTT (Blondeau and Dellu-
Hagedorn 2007), delay discounting impulsivity, and impul-
sive behaviour on a stop-signal reaction time task (Robinson
et al. 2009). Although the primary mechanism of action of
atomoxetine is well understood, the downstream effects of
increased synaptic NA function are poorly characterised.
These effects may involve postsynaptic $\alpha2$-adrenoceptors as
guanfacine, a selective $\alpha2$-adrenoceptor agonist, possesses
clinical efficacy in ADHD (Scahill et al. 2001) and improves
response inhibition in rodents (Franowicz et al. 2002). In
the present study, therefore, we compared the effects of
atomoxetine and guanfacine in LI and HI rats, specifically
to investigate whether these compounds produce quantita-
tively similar effects on spontaneously high levels of
impulsivity in rats.

Methods and materials

Subjects

Subjects were three cohorts of male Lister hooded rats
(Charles River, Margate, UK) weighing 275–300 g at the
start of each experiment and maintained at 85% of their
free-feeding weight. Subjects were housed initially in
groups of four with controlled temperature and humidity
conditions under an alternating light/dark cycle (red lights
on from 7.30 a.m. to 7.30 p.m.) and with water available ad
libitum. The first cohort of rats (eight HI, seven LI) was
treated with methylphenidate, atomoxetine and guanfacine,
in that order, and each following a washout period of
1 week. The second cohort was treated with quinpirole
(eight HI, eight LI), whilst the third cohort (seven HI, seven
LI) was treated with sumanirole and GBR 12909 (again
separated by a 1-week washout period). Three rats were

excluded from the study (two LI rats from the second and third cohorts and one HI rat from the first cohort) due to unstable performance on the 5CSRTT prior to the drug challenges.

Behavioural training

Subjects were trained to detect the spatial location of a brief light stimulus presented in one of five open apertures and to refrain from responding at inappropriate times, as described previously (Bari et al. 2008). A correct response was rewarded with the delivery of a single 45-mg sucrose pellet in the rear magazine (Noyes dustless pellets, 45 mg; Sandown Scientific, UK). An incorrect nose poke response in a non-illuminated aperture or a failure to respond within the set time period resulted in no reward delivery and a timeout period of 5 s that was occasioned by the house light being extinguished. A premature response, taken as a measure of impulsivity (Robbins 2002), was recorded for responses made in any aperture before the visual cue light had been presented; again, this led to a 5-s timeout period. Subjects were trained to a level of at least 80% accuracy with an intertrial interval (ITI) of 5 s, stimulus duration (SD) of 0.5 s and a limited hold period of 5 s to make a response. Each cohort of rats then underwent a screening procedure to select rats exhibiting extreme impulsivity phenotypes (LI and HI). During these sessions the ITI was extended to 7 s to promote the occurrence of a premature response (see Dalley et al. 2007). The long ITI (LITI) session consisted of 100 trials and was followed by two baseline days (ITI 5 s, SD 0.5 s) and two further days where rats remained in their home cage. This schedule was repeated three times at weekly intervals. The number of premature responses for each LITI session was averaged and ranked. Rats were designated high or low impulsive rats based on the criteria of premature responses greater than or equal to 50 premature for all three LITI sessions ('HI') or premature responses less than or equal to 30 for all LITI sessions ('LI'), as adopted previously (Dalley et al. 2007). Rats were then individually housed and stabilised on the 5CSRTT for five consecutive days prior to drug testing.

Drugs

(R)-Quinpirole, guanfacine hydrochloride and methylphenidate hydrochloride were commercially sourced (Sigma Aldrich, UK) and dissolved in 0.9% saline. Atomoxetine hydrochloride was a generous gift from Eli Lilly Inc. and was dissolved in 0.01 M phosphate-buffered saline. Sumanirole ((R)-5,6-dihydro-5-(methylamino)-4H-imidazo [4,5,1-ij]quinolin-2(1H)-one (Z)-2-butenedioate) was synthesized by Mu-Fa Zou in the Medicinal Chemistry Section, NIDA-IRP, NIH, Baltimore, USA, using the published synthesis (Romero et al. 1997) and was dissolved in 0.01 M phosphate-buffered saline. GBR 12909 dihydrochloride was purchased from Sigma (Germany) and dissolved in distilled deionised water.

Behavioural testing

Drugs were injected in a volume of 1 ml/kg using a Latin square design. Methylphenidate, atomoxetine, guanfacine and GBR 12909 were administered intraperitoneally, 20 min before behavioural testing on the 5CSRTT. Quinpirole and sumanirole were injected subcutaneously 90 and 15 min, respectively, before behavioural testing. Test sessions consisted of 100 trials, an SD of 0.5 s and an ITI of 5 s and were generally 25 min in duration.

Data analysis

Behavioural data were subjected to a repeated measures analysis of variance (ANOVA) using the SPSS statistical package (v19). Significant departures from sphericity were adjusted using a Greenhouse–Geisser correction of degrees of freedom to provide more conservative probabilities. Significant main effects and interactions were further analysed using ANOVA and SIDAK-corrected pairwise comparisons. A significance level of $p < 0.05$ was used for all analyses. Data are presented as means±SEM.

Results

Quinpirole

Quinpirole produced a significant decrease in premature responding on the 5CSRTT (dose—$F_{(1.903, 24.736)} = 15.237$, $p < 0.001$; Fig. 1a). Planned post hoc tests revealed that premature responding was significantly reduced relative to the vehicle condition following the administration of 0.01, 0.03 and 0.1 mg/kg for both LI and HI rats. Quinpirole also significantly increased the number of omissions (dose—$F_{(4, 52)} = 33.132$, $p < 0.001$; Fig. 1b). This effect was differentially expressed in HI and LI rats (group × dose interaction—$F_{(4, 52)} = 3.0$, $p < 0.027$) with omissions significantly lower in HI rats following a dose of 0.03 mg/kg ($p < 0.001$). Omissions significantly increased relative to the vehicle condition following the highest dose of quinpirole in HI rats and following 0.03 and 0.1 mg/kg in LI rats (all $p < 0.005$). In addition, quinpirole significantly increased magazine collection latencies (dose—$F_{(4, 52)} = 46.03$, $p < 0.001$; Fig. 3a) and correct response latencies (dose—$F_{(4, 52)} = 3.755$, $p < 0.01$; Fig. 3b) but had no significant effect on attentional accuracy (Fig. 3c).

