The dopamine D₃ receptor antagonist, S33138, counters cognitive impairment in a range of rodent and primate procedures

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Abstract

Although dopamine D₃ receptor antagonists have been shown to enhance frontocortical cholinergic transmission and improve cognitive performance in rodents, data are limited and their effects have never been examined in primates. Accordingly, we characterized the actions of the D₃ receptor antagonist, S33138, in rats and rhesus monkeys using a suite of procedures in which cognitive performance was disrupted by several contrasting manipulations. S33138 dose-dependently (0.01–0.63 mg/kg s.c.) blocked a delay-induced impairment of novel object recognition in rats, a model of visual learning and memory. Further, S33138 (0.16–2.5 mg/kg s.c.) similarly reduced a delay-induced deficit in social novelty discrimination in rats, a procedure principally based on olfactory cues. Adult rhesus monkeys were trained to perform cognitive procedures, then chronically exposed to low doses of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine which produced cognitive impairment without motor disruption. In an attentional set-shifting task of cognitive flexibility involving an extra-dimensional shift, deficits were reversed by S33138 (0.04 and 0.16 mg/kg p.o.). S33138 also significantly improved accuracy (0.04 and 0.16 mg/kg p.o.) at short (but not long) delays in a variable delayed-response task of attention and working memory. Finally, in a separate set of experiments performed in monkeys displaying age-related deficits, S33138 significantly (0.16 and 0.63 mg/kg p.o.) improved task accuracies for long delay intervals in a delayed matching-to-sample task of working memory. In conclusion, S33138 improved performance in several rat and primate procedures of cognitive impairment. These data underpin interest in D₃ receptor blockade as a strategy for improving cognitive performance in CNS disorders like schizophrenia and Parkinson’s disease.

Key words: Attention, cognition, dopamine D₃ receptor, executive function, frontal cortex.

Introduction

Management of the cognitive impairment associated with psychiatric and neurological disorders like schizophrenia, major depression and Parkinson’s disease remains a major challenge (Austin et al. 2001; Keefe et al. 2006; Nuechterlein et al. 2004; Young et al. 2009; Zgaljardic et al. 2004) and, among numerous therapeutic strategies, there remains considerable interest in dopaminergic mechanisms. This is not surprising in view of the key role of dopamine (DA) in the control of cognition via actions expressed both in the prefrontal cortex (PFC) and in subcortical structures like the striatum, nucleus accumbens and hippocampus (Cools, 2008; Dalley et al. 2004; El Ghundi et al. 2007; Nieoullon, 2002). An optimal level of D₃ receptor stimulation in PFC facilitates working memory, and recruitment of D₃ (and/or D₄) receptors in the...
hippocampus participates in processes underlying learning and memory formation (Di Cara et al. 2007; El-Ghundi et al. 2007; Williams & Castner, 2006). Cortical populations of D3 receptors also affect mnemonic function, but their influence remains poorly defined (Bernaerts & Tirelli, 2003; Woolley et al. 2008). More recently, interest has focused on the apparently contrasting roles of D3 compared to closely related D2 receptors. Activation of D2 sites (over a defined dose range) in the PFC and subcortical regions favours cognitive processes, whereas their pharmacological blockade or genetic inactivation is generally deleterious (Cools, 2008; El Ghundi et al. 2007; Glickstein et al. 2002, 2005; Loiseau & Millan, 2009; Millan et al. 2007).

Intriguingly, however, mimicking the differential roles of D2 receptors has undergone comprehensive pre-clinical testing and is both safe and well-tolerated, rendering it appropriate for studies in primates. Therefore, we first, examined the actions of S33138 in a novel object recognition (NOR) procedure in rats where, in contrast to social recognition (olfactory cues), visual cues predominate (Dere et al. 2007; Winters et al. 2008). Second, we evaluated its effects in a model of social novelty discrimination (SND), whereby a mature subject must differentiate a familiar from a novel juvenile (Pichat et al. 2007; Terranova et al. 2005). Third, we characterized the actions of S33138 in rhesus monkeys chronically pre-exposed to low doses ('CLD') of the dopaminergic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This treatment ('CLD-MPTP') leads to marked deficits in attention, speed of processing and executive function, although monkeys fully retain their ability to perform cognitive tasks (Decamp & Schneider, 2004; Schneider & Kovelowski, 1990; Schneider et al. 1994). Fourth, the actions of S33138 were evaluated in a delayed matching-to-sample (DMTS) task of working memory in aged rhesus monkeys, a model of ‘natural’ cognitive impairment (Buccafusco, 2008; Buccafusco et al. 2004).

Materials and methods

NOR in rats

The procedure was conducted in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986 with local ethical committee approval (University of Nottingham). Male Lister Hooded rats weighing 170 g were purchased from Charles River (UK). The procedure was adapted from Bianchi et al. (2006). Briefly, rats were habituated to the test arena for 1 h, 24 h before the test. On the test day, during the
familiarization trial (T1), two identical bottles were placed in the arena and the rat was allowed to explore them for 3 min. During the choice trial (T2), conducted 4 h later, one object was replaced with a novel unfamiliar object and the rat was allowed to explore the objects again for 3 min. During each trial, exploration of the objects was recorded. S33138 was injected 30 min prior to T1. A NOR ratio (exploration of novel object – exploration of familiar object/total object exploration) was calculated. Data were analysed by two-way ANOVA with exploration of the novel and familiar objects the repeated within-subject factors, and treatment the between-subjects factor. NOR ratio and times of investigation during T1 and T2 were analysed by one-way ANOVA followed by Dunnett’s test. In dose–response studies, each animal received doses of drug (vehicle) in a pseudo-random order at several day intervals to minimize inter-trial variation.

**SND in rats**

Procedures conformed to European (86/609-EEC) and French (87/848) decrees for the care and use of laboratory animals. The procedure was adapted from Terranova et al. (2005). Male Wistar adult (240–260 g) and juvenile (21 d) animals were employed: they were purchased from Janvier (France). Half of the juveniles were identified on their tails, coat and head with a small, non-odorous black mark. On the test day, a juvenile (‘white’ or ‘black’, pseudo-randomly chosen), was placed into the adult home cage for a 5-min period (P1) and time spent by the adult in social investigation of the juvenile was recorded. Thirty minutes later, this juvenile was reintroduced for a second 5-min period (P2), together with a novel juvenile.

