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Regular research article

Selective blockade of dopamine D₃ receptors enhance while D₂ receptor antagonism impairs social novelty discrimination and novel object recognition in rats: a key role for the prefrontal cortex.

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Abbreviations: ANOVA (analysis of variance); NOR (novel object recognition); PCP phencyclidine; PFC (prefrontal cortex); SND (social novelty discrimination).

Abstract

Dopamine D₃ receptor antagonists exert pro-cognitive effects in both rodents and primates. Accordingly, this study compared the roles of dopamine D₃ versus D₂ receptors in social novelty discrimination (SND), which relies on olfactory cues, and novel object recognition (NOR), a visual recognition task. The dopamine D₃ receptor antagonist, S33084 (0.04-0.63 mg/kg), caused a dose-related reversal of delay-dependent impairment in both SND and NOR procedures in adult rats. Furthermore, mice genetically deficient in dopamine D₃ receptors displayed enhanced discrimination in the SND task compared to wild-type controls. In contrast, acute treatment with the preferential dopamine D₂ receptor antagonist, L741,626 (0.16-5.0 mg/kg), or with the dopamine D₃ agonist, PD128,907 (0.63-40 µg/kg), caused a dose-related impairment in performance in rats in both tasks after a short inter-trial delay. Bilateral microinjection of S33084 (2.5 µg/side) into the prefrontal cortex (PFC) of rats increased SND and caused a dose-related (0.63-2.5 µg/side) improvement in NOR, while intra-striatal injection (2.5 µg/side) had no effect on either. In contrast, bilateral microinjection of L741,626 into the PFC (but not striatum) caused a dose-related (0.63-2.5 µg/side) impairment of NOR. These observations suggest that blockade of dopamine D₃ receptors enhances both SND and NOR, whereas D₃ receptor activation or antagonism of dopamine D₂ receptors impairs cognition in these paradigms. Furthermore, these actions are mediated, at least partly, by the PFC. These data have important implications for exploitation of dopaminergic mechanisms in the treatment of schizophrenia and other CNS disorders, and support the potential therapeutic utility of dopamine D₃ receptor antagonism.

Introduction

The dopaminergic system plays a key role in a diverse array of physiological processes including neuroendocrine function, motor behavior, emotion and cognitive function. Similarly, dysfunction of dopaminergic pathways and the closely related dopamine D₂ and D₃ receptors are implicated in the pathophysiology of Parkinson's disease, ADHD and schizophrenia, as well as substance abuse (Heidbreder and Newman, 2010; Joyce, 2001). A high density of dopamine D₂ receptors occurs in rodent and primate caudate putamen and nucleus accumbens, but they are also found in many other brain regions at lower levels (Heidbreder and Newman, 2010; Joyce, 2001; Sokoloff *et al*, 2006). Dopamine D₃ receptors are less abundant than their D₂ counterparts in the brain and are principally located in mesolimbic regions like the ventral striatum, islands of Calleja, nucleus accumbens, globus pallidus and thalamus, but they are also found in the frontal and other cortical regions as well as the cerebellum (Herroelen *et al*, 1994; Meador-Woodruff *et al*, 1996; Sokoloff *et al*, 2006; Sokoloff *et al*, 1990). PET studies in mice and baboons, using the preferential D₃ ligand, [¹¹C](+)-PHNO, in the presence and absence of the D₃ receptor antagonist, SB277,011, confirm the D₃ receptor is more highly expressed than the D₂ in the ventral pallidum, substantia nigra, thalamus and habenula, while binding in the dorsal striatum is almost exclusively that of the D₂ receptor (Rabiner *et al*, 2009).

Selective antagonists exist for characterising the roles of dopamine D₃ and D₂ receptor subtypes (Boeckler and Gmeiner, 2006; Ehrlich *et al*, 2009; Joyce and Millan, 2005). The dopamine D₃ receptor antagonists, SB277,011 and S33084, have high selectivity for the dopamine D₃ over the D₂ receptor (over 100 fold in ligand binding studies, Millan *et al*, 2000a; Millan *et al*, 2000b). Furthermore, L741,626 is currently the most preferential D₂ receptor antagonist available, having between fifteen and forty fold selectivity for the rat dopamine D₃ relative to the D₂ receptor in radioligand binding (Cussac *et al*, 2000; Kulagowski *et al*, 1996; Millan *et al*, 2004; Reavill *et al*, 2000) and functional assays (Grundt *et al*, 2007) with both native and cloned receptors. No data is currently available in the mouse to confirm a similar selectivity. In the current behavioural studies doses were carefully selected to enable predominant D₂ receptor blockade to be achieved based on previous *in vivo* work (Millan *et al*, 2004).

Together with dopamine D₃ receptor agonists, such as PD128,907 (Pugsley *et al*, 1995), these are useful pharmacological tools to specifically characterise the location and function of these receptors (Boeckler and Gmeiner, 2006).

We previously demonstrated that the antagonist, S33138, which has approximately 25-fold higher affinity for the D₃ than the D₂ receptor (Millan *et al*, 2008), has pro-cognitive effects in two rat models of cognition, social novelty discrimination (SND) and novel object recognition (NOR) (Millan *et al*, 2010). Interestingly, by using an extended dose range of S33138 the NOR data showed an inverted-U shape consistent with increasing occupancy of D₂ receptor opposing the effect of D₃ receptor antagonism on cognitive performance. Additionally, both S33138 and S33084 reversed the impairment in NOR produced by social isolation rearing of rat pups (a neurodevelopmental model of schizophrenia) while L741,626 was ineffective (Watson *et al*, 2011). These pro-cognitive effects of D₃ receptor blockade are consistent with other reports that SB277,011 prevents scopolamine-induced impairment of spatial learning and memory in the Morris water maze (Laszy *et al*, 2005). Furthermore, in a rat social recognition paradigm, both S33084 and SB277,011 enhanced performance accompanied by elevation of frontal cortex acetylcholine overflow measured by microdialysis. In contrast, L741,626 had amnesic properties and no effect on cortical acetylcholine release (Millan *et al*, 2007). Furthermore microinjection of S33084 or SB277,011 into the prefrontal cortex (PFC), but not the nucleus accumbens or striatum, improved social recognition while L741,626 was without effect (Loiseau and Millan, 2009). Finally, mice genetically deficient in the D₃ receptor show increased cognitive flexibility in the attentional set-shifting task and improved retention in a passive avoidance test (Glickstein *et al*, 2005; Micale *et al*, 2010).

The current study further characterises the opposing roles of dopamine D₃ and D₂ receptors using the selective D₃ receptor antagonist, S33084, the preferential D₂ receptor antagonist, L741,626, and the selective D₃ agonist, PD128,907 in two rodent models of cognition (SND and NOR). This is the first systematic evaluation of the comparative role of dopamine D₂ and D₃ receptors in these behavioral tasks performed by altering the delay between trials such that rats are able (with short) or unable (with long delay) to discriminate; allowing characterisation of drugs exerting both positive and detrimental effects on cognitive performance. Furthermore, we previously showed that social recognition is modulated, by dopamine D₃ receptors in the PFC (Loiseau and Millan, 2009) probably reflecting its important role in the top-down control of cognitive processing, which is disrupted in schizophrenia (Baluch and Itti, 2011; Klinkenberg *et al*, 2011; Noudoost *et al*, 2010; Rossi *et al*, 2009; Sarter *et al*, 2009) and its well-establish role on working memory. Finally

amphetamine-induced improvement in NOR in an *Fmr1* knockout mouse model of Fragile X syndrome correlated to the increase in PFC but not striatal dopamine release measured by microdialysis (Ventura *et al*, 2004). Hence, the role of the PFC in SND and NOR was investigated by discrete injection of selective compounds compared to the effect of control injections into the striatum, a region known to be involved in procedural memory but not object discrimination or social recognition. Finally, performance of mice deficient in dopamine D₃ receptors (*DRD3*^{-/-}) was examined in the SND task to further characterise the role of this receptor.

Methods

Social Novelty Discrimination

The SND procedure was adapted from Terranova et al. (2005) and previously used in this laboratory (Millan *et al*, 2010). Group-housed adult (240–260 g) and juvenile (21 day old) male Wistar rats purchased from Janvier (France) were isolated two days before testing while juveniles were group-housed throughout. Half of the juveniles were marked on tail, coat and head with non-odorous black dye. On the test day, a juvenile (unmarked ‘white’ or marked ‘black’, chosen in a pseudo-random manner) was placed into the adult home cage for 5 min (P1) and time spent by the adult in social investigation of the juvenile recorded. There was either no-delay or a thirty minute delay between the end of P1 and the juvenile being reintroduced for a second 5 min period (P2) together with another novel juvenile. During P2, the time spent investigating each juvenile (P2 novel and familiar) was recorded in seconds. The SND ratio (investigation of the novel juvenile/investigation of the familiar juvenile) was calculated for P2 measurements. Investigation times during P2 were 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 was analysed using Mann-Whitney or Kruskal-Wallis followed by Dunn’s post-hoc tests. Total investigation times during P1 and P2 were also analysed by one-way ANOVA followed by Dunnett’s *t* or Fishers LSD post hoc tests. As several groups have shown the most pronounced interaction between adult and juvenile rats occurs within the first few minutes of P2 in the SND paradigm (Engelmann *et al*, 1995), supplementary information (Figure S1) of the time course of the P2 interaction in an additional group of drug free rats recorded 30 min after P1 shows that the SND ratio is suppressed in the first 2 to 3 min to the same extent as over 5 min. Therefore the

use of a 5 min P2 observation period does not mask an initial juvenile preference, validating the use of this protocol for all studies presented herein.

Mutant Mice

Mice genetically deprived of dopamine D₃ receptors (D₃ knockout mice; D₃-KO) were obtained from the Jackson Laboratory (B6.129S4-Drd3^{Tm1Dac}/J) (Accili *et al.*, 1996). Homozygous mice were generated on a pure genetic background and were compared to C57BL/6J wildtype (WT) mice. Although several distinct lines of D₂ receptor knockout mice are available they frequently develop hypertension and pituitary tumors (the latter resulting in elevated circulating glucocorticoid levels) and often show impaired motor function and gait (Holmes *et al.*, 2004), features dependent on the background strain (Waddington *et al.*, 2005). Although D₂ knockout mice show impaired autoreceptor function, neither the synaptic level nor tissue dopamine content appear to alter, suggesting that developmental compensations including elevation in striatal dopamine transporter levels may compromise interpretation of which phenotypic alterations are specifically due to change in D₂ receptor expression in these mice (Holmes *et al.*, 2004; Waddington *et al.*, 2005). Therefore the current study used dopamine D₂ receptor selective ligands instead of D₂^{-/-} mice. Several groups have used D₂^{-/-} mice for cognitive studies, and these show impaired reversal learning in an attentional set-shifting task (DeSteno and Schmauss, 2009), opposite to D₃^{-/-} which have improved reversal learning in a two choice perceptual discrimination task (Glickstein *et al.*, 2005). Thus future studies using D₂ mutant mice (either knockout or over expression ((Li *et al.*, 2011)) would be valuable to confirm the current pharmacological evaluation.

Transgenic mice were isolated the day before the SND test which was performed as described above.

Surgery

The surgical procedure for implanting bilateral stainless steel guide cannulae above the PFC or striatum was adapted from Chudasama and Robbins (2004) and was exactly as employed in our previous studies (Loiseau and Millan, 2009). As a control, equivalent microinjection was performed into the striatum because of the known very high level of expression of the dopamine D₃ receptor in the striatum (Bouthenet *et al.*, 1991; Diaz *et al.*, 2000) and evidence that this area is not involved in the regulation of either SND or NOR.