Fig. 1 a–f Effects of the DA D2/D3R agonist quinpirole, the DA D2R agonist sumanirole and the selective DA reuptake inhibitor GBR 12909 on the number of premature responses and number of omissions made by high and low impulsive rats on the five-choice serial reaction time task. There were significant main effects of quinpirole and sumanirole on premature responses and omissions (quinpirole: premature responses $F_{(1.903, 24.736)}=15.237$, $p<0.001$; omissions $F_{(4, 52)}=33.132$, $p<0.001$; sumanirole: premature responses $F_{(3, 33)}=16.14$, $p<0.001$; omissions $F_{(3, 33)}=6.123$, $p<0.005$) and a significant main effect of GBR 12909 on premature responding [$F_{(2.250, 27.00)}=3.618$, $p<0.05$].*$p<0.05$, pairwise comparisons of mean responding (vehicle vs dose) independent of impulsivity phenotype. #$p<0.05$, pairwise comparisons of mean responding (vehicle vs dose, in high impulsive rats). Data are means±SEM
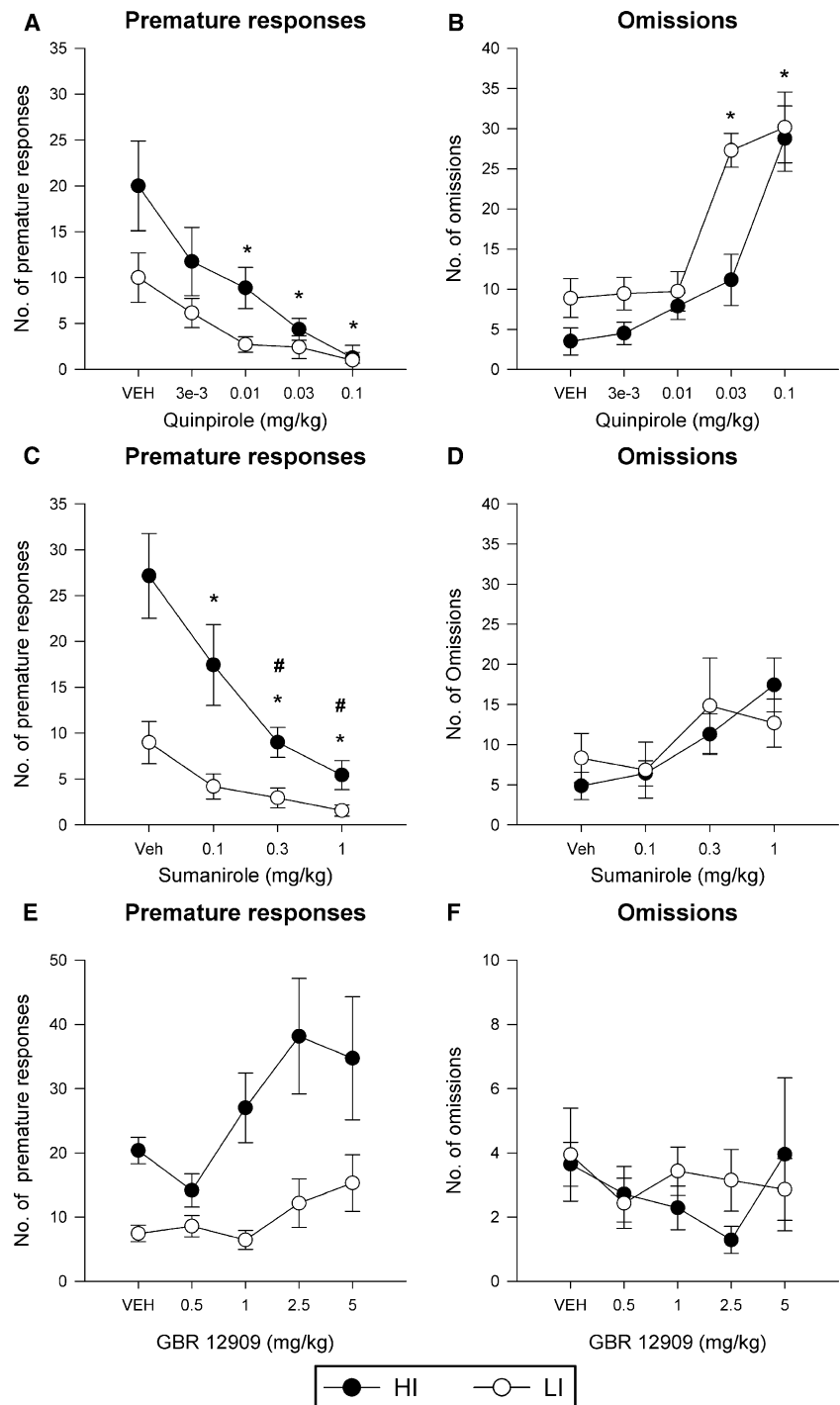
Sumanirole

Sumanirole significantly reduced premature responding on the 5CSRTT (dose—$F_{(3, 33)}=16.14$, $p<0.001$; Fig. 1c), an effect that was dependent on baseline levels of impulsivity (group × dose interaction—$F_{(3, 33)}=5.102$, $p=0.005$). Thus, unlike LI rats, premature responses were significantly reduced relative to the vehicle condition following all doses of

sumanirole tested in HI rats (0.1 mg/kg, $p<0.05$; 0.3 mg/kg, $p<0.005$; 1.0 mg/kg, $p<0.001$). Premature responses were significantly higher in HI rats compared with LI rats for all but the highest dose of sumanirole evaluated. Omissions also significantly increased following the administration of sumanirole (dose—$F_{(3, 33)}=6.123$, $p<0.005$; Fig. 1d); however, this effect was less marked than in rats treated with quinpirole (compare

Fig. 1b). In addition, sumanirole increased magazine collection latencies ($F_{(1.213, 13.342)}=4.839$, $p<0.05$; Fig. 3d) and correct response latencies ($F_{(1.69, 18.595)}=22.056$, $p<0.001$; Fig. 3e) and like quinpirole had no significant effect on accuracy (Fig. 3f).

GBR-12909

The effects of the selective DAT inhibitor GBR 12909 on behavioural performance on the 5CSRTT are shown in Figs. 1 and 3. GBR 12909 significantly increased premature responding (dose—$F_{(2.250, 27.00)}=3.618$, $p<0.05$; Fig. 1e), an effect that did not significantly interact with baseline levels of impulsivity, although there was a tendency for this compound to reduce impulsivity in HI rats at the lowest dose tested. No other behavioural measures were significantly affected by GBR 12909, including omissions, response latencies and accuracy.