During P2, times of investigation of each juvenile (P2 novel and P2 familiar) were recorded. S33138 was injected 30 min prior to P1. A SND ratio (investigation of the novel juvenile/investigation of the familiar juvenile) was calculated and analysed by two-way ANOVA with exploration of novel and familiar juveniles as the repeated within-subjects factor, and treatment as the between-subjects factor. SND ratio and total times of investigation during P1 and P2 were analysed by one-way ANOVA followed by Dunnett’s t test.

**Assessment of cognitive performance in CLD-MPTP-treated monkeys**

The procedures employed were as previously described (Decamp & Schneider, 2004; Schneider et al. 1994, 2000, 2003). Experiments were conducted on six adult, male rhesus monkeys (8–10 kg) pre-trained to perform tasks (reinforced by a sugar pellet) reflecting attention, executive function and working memory. Animals were administered low doses of the neurotoxin MPTP (0.05–0.15 mg/kg p.o., 2–3 times per week) over approximately 4 months to produce cognitive deficits, but no Parkinson-like motor impairment, as confirmed by behavioural observations, normal behaviour in cognitive tests, and response-time data acquired during performance of certain computerized tasks described below (not shown). Cognitive performance was assessed by a previously validated computer-controlled test battery that included a variable delayed response (VDR) task and an ASST. Baseline (MPTP/vehicle) values were always determined the day prior to receiving S33138 and values averaged for data presentation (Tables 3, Fig. 3). All animals received each dose of S33138 (and vehicle) in a quasi-random order. Animals were allowed a minimum of 3 d between tests.

In the VDR procedure, a cue appeared on the right or left side of a touch-sensitive screen for 2 s, it was then extinguished for variable delay periods, and then identical left and right choice stimuli were presented. A response was rewarded when the cue located on the same side as where the cue appeared was touched. Five different delay lengths were randomly distributed in blocks of eight trials over a 40-trial daily testing session. The range of delays was 2–60 s, and a range of delays up to chance performance was determined for each individual to define its spatial working-memory abilities. The side of cue presentation was distributed quasi-randomly over the 40-trial session. All animals were trained and tested in the morning and food restricted overnight prior to testing. The percentage of correct responses was recorded and performance after MPTP exposure compared to performance prior to exposure by paired t tests. The effects of S33138 (0.04, 0.16, 0.63 mg/kg p.o.) were analysed independently for each delay and each time (1 and 24 h) by one-way ANOVA followed by Dunnett’s test comparisons of MPTP/S33138 vs. MPTP/vehicle-treated subjects.

The ASST is based on the Wisconsin Card Sorting Test and the Cambridge Automated Neuropsychological Test Battery (CANTAB) (Decamp & Schneider, 2004). Briefly, subtests were evaluated: simple discrimination (SD), simple discrimination reversal (SDR), compound discrimination (CD) and intra/extra-dimensional shift (IDS/EDS). Each session started with the SD and continued to the next subtest if, and only if the criterion of six consecutive positive responses was achieved. A maximum of 200 sessions was presented in single daily sessions. For each subtest, the number of sessions needed to reach criterion...
was expressed as a percentage change from pre-MPTP baseline values (see Results section). The effects of MPTP were compared to baseline values using paired t tests. The effects of S33138 in CLD-MPTP subjects were analysed for each task separately by one-way ANOVA followed by Dunnett’s test. Given the limitations of this task for repetitive drug testing, each drug dose was evaluated only 1 h after administration, and there was 3–4 wk between tests.

**DMTS procedure in aged monkeys**

Well-trained female rhesus (n = 6) macaques aged 17–33 yr and weighing 6.4–10.6 kg served as subjects. DMTS testing was conducted once each weekday, and all animals received each dose of S33138 (and vehicle) in a quasi-random order. All procedures were approved by the Medical College of Georgia Institutional Animal Care and Use Committee and are consistent with AAALAC guidelines. The test was performed as previously described (Buccafusco, 2008; Buccafusco et al. 2004). Test panels attached to the animals’ home cages presented the task by use of a computer-automated touch-screen system. A trial was initiated by presentation of a stimulus (red, blue, or yellow rectangles). The sample rectangle remained in view until the monkey touched it to initiate a delay (retention) interval after which two choice rectangles appeared. One colour matched the stimulus. Only a correct (matching) choice was reinforced (by a flavoured pellet). The inter-trial interval was 5 s and each session consisted of 96 trials. Three different presentation sequences were rotated through each daily session to prevent subjects from memorizing the first of several trials. The duration for each delay interval was adjusted for each subject until three levels of group performance accuracy were approximated: zero delay (85–100% of trials answered correctly); short delay (75–84% correct); medium delay (65–74% correct) and long delay (55–64% correct). Percent correct responses were subdivided according to the delay interval. Several testing precautions were incorporated into the presentation of the matching problem. First, the various combinations of stimulus colour (e.g. red, green, yellow) were arranged so that each of the three colours appeared an equal number of times as a sample; each colour appeared an equal number of times on the two choice keys; and each colour appeared an equal number of times in combination with each other colour. Similarly, when two colours (e.g. green/yellow) appeared in combination, each colour was counterbalanced between left and right in a non-predictable pattern. Thus, correct responses were arranged so that simplistic strategies such as position preference, left/right alternation, or even double left/right alternation resulted in chance levels of accuracy (i.e. ~50% correct). Finally, all stimulus-counterbalancing procedures were matched to length of delay. This (more detailed information) has been included in previous papers (e.g. Terry et al. 2005). The raw data (% trials correct) were analysed employing a multi-factorial ANOVA with repeated measures. An orthogonal multi-comparison t test was used to compare individual means.