Adult rats were anaesthetised by an intraperitoneal (i.p.) injection of chloral hydrate (400 mg/kg, as 10 ml/kg) and placed in a stereotaxic frame (David Kopf Instruments, Phymep,

Paris). Guide cannulae, consisting of two 22-gauge metal tubes (inner diameter: 0.39 mm) 1.5 mm apart (centre to centre), projecting 3.5 mm from a plastic pedestal for the PFC and 5.0 mm apart and projecting 5.0mm for the striatum (Plastics One, USA) were mounted on a stereotaxic frame and lowered at the following coordinates from Bregma: PFC; AP:+3.0, L: \pm 0.7, DV: -2.3, striatum; AP:+0.5, L: \pm 2.5, DV: -4.0 (Paxinos and Watson, 1997). The cannulae were fixed with dental cement to adjacent stainless steel screw anchors. Stylets (Plastics One, USA) placed in the guide cannulae prevented occlusion prior to drug injection. Animals were housed individually and allowed to recover for at least one week before testing.

Microinfusion

After post-operative recovery, rats were handled to minimize any stress associated with the drug infusion procedure. On the test day, rats were gently restrained while stylets were removed and replaced with a sterile 28-gauge (inner diameter: 0.18 mm, outer diameter: 0.36 mm) stainless steel injector extending 1.0mm beyond the tip of the guide cannulae (Plastics One, USA). Injectors were connected (Plastics One, USA) to 10 μ l precision syringes (Hamilton, Phymep, Paris) mounted in an infusion pump (Harvard Apparatus, Holliston, MA) and 1 μ l drug or vehicle infused bilaterally over 2 min, 5 min before the P1 or familiarisation trial. The injectors were left in place for 2 min before being removed.

Histology

After behavioral testing, brains were removed and frozen for histological verification of the injection position from coronal brain sections according to the atlas of Paxinos and Watson (1997). Figure 1 illustrates the location of injections sites in the striatum and PFC.

Novel Object Recognition

Male Lister Hooded rats (Charles River, UK) housed in groups of four were used for novel object recognition (NOR) using a two trial object discrimination task adapted from Ennaceur and Delacour (1988) routinely in use in our laboratory (Bianchi *et al*, 2006; Bianchi *et al*, 2009; King *et al*, 2009; Lapiz *et al*, 2000). Rats (initially weighing approximately 200 g) were habituated to the test arena (a 39.0 x 23.5 x 24.5 cm perspex cage) for 1 h, the day before each NOR test. On the test day, animals were re-acclimatised to the arena for 3min,

returned to their home cage while two identical objects (8 cm high, 5 cm diameter water-filled, plastic bottles covered with white masking tape) were secured by Blu-Tack® to the floor in opposite corners of the arena (5 cm from the side and 10 cm from the back). The rat was allowed to explore both objects for 3 min during the first familiarisation trial and the time (s) spent exploring each object recorded with stop watches. Animals were returned to their home cage for an variable inter-trial interval before reintroduction into the cage with a copy of an original (familiar) object and one novel object (identical white masking taped plastic bottle covered with four prominent 1.2 cm black horizontal stripes) for a second 3 min trial. During the second choice trial, exploration of each object was recorded again separately. The location of the novel object was varied in a pseudorandom order within groups. Exploratory behavior was defined as sniffing, touching and direct attention to the object, with active vibrissae whilst the nose was within 1 cm of the object. Climbing on or chewing the object were not considered exploratory behaviors and not recorded. Each study was performed in a separate cohort of rats (n=12 per group) with each rat receiving every dose or combination of drugs in a pseudorandom order. This was done to minimise the influence of inter-individual variability and reduce the number of animals required. All behavioral studies were conducted by a single experienced examiner who was unaware of the treatment given. All behavioral apparatus was cleaned using 20 % ethanol between trials and objects within trials to remove odour cues. Data was analysed by two-way ANOVA with exploration of the novel and familiar objects the repeated within-subject factors and treatment the between-subject factor, with Bonferroni post-hoc tests to determine significant differences in exploration between novel and familiar objects. As variation in exploration of individual objects between rats can confound interpretation, the choice trial raw data was converted to discrimination ratio ($d_2 \text{ score} = (\text{novel object} - \text{familiar object}) / (\text{novel object} + \text{familiar object})$) values which were analysed by two-way ANOVA followed by appropriate Dunnett's *t* or LSD post-hoc tests to determine changes in object exploration pattern independent of actual object exploration times. All values are presented as mean \pm s.e.m. and $P < 0.05$ was considered significant. Surgical and microinfusion techniques were as described for the SND procedure except that anaesthesia was induced with 3.5 % isoflurane and maintained with 2 % in 33 % O₂ and 66 % N₂O.

SND procedures conform to European (86/609-EEC) and French (87/848) decrees for the care and use of laboratory animals and NOR studies were conducted after approval by the

University of Nottingham local ethical review committee and in accordance with the Home Office Animals (Scientific Procedures) 1986 ACT.

Drugs

S33084 (0.01-0.63 mg/kg) and L-741,626 (0.063-5 mg/kg) were dissolved in saline with lactic acid before adjusting to neutral pH. PD128,907 (0.63-40.0 µg/kg) was dissolved in saline. All drugs were injected (1ml/kg, s.c.) 30 min prior to each test (P1 in SND and familiarisation trial in NOR). In experiments where two drugs were administered; L741,626/ vehicle was injected 45 min prior and S33084/ vehicle 30 min prior to the first trial, S33084/ vehicle 45 min prior followed by PD128,907/ vehicle 30 mins prior to the first trial. All drug doses are expressed in terms of the base. For the microinfusion studies S33084 was dissolved in artificial cerebrospinal fluid (aCSF) containing 10 % hydroxypropyl-β-cyclodextrine (Sigma, Chesnes, France) and L741,626 in aCSF containing 5 % dimethyl sulfoxide (Riedel-de Haën, Germany) and 5% cremophor EL (Sigma, Chesnes, France). All drugs were provided by Institut de Recherches Servier.

Results

Effect of dopamine D₃ and D₂ antagonists on delay dependent-impairment of social novelty discrimination.

As expected by using a 30 min delay between P1 and P2 in the SND task, vehicle-treated rats spent an equal time investigating the novel and familiar juvenile during P2, consistent with an inability to perform social discrimination with this inter-trial delay (Fig 2a). Following S33084 injection two-way repeated measures ANOVA on the P2 data showed a significant juvenile x treatment interaction ($F_{(3,19)}=5.15$, $P<0.01$). The preferential dopamine D₃ antagonist, S33084 caused a progressive increase in social discrimination ($H=9.778$, 3, $N=23$, $P<0.05$) which reached significance from vehicle (Fig 2b, $P<0.05$) with the highest dose (0.63 mg/kg). Moreover, since S33084 did not significantly alter the total investigation of the juveniles during either P1 or P2 (Table 1) this increase in SND was clearly due to a redistribution of investigative behavior to the novel rat rather than a non-specific change in exploratory behavior or sedation.

Treatment with the preferential dopamine D₂ receptor antagonist, L741,626, did not significantly affect social discrimination after a 30 min delay (Fig 2d) in SND. At all doses rats spent an equal time investigating the novel and familiar juvenile (Fig 2c), such that ANOVA showed there was no significant juvenile x treatment interaction. However it should be noted that total investigative activity during both P1 and P2 was reduced in a dose-related manner by L741,626 (Table 1, P1; $F_{(3,20)}=6.4$ $P<0.01$, P2; $F_{(3,20)}=15.15$, $P<0.001$).

The reversal of the social discrimination impairment produced by S33084 (0.63 mg/kg) using a 30 min inter-trial delay was blocked by pre-treatment with L741,626 (2.5 mg/kg) (Fig 2f, S33084 treatment; $F_{(1,31)}=6.37$, $P<0.05$, L741,626 treatment; $F_{(1,31)}=5.82$, $P<0.05$, S33084 x L741,626 interaction $F_{(1,31)}=4.65$, $P<0.05$). In this group there was significant juvenile x S33084 interaction ($F_{(1,31)}=6.94$, $P<0.05$) and a significant juvenile x L741,626 interaction ($F_{(1,31)}=6.94$, $P<0.05$). Thus groups treated with vehicle/vehicle and L741,626/vehicle spent an equal time investigating both juveniles while the vehicle/S33084 treated group spent significantly longer investigating the novel than the familiar juvenile (Fig 2e). Interestingly when given in combination (L741,626/S33084) the preferential exploration of the novel juvenile was prevented. Neither drug at the doses used had any significant effect on total investigation activity during P1 (Table 1). However, during P2, while S33084 treatment had no effect, L741,626 did slightly reduce total investigatory activity which reached significance, as shown in Table 1 (S33084; not significant, L741,626; $F_{(1,31)}=6.51$, $P<0.05$; S33084 x L741,626 not significant).

Effect of dopamine D₂ and D₃ antagonists and a dopamine D₃ agonist on social novelty discrimination with no inter-trial interval.

With no delay between P1 and P2 in the SND task vehicle treated rats naturally spend more time exploring the novel than the familiar juvenile, as seen in Fig 3a. The preferential dopamine D₃ receptor antagonist, S33084 had no significant affect on this distribution of investigation between the juveniles (object x S33084 treatment; not significant), such that with all doses rats preferentially explored the novel over the familiar juvenile (Fig 3b). Furthermore, the total social investigation during P1 and P2 was unaffected by S33084 (Table 1).

The preferential dopamine D₂ receptor antagonist, L741,626, caused a progressive impairment in the ability of rats to discriminate the novel juvenile in this task (Fig 3d, $H=16.11$, 3, N=29, $P<0.01$) which reached significance with the highest dose (2.5 mg/kg).

Two-way ANOVA performed on the P2 data showed a significant juvenile x L741,626 interaction ($F_{(3,25)}=7.03$, $P<0.01$). And post-hoc analysis showed that after the highest dose (2.5 mg/kg) of L741,626 rats spent an equal time investigating each juvenile (Fig 3c). Although L741,626 had a significant effect on total social investigatory behavior during P1 ($F_{(3,25)}=4.30$, $P<0.05$) this was not dose-dependent and no significant effect was seen during P2 (Table 1).

After administration of the dopamine D₃ receptor agonist, PD128,907, two-way repeated measures ANOVA performed on the investigation of the novel and familiar juvenile during P2 showed a significant juvenile x PD128,907 treatment interaction ($F_{(4,29)}=7.55$, $P<0.001$). Post-hoc analysis showed that there was no significant difference between the amount of investigation of the novel or familiar juvenile with the two highest doses tested (10 and 40 µg/kg, Fig 3e). The ratio data (Fig 3f) confirmed a significant treatment effect ($H=14.95$, 4, $N=34$, $P<0.01$), however only the 10.0 µg/kg dose significantly reduced the discrimination ratio from that in vehicle treated rats ($P<0.01$). PD128,907 had no significant effect on total social investigatory activity in either trial.

Effect of bilateral microinjection of dopamine D₂ and D₃ antagonists into the prefrontal cortex or striatum on social novelty discrimination.

Bilateral microinjection of the preferential dopamine D₃ receptor antagonist, S33084 (2.5µg/side) into the PFC had no significant effect on total investigatory activity in either P1 or P2 (Table 1). After microinjection into the PFC S33084 caused a significant juvenile x treatment interaction ($F_{(1,9)}=30.24$, $P<0.001$), such that it significantly improved SND after a 30 min delay (Fig 4a and b, $U=0.00$, $P<0.01$). In contrast bilateral injection of S33084 (2.5 µg/side) into the striatum had no significant effect on social novelty discrimination such that rats showed equal investigation of both juveniles (Fig 4c) and the SND ratio remained at chance level for both vehicle and S33084 groups (Fig 4d). As with microinjections into the PFC, injection into the striatum had no significant effect on total time spent investigating juveniles during P1 or P2 (Table 1).

Performance of mutant DRD3^{-/-} mice in the social novelty discrimination task and the effect of a dopamine D₃ receptor antagonist.

After a 30 min delay, mice deficient in dopamine D₃ receptors spent significantly more time investigating the novel than the familiar juvenile (Fig 5a, juvenile x genotype; $F_{(1,10)}=25.13$,

$P < 0.001$). Correspondingly the SND ratio was significantly higher in the DRD3^{-/-} than the wild type mice (Fig 5b, $U = 0.50$, $P < 0.01$). Furthermore, the DRD3^{-/-} mice showed no significant change in total juvenile investigatory activity during P1 or P2 showing the specificity of the behavioral change observed.