Atomoxetine

Atomoxetine produced a significant reduction in premature responding (dose—$F_{(4, 52)}=5.312$, $p=0.001$; group × dose [$F_{(4, 52)}=2.539$ $p=0.051$]; Fig. 2a), and a significant increase in the number of omissions [$F_{(2.290, 29.766)}=6.375$, $p<0.005$; Fig. 2b]. These effects were inseparable with doses of atomoxetine affecting premature responding in HI rats also significantly increasing omissions (i.e., 1 and 3 mg/kg). Atomoxetine also significantly slowed food collection latencies ($F_{(4, 52)}=6.069$, $p<0.001$; Fig. 4b) but had no significant effect on correct response latencies (Fig. 4a) and attentional accuracy (Fig. 4c).

Guanfacine

Premature responding on the 5CSRTT was also significantly reduced by the alpha-2 adrenoceptor agonist guanfacine (dose—$F_{(4, 48)}=5.297$, $p=0.001$; Fig. 2c). This was significant (vs the vehicle control condition) for HI and LI rats at the two highest doses of guanfacine tested (0.3 and 1 mg/kg). Guanfacine also significantly increased the number of omissions (dose—$F_{(1.822, 21.865)}=4.170$, $p<0.05$; Fig. 2d) and correct response latencies (dose: $F_{(1.445, 17.345)}=7.472$, $p<0.01$, Fig. 4e), which reached statistical significance at the highest dose tested for both HI and LI groups combined (vehicle vs. 1 mg/kg, $p<0.05$). Unlike the other compounds evaluated in this study guanfacine also significantly affected attentional accuracy (dose $F_{(1.937, 23.249)}=5.812$, $p<0.01$; Fig. 4f) with the highest dose (1 mg/kg) significantly reducing accuracy in LI and HI rats relative to the vehicle control condition.
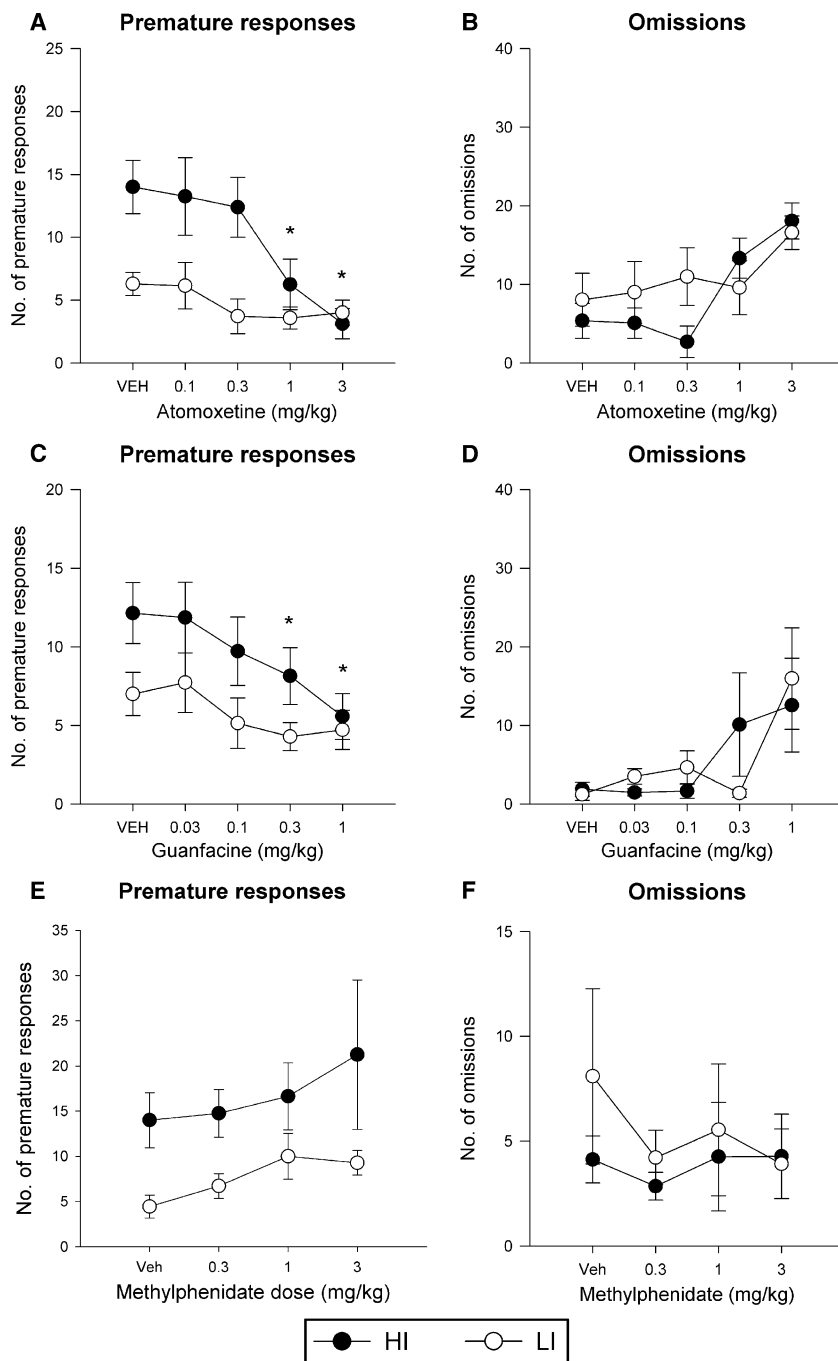
Methylphenidate

Methylphenidate had no significant effect on premature responding (Fig. 3e) or other behavioural measures on the 5CSRTT, including omissions (Fig. 3f), magazine collection latency (Fig. 4g), correct response latency (Fig. 4h) and attentional accuracy (Fig. 4i). However, there was a clear tendency of this compound to increase premature responding in both the LI and HI groups despite the evidently large inter-individual variation in responsiveness of HI rats, especially at the highest dose tested.