**Drug administration schedules**

Drug doses are expressed in terms of the base. In rodent procedures, S33138 (0.01–2.5 mg/kg s.c.) was dissolved in saline and administered at 1 ml/kg, 30 min prior to testing. For the CLD-MPTP model, S33138 (0.04, 0.16, and 0.63 mg/kg p.o., in quasi-random order) was diluted in sugar water and administered in a volume of 2 ml/kg while the animal was in its home cage. After administration of S33138, neither drug nor vehicle were given for at least 3 d, or longer for the ASST (see above). Animals were tested 1 h and 24 h after administration, except for the ASST (1 h). In aged monkeys (DMTS), S33138 (0.011, 0.045, 0.18, and 0.72 mg/kg p.o.) was dissolved in water (0.3 ml) and pipetted onto a sugar cube, and given to the subject for consumption 1 h prior to DMTS testing. Three vehicle (water on sugar cube) sessions were run during the study. Animals were tested 1 h and 24 h after administration.

**Results**

**NOR in rats (Table 1, Fig. 1)**

Activity in the NOR procedure reflects an influence on declarative memory, with certain authors suggesting a relationship with human episodic memory (Aggleton & Brown 2006; Dere et al. 2007; Winters et al. 2008). As previously described (Bianchi et al. 2006), after a 4 h inter-trial delay, vehicle-treated rats spent equivalent time in exploring the familiar vs. the novel object during the second choice trial, consistent with natural forgetting (Table 1). Two-way ANOVA performed on exploration during the choice trial showed a significant object x treatment interaction (p < 0.01). S33138 reversed the delay-induced deficit of NOR [F(5, 93) = 5.02, p < 0.001], with a maximal effect at 0.16 mg/kg (Fig. 1). S33138 did not modify total exploration of both objects during either the familiar or choice trials, excluding a non-specific confounding behavioural effect on object exploration: rather it exerted a specific
effect on cognitive processes. However, since S33138 was injected before acquisition, further studies are needed to clarify its influence upon different stages of mnemonic processing: encoding, consolidation, storage and retrieval (Dere et al., 2007; Winters et al., 2008).

### SND in rats (Table 2, Fig. 2)

In distinction to NOR, which is dependent upon visual cues, SND principally exploits chemosensory cues (Davis, 2004). This procedure taxes selective attention (Pichat et al., 2007; Terranova et al., 2005), and a significant involvement of memory per se is unlikely. Thus, with a 30-min delay, no impairment of identification was seen when the novel juvenile was presented alone during the second session (not shown). With a short period P1 (5 min), vehicle-treated animals spent equivalent time investigating the familiar and the novel juvenile during P2, consistent with a lack of social discrimination. Two-way ANOVA performed on exploration of novel and familiar juveniles during P2 showed a significant juvenile x treatment interaction ($p < 0.01$). S33138 provoked a dose-dependent increase of social discrimination [$F(4, 43) = 5.30$, $p < 0.001$], reaching statistical significance at the doses of 0.63 and 2.5 mg/kg (Fig. 2), whereas it had no significant effect on total investigation of juveniles during either P1 or P2 (Table 2).

### VDR task in CLD-MPTP-treated monkeys (Table 3, Fig. 3)

In the VDR task, short and intermediate delays are more heavily attention-loaded than longer delays, which reflect a greater component of working memory (Decamp & Schneider, 2004). Prior to MPTP exposure, short delay trials were performed significantly better than long delay trials. Following exposure to MPTP, all subjects exhibited a statistically significant ($p < 0.05$) decline in VDR task performance (% correct responses) with all delays except the longest (Table 3, Fig. 3). For the shortest delay (D1), S33138 significantly improved % correct responses when testing was performed at 1 h [$F(3, 35) = 5.17$, $p < 0.01$] as well as at 24 h [$F(3, 35) = 10.23$, $p < 0.01$] after treatment, with significant effects at the doses of 0.04 and 0.16 mg/kg (Fig. 3). For all other delays, despite tendencies at short and intermediate delays (D2 and D3), these analyses revealed no statistically significant effects on performance except for the dose of 0.16 mg/kg at D2 (Table 3).

### Cognitive flexibility: ASST in CLD-MPTP-treated monkeys (Fig. 4)

For CLD-MPTP monkeys, particular interest was given to measures of ‘cognitive flexibility’: that is, the

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### Table 1. Reversal by S33138 of a deficit in novel object discrimination induced by a prolonged inter-trial delay (4 h) in rats

<table>
<thead>
<tr>
<th>Dose of S33138 (mg/kg)</th>
<th>n</th>
<th>T1 (s)</th>
<th>Total T2 (s) Familiar + novel</th>
<th>T2 (s) Familiar</th>
<th>T2 (s) Novel</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Vehicle)</td>
<td>24</td>
<td>19.50±2.29</td>
<td>15.58±2.01</td>
<td>7.88±1.17</td>
<td>7.71±1.07</td>
</tr>
<tr>
<td>0.01</td>
<td>12</td>
<td>24.50±2.54</td>
<td>17.50±1.43</td>
<td>6.33±0.48</td>
<td>11.17±1.51</td>
</tr>
<tr>
<td>0.04</td>
<td>12</td>
<td>24.92±3.35</td>
<td>14.17±2.12</td>
<td>4.42±0.85</td>
<td>9.75±1.63</td>
</tr>
<tr>
<td>0.16</td>
<td>23</td>
<td>24.84±1.89</td>
<td>17.26±1.61</td>
<td>5.18±0.84</td>
<td>12.09±1.16</td>
</tr>
<tr>
<td>0.63</td>
<td>11</td>
<td>21.18±2.44</td>
<td>15.18±2.29</td>
<td>4.83±0.84</td>
<td>10.00±1.77</td>
</tr>
<tr>
<td>2.5</td>
<td>12</td>
<td>13.83±1.98</td>
<td>13.58±1.88</td>
<td>5.00±0.90</td>
<td>8.58±1.31</td>
</tr>
</tbody>
</table>

T1, Familiarization trial; T2, choice trial.