After systemic injection with S33084 (0.63 mg/kg s.c.) wild type mice spent significantly more time investigating the novel than the familiar juvenile (Fig 5c). DRD3^{-/-} were able to discriminate the novel juvenile and S33084 treatment had no additional effect on this behavior. There was no significant main effect of genotype or drug injection on total investigation behavior during either P1 or P2 (Table 1), although there was a genotype x drug interaction in P2 ($F_{(1,10)} = 4.89$, $P < 0.05$) and wild type mice treated with S33084 had reduced total P2 compared to vehicle controls, they still preferentially explored the novel juvenile.

Effect of dopamine D₃ and D₂ antagonists on delay dependent-impairment of novel object recognition.

The current study used NOR to distinguish the effects of dopamine D₃ and D₂ receptor antagonists on recognition memory impaired by using a 4h inter-trial interval, a model of natural forgetting.

In dose-response studies with both the preferential dopamine D₃ receptor antagonist, S33084, and the preferential dopamine D₂ antagonist, L741,626, all treatment groups spent an equal amount of time exploring the two identical objects during the familiarisation trial (T1, data not shown), confirming that rats had no innate spatial preference for either object location. Total object exploration during the familiarisation trial was also unaffected by treatment with either drug (see Table 2). As expected in both studies rats treated with vehicle were unable to discriminate the novel from the familiar object in the choice trial following a 4h ITI, spending an equivalent amount of time exploring each object (Fig 6a and c). Following treatment with S33084 repeated-measures ANOVA revealed a significant main effect of object ($F_{(1,44)} = 26.19$, $P < 0.001$) and a significant object x treatment interaction ($F_{(3,44)} = 4.04$, $P < 0.05$) in the pattern of object exploration during the choice trial (Fig 6a). S33084 caused a redistribution of object exploration; producing a dose-related and significant increase in exploration of the novel over the familiar object without altering total exploration in the choice trial. In confirmation of this increase in NOR there was a concomitant increase in the d2 score (Fig 6b, $F_{(3,44)} = 15.36$, $P < 0.001$), consistent with it being due to redistribution of exploration time without any change in total object exploration during the choice trial (Table

2). In contrast, after treatment with L741,626, there was no significant main effect of object or treatment, nor any object x treatment interaction (Fig 6c). Irrespective of the dose of L741,626 used rats were unable to discriminate the novel from the familiar object such that the discrimination ratio (d2) for all groups was unaltered and remained close to chance (Fig 6d).

After the combined treatment of L741,626 (0.63 mg/kg) and S33084 (0.16 mg/kg) there was no significant effect of either drug (alone or in combination) on the total exploration of the objects during the familiarisation trial (Table 2). One rat was excluded from the data due to not performing the task (spending <1 s attending the objects). Statistical analysis of object exploration during the choice trial revealed significant main effects of object ($F_{(1,40)}=25.81$, $P<0.001$), an object x S33084 treatment interaction ($F_{(1,40)}=22.03$, $P<0.001$), object x L741,626 treatment interaction ($F_{(1,40)}=6.09$, $P<0.05$) and object x S33084 treatment x L741,626 treatment interaction ($F_{(1,40)}=8.15$, $P<0.01$) (Fig 6e). Analysis of the d2 score (Fig 6f) revealed a significant main effect of S33084 ($F_{(1,40)}=25.34$, $P<0.001$), but not L741,626 and a significant S33084 x L741,626 interaction ($F_{(1,40)}=5.77$, $P<0.05$) with both vehicle/vehicle and L741,626/vehicle treated groups being unable to discriminate the novel from the familiar object while, as with the previous experiment, S33084 restored discrimination. Although rats showed a significant degree of discrimination of the novel over familiar object after combined treatment of S33084 and L741,626 using the d2 score ($P<0.01$ compared to vehicle/ vehicle) this was significantly lower than that of the S33084/vehicle group ($P<0.01$). Although there was a significant effect of L741,626 ($F_{(1,40)}=6.79$, $P<0.05$) on total object exploration during the choice trial there was no effect of S33084 nor any S33084 x L741,626 interaction and no group had significantly different total exploration times from any other (Table 2).

Effect of dopamine D₂ antagonist and dopamine D₃ agonist on novel object recognition with a short inter-trial interval.

The effect of the preferential dopamine D₂ antagonist, L741,626, and the dopamine D₃ agonist, PD128,907, was examined on NOR using a short (2min inter-trial interval) delay, so that control rats could readily discriminate the novel from the familiar object, and any amnesic properties of the compounds could be evaluated.

Neither L741,626 nor PD128,907 had any significant effect on the total exploration of both objects during the familiarisation trial (Table 2) or on the distribution of exploration across

both objects which were explored equally (data not shown). With both compounds vehicle treated rats were able to discriminate the novel from the familiar object (Fig 7a-d). Furthermore, in both cases there was a significant main effect of object (L741,626; $F_{(1,44)}=16.53$, $P<0.001$; PD128,907; $F_{(1,44)}=24.31$, $P<0.001$), and a significant object x treatment interaction (L741,626; $F_{(3,44)}=30.31$, $P<0.001$; PD128,907; $F_{(3,44)}=6.87$, $P<0.01$) during the second choice trial. Such that both L741,626 and PD128,907 caused a dose-related impairment in performance (d2 score: L741,626; $F_{(3,44)}=6.95$, $P<0.01$, PD128,907; $F_{(3,44)}=6.10$, $P<0.01$). The inability to distinguish the novel from the familiar object resulted from a redistribution of exploration time between the objects rather than a global reduction in exploration since total object exploration during the choice trial was not significantly affected by either drug (Table 2).

Treatment with the dopamine D₃ receptor antagonist, S33084 (0.16 mg/kg), produced no significant enhancement in discrimination, which was not surprising since after a short inter-trial interval rats were already able to discriminate the novel object (Fig 7e and f). As previously seen, rats treated with PD128,907 (2.5 µg/kg) alone (after vehicle) were unable to discriminate between the objects in the choice trial. In contrast, rats treated with S33084 prior to PD128,907 retained the ability to discriminate the objects, as seen in Fig 7e where exploration of the novel is significantly higher than the familiar object except in the vehicle/PD128,907 treatment combination ($P<0.001$ repeated-measure ANOVA; object; $F_{(1,44)}=89.41$, $P<0.001$; object x S33084 treatment interaction; $F_{(1,44)}=8.23$, $P<0.01$, object x PD128,907 treatment interaction; $F_{(1,44)}=3.26$, $P=0.08$, object x S33084 x PD128,907 treatment interaction; not significant). In agreement with the raw exploration data the d2 score for the vehicle/ PD128,907 group was significantly lower than all other drug combinations in that group (Fig 7f, $P<0.01$ compared to all others, two-way ANOVA; S33084 treatment; $F_{(1,44)}=7.59$, $P<0.01$, PD128,907 treatment; not significant, S33084 x PD128,907 treatment interaction; $F_{(1,44)}=6.49$, $P<0.05$). Neither drug treatment had any significant effect on the distribution of exploration of the two familiar objects during the familiarisation trial, which were explored equally in all groups (data not shown). Neither drug significantly affected total object exploration during either the familiarisation or choice trials thus changes in object discrimination reflect a redistribution of exploration between the two objects (Table 2).

Effect of bilateral prefrontal cortical or striatal microinjection of dopamine D₂ and D₃ receptor antagonists on novel object recognition

Bilateral microinjection of the dopamine D₃ receptor antagonist, S33084, into the PFC had no significant effect on either the distribution of object exploration (data not shown) or total object exploration during the familiarisation trial (Table 2) although the latter appeared to be slightly higher than in other experiments. After a 4 h inter trial-interval vehicle treated rats were unable to discriminate the novel and familiar objects (Fig 8a) during the choice trial. However, both doses of S33084 (0.63 and 2.5 µg/side) significantly increased novel object discrimination ($P < 0.05$ and 0.01 respectively compared to vehicle, Fig 8b). Repeated-measures ANOVA of the choice trial data showed a significant main effect of object ($F_{(1,21)} = 27.92$, $P < 0.001$) and a significant object x treatment interaction ($F_{(2,44)} = 5.53$, $P < 0.05$). Similarly when expressed as the d2 score there was a main effect of treatment ($F_{(2,21)} = 6.52$, $P < 0.01$) confirming that PFC microinjection of S33084 reverse a delay-induced impairment in object discrimination. S33084 treatment had no main effect on total choice trial object exploration showing that the improvement in novel object exploration resulted from a shift in attention away from the familiar object (Table 2).

Bilateral PFC microinjection of the dopamine D₂ antagonist, L741,626, had no significant effect on the distribution of exploration of the two objects during the familiarisation trial (data not shown) or on the total object exploration (Table 2) during either the familiarisation or choice trials. By using a 2 min inter-trial as expected vehicle treated rats spent more time exploring the novel than the familiar object during the choice trial (Fig 8c). Drug treatment significantly altered this pattern of object exploration (object; $F_{(1,32)} = 37.52$, $P < 0.001$, object x treatment interaction; $F_{(3,32)} = 13.81$, $P < 0.001$) producing a dose-dependent (0.63 to 5.0 µg/side) reduction in novel versus familiar object exploration. L741,626 significantly reduced the d2 ratio at all doses compared to that of vehicle controls (Fig 8d, treatment; $F_{(3,32)} = 17.56$, $P < 0.001$) consistent with it impairing visual recognition memory. In contrast microinjections of S33084 and L741,626 into the striatum had no effect on NOR performance (Fig 8e and f). With a long inter-trial interval microinjections of S33084 (2.5 µg/side) into the striatum did not restore performance and the d2 score remained unaltered (Fig 8e). Both vehicle and S33084 treated animals explored the novel and familiar object to a similar extent during the choice trial (data not shown). With a two minute inter-trial interval microinjections of L741,626 (2.5 µg/side) into the striatum caused no impairment of novel object discrimination (Fig 8f). Rats given intra-striatal L741,626 preferentially explored the novel compared to the familiar object (data not shown; object; $F_{(1,17)} = 54.32$, $P < 0.001$) but this was not affected by drug (object x treatment interaction, not significant). Microinjection of S33084 or L741,626

into the striatum had no effect on total object exploration during either the familiarisation or choice trials (Table 2).

Discussion

The current study is the first systematic comparison of the contrasting role of dopamine D₃ and D₂ receptors in two rodent models of learning and memory; the SND and NOR tests using selective ligands and a D₃ knockout mouse. The results support the proposal that blockade of dopamine D₃ receptors enhances cognitive function, while activation of the dopamine D₃ receptor with agonists or blockade of dopamine D₂ receptors impairs learning and memory in these paradigms. Furthermore, this is the first study using microinjection studies to identify a potentially important brain site of the differential actions of dopamine D₂ and D₃ receptor ligands in two complimentary cognitive behavioral tasks. The data suggest that dopamine D₂ and D₃ receptors located in the PFC have opposing roles in modulation performance of both SND and NOR while the striatum has a much smaller or negligible role in these procedures.

Effects of dopamine antagonists on social novelty discrimination

The SND paradigm utilises an innate behavioral response to investigate novel conspecifics as an index of the ability of rodents to discriminate a previously investigated familiar from a novel juvenile rat or mouse (Engelmann *et al*, 1995; Ferguson *et al*, 2002). The primary salient cue for social discrimination is olfactory and the task examines short-term memory involving a strong attentional component (Ferguson *et al*, 2002). SND is disrupted by extending the inter-trial interval, other parametric modification (reducing the time available to investigate the juvenile during P1), acute injections of phencyclidine (PCP) and neonatal treatment with PCP (Engelmann *et al*, 1995; Terranova *et al*, 2005). SND is thought to be a test of social recognition, in a separate cognitive domain from the visual learning and memory involved in NOR (Young *et al*, 2009) described below. SND involves the evaluation and response to social cues, the accessory olfactory bulb and the neuropeptides oxytocin and vasopressin which enhance acquisition and consolidation respectively (Insel and Fernald, 2004); none of which are involved in NOR. Indeed oxytocin knock-out mice show specific deficits in SND without any alteration in non-social memory (Ferguson *et al*, 2000; Goodson and Thompson, 2010) confirming that SND and NOR map to different cognitive domains. In

the delay-induced deficit protocol used herein, the delay alone (30 min) is insufficient to induce natural forgetting of the juvenile presented in P1, since the adult rat can successfully discriminate the novel from the familiar juvenile if their movement is restricted in mesh cages on opposite sides of the arena in P2 (Terranova et al, 2005). Thus the free movement of the juvenile rats necessitates investment of sustained and selective attention as well as effective working memory (Engelmann et al, 1995; Terranova et al, 2005) which are both essential to enable performance of the SND task.