Discussion

The main findings of this study demonstrate divergent functional roles of the dopaminergic and noradrenergic systems in the modulation of impulsive behaviour and attentional performance in rats and provide additional support for the notion that DA receptor dysfunction associates with extreme impulsivity endophenotypes on the 5CSRTT. Selective inhibition of the NA and DA transporters by atomoxetine and GBR 12909, respectively, decreased and increased impulsive behaviour on the 5CSRTT, whereas methylphenidate, a compound used widely to treat ADHD, was without significant effect. Activation of DA D2-like receptors by the D2/3 receptor agonist quinpirole and the selective D2 receptor agonist sumanirole resulted in a dose-dependent decrease in impulsivity that, at lower doses (0.01 mg/kg quinpirole and 0.1 mg/kg sumanirole), was selectively mediated and devoid of effects on attentional accuracy as well as the speed and rate of responding (Table 1). Higher doses of quinpirole and sumanirole resulted in more general effects on performance and behavioural output. However, these non-specific effects differed between HI and LI rats with sumanirole producing less pronounced effects on omissions (a gross measure of inattention) than quinpirole. Moreover, the slowing of responding produced by higher doses of quinpirole and the concomitant rise in omissions was blunted in high impulsive rats compared with low impulsive rats. By contrast, the selective alpha-2 adrenoceptor agonist, guanfacine, produced qualitatively and quantitatively similar effects on behavioural performance in rats selected for high and low impulsivity compared with atomoxetine. Taken together, the present results indicate that high impulsivity in rats can be remediated by direct and indirect NA and DA agonists. These effects appear dissociable from the potential confounding influence of behavioural activation mechanisms, specifically following low doses of quinpirole and sumanirole.

Previous research has established that high impulsivity of rats on the 5CSRTT predicts the escalation of cocaine,

nicotine and sucrose self-administration (Dalley et al. 2007; Diergaarde et al. 2008, 2009), and also a tendency towards compulsive cocaine seeking and to relapse (Belin et al. 2008; Economidou et al. 2009). High impulsive rats also show reduced DA D2/3 receptor binding in the ventral striatum, including the NAcb, but not the dorsal striatum (Dalley et al. 2007) and reduced dopamine release in the nucleus accumbens core (Diergaarde et al. 2008). High impulsivity is remediated by the DA D2/3 receptor antagonist nafadotride infused in the nucleus accumbens

core but exacerbated by infusions of the same compound in the shell subregion (Besson et al. 2010). This evidence together with findings that DA D2 receptor antagonism in the nucleus accumbens core reduces impulsivity produced by systemic amphetamine as well as selective PFC lesions (Pattij et al. 2007; Pezze et al. 2009) highlights an important role of DA-ergic inputs to the nucleus accumbens core in regulating impulsive-like behaviour.

Similar to recently reported effects of full and partial D2/3 receptor agonists in selected impulsive rats (Besson et
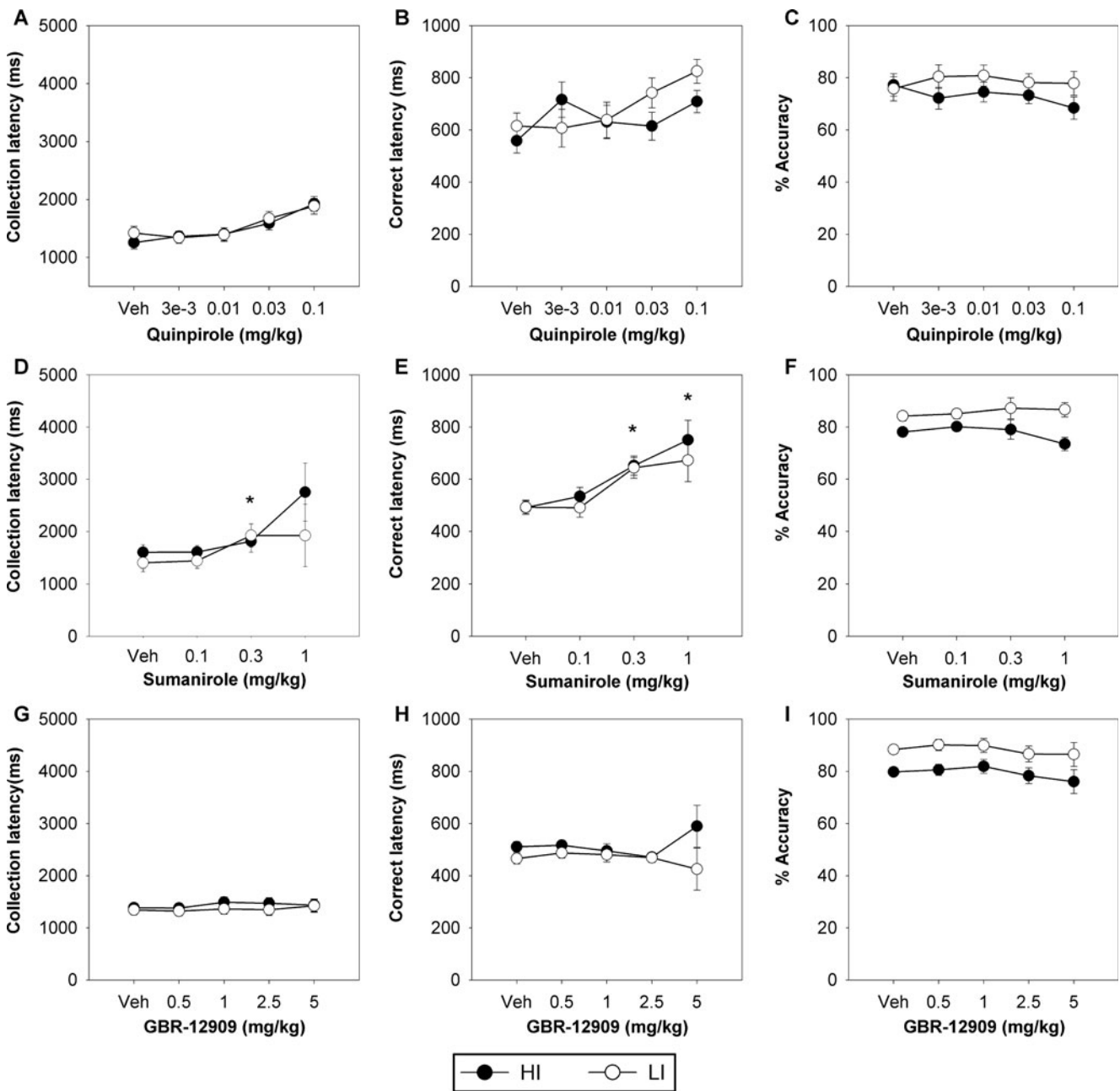
Fig. 3 a–i Effects of quinpirole, sumanirole and GBR-12909 on food collection latencies, correct response latencies and attentional accuracy on the five-choice serial reaction time task. There were significant main effects of quinpirole and sumanirole on food collection latencies and correct response latencies (quinpirole: magazine latencies $F_{(4, 52)}=46.03$, $p<0.001$; correct response latencies $F_{(4, 52)}=3.755$, $p<0.01$; sumanirole: magazine latencies $F_{(1.213, 13.342)}=4.839$, $p<0.05$; correct response $F_{(1.69, 18.595)}=22.056$, $p<0.001$). *$p<0.05$, pairwise comparisons of mean responding (vehicle vs dose). Data are means±SEM