Data are expressed as the mean ± S.E.M. of exploration duration. S33138 was administered s.c., 30 min before T1. For statistical analyses, see Results section and Fig. 1 legend.
ability to adapt behaviour in the face of altered environmental demands, to learn a new rule, and to suppress a previously learned response. SDR is a measure of cognitive flexibility and attentional set-shifting refers to a new phase of learning in which attention is shifted from one (no longer relevant) stimulus to another (new relevant) stimulus (Boulougouris & Tsaltas, 2008; Chudasama & Robbins, 2006; Decamp & Schneider, 2004). In the present paradigm, following a compound discrimination procedure, the IDS involved a change in line orientation or a different shape, while the EDS represented a switch from line to shape (or vice versa) (Decamp & Schneider, 2004). Baseline, pre-MPTP number of sessions to reach criterion (six consecutive correct sessions) for the various cognitive subtests were as follows: SD = 17 ± 3; SDR = 24 ± 7; CD = 22 ± 6; IDS = 38 ± 15 and EDS = 28 ± 9. Neither SD nor CD performance were significantly affected by CLD-MPTP, and S33138 exerted no effect. Inspection of Fig. 4 suggests that S33138 reduces the MPTP-induced deficit in SDR, but this effect just missed statistical significance [F(2,11) = 3.52, p = 0.07]. In the IDS procedure, there was an overall significant effect of S33138 [F(2,11) = 4.42, p < 0.05], but no significant influence of MPTP. Finally, in the EDS procedure, MPTP elicited a ~4-fold and significant increase in the number of session needed to make six consecutive correct responses, and S33138 (0.04 and 0.16 mg/kg) reversed this MPTP-induced impairment, yielding significant improvements in EDS [F(2,11) = 11.1, p < 0.005] (Fig. 4). However, the highest dose (0.63 mg/kg) disrupted performance and, in most cases, animals only minimally performed the task data (not shown). Collectively, the data from the SDR and EDS shift components suggest that low doses of S33138 counter CLD-MPTP-induced deficits in cognitive flexibility.

### Table 2. Improvement by S33138 of social novelty discrimination in rats in a paradigm with a 30-min inter-trial interval

<table>
<thead>
<tr>
<th>Dose of S33138 (mg/kg)</th>
<th>n</th>
<th>P1 (s)</th>
<th>Total P2 (s)</th>
<th>P2 (s)</th>
<th>P2 (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Familiar + novel</td>
<td>Familiar</td>
<td>Novel</td>
</tr>
<tr>
<td>0 (Vehicle)</td>
<td>15</td>
<td>103.47 ± 6.83</td>
<td>83.87 ± 6.02</td>
<td>40.53 ± 3.50</td>
<td>43.33 ± 2.80</td>
</tr>
<tr>
<td>0.16</td>
<td>7</td>
<td>117.43 ± 10.93</td>
<td>64.86 ± 6.28</td>
<td>30.57 ± 3.08</td>
<td>34.29 ± 3.64</td>
</tr>
<tr>
<td>0.31</td>
<td>6</td>
<td>87.50 ± 4.04</td>
<td>62.67 ± 10.75</td>
<td>28.67 ± 6.17</td>
<td>34.00 ± 6.49</td>
</tr>
<tr>
<td>0.63</td>
<td>8</td>
<td>101.00 ± 10.15</td>
<td>78.50 ± 9.83</td>
<td>26.25 ± 3.19</td>
<td>52.25 ± 8.06</td>
</tr>
<tr>
<td>2.5</td>
<td>8</td>
<td>94.38 ± 9.71</td>
<td>59.25 ± 7.12</td>
<td>20.13 ± 3.25</td>
<td>39.13 ± 4.60</td>
</tr>
</tbody>
</table>

P1, First trial (familiar juvenile only); P2, second trial (both juveniles).

Data are expressed as the mean ± S.E.M. of duration of active social investigation. S33138 was administered s.c., 30 min before P1. For statistical analyses, see Results section and Fig. 2 legend.

### DMTS task in aged monkeys (Fig. 5)

The DMTS procedure is a well-established model of working memory where task difficulty can be manipulated by employing variable delay intervals. It was undertaken in 10 aged animals showing a natural deficit relative to younger peers (Buccafusco, 2008; Buccafusco et al. 2004). Performance accuracies during vehicle sessions in the standard DMTS task were as follows: zero delay, 97.5% trials correct; short delay, 81.8%; medium delay, 69.1%, and long delay, 61.4%. Thus, as a group, baseline accuracies fell within the targeted ranges for each delay interval. Task accuracies obtained after vehicle and after each of the four doses of S33138 are presented as a function of dose for each delay interval in Fig. 5. There was overall a statistically significant increase in task accuracies 1 h after S33138 administration [F(4,9) = 3.14, p = 0.016].
and a dose-dependent improvement in DMTS performance was associated with long-delay trials. Statistically significant increases in accuracy were seen for both the 0.16 mg/kg ($t = 2.0, p = 0.047$) and the 0.64 mg/kg ($t = 3.44, p = 0.0007$) doses. Moreover, there was a statistically significant increase in task accuracy obtained with the 0.04 mg/kg dose during medium delay trials ($t = 2.33, p = 0.021$). During sessions run 24 h after testing (Fig. 5), the influence of S33138 upon task accuracies was less pronounced and no longer statistically significant [$F(4, 9) = 0.426, p = 0.79$], probably reflecting higher variability.