In the current study, selective antagonism of the dopamine D₃ receptor by S33084 caused a progressive improvement in SND with increasing doses when the inter-trial interval was sufficiently long to reduce discrimination from maximal. In contrast, the dopamine D₃ agonist, PD128,907, and the D₂ antagonist, L741,626, both impaired SND when this was maximal following a short inter-trial delay. Consistent with the proposal that dopamine D₂ and D₃ receptors have opposing modulatory effects on learning and memory (see the Introduction), the improvement in SND seen with S33084 was also reversed by prior treatment with L741,626. Microdialysis studies show that systemic administration the dopamine D₂ and D₃ compounds utilised herein alter neurotransmitter release for at least 2 h (Millan *et al*, 2000b; Millan *et al*, 2004), so active CNS concentrations will be present during both acquisition and consolidation. However, S33084 (0.63 mg/kg) produces an equivalent reversal of the delay-induced impairment when administered one minute after P1 (data not shown), suggesting that the effects of D₃ antagonists are unlikely to be the result from improved acquisition, but rather augmentation of the rat's exploitation of information acquired during P2. Although few compounds with selectivity for individual dopamine receptors have been used in this paradigm, the enhanced SND produced by S33084 is comparable to that seen with the dopamine D₃ receptor antagonist, S33138 (Millan *et al*, 2010). Further, in light of the data presented herein, it is likely that the reversal of SND disruption by the mixed dopamine D₂ receptor antagonist and 5-HT_{1A} receptor agonist, SSR181507, is due to its activation of 5-HT_{1A} receptors (Terranova *et al*, 2005). It is however noteworthy that both the atypical antipsychotics, clozapine and amisulpiride, and to a lesser extent (at one dose) the typical antipsychotic, haloperidol, reverse a time-delay induced SND deficit (Terranova *et al*, 2005) while these drugs have limited efficacy against the cognitive deficits of schizophrenia (Keefe *et al*, 2007), so any translational relevance of such findings needs to be made with caution.

Transgenic mice lacking the dopamine D₃ receptor (DRD3^{-/-}) showed a greater spontaneous discrimination of a novel juvenile than wild type controls in SND in the current study. Furthermore, while S33084 improved SND performance in wild type mice it had no effect on behavior in DRD3^{-/-} mice. Consistent with having an elevated basal social memory, DRD3^{-/-} mice show enhanced performance in a step-through passive-avoidance test (Micale *et al*, 2010) and set-shifting performance in an attentional set-shifting task accompanied by increased *c-fos* expression in the anterior cingulate, prelimbic and infralimbic cortices compared to wild type (Glickstein *et al*, 2005). In contrast dopamine D₂ receptor knock-out mice are impaired in the first compound discrimination of the attentional set-shifting task and have significantly lower *c-fos* expression compared to wild type (Glickstein *et al*, 2005). Interestingly in a delayed alternation spatial working memory task both DRD2^{-/-} and DRD3^{-/-} mutant mice were impaired compared to wild type (Glickstein *et al*, 2002). However this response was fully (DRD2^{-/-}) and partially (DRD3^{-/-}) rescued by repeated dopamine D₁ receptor agonist treatment which caused a parallel increase in *c-fos* expression in PFC neurones, consistent with PFC dopamine D₁ receptor activation restoring working memory deficits in both these mutants. Collectively this data suggests that dopamine D₂ and D₃ receptors may have distinctive roles in particular learning and memory tasks reflecting their distinct pattern of neuronal distribution.

The role of dopamine D₃ receptors in cognitive functions has recently been highlighted in a model of neurofibromatosis, a genetic developmental disorder associated with tumor predisposition and cognitive deficits. Mice carrying a heterozygous null mutation of the *Nf1* gene (*NF1*^{+/-}) associated with neurofibromatosis exhibit spatial working memory deficits (Costa *et al*, 2002). Network analysis of gene expression in these mutant mice suggests that cognitive deficits may relate to alterations in the trafficking of complexes involving neurofibromin (NF1), amyloid precursor protein (APP) and the dopamine D₃ receptor (Donarum *et al*, 2006). Furthermore both levels of APP protein and mRNA were significantly increased while neurofibromin (NF1) levels were decreased compared to wild type mice in dopamine D₃ knockout subjects (Castorina *et al*, 2011). Hence, these KO studies indicate a potentially broader relevance of dopamine D₃ receptors in cognition and CNS disorders

Effects of dopamine antagonists on recognition memory

NOR is a form of visual recognition memory dependent on spontaneous innate preference of rats to investigate novel objects removing the requirement for training, motivational food

reward or aversive stimuli (Ennaceur and Delacour, 1988). The task has translational relevance to the visual recognition memory impairments seen in schizophrenia (Young *et al*, 2009). NOR is altered by increasing the inter-trial interval (King *et al*, 2004; Sutcliffe *et al*, 2007), pretreatment with NMDA receptor antagonists such as PCP or MK-801 (Grayson *et al*, 2007), muscarinic receptor antagonists, such as scopolamine (Woolley *et al*, 2003), and neurodevelopmental interventions such as isolation rearing of pups from weaning (Bianchi *et al*, 2006; Fone and Porkess, 2008; Jones *et al*, 2011). Collectively these data suggest that NOR is regulated by both cholinergic and glutamatergic mechanisms. Consistent with the current report one previous study showed that dopamine D₂/D₃ receptor antagonist, raclopride, impairs NOR (Woolley *et al*, 2003). The present study extends these findings showing that blockade of the dopamine D₃ receptor enhances, while D₃ receptor activation or D₂ receptor blockade impairs NOR, consistent with SND studies. Although comparable pharmacological effects are seen in both NOR and SND tasks, the former appears to be more sensitive, requiring approximately four-fold lower doses to achieve significant mnemonic or amnesic activity than required in the SND task. However, as discussed below, when S33084 was administered into the PFC it showed an apparently equivalent dose effect on both behaviors suggesting that the different systemic potency reflects divergent post-dopaminergic anatomical pathways mediating the two behavioral changes. We have also characterised other drug classes, including mGluR, 5-HT₆ and NMDA ligands across the two procedures and the difference in potency is inconsistent but not a unique feature of drugs interacting with the dopamine D₃ receptor. Previously we demonstrated another, less selective, dopamine D₃ antagonist, S33138, reverses delay-dependent impairments in NOR in the rat (Millan *et al*, 2010). Furthermore S33138 and S33084 both reverse a social isolation rearing deficit in NOR (Watson *et al*, 2011), and this study shows the dopamine D₃ agonist, PD128,907 impairs object recognition in group-housed controls. In the marmoset, the dopamine D₃ receptor agonist, 7-OH-DPAT, impaired visual object discrimination performance (Smith *et al*, 1999) and passive avoidance learning in mice (Ukai *et al*, 1997), supporting the amnesic properties of D₃ receptor activation across species. Of note S33084 reverses the PD128,907-induced impairment in NOR, presumably through common receptor antagonism. While blockade of D₃ receptors enhanced NOR, antagonism of D₂ receptors with L741,626 impaired NOR after short inter-trial delays. Furthermore, L741,626 attenuated the enhancement of NOR produced by S33084. It also impaired NOR in group-housed rats using a 2 h hour inter-trial interval (Watson *et al*, 2011) and blockade of D₂ sites presumably explains why the mixed D₂/D₃ antagonists, raclopride and eticlopride, both impaired recognition memory in rats (Besheer *et*

al, 1999; Woolley *et al*, 2003). Of note the marked effects of the drugs used (or genetic alteration) on discrimination in NOR (and SND) cannot be explained by any change in total T2 (or P2, respectively) and are consistent with these being due to changes in learning and memory rather than altered motivation to perform the task. As drugs were administered before the first habituation trial in the present work, the stage(s) of learning and memory (encoding, consolidation or retrieval) affected by D₃ and D₂ receptors warrants evaluation in future studies.

CNS sites of action of dopamine D₃ and D₂ receptors ligands

To attempt to identify the CNS location of dopamine D₃ and D₂ receptors involved in the current cognitive effects, discrete microinjection studies were performed. SND was enhanced by bilateral injections of the D₃ receptor antagonist, S33084, into the PFC while injection into the striatum had no effect. These results support previous studies in the social recognition task where S33084 reversed delay-induced impairments when injected into PFC but not into the striatum or nucleus accumbens (Loiseau and Millan, 2009). In contrast, bilateral microinjection of the preferential dopamine D₂ receptor antagonist, L741,626 into the PFC did not reverse the impairment in social recognition. Although the neural pathways underlying SND have not been well defined, the behavior relies on chemosensory cues to form an ‘olfactory signature’ for the familiar juvenile, and areas known to be involved in social cognition include the piriform, entorhinal and perirhinal cortices and the amygdaloid complex (Davis, 2004; Ferguson *et al*, 2002). These areas contain low levels of dopamine D₃ receptors but further work would be needed to specifically exclude these regions in the effect of dopamine D₃ antagonists on SND.

Low doses of psychostimulants such as methylphenidate, which improve attention in the rodent, elevate PFC dopamine release measured by microdialysis, consistent with this being involved in attention (Berridge *et al*, 2006). PET studies in baboons show dopamine D₃ receptor-mediated regional cerebral blood flow responses are restricted to prefrontal and limbic cortices (Black *et al*, 2002), and performance in the PFC-dependent attentional set-shifting task is improved in dopamine D₃^{-/-} receptor mice (Glickstein *et al*, 2005). A distributed network of neurones in the prefrontal and parietal cortex is involved in the top-down regulation of attention, regulating eye movement and biasing sensory processing in favour of behaviorally relevant information, so improving saliency (Noudoost *et al*, 2010; Rossi *et al*, 2009). Recent human fMRI studies show that disruption of PFC activation

(mimicking the disruption seen of GABA-driven neural synchrony, attention and working memory in schizophrenia (Gonzalez-Burgos *et al*, 2010)) diminishes selective attention and subsequent recognition memory consistent with this top-down regulation of visual processing and working memory (Rauss *et al*, 2011; Zanto *et al*, 2011). Collectively these studies in mice, rats and primates support the importance of PFC dopamine D₃ receptor activation in the control of cognitive processes involving attentional mechanisms. In a repeated amphetamine-induced model of psychosis and cognitive impairment, sustained attention in an operant task was accompanied by decreased PFC acetylcholine release (Sarter *et al*, 2009). It is well established that the PFC dopamine D₃ receptors modulate cholinergic neurotransmission (Lacroix *et al*, 2003; Millan *et al*, 2007) consistent with this being a potential mechanism for the observed cognitive effects seen in this study. In addition, there is evidence that dopamine D₃ receptors are located on astrocytes (Choi *et al*, 2006) where they may regulate release of D-serine which in turn activates NMDA receptors and could also enhance NOR and SND (Fossat *et al*, 2011).