al. 2010; Winstanley et al. 2010), systemic administration of sumanirole and quinpirole resulted in a dose-dependent reduction in impulsivity. Although floor effects in the low impulsive group may have had a bearing on these results, low doses of sumanirole were effective in selectively reducing premature responding in high impulsive rats. Sumanirole is a highly selective D2 receptor agonist possessing a 200-fold greater affinity for D2 than D1, D3

and D4 receptors (McCall et al. 2005) and thus may be a useful compound to determine whether D2 rather than D3 receptors are selectively disrupted in high impulsive rats. Our results show that sumanirole and quinpirole produce a broadly equivalent profile of effects on impulsivity, omissions and speed of responding with one exception; high impulsives were less sensitive to the effects of quinpirole on omissions. The origin of this possible divergent response is
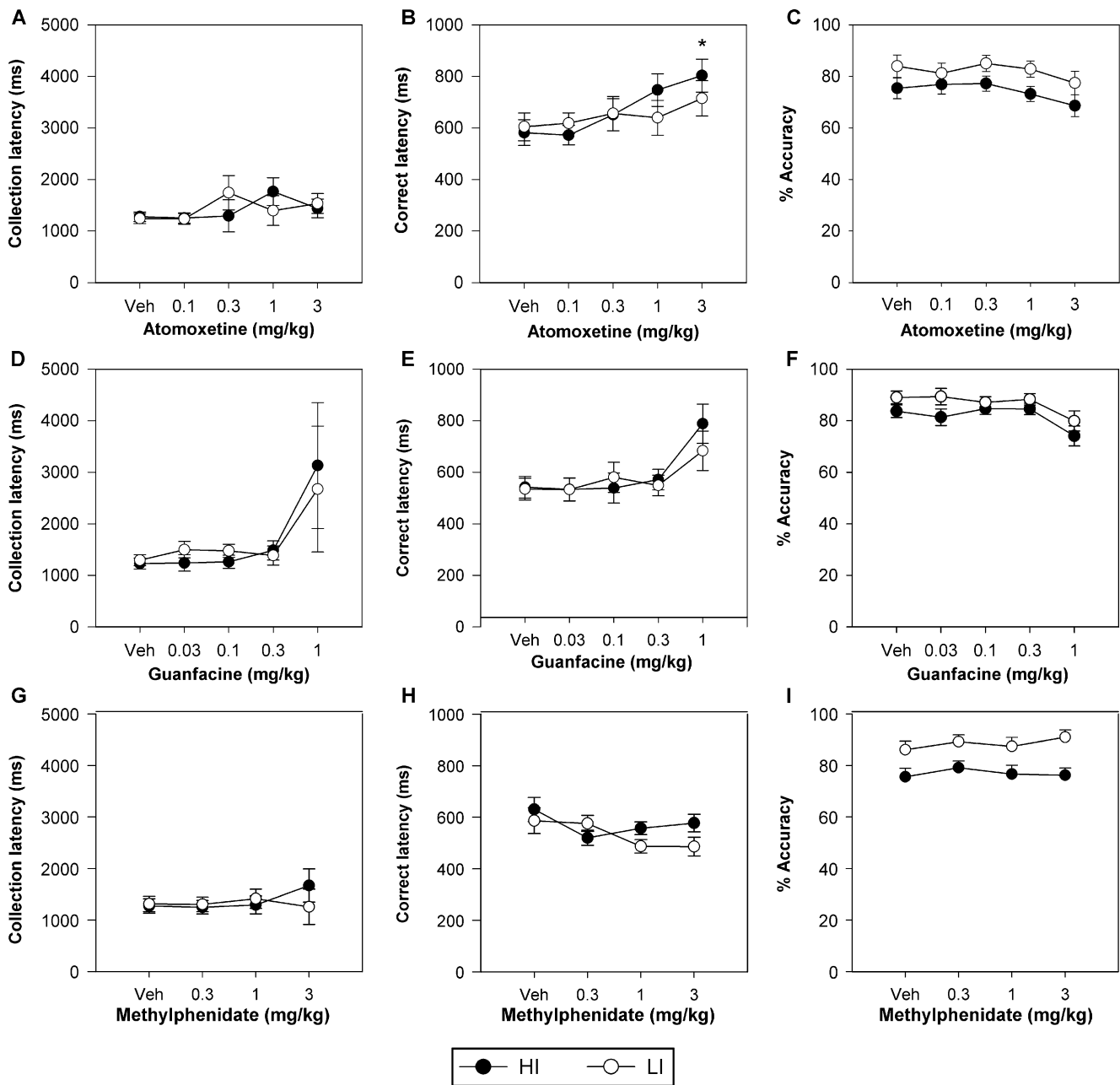
Fig. 4 a–i Effects of atomoxetine, guanfacine and methylphenidate on food collection latencies, correct response latencies and attentional accuracy on the five-choice serial reaction time task. There was a significant main effect of atomoxetine on food collection latencies ($F_{(4, 52)}=6.069$, $p<0.001$) and significant main effects of guanfacine on correct response latencies ($F_{(1.445, 17.345)}=7.472$, $p<0.01$) and attentional accuracy ($F_{(1.937, 23.249)}=5.812$, $p<0.01$). *$p<0.05$, pairwise comparisons of mean responding (vehicle vs dose). Data are means±SEM

unknown and plausibly may also have been detected in rats treated with higher doses of sumanirole. Previous research indicates that the direct administration of quinpirole in the nucleus accumbens core, an area with a low abundance of D3 receptors (Bouthenet et al. 1991), does not increase omissions even after relatively high doses (Pezze et al. 2009). Indeed, microinfusions of the D2 receptor antagonist eticlopride in the core leads to a significant increase in

omissions on the 5CSRTT (Pattij et al. 2007). Moreover, although quinpirole likewise increased omissions on this task when infused in the orbitofrontal cortex (OFC), this effect was no different between low and high impulsive rats (Winstanley et al. 2010). Thus, the differential increase in omissions induced by quinpirole in HI and LI rats appears to be mediated by structures other than the nucleus accumbens core and OFC.