**Discussion**

**Evaluation of the influence of S33138 upon cognitive performance in rats and primates**

The present observations amplify previous studies in demonstrating that S33138 exerts potent ‘pro-cognitive’ properties across a broad range of paradigms (Table 4). These are the first published data on the effects of a D₃ receptor antagonist in monkeys. Apart from the desire to limit experimentation, the lack of previous reports may be explained by the need for a thorough safety dossier, which was available for S33138. It was also particularly appropriate for these studies. First, its pharmacological profile has been thoroughly characterized, it is metabolically stable and possesses a substantial half-life (around 12 h p.o.) in rodents and primates (Millan et al. 2008a; Millan & Brocco, 2008; Thomasson-Perret et al. 2008). This may at least partially explain the activity of S33138 in certain primate cognitive procedures 24 h after administration. Although similar ‘protracted’ pro-cognitive actions of other drugs (like nicotinic agents) have been attributed to ‘adaptive processes’ (Buccafusco et al. 2005, 2009), there is currently no evidence for this intriguing possibility with S33138. Second, studies in rodents and primates have established that doses of 0.01–0.63 mg/kg i.p. and p.o. selectively occupy D₃ receptors (Millan et al. 2008a,c): this dose range corresponds to those active in the present procedures (Table 4). Third, at D₃ receptor-selective doses, S33138 is well-tolerated and causes no disruption of motor performance, endocrine secretion or autonomic-cardiovascular function (Millan et al. 2008a; Millan & Brocco, 2008; Thomasson-Perret et al. 2008).

**NOR in rats**

By analogy to stimulation of D₃ receptors (Besheer et al. 1999), blockade of D₃ receptors by S33138 reversed a delay-induced impairment in NOR. U-shaped dose–response curves are well-known for D₃ agonists (Williams & Castner, 2006). Nonetheless, the inversion of the S33138 dose–response curve presumably reflects onset of D₃ receptor antagonism since a further selective D₃ receptor antagonist, S33084, similarly improved NOR whereas the D₂ antagonist, L741,626 impaired NOR and blunted the effects of D₃ blockade (Loiseau et al. 2009). Interestingly, at identical doses, S33138 also reversed the deficits in NOR provoked by post-weaning isolation of rats (D. J. G. Watson,

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**Table 3. Influence of S33138 on variable delayed response performance in chronic, low dose 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys**

<table>
<thead>
<tr>
<th>Treatment (dose)</th>
<th>Delay 2 (5 s)</th>
<th>Delay 3 (10 s)</th>
<th>Delay 4 (24–45 s)</th>
<th>Delay 5 (46–60 s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-MPTP/Vehicle</td>
<td>91.67 ± 1.31</td>
<td>90.12 ± 1.62</td>
<td>65.70 ± 4.45</td>
<td>58.87 ± 1.97</td>
</tr>
<tr>
<td>Post-MPTP/Vehicle</td>
<td>64.87 ± 2.45</td>
<td>60.07 ± 3.07</td>
<td>46.36 ± 2.98*</td>
<td>50.35 ± 2.56</td>
</tr>
<tr>
<td>1 h (S33138, mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-MPTP/0.04</td>
<td>75.69 ± 8.24</td>
<td>67.01 ± 4.76</td>
<td>49.65 ± 5.63</td>
<td>49.31 ± 5.81</td>
</tr>
<tr>
<td>Post-MPTP/0.16</td>
<td>73.61 ± 6.33</td>
<td>69.10 ± 9.18</td>
<td>53.82 ± 4.82</td>
<td>58.68 ± 5.80</td>
</tr>
<tr>
<td>Post-MPTP/0.63</td>
<td>67.74 ± 7.81</td>
<td>56.25 ± 3.95</td>
<td>47.93 ± 6.59</td>
<td>42.19 ± 8.61</td>
</tr>
<tr>
<td>24 h (S33138, mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-MPTP/0.04</td>
<td>68.06 ± 3.56</td>
<td>65.63 ± 3.52</td>
<td>57.99 ± 6.75</td>
<td>47.92 ± 3.84</td>
</tr>
<tr>
<td>Post-MPTP/0.16</td>
<td>79.51 ± 4.00†</td>
<td>63.89 ± 5.37</td>
<td>65.28 ± 6.03</td>
<td>55.21 ± 5.71</td>
</tr>
<tr>
<td>Post-MPTP/0.63</td>
<td>71.88 ± 5.76</td>
<td>59.72 ± 7.56</td>
<td>54.17 ± 7.85</td>
<td>54.17 ± 4.47</td>
</tr>
</tbody>
</table>

S33138 was administered p.o. and doses are indicated in mg/kg. Post-MPTP/vessel values correspond to test sessions performed immediately before administration of S33138. For data with delay 1 (2 s), see Fig. 3. All data are means ± S.E.M. of percent correct responses acquired from all six monkeys tested.

* Indicates the significance of pre-MPTP/vehicle vs. post-MPTP/vehicle values in paired Student’s $t$ tests, $p < 0.05$.

† Indicates the significance of MPTP/S33138 vs. MPTP/vehicle differences in Dunnett’s test following ANOVA, $p < 0.05$. 

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## References

Brocco, 2008; Thomasson-Perret et al. 2008.

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* Indicates the significance of pre-MPTP/vehicle vs. post-MPTP/vehicle values in paired Student’s $t$ tests, $p < 0.05$.

† Indicates the significance of MPTP/S33138 vs. MPTP/vehicle differences in Dunnett’s test following ANOVA, $p < 0.05$. 

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By analogy to stimulation of D₃ receptors (Besheer et al. 1999), blockade of D₃ receptors by S33138 reversed a delay-induced impairment in NOR. U-shaped dose–response curves are well-known for D₃ agonists (Williams & Castner, 2006). Nonetheless, the inversion of the S33138 dose–response curve presumably reflects onset of D₃ receptor antagonism since a further selective D₃ receptor antagonist, S33084, similarly improved NOR whereas the D₂ antagonist, L741,626 impaired NOR and blunted the effects of D₃ blockade (Loiseau et al. 2009). Interestingly, at identical doses, S33138 also reversed the deficits in NOR provoked by post-weaning isolation of rats (D. J. G. Watson,
unpublished observations), a developmental model of schizophrenia (Bianchi et al. 2006). Although few data are available for D₂/D₃ antagonists, sulpiride, raclopride, eticlopride and haloperidol were either inactive (Besheer et al. 1999) or impaired NOR using short inter-trial intervals (Terry et al. 2007). It may be deduced that their negative impact reflects blockade of D₂ sites. Indeed, loss of perirhinal cortex D₂ receptors was associated with NOR deficits in a genetic model of Huntington’s disease (Cummings et al. 2006).