Discrete bilateral microinjections of S33084 into the PFC caused a dose-related reversal of delay-induced impairment of NOR while bilateral microinjection of the L741,626 into the same region impaired NOR. Of note, systemic administration of the dopamine D₁ agonist SKF 8129, which improves NOR memory after a 4 h delay (Hotte *et al*, 2005), increases phosphorylation of CREB and dopamine and cAMP-regulated phosphoprotein 32 kDa (DARPP-32) two substrates linked to the D₁ transduction system in the PFC (Hotte *et al*, 2006). Additionally, bilateral microinjection of the dopamine D₁ receptor antagonist, SCH23390, into the PFC of mice altered recognition memory and increased ERK1/2 phosphorylation therein (Nagai *et al*, 2007). Facilitation of NMDA receptor-dependent neurotransmission by the selective deletion of forebrain glycine transporter 1 (GlyT1) also enhances object recognition (Singer *et al*, 2007) and social cognition (Shimazaki *et al*, 2010). Collectively this suggest that although the PFC may not be critical for task performance, it exerts a powerful modulatory effect on NOR. Lesion and pharmacological studies have established the importance of medial temporal lobe structures such as the perirhinal and entorhinal cortices in the mediation of recognition memory (Abe *et al*, 2004; Aggleton *et al*, 1997; Ennaceur and Aggleton, 1997), though the role of the hippocampus is more contentious (Ainge *et al*, 2006; Gould *et al*, 2002; Hammond *et al*, 2004). As there is little dopamine D₃ receptor expression in the hippocampus this is unlikely to be an important site of action in the current study but further studies would be required to exclude this.

The apparent amnesic property of dopamine D₂ receptor blockade has significant implications for the treatment of schizophrenia. Antagonism at dopamine D₂ receptors is a common pharmacological mechanism of existing antipsychotic drugs which do not effectively treat the cognitive deficits seen in schizophrenia (Keefe *et al*, 2007). One strategy to improve cognitive therapy with antipsychotic drugs is to develop optimised dopamine D₃ and D₂ antagonists like S33138 (Joyce and Millan, 2005). However the potential therapeutic benefit of targeting dopamine D₃ receptors is not restricted to the treatment of cognitive dysfunction in schizophrenia (Millan and Brocco, 2008) but also in other common CNS disorders such as Parkinson's disease, Alzheimer's disease and as mentioned above neurofibromatosis and autism-related disorders.

Conclusions

In conclusion, the current data show that selective antagonism of dopamine D₃ receptor reverses delay-induced impairment of both visual recognition and SND memory. In contrast, both activation of dopamine D₃ receptors and antagonism of dopamine D₂ receptors impair NOR and SND performance. Furthermore, supporting previous work, these cognitive effects are mediated, at least in part, by the PFC which likely exerts its actions by top-down control of cognitive (attentional and other) processes integrated in other regions processing social-olfactory (such as amygdala and lateral septum) and visual-recognition (perirhinal and entorhinal cortex) cues. These structures have relatively few D₃ (and D₂) receptors, but any role in the control of SND and NOR by dopamine D₃ and D₂ receptor ligands remains to be examined. It would be worthwhile to compare the role of dopamine D₃ and D₂ receptors in the regulation of other cognitive tasks regulated by the PFC such as attentional set-shifting and other procedures interrogating executive function to further delineate the importance of this structure in the changes in learning and memory observed and to confirm the differential involvement of dopamine D₂ and D₃ receptors by evaluation of behavior in D₂ mutant mice.

Disclosure/Conflict of Interest

David J.G. Watson, Florence Loiseau, Mark J. Millan, Charles A Marsden and Kevin C.F. Fone 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 three 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 made by David J.G. Watson, Charles A. Marsden and Kevin C.F. Fone was financially supported by Servier.

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Supplementary information is available at the *Neuropsychopharmacology* website.

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Titles and Legends to Figures

Figure 1 Position of injection sites in the rat PFC (upper, +3.2mm from Bregma) and striatum (lower diagram, +0.48mm from Bregma) of rats used for behavioural studies on coronal sections taken from Paxinos and Watson (Paxinos and Watson, 1997) together with a schematic representation of the injector.

Figure 2 Dose-response effect of, and interaction between the preferential dopamine D₃ receptor antagonist, S33084, and the preferential dopamine D₂ receptor antagonist, L741,626 on social novelty discrimination (SND) in the rat. Data in panels a, c and e shows time spent investigating the novel compared to the familiar juvenile in P2 following a 30min inter-trial interval (mean ± s.e.m. of juvenile exploration). *** $P < 0.001$ from the novel juvenile at the same dose in the same treatment group (Bonferroni post-hoc). Panels b, d and f show the SND ratio (novel/familiar) for the same behaviour, * $P < 0.01$ compared to vehicle, ** $P < 0.01$ compared to vehicle/vehicle group, ++ $P < 0.01$ compared to vehicle/S33084 group. Numbers in parentheses indicate number of rats in each group. Note the D₃ receptor antagonist restores a time delay-induced reduction in social novelty discrimination (a and b) which is prevented by the D₂ receptor antagonist (e and f), while the latter has no effect on its own at this inter-trial interval (c and d).

Figure 3 Dose-response effects of the preferential dopamine D₃ receptor antagonist, S33084, the preferential dopamine D₂ receptor antagonist, L741,626 and the dopamine D₃ agonist, PD128,907, on social novelty discrimination (SND) with no inter-trial delay in the rat. Data in panels a, c and e shows time spent investigating the novel compared to the familiar juvenile in P2 (mean ± s.e.m. of juvenile exploration). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ from the novel juvenile at the same dose in the same treatment group (Bonferroni post-hoc following ANOVA). The SND ratio (novel/familiar) is shown in panels b, d and f. * $P < 0.05$ and, ** $P < 0.01$ compared to vehicle. Numbers in parentheses indicate the number of rats in each group. Note that while the D₃ receptor antagonist has no effect on social novelty discrimination, the D₂ receptor antagonist and D₃ receptor agonist both impair recognition of the novel juvenile in P2.

Figure 4 Effect of bilateral microinjection of the preferential dopamine D₃ receptor antagonist S33084 into the prefrontal cortex (a) or striatum (c) on social novelty discrimination (SND) in the rat. Bars show juvenile exploration (sec, mean \pm s.e.m.) following a 30 min inter-trial interval. *** P <0.001 from the novel juvenile in the same treatment group (Bonferroni post-hoc following ANOVA). Panels **b** and **d** show the SND ratio (novel/familiar) following microinjection of S33084 (2.5 μ g/side) into the prefrontal cortex and striatum respectively. ** P <0.01 compared to vehicle. Numbers in parentheses indicate the number of rats in each group. Note that S33084 only attenuated social novelty discrimination when injected into the prefrontal cortex and not into the striatum.

Figure 5 Comparison of the performance of dopamine D₃ receptor deficient (DRD3 -/-) to wild type (WT) mice on social novelty discrimination (SND) in drug free panels a and b and administration of the preferential dopamine D₃ receptor antagonist, S33084. Bars show juvenile exploration (sec, mean \pm s.e.m., n=6) following a 30 min inter-trial interval (panels **a** and **c**). * P <0.05, ** P <0.01, *** P <0.001 from the novel juvenile at the same dose in the same treatment group (Bonferroni post-hoc). The SND ratio (novel/familiar time) is shown in panels **b** and **d** ** P <0.01 compared to vehicle. * P <0.05 compared to all other groups. Note that DRD3 knock-out mice were able to distinguish between the familiar and novel juveniles after a 30 min inter-trial interval when wild type mice were unable to discriminate.

Figure 6 Dose-response effect and interaction between the preferential dopamine D₃ receptor antagonist, S33084, and the preferential dopamine D₂ receptor antagonist, L741,626 on performance in the novel object recognition task, using a 4 h inter-trial interval in the rat. Data in panels **a**, **c** and **e** shows the actual time spent investigating the novel compared to the familiar object in the second choice trial (sec, mean \pm s.e.m., n=11-12). *** P <0.001 from the novel object at the same dose within the same treatment group (Bonferroni post-hoc following ANOVA). Exploration times were also used to calculate the d2 score (see methods for definition) as an index of preferential object exploration in the choice trial and are shown **b**, **d** and **f**. (**b**) * P <0.05 from the novel object at the same dose. (**f**) ** P <0.01 and *** P <0.001 compared to vehicle/ vehicle group, ++ P <0.01 compared to vehicle/ S33084. Note that the D₃ receptor antagonist was able to restore a time induced

natural-forgetting (**a** and **b**) in the novel object discrimination paradigm which was prevented by co-administration of the D₂ receptor antagonist (**e** and **f**) while the latter had no effect on its own (**c** and **d**).

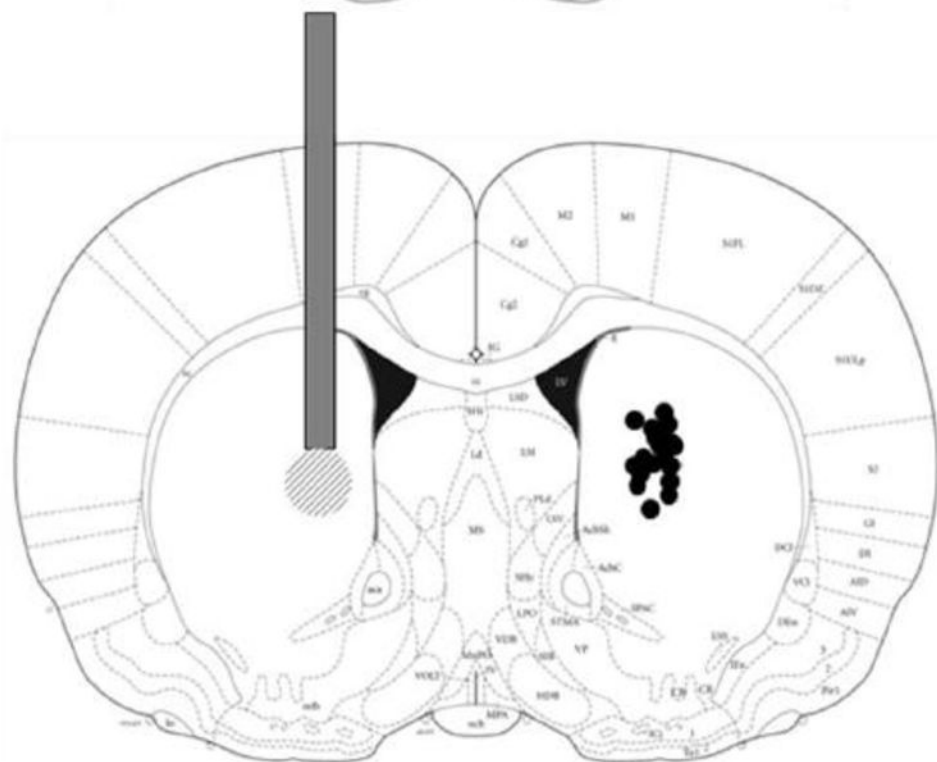
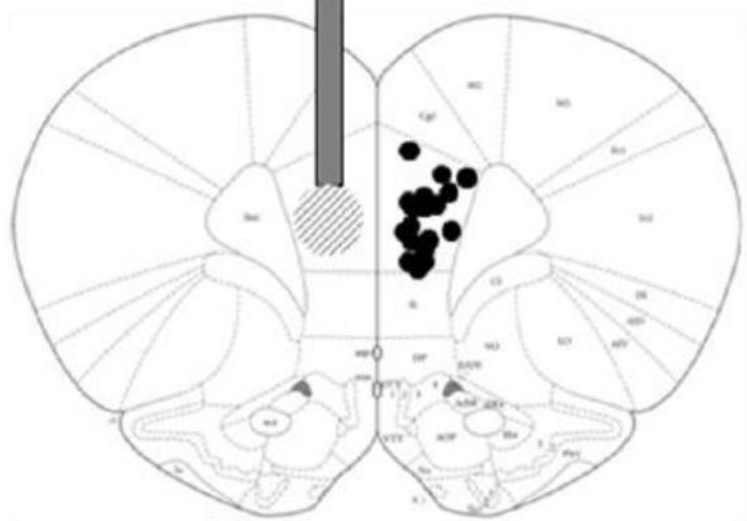
Figure 7 Examination of the dose-response effects and interaction between the preferential dopamine D₂ receptor antagonist, L741,626, and the dopamine D₃ receptor agonist, PD128,907 on novel object recognition task, using a 2min inter-trial interval in the rat. Data in panels **a**, **c** and **e** shows the time spent investigating the novel compared to the familiar object in the choice trial (sec, mean \pm s.e.m., n=12). ** $P < 0.01$ and *** $P < 0.001$ from the novel object at the same dose within the same treatment group (Bonferonni post-hoc). Panels **b**, **d** and **f** show the derived d2 score (see methods for definition). (**b** and **d**) ** $P < 0.01$ compared to vehicle, *** $P < 0.01$ compared to vehicle, (**f**) ** $P < 0.01$ compared to vehicle/ vehicle group, ++ $P < 0.01$ compared to vehicle/ PD128,907, Bonferonni post-hoc following ANOVA. The D₂ receptor antagonist (**a** and **b**) and the D₃ receptor agonist (**c** and **d**) both impaired object discrimination and the effect of the latter was prevented by pretreatment with a D₃ receptor antagonist (**e** and **f**).