The neural loci underlying the reduction in impulsive behaviour following quinpirole and sumanirole administration remain to be clarified but putatively may involve the PFC (including the OFC), nucleus accumbens and DA neurons in the ventral tegmental area (Dalley et al. 2011). In a recent study, intra-OFC quinpirole administration in low and high impulsive rats produced a generalized disruption in performance on the 5CSRTT with effects on premature responding, accuracy, omissions and response latencies (Winstanley et al. 2010). Such effects suggest that D2/3 receptors in this region do not play a selective role in impulse control. The disruptive effects of intra-OFC quinpirole in this setting could potentially be mediated through effects on postsynaptic D2/3 receptors since mesocortical DA neurons are deficient in (or lack) presynaptic autoreceptors (Bannon et al. 1982; Chiodo et al. 1984). However, the same is not true of mesolimbic DA neurons innervating the nucleus accumbens, which are subject to regulation by D2/3 autoreceptors (Benoit-Marand et al. 2001; Schmitz et al. 2002). Thus, the decrease in impulsivity caused by low-dose quinpirole and sumanirole may be due, in part, to diminished DA release in the nucleus accumbens, leading in turn to a reduction in behavioural activation (Robbins and Everitt 2007). A plausible candidate for this effect is the postsynaptic D1 receptor as blockade of these receptors in the core, but not the shell, of the nucleus accumbens reduced impulsivity on the 5CSRTT (Pattij et al. 2007), whereas administration of the D1 receptor agonist SKF 38393 in the core had the opposite effect (Pezze et al. 2007).

Systemic administration of atomoxetine, a selective NA reuptake inhibitor (Bymaster et al. 2002), and guanfacine, a selective alpha-2 adrenoceptor agonist (Arnsten et al. 2007), resulted in a dose-dependent decrease in impulsivity but at doses which also increased omissions and adversely affected the speed of responding. Such non-specific effects are consistent with reports of sedation in animal studies (Milstein et al. 2010) and clinical subgroups treated with guanfacine (Biederman et al. 2004). At higher doses, atomoxetine also appeared to exhibit sedative effects as reflected by increased response latencies, omissions as well as reduced general activity (present study and see Blondeau and Dellu-Hagedorn 2007). However, lower doses have been shown to selectively reduce impulsivity on a broad

Table 1 Summary of the main behavioural effects of direct and indirect noradrenergic and dopaminergic agonists on the behavioural performance of rats on the five-choice serial reaction time task

Drug		Premature	Omissions	Collection latency (ms)	Correct latency (ms)	Accuracy
Quinpirole	HI	↓	↑	↑	↑	-
	LI	↓	↑	↑	↑	-
Sumanirole	HI	↓	↑	↑	↑	-
	LI	↓	-	↑	↑	-
GBR 12909	HI	↑	-	-	-	-
	LI	↑	-	-	-	-
Atomoxetine	HI	↓	↑	-	↑	-
	LI	-	-	-	-	-
Guanfacine	HI	↓	↑	↑	↑	↓
	LI	-	↑	↑	↑	↓
Methylphenidate	HI	-	-	-	-	-
	LI	-	-	-	-	-

↑ Increase with respect to vehicle, ↓ with respect to vehicle, − no change with respect to vehicle. Note that the effects on premature responding for quinpirole and sumanirole occur at doses with no effects on response latencies or omissions.

range of tasks, including the 5CSRTT (Blondeau and Dellu-Hagedorn 2007; Robbins and Everitt 2007; Navarra et al. 2008), stop-signal reaction time task and the delay discounting paradigm (Robinson et al. 2008). The lack of selective effects of atomoxetine in the present study may be due to the inclusion of high impulsive rats, which exhibited a more pronounced response than low impulsive rats (in terms of omissions and response latencies) to a moderate dose of atomoxetine (1 mg/kg). Atomoxetine and guanfacine are postulated to facilitate PFC function and improve sustained attention by indirectly or directly stimulating postsynaptic alpha-2 adrenoceptors (Arnsten et al. 2007; Sagvolden 2006; Bymaster et al. 2002) reflected in the similar behavioural effects of these compounds in the present study.

The primary effects of methylphenidate in the brain are to increase NA and DA neurotransmission by blocking the NA and DA transporters (Elia et al. 1999; Kutcher et al. 2004; Solanto 2002; McKittrick and Abercrombie 2007). Consistent with earlier findings (van Gaalen et al. 2006), selective blockade of the DA transporter by GBR 12909 in the present study resulted in a significant increase in premature responding. Based on previous evidence, this effect was most likely mediated by the activation of D1 and D2 receptors in the nucleus accumbens, in particular within its core subregion. Thus, in addition to the prominent role for D1 receptors in impulsivity (see above), D2 receptors also powerfully modulate the effects of stimulant drugs such as *d*-amphetamine on impulse control (van Gaalen et al. 2006). This interaction has recently been localised to the nucleus accumbens core as demonstrated by the complete blockade of *d*-amphetamine-induced impulsivity on the 5CSRTT by intra-core infusions of the D2 receptor antagonist eticlopride (Pattij et al. 2007). Moreover, the D2 receptor antagonist sulpiride strongly attenuates the elevation in premature responding induced by PFC lesions when directly administered in the nucleus accumbens core (Pezze et al. 2009).

In the present study, methylphenidate did not significantly affect premature responding in high and low impulsive rats. Previous studies report increased impulsivity in rats treated with methylphenidate when tested with the usual constant ITI in the 5CSRTT (Milstein et al. 2010; Navarra et al. 2008; Blondeau and Dellu-Hagedorn 2007), and our present findings indicate that this was indeed the case for low impulsive rats with a near doubling in the frequency of premature responses following the administration of methylphenidate. In the less common variant of the task when premature responses are recorded but not punished, methylphenidate has the opposite effect and reduces impulsivity (Bizarro et al. 2004). Earlier evidence also indicates that low-dose methylphenidate improves visual attention and *lowers* impulsivity in rats selected for poor baseline

performance on the 5CSRTT (Puumala et al. 1996). Thus, the effects of methylphenidate on impulse control are evidently dependent on baseline levels of impulsivity, consistent with the rate-dependent effects of this compound in children (Robbins and Sahakian 1979). Such a relationship seen in this study with methylphenidate suggests at least two mechanisms, which may not be mutually exclusive. Firstly, the effects of NA, which functions to inhibit impulsive responding, may be greater in high impulsive rats or at least sufficient to cancel out the DA-enhancing effects of methylphenidate. Secondly, the behavioural activating effects of DA are blunted in high impulsive rats. This second mechanism would be compatible with the finding of reduced D2/3 receptor availability in high impulsive rats (Dalley et al. 2007) if indeed such effects were localised to the nucleus accumbens core. Finally it should be noted that we observed a large inter-individual response to methylphenidate in high impulsive rats, especially at higher doses. This variation may reflect differences in the extent of the primary neurochemical deficit in hyper-impulsive rats in terms of NA and DA function or polymorphisms of the NA and DA transporter genes which may underlie variation in the clinical response to methylphenidate (Roman et al. 2004; Yang et al. 2004).