The latter observations are in accord with evidence for a crucial role of the perirhinal cortex in all stages (encoding to retrieval) of NOR, and specifically for novel-familiar discrimination (Aggleton & Brown, 2006; Barker et al. 2007; Dere et al. 2007; Winters et al. 2008). Accordingly, inactivation of the perirhinal cortex impairs NOR, at least partly due to interference with cholinergic mechanisms (Aggleton & Brown, 2006; Warburton et al. 2003; Winters et al. 2008; Winters & Bussey, 2005). However, there is currently no evidence that D₃ antagonists exert actions in the perirhinal cortex. A role for S33138 in the hippocampus also seems unlikely, despite its importance in NOR recollection (Aggleton & Brown 2005; Dere et al. 2007; Sauvage et al. 2008; Winters et al. 2008). By contrast, in addition to attentional ‘supervision’ (Dalley et al. 2004; Seamans & Yang, 2004), recent studies suggest a more important role of the PFC in NOR than previously imagined. Moreover, reflecting their reciprocal interconnections, the PFC and perirhinal
cortex operate as an integrated network for NOR (Akirav & Maroun, 2006; Barker et al., 2007; Pihlajamäki et al., 2005). Since cholinergic mechanisms are involved (Kozak et al., 2006; Sarter et al., 2003), induction by S33138 of frontocortical ACh release (Millan et al., 2008a) may contribute to its facilitation of NOR.

SND in rats

Supporting a role of D₃ receptor blockade in the improvement of SND by S33138, its actions are mimicked by other D₃ antagonists, whereas the D₄ antagonist, L741,626, disrupts SND (Loiseau et al., 2009). These observations mirror findings obtained in a model of social recognition (Millan et al. 2007). In the SND procedure, both novel and familiar juveniles move freely and quickly, implying an important role for attention (Engelmann et al. 1995; Terranova et al., 2005), whereas delay-induced impairment of social recognition incorporates a component of ‘long-term’ memory (Di Cara et al., 2007; Millan et al., 2007). SND may also be differentiated from NOR by its dependence on chemosensory cues which are processed via olfactory structures before accessing the amygdaloid complex, piriform and entorhinal cortices, then perirhinal cortex (Davis, 2004). One possible locus of action of S33138 in the SND test is the PFC since injection of S33138 into this region improves social recognition (Millan et al., 2008a). Further, PFC release of DA is accelerated by exposure to a novel juvenile (Feenstra et al., 2000). However, juvenile discrimination is associated with even more pronounced DA release in the nucleus accumbens (Feenstra et al., 2000), so this D₃ receptor-rich region may also be involved in the actions of S33138. As for the NOR procedure, it remains to be seen which stage of mnemonic processing is affected by S33138.

VDR tasks in monkeys

In CLD-MPTP-treated monkeys, performance in the VDR task was impaired (Schneider et al., 2003), and S33138 significantly countered deficits at the shortest delays. Further work would be justified to determine whether this effect of S33138 – and other D₃ antagonists – upon performance in this model reflects an influence upon working memory or, more likely, attention. In any event, the present data are intriguing in the light of studies showing effects of other drug classes upon attentional components of the VDR task: for example, Glycine₉ receptor partial agonists (Schneider et al., 2000) and α₇ nicotinic receptor agonists (Schneider et al., 2003). Underlining interest in the effects of S33138, D₁ and D₃ receptor agonists are...
Table 4. Overview of the influence of S33138 upon cognitive functions in rodents and primates

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Perturbation</th>
<th>Doses tested</th>
<th>Route</th>
<th>Species</th>
<th>Minimal effective dose</th>
<th>Effect</th>
<th>Principle cognitive domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social recognition*</td>
<td>Delay (2 h)</td>
<td>0.04–2.5</td>
<td>s.c.</td>
<td>Rat</td>
<td>0.63</td>
<td>↓ Deficit</td>
<td>Mainly olfactory, memory (delay) and attention (scopolamine)</td>
</tr>
<tr>
<td></td>
<td>Delay (2 h)</td>
<td>0.16–2.5 µg</td>
<td>i.c.</td>
<td>Rat</td>
<td>2.5 µg (PFC)</td>
<td>↓ Deficit</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scopolamine (1.25)</td>
<td>0.04–0.63</td>
<td>s.c.</td>
<td>Rat</td>
<td>0.16</td>
<td>↓ Deficit</td>
<td></td>
</tr>
<tr>
<td>5-Choice serial reaction time*</td>
<td>None</td>
<td>0.16–2.5</td>
<td>s.c.</td>
<td>Rat</td>
<td>&gt; 2.5</td>
<td>No disruption of performance</td>
<td>Attention (accuracy), Speed of processing (latency)</td>
</tr>
<tr>
<td>Passive avoidancec</td>
<td>Scopolamine (0.16)</td>
<td>0.16–2.5</td>
<td>s.c.</td>
<td>Mouse</td>
<td>0.63</td>
<td>↓ Deficit</td>
<td>Associative learning</td>
</tr>
<tr>
<td></td>
<td>Dizocilpine (0.04)</td>
<td>0.63–2.5</td>
<td>s.c.</td>
<td>Mouse</td>
<td>2.5</td>
<td>↓ Deficit</td>
<td></td>
</tr>
<tr>
<td>Object recognition</td>
<td>Isolation rearingb</td>
<td>0.16–2.5</td>
<td>s.c.</td>
<td>Rat</td>
<td>0.16</td>
<td>↓ Deficit</td>
<td>Visual, declarative memory, attention</td>
</tr>
<tr>
<td></td>
<td>Delay (4 h)</td>
<td>0.16–2.5</td>
<td>s.c.</td>
<td>Rat</td>
<td>0.16</td>
<td>↓ Deficit</td>
<td></td>
</tr>
<tr>
<td>Social discrimination</td>
<td>Delay (30 min)</td>
<td>0.16–2.5</td>
<td>s.c.</td>
<td>Rat</td>
<td>0.63</td>
<td>↓ Deficit</td>
<td>Selective attention (mainly olfactory)</td>
</tr>
<tr>
<td>Variable delayed response</td>
<td>Chronic, low-dose MPTP</td>
<td>0.04–0.63</td>
<td>p.o.</td>
<td>Rhesus monkey</td>
<td>0.04</td>
<td>↓ Deficit with short delays</td>
<td>Attention</td>
</tr>
<tr>
<td>Attentional set shifting</td>
<td>Chronic, lowdose MPTP</td>
<td>0.04–0.63</td>
<td>p.o.</td>
<td>Rhesus monkey</td>
<td>0.04</td>
<td>↓ EDS deficit</td>
<td>Executive function (cognitive flexibility)</td>
</tr>
<tr>
<td>Delayed matching to sample</td>
<td>Aging (27 years)</td>
<td>0.01–0.72</td>
<td>p.o.</td>
<td>Rhesus monkey</td>
<td>0.16</td>
<td>↓ Deficit with long delays</td>
<td>Working memory</td>
</tr>
</tbody>
</table>

MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; EDS, extradimensional shift. Doses of scopolamine and dizocilpine are in mg/kg s.c.; i.c. signifies bilateral microinjection into prefrontal cortex. Note active dose ranges correspond well to those defined as blocking D3 but not D2 receptors in primates and rodents (Millan et al. 2008a–c).

*a Millan et al. (2007); b D. J. G. Watson et al. (unpublished observations); c Millan & Brocco (2008).
ineffective, while l-dopa worsens VDR performance, possibly due to ‘overdosage’ of normosensitive D2/D3 receptors (Cools et al. 2008; Decamp & Schneider, 2009; Schneider et al. 1994). The doses of S33138 which attenuated the influence of MPTP upon D1 in the VDR procedure were low, corresponding well to those blocking D3 receptors in marmosets (Millan et al. 2008a,c), and active in SNd (and social recognition) procedures (Table 4). In view of positive actions of nicotinic agonists in VDR procedures (Decamp & Schneider 2009; Schneider et al. 2003), and of the role of PFC pools of ACh in controlling attention (Dalley et al. 2004; Robbins & Roberts, 2007; Sarter et al. 2003), the PFC may well be involved in the actions of S33138 (Millan et al. 2008a). However, other structures may be implicated, such as the nucleus accumbens (Mele et al. 2004; Pezze et al. 2007) that, together with the PFC (and amygdala), constitute a cerebral network regulating goal-directed behaviours, and investment of effort into cognitive and motor tasks (Goto & Grace, 2008; Salamone et al. 2007). Further, dopaminergic mechanisms in the nucleus accumbens modulate ACh release in PFC (Brooks et al. 2007).

**Attentional set-shifting procedure in monkeys**

Confirming prior work (Decamp & Schneider, 2004, 2009), CLD-MPTP-treated monkeys performed poorly in two tests of cognitive flexibility, reversal learning and an EDS-ASST shift, deficits abrogated by S33138. Neural circuits involved in ASST are not identical to those implicated in reversal learning (Brown & Bowman, 2002; Boulougouris et al. 2007; Dalley et al. 2004; Robbins & Roberts, 2007). Nonetheless, both tasks share the need for learning of new strategies and inhibition of previously learned rules. Thus, it is interesting to collectively consider studies of these paradigms that suggest an enhancement of cognitive flexibility by D3 but not D2 receptor blockade. DA release in the medial PFC is elevated during reversal learning (Van der Meulen et al. 2007), and lesions of the PFC disrupt the EDS component of the ASST (Birrell & Brown, 2000), supporting a role for DA in the PFC in the control of cognitive flexibility (Robbins & Roberts, 2007; Tunbridge et al. 2004), an assertion underpinned by imaging studies in humans (Dodds et al. 2008). Furthermore, ASST in rats was impaired by injection of eticlopride into the PFC (Floresco et al. 2006b), and it also blocked reversal learning upon introduction into the orbitofrontal cortex (Calaminus & Hauber, 2008). Sulpiride interfered with reversal learning in rats (Winter et al. 2009) and disrupted an EDS-ASST in healthy humans (Mehta et al. 1999), while raclopride perturbed reversal learning in monkeys (Lee et al. 2007), and haloperidol both interfered with reversal learning in marmosets (Ridley et al. 1981) and perturbed the reversal learning phase of an EDS-ASST procedure in mice (De Steno & Schmauss, 2009). Supporting the notion that the action of these mixed D2/D3 antagonists reflects D3 receptor blockade, similar deficits in EDS-ASST and reversal learning were seen in mice genetically deprived of D3 receptors: conversely, a facilitation was seen in the reversal learning component of an EDS-ASST task in mice lacking D3 receptors (Glickstein et al. 2005; Kruzich et al. 2006). Moreover, preferential D3 receptor agonists compromise performance in reversal learning tasks in marmosets and rats: although the mildly preferential D3 antagonist, nafadotride, did not block the actions of quinpirole, more selective D3 antagonists would be needed to confirm the role of D3 receptors (Boulougouris et al. 2009; Joyce & Millan, 2005; Smith et al. 1999). In any event, D3 knockout mice performed a reversal learning phase of an EDS-ASST task better than wild-type counterparts, and c-fos expression was higher in the PFC (Glickstein et al. 2005). Finally, the intensity of engrailed-2 gene expression in PFC correlated with performance of ASST in mice, and was reduced in D2 receptor KO mice that showed deficits in this task (De Steno & Schmauss, 2009).