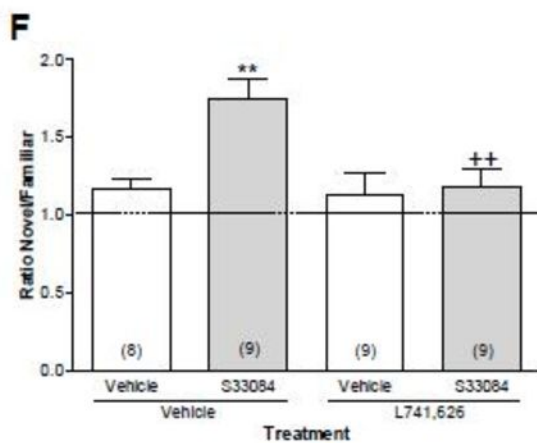
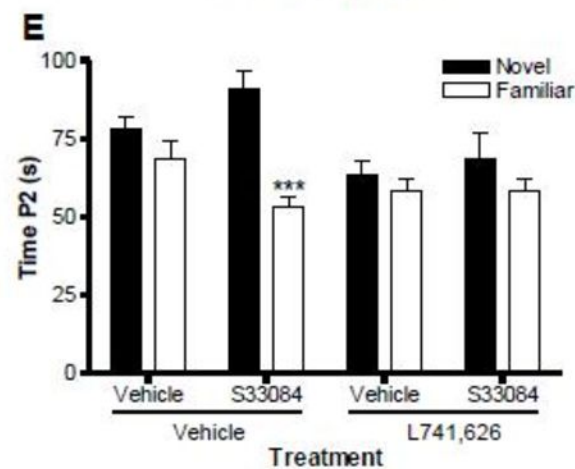
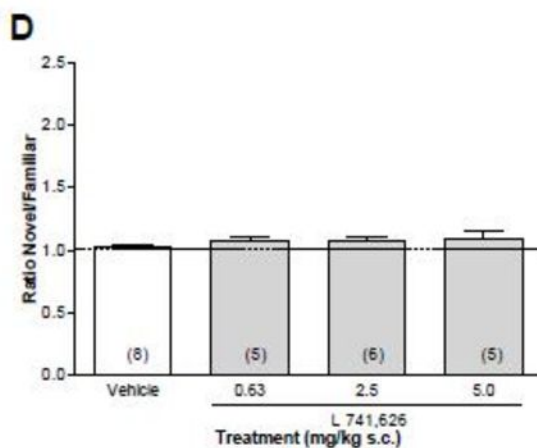
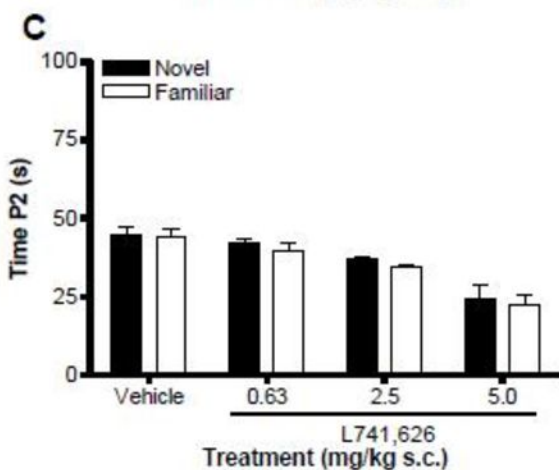
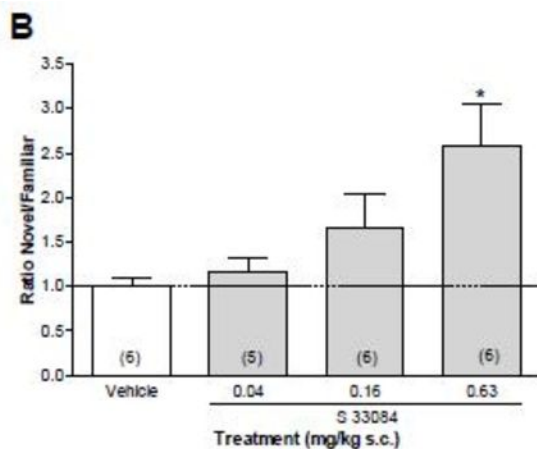
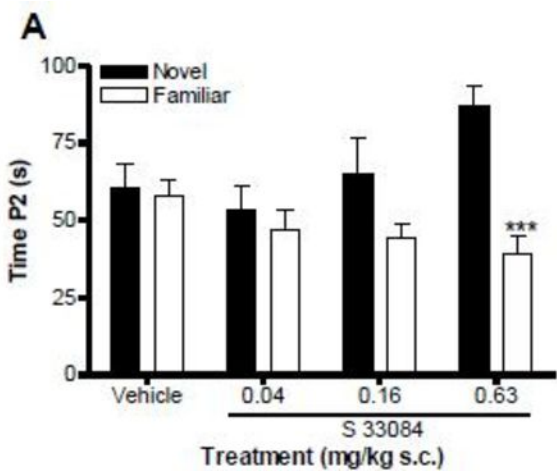
Figure 8 Comparison of the effect of bilateral microinjection of the preferential dopamine D₃ receptor antagonist, S33084 and the preferential dopamine D₂ receptor antagonist, L741,626 into the rat prefrontal cortex and striatum on novel object recognition using a 4h and 2min inter-trial interval respectively. Panels **a** and **c** show the time spent (mean \pm s.e.m., n=8-9) investigating the novel compared to the familiar object in the choice trial following drug injection into the prefrontal cortex. ** $P < 0.01$ and *** $P < 0.001$ from the novel object at the same dose within the same treatment group (Bonferonni post-hoc). Exploration times were used to calculate the d2 score (see methods for definition) (**b** and **d**) and evaluate preferential object exploration as an index of recognition memory. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared to vehicle following ANOVA. Panels **e** and **f** show the derived d2 score from the choice trial exploration times after injection into the striatum of S33084 using a 4h inter-trial interval or L741,626 using a 2 min inter-trial interval, respectively. When injected into the prefrontal cortex the two antagonists had opposite effects on object discrimination such that the D₃ receptor antagonist reversed natural forgetting (**a** and **b**) while the D₂ antagonist impaired discrimination (**c** and **d**). In contrast,

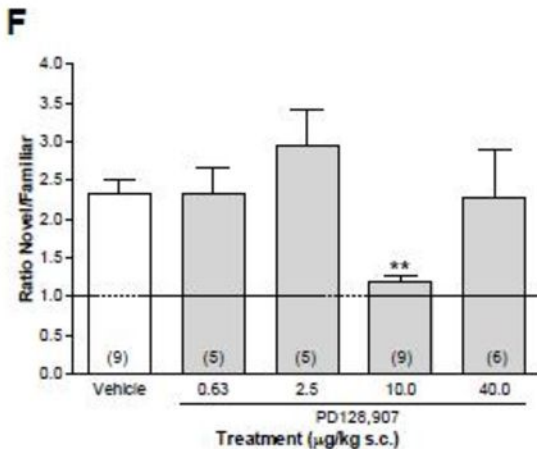
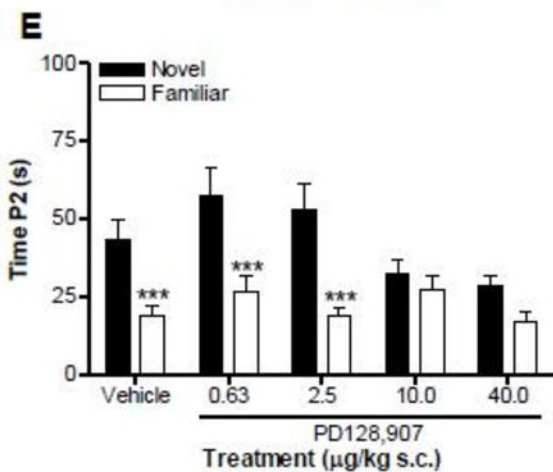
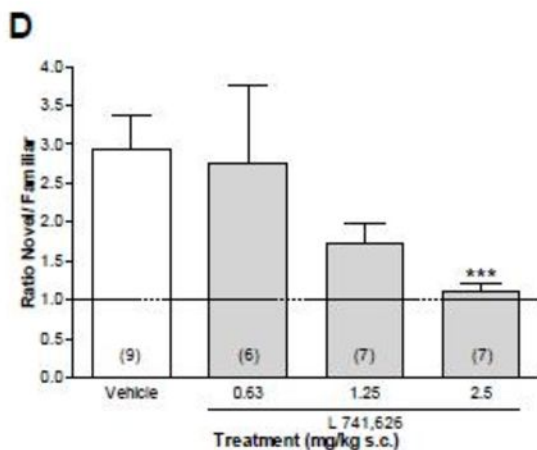
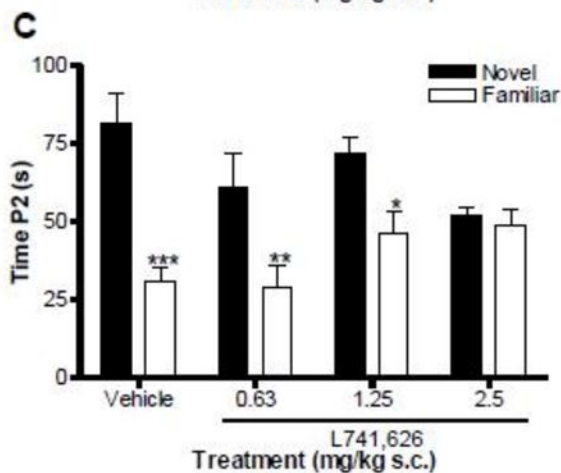
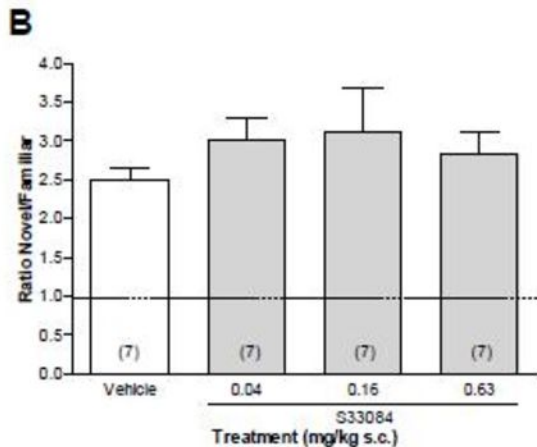
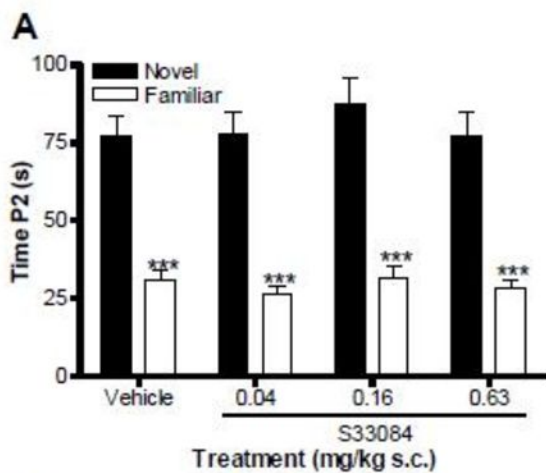
neither drug had any effect on novel object recognition when injected into the striatum (**e** and **f**).