In conclusion, the main findings of this study indicate that dopaminergic and noradrenergic mechanisms have divergent functional roles in the modulation of hyper-impulsivity in rats on the five-choice serial reaction time task. In contrast to the direct and indirect NA receptor agonists guanfacine and atomoxetine, the selective D2/3 receptor agonists sumanirole and quinpirole attenuated impulsivity on this task at doses that did not produce excessive sedation. The differential effects of quinpirole in high impulsive rats are consistent with D2/3 receptor dysfunction in this subset of animals, which may have relevance for the elucidation of neural vulnerability mechanisms underlying stimulant addiction.

References

Arnsten AF, Scahill L, Findling RL (2007) Alpha2-adrenergic receptor agonists for the treatment of attention-deficit/hyperactivity disorder: emerging concepts from new data. J Child Adolesc Psychopharmacol 17(4):393–406

Bannon MJ, Reinhard JF, Bunney EB, Roth RH (1982) Unique response to antipsychotic drugs is due to absence of terminal autoreceptors in mesocortical dopamine neurones. Nature 296 (5856):444–446

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

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

Benoit-Marand M, Borrelli E, Gonon F (2001) Inhibition of dopamine release via presynaptic D2 receptors: time course and functional characteristics in vivo. J Neurosci 21(23):9134–9141

Besson M, Belin D, McNamara R, Theobald DE, Castel A, Beckett VL, Crittenden BM 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(2):560–569

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

Biederman J, Spencer T, Wilens T (2004) Evidence-based pharmacotherapy for attention-deficit hyperactivity disorder. Int J Neuropsychopharmacol 7(1):77–97

Bizarro L, Patel S, Murtagh C, Stolerman IP (2004) Differential effects of psychomotor stimulants on attentional performance in rats: nicotine, amphetamine, caffeine and methylphenidate. Behav Pharmacol 15(3):195–206

Blondeau C, Dellu-Hagedorn F (2007) Dimensional analysis of ADHD subtypes in rats. Biol Psychiatry 61(12):1340–1350

Booze RM, Wallace DR (1995) Dopamine D2 and D3 receptors in the rat striatum and nucleus accumbens: use of 7-OH-DPAT and [125I]-iodosulpride. Synapse 19(1):1–13

Bouthenet M, Souil E, Martres M, Sokoloff P, Giros B, Schwartz J (1991) Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D2 receptor mRNA. Brain Res 564(2):203–219. doi:10.1016/0006-8993(91)91456-B

Bymaster FP, Katner JS, Nelson DL, Hemrick-Luecke SK, Threlkeld PG, Heiligenstein JH, Morin SM et al (2002) Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/ hyperactivity disorder. Neuropsychopharmacology 27(5):699–711

Caballero J, Nahata MC (2003) Atomoxetine hydrochloride for the treatment of attention-deficit/hyperactivity disorder. Clin Ther 25 (12):3065–3083

Chamberlain SR, Del Campo N, Dowson J, Müller U, Clark L, Robbins TW, Sahakian BJ (2007) Atomoxetine improved response inhibition in adults with attention deficit/hyperactivity disorder. Biol Psychiatry 62(9):977–984

Chamberlain SR, Sahakian BJ (2007) The neuropsychiatry of impulsivity. Curr Opin Psychiatry 20(3):255–261

Chiodo L, Bannon M, Grace A, Roth R, Bunney B (1984) Evidence for the absence of impulse-regulating somatodendritic and synthesis-modulating nerve terminal autoreceptors on subpopulations of mesocortical dopamine neurons. Neuroscience 12(1):1–16

Curran EJ, Watson SJ (1995) Dopamine receptor mRNA expression patterns by opioid peptide cells in the nucleus accumbens o the rat: a double in situ hybridization study. J Comp Neurol 361(1):57–76

Dalley JW, Everitt BJ, Robbins TW (2011) Impulsivity, compulsivity, and top-down cognitive control. Neuron 69(4):680–694

Dalley JW, Fryer TD, Brichard L, Robinson ESJ, Theobald DEH, Laane K, Pena Y et al (2007) Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. Science 315 (5816):1267–1270

Diergaarde L, Pattij T, Nawijn L, Schoffelmeer AN, De Vries TJ (2009) Trait impulsivity predicts escalation of sucrose seeking and hypersensitivity to sucrose-associated stimuli. Behav Neurosci 123 (4):794–803

Diergaarde L, Pattij T, Poortvliet I, Hogenboom F, de Vries W, Schoffelmeer ANM, De Vries TJ (2008) Impulsive choice and impulsive action predict vulnerability to distinct stages of nicotine seeking in rats. Biol Psychiatry 63(3):301–308

Dougherty DD, Bonab AA, Spencer TJ, Rauch SL, Madras BK, Fischman AJ (1999) Dopamine transporter density in patients with attention deficit hyperactivity disorder. Lancet 354 (9196):2132–2133

Economidou D, Pelloux Y, Robbins TW, Dalley JW, Everitt BJ (2009) High impulsivity predicts relapse to cocaine-seeking after punishment-induced abstinence. Biol Psychiatry 65(10):851–856

Elia J, Ambrosini PJ, Rapoport JL (1999) Treatment of attention-deficit–hyperactivity disorder. N Engl J Med 340(10):780–788

Ernst M, Zametkin AJ, Matochik JA, Jons PH, Cohen RM (1998) DOPA decarboxylase activity in attention deficit hyperactivity disorder adults. A [fluorine-18]fluorodopa positron emission tomographic study. J Neurosci 18(15):5901–5907

Fernando ABP, Robbins TW (2011) Animal models of neuropsychiatric disorders. Annu Rev Clin Psychol 7(1):110301095455042

Franowicz JS, Kessler LE, Borja CMD, Kobilka BK, Limbird LE, Arnsten AFT (2002) Mutation of the alpha 2A-adrenoceptor impairs working memory performance and annuls cognitive enhancement by guanfacine. J Neurosci 22(19):8771–8777

van Gaalen MM, van Koten R, Schoffelmeer AN, Vanderschuren LJ (2006) Critical involvement of dopaminergic neurotransmission in impulsive decision making. Biol Psychiatry 60(1):66–73