Thus, in harmony with the present studies of S33138, the above observations support a role for D3 receptor blockade in favouring cognitive flexibility, whereas D2 receptor antagonism is unfavourable. A role for the PFC appears likely, but its operation is dependent upon circuits interlinking it with the thalamus and the basal ganglia (Block et al. 2007; Cools et al. 2009; Dodd et al. 2008; Packard & Knowlton, 2002). The dorsal (associative) striatum is of special interest since a marked depletion of DA levels is seen following CLD-MPTP treatment, whereas DA loss is less extensive in the putamen – explaining preservation of motor performance (Schneider, 1990). Striatal DA depletion impaired reversal learning in rats (Cools et al. 2009; Crofts et al. 2001; O’Neill & Brown, 2007). However, a more likely locus of action for S33138 is the receptor-rich globus pallidus linked to associative basal ganglia circuits (Cools, 2008; Packard & Knowlton, 2002). Lesioning the globus pallidus impairs ASST (Demakis et al. 2003), and alterations in pallidal D3 receptor expression have been related to cognitive deficits in Parkinson’s disease (Boileau et al. 2009). Since dopaminergic mechanisms in the nucleus accumbens regulate both reversal learning and ASST (Block et al. 2007; Dodd et al. 2008; Floresco et al. 2006a), this is also a possible locus of action of S33138.
As regards other mechanisms, CLD-MPTP is associated with a decrease of noradrenaline (NA) levels in PFC (Schneider, 1990) and increases in NA levels improve ASST performance (Lapiz & Morilak, 2006; Seu et al. 2009) while lesions of frontocortical NA input impair it (McGaughy et al. 2008). Nonetheless, S33138 does not affect dialysis levels of NA in frontal cortex so this mechanism is unlikely to be involved. Finally, cholinergic mechanisms in the caudate underpin ASST (Chen et al. 2004; Ragozzino, 2003), but S33138 does not affect ACh levels in this structure (Millan et al. 2008a).

**DMTS procedure**

S33138 improved task accuracies in the DMTS model, mainly during long-delay trials. Although attention and working memory reciprocally interact (Awh et al. 2006), this observation suggests an effect mainly on working-memory retention and/or retrieval in this procedure (Buccasfusco, 2008). This observation contrasts to the lack of influence of S33138 at long delays in CLD-MPTP-treated monkeys performing a VDR task. The difference may reflect contrasting age- vs. neurotoxin-induced impairments, and different neurochemical underpinnings of deficits. In fact, by analogy to D₃ receptor deletion, working-memory performance was decreased in mice genetically lacking D₃ receptors, but the procedure used was dependent upon optimal operation of D₁ receptors (Glickstein et al. 2002) and a subsequent report found no difference (Chourbaji et al. 2008). Moreover, D₃ antagonists countered pharmacologically induced working-memory deficits in a radial maze in rats (Laszy et al. 2005). In view of the importance of cholinergic mechanisms in working memory (Briand et al. 2007; Chudasama & Robbins, 2006) and the DMTS paradigm (Buccasfusco, 2008), the enhancement by S33138 of cholinergic input to the PFC is probably implicated. However, despite the role of PFC D₁ receptors, the roles of D₂ and D₃ receptors in working memory remain to be established (Floresco & Magyar, 2006; Seamans & Yang, 2004; Williams & Castner, 2006). Dopaminergic mechanisms in the caudate are also implicated in DMTS performance (Cools et al. 2009; Kesner & Gilbert, 2006; Landau et al. 2009), but a more attractive possibility is the D₃ receptor-rich globus pallidus, a component of cortico-basal ganglia circuits controlling working memory (Mcnab & Klingberg, 2008). Moreover, the cerebellum also has a high level of D₃ receptors (lobules 8–10) and similarly participates in circuits controlling working memory (Strick et al. 2009). Changes in the cerebral density of D₃ receptors have been reported in old rats (Valerio et al. 1994), so further studies with S33138 in aged subjects would be of interest.

Although previous work suggested a sexual dimorphism in the DNMS response to the acetylcholinesterase inhibitor, donepezil (Buccasfusco et al. 2003), S33138 yielded similar results in male and female primates. Indeed, there is no a priori reason why blockade of D₃ receptors should differentially influence cognition in males vs. females. Nonetheless, gender differences in cognition have been noted in humans and they are of potential relevance to psychiatric disorders and their control (Koch et al. 2007; Guillem et al. 2009). Further, the preponderance of male-only experimental studies was recently evoked by Hughes (2007). Hence, attention to gender in future studies of D₃ antagonists and other classes of agent would be of interest.

**Concluding comments**

As summarized in Table 4, S33138 improves measures of working memory, declarative memory, cognitive flexibility and attention in a broad palette of rat and primate models incorporating pharmacological (scopolamine), disease-related (CLD-MPTP) and natural (delay and aged-related) cognitive deficits. It is likely that the PFC and enhanced frontocortical cholinergic neurotransmission participate in certain effects of S33138, but not all. Thus, its precise mechanisms of action await further characterization, and a role for D₃ receptor-rich structures like the globus pallidus, accumens – and even cerebellum – justifies exploration. The actions of S31338 are expressed over a consistent and low range of doses corresponding to those selectively occupying D₁ receptors (Table 4; Millan et al. 2008a, c). Moreover, selective D₃ antagonists reproduce its pro-cognitive actions in rodents, whereas preferential D₂ antagonists are inactive or provoke cognitive impairment. Clearly, it would be instructive to extend such comparative work to primates. In any event, collectively with other pharmacological and gene knockout studies, it may be concluded that: (1), the roles of D₁ receptors in cognitive processing differ to those of their D₂ counterparts and (2), D₃ receptor blockade is a promising strategy for improving cognitive performance in schizophrenia, Parkinson’s disease and other CNS disorders.

**Acknowledgements and dedication**

We thank Sylvie Girardon for excellent technical assistance, and Marianne Soubeyran for excellent secretarial help. Sadly, during the revision of this paper,
one of our co-authors, Jerry Buccafusco, passed away. We take this opportunity to express our appreciation of his generosity, reactivity, openness, expertise and scientific rigour, as revealed not only in the preparation of this article and the performance of the underlying studies, but also in our other work and collaborations with him. He will be sadly missed.

Statement of Interest

Mark J. Millan, Florence Loiseau, Nitza Thomasson-Perret and Elisabeth Mocaer declare that, except for income received from their primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest. The contribution to this work by Motac Cognition, Inc. and received no compensation from Servier. The contribution to this work by David J. G. Watson, Kevin C. F. Fone, and Jerry J. Buccafusco was financially supported by Servier.

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