Table 1 Effect of dopamine D₃ and D₂ receptor ligands on social novelty discrimination in Wistar rats and dopamine D₃ deficient mutant mice. Numbers in parenthesis indicate the number of animals in each treatment group. P1, first trial (familiar juvenile only); P2, second trial (both juveniles); PFC; prefrontal cortex, WT; wild type. Data are expressed as the mean \pm s.e.m. duration (sec) of active social investigation. * $P < 0.05$ from vehicle, vehicle/vehicle or WT/vehicle respectively following ANOVA.

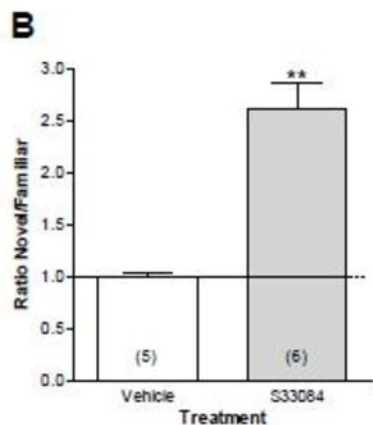
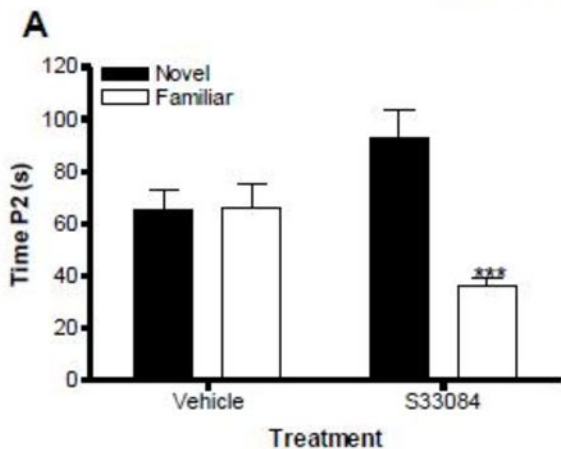
Table 2 Effect of dopamine D₃ and D₂ receptor compounds on novel object recognition in adult male Lister Hooded rats. See text for statistical analysis, n=12 unless otherwise indicated by a number in parenthesis. T1, first (familiarisation) trial; T2, second (choice) trial. PFC; prefrontal cortex. Data are expressed as the mean \pm s.e.m. duration (sec) of active object exploration. \$ ANOVA showed a main effect of L741,626 on total T2 exploration time ($F_{(1,40)}=6.79$, $P < 0.05$) but there is no significant post-hoc difference between any of the treatment combinations.



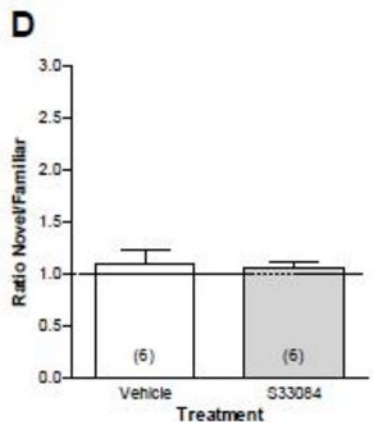
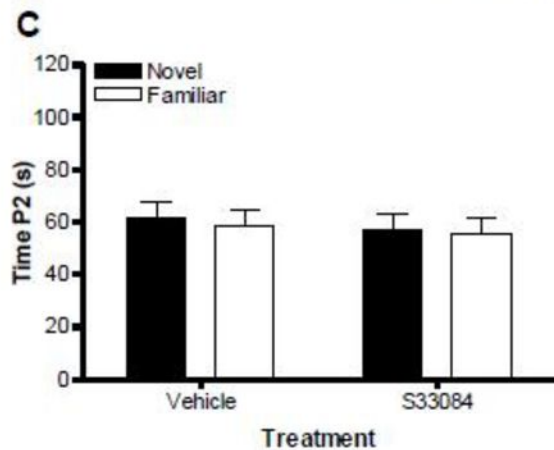


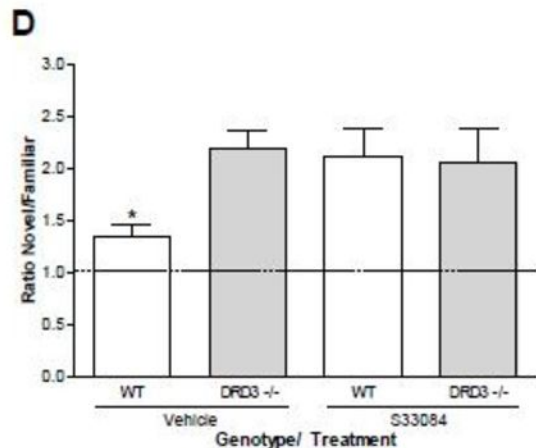
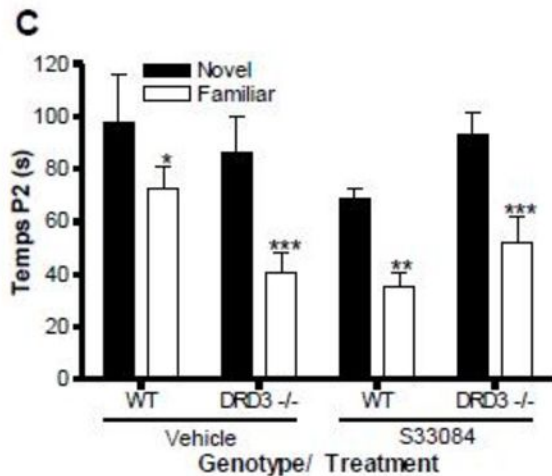
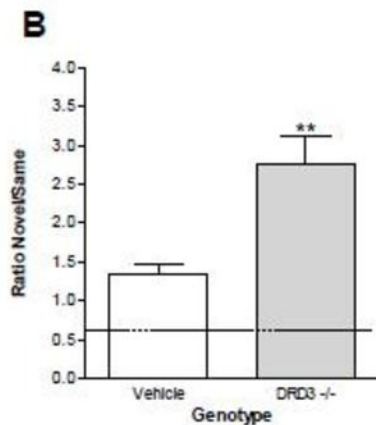
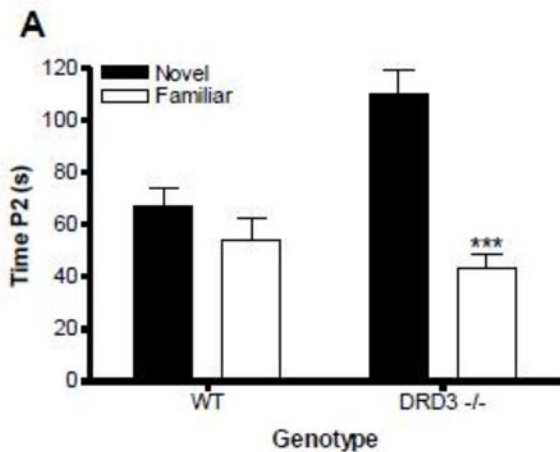


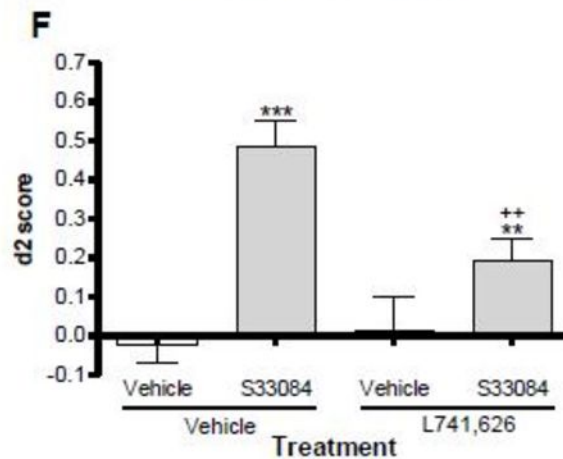
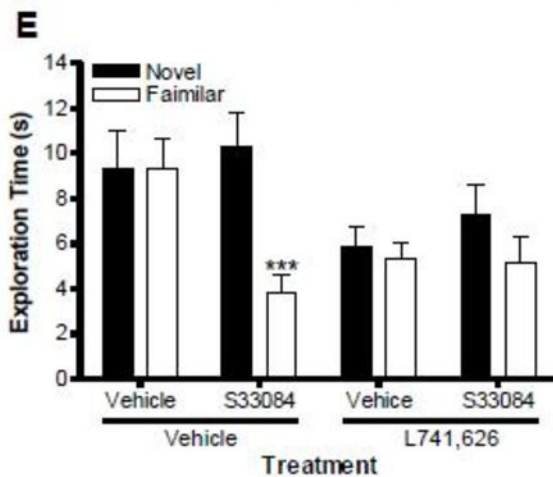
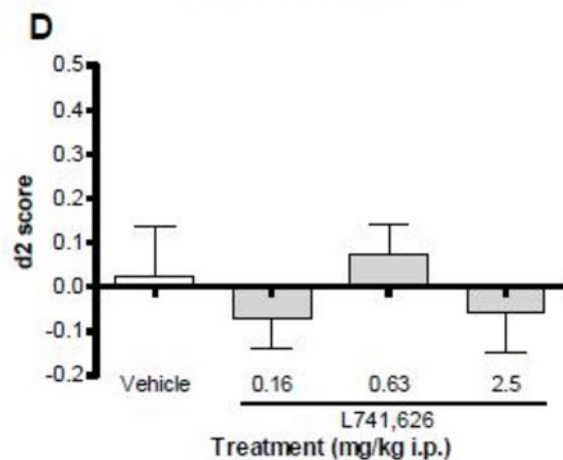
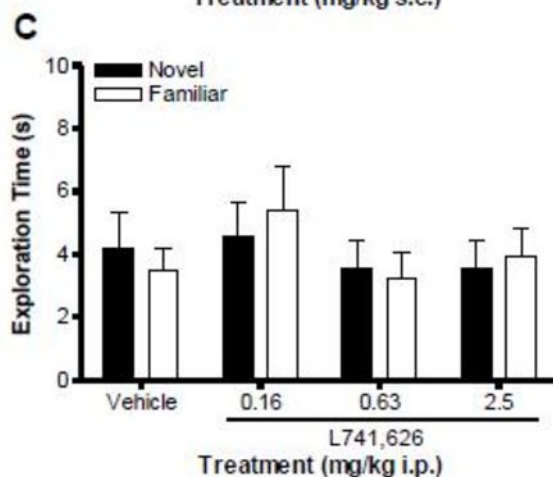
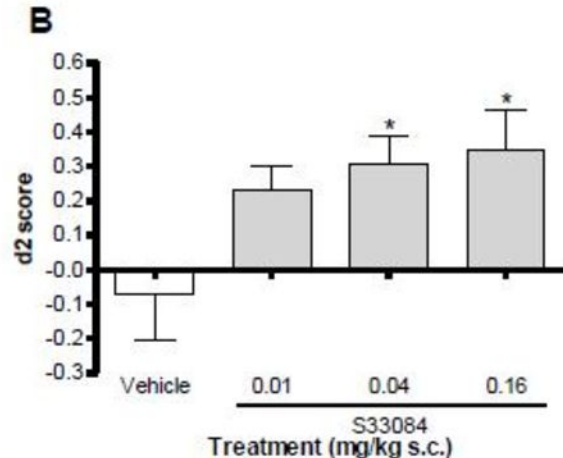
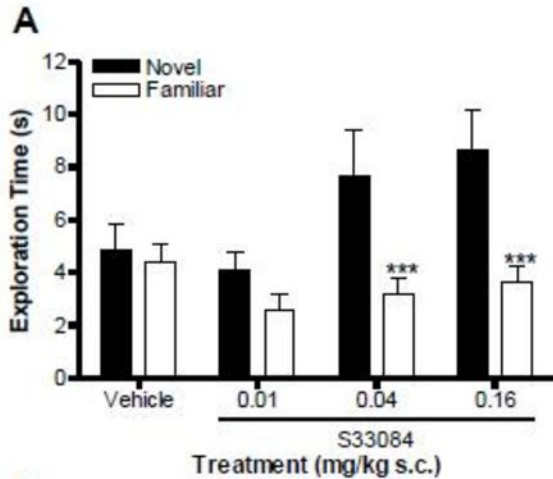
Frontal Cortex