Heikkila RE, Manzino L (1984) Behavioral properties of GBR 12909, GBR 13069 and GBR 13098: specific inhibitors of dopamine uptake. Eur J Pharmacol 103(3–4):241–248

Kratochvil CJ, Heiligenstein JH, Dittmann R, Spencer TJ, Biederman J, Wernicke J, Newcorn JH et al (2002) Atomoxetine and methylphenidate treatment in children with ADHD: a prospective, randomized, open-label trial. J Am Acad Child Adolesc Psychiatry 41(7):776–784

Krause K, Dresel SH, Krause J, Kung HF, Tatsch K (2000) Increased striatal dopamine transporter in adult patients with attention deficit hyperactivity disorder: effects of methylphenidate as measured by single photon emission computed tomography. Neurosci Lett 285(2):107–110

Kutcher S, Aman M, Brooks SJ, Buitelaar J, van Daalen E, Fegert J, Findling RL et al (2004) International consensus statement on attention-deficit/hyperactivity disorder (ADHD) and disruptive behaviour disorders (DBDs): clinical implications and treatment practice suggestions. Eur Neuropsychopharmacol 14 (1):11–28

McCall RB, Lookingland KJ, Bédard PJ, Huff RM (2005) Sumanirole, a highly dopamine D2-selective receptor agonist: in vitro and in vivo pharmacological characterization and efficacy in animal models of Parkinson's disease. J Pharmacol Exp Ther 314 (3):1248–1256

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(5):1247–1256

Milstein JA, Dalley JW, Robbins TW (2010) Methylphenidate-induced impulsivity: pharmacological antagonism by beta-adrenoreceptor blockade. J Psychopharmacol 24(3):309–321

Moeller FG, Barratt ES, Dougherty DM, Schmitz JM, Swann AC (2001) Psychiatric aspects of impulsivity. Am J Psychiatry 158 (11):1783–1793

Navarra R, Graf R, Huang Y, Logue S, Comery T, Hughes Z, Day M (2008) Effects of atomoxetine and methylphenidate on attention

and impulsivity in the 5-choice serial reaction time test. Prog Neuropsychopharmacol Biol Psychiatry 32(1):34–41

Newcorn JH, Halperin JM, Jensen PS, Abikoff HB, Arnold LE, Cantwell DP, Conners CK et al (2001) Symptom profiles in children with ADHD: effects of comorbidity and gender. J Am Acad Child Adolesc Psychiatry 40(2):137–146

Pattij T, Janssen MC, Vanderschuren LJ, Schoffelmeer AN, van Gaalen MM (2007) Involvement of dopamine D1 and D2 receptors in the nucleus accumbens core and shell in inhibitory response control. Psychopharmacology 191(3):587–598

Pezze MA, Dalley JW, Robbins TW (2009) Remediation of attentional dysfunction in rats with lesions of the medial prefrontal cortex by intra-accumbens administration of the dopamine D2/3 receptor antagonist sulpiride. Psychopharmacology 202(1–3):307–313

Pezze MA, 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(2):273–83

Potenza MN (2007) To do or not to do? The complexities of addiction, motivation, self-control, and impulsivity. Am J Psychiatry 164(1):4–6

Puumala TT, Ruotsalainen S, Jakala P, Koivisto E, Riekkinen P Jr, Sirvio J (1996) Behavioral and pharmacological studies on the validation of a new animal model for attention deficit hyperactivity disorder. Neurobiol Learn Mem 66: 198–211

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

Robbins TW, Everitt BJ (2007) A role for mesencephalic dopamine in activation: commentary on Berridge (2006). Psychopharmacology 191(3):433–437

Robbins TW, Sahakian BJ (1979) "Paradoxical" effects of psychomotor stimulant drugs in hyperactive children from the standpoint of behavioural pharmacology. Neuropharmacology 18(12):931–950

Robinson E, Eagle D, Economidou D, Theobald D, Mar A, Murphy E, Robbins T et al (2009) Behavioural characterisation of high impulsivity on the 5-choice serial reaction time task: specific deficits in 'waiting' versus 'stopping'. Behav Brain Res 196(2):310–316

Robinson ESJ, Eagle DM, Mar AC, Bari A, Banerjee G, Jiang X, Dalley JW et al (2008) Similar effects of the selective noradrenaline reuptake inhibitor atomoxetine on three distinct forms of impulsivity in the rat. Neuropsychopharmacology 33(5):1028–1037

Roman T, Rohde LA, Hutz MH (2004) Polymorphisms of the dopamine transporter gene: influence on response to methylphenidate in attention deficit-hyperactivity disorder. Am J Pharmacogenomics 4(2):83–92

Romero AG, Darlingotn WH, McMillan MW (1997) Synthesis of the selective D$_2$ receptor agonist PNU-95666E from D-phenylalanine using a sequential oxidative cyclization strategy. J Org Chem 62:6582–6587

Sagvolden T (2006) The alpha-2A adrenoceptor agonist guanfacine improves sustained attention and reduces overactivity and impulsiveness in an animal model of attention-deficit/hyperactivity disorder (ADHD). Behav Brain Funct 2:41–41

Scahill L, Chappell PB, Kim YS, Schultz RT, Katsovich L, Shepherd E, Arnsten AF et al (2001) A placebo-controlled study of guanfacine in the treatment of children with tic disorders and attention deficit hyperactivity disorder. Am J Psychiatry 158(7):1067–1074

Schmitz Y, Schmauss C, Sulzer D (2002) Altered dopamine release and uptake kinetics in mice lacking D2 receptors. J Neurosci 22(18):8002–8009

Solanto MV (2002) Dopamine dysfunction in AD/HD: integrating clinical and basic neuroscience research. Behav Brain Res 130(1–2):65–71

Volkow ND, Wang G, Kollins SH, Wigal TL, Newcorn JH, Telang F, Fowler JS et al (2009) Evaluating dopamine reward pathway in ADHD: clinical implications. JAMA 302(10):1084–1091

Winstanley CA, Zeeb FD, Bedard A, Fu K, Lai B, Steele C, Wong AC (2010) Dopaminergic modulation of the orbitofrontal cortex affects attention, motivation and impulsive responding in rats performing the five-choice serial reaction time task. Behav Brain Res 210(2):263–272

Yang L, Wang Y, Li J, Faraone SV (2004) Association of norepinephrine transporter gene with methylphenidate response. J Am Acad Child Adolesc Psychiatry 43(9):1154–1158