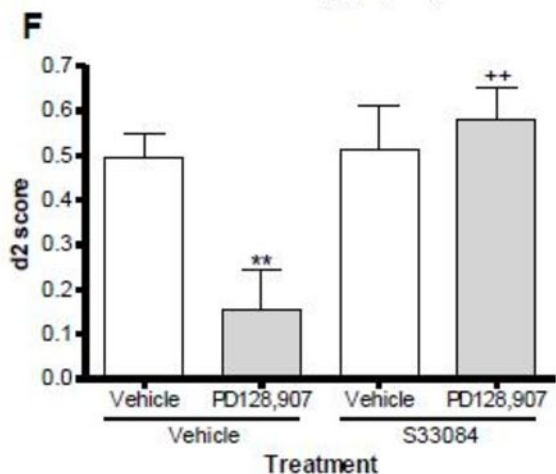
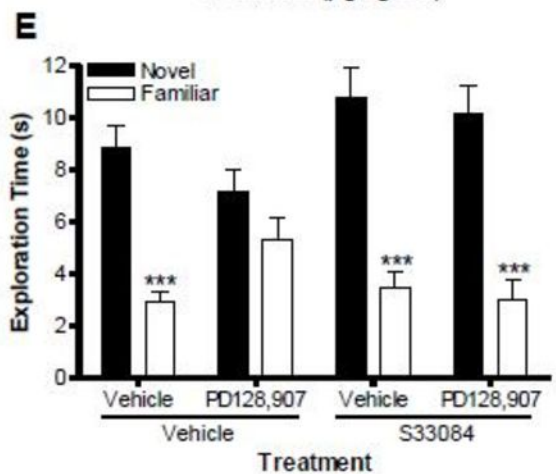
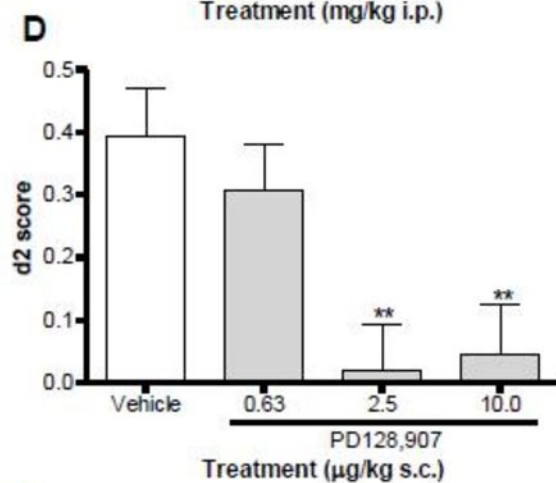
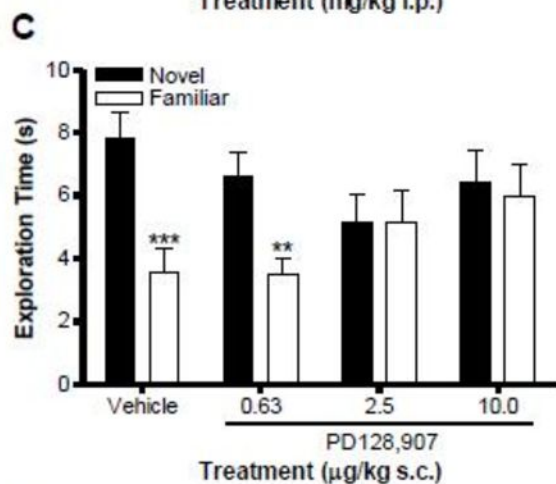
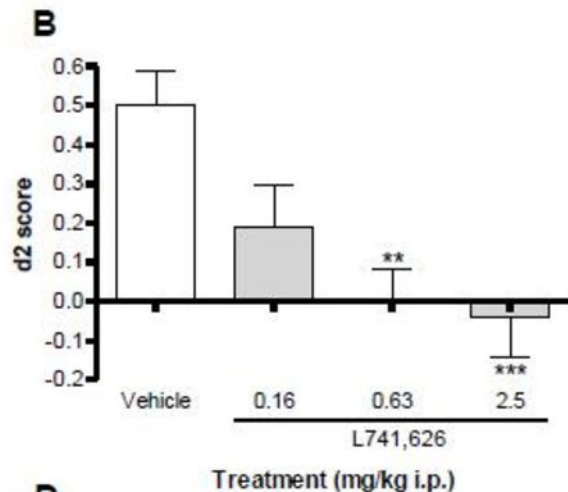
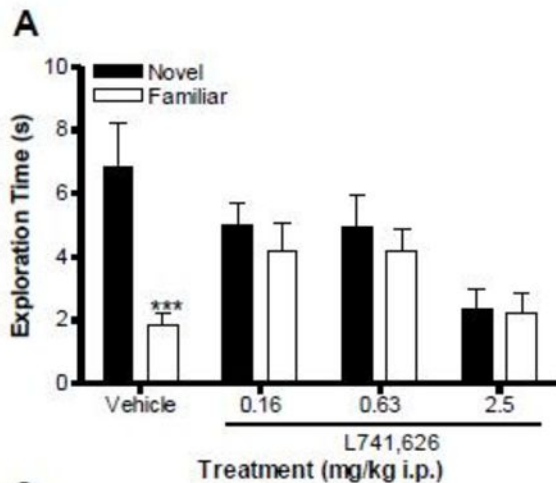


Striatum

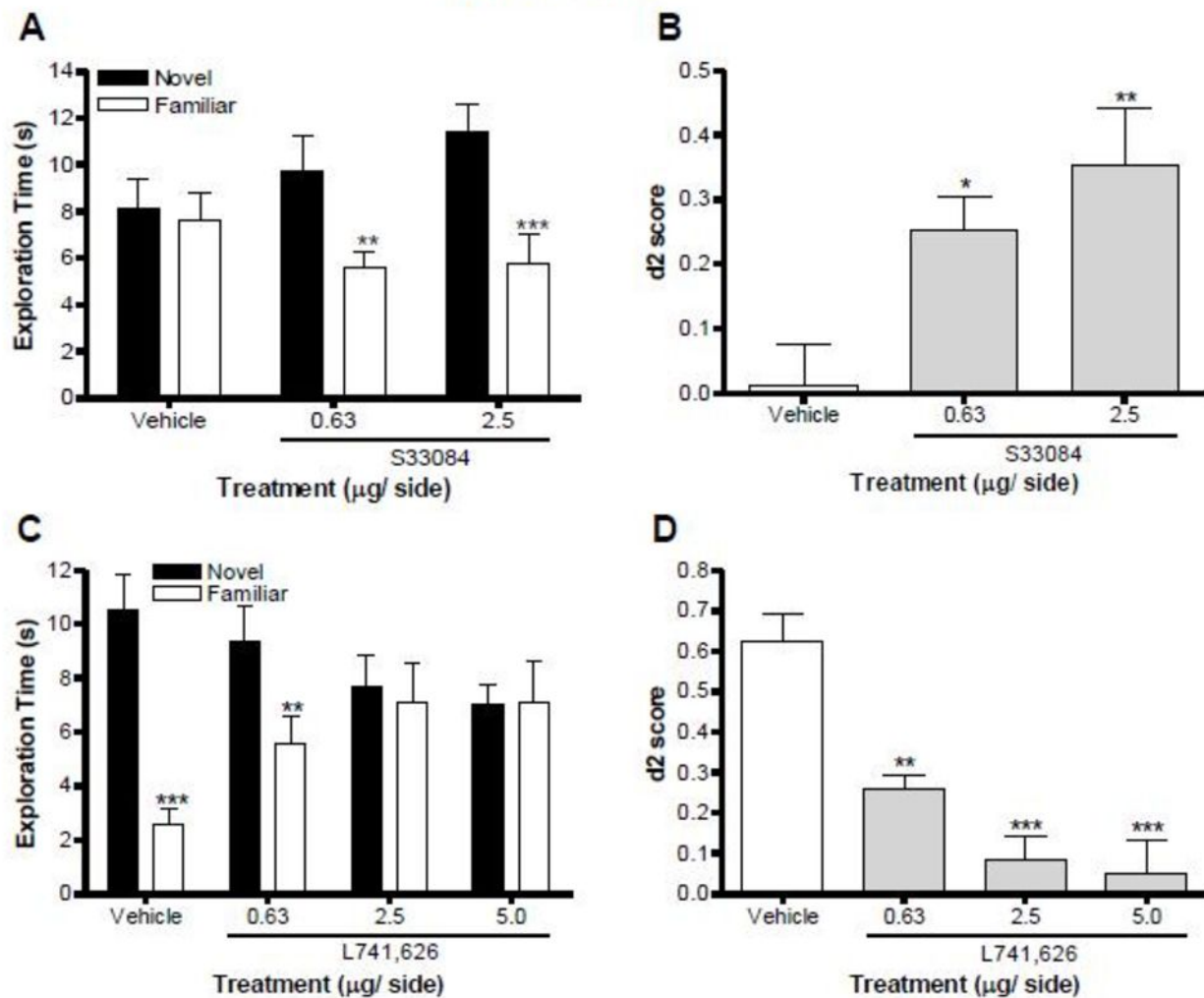








Frontal Cortex



Striatum

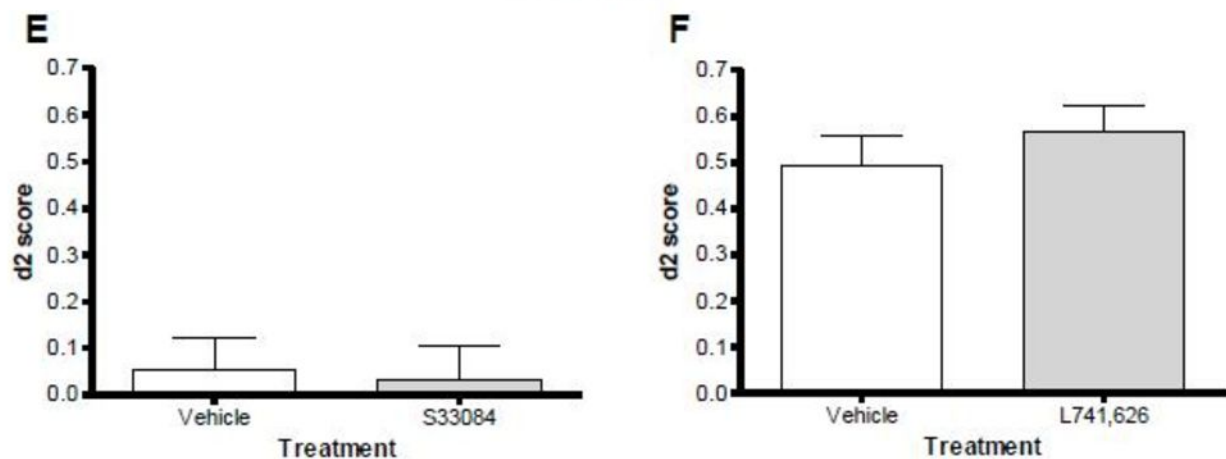


Table 1 Social Novelty Discrimination

Drug/ Dose		Inter-trial Interval	P1 Total Juvenile Exploration (s)	P2 Total Juvenile Exploration (s)
S33084	Vehicle (6)	30min	109.8 (10.6)	118.2 (13.8)
	0.04 mg/kg (5)		99.9 (5.6)	100.0 (13.0)
	0.16 mg/kg (6)		93.0 (8.9)	109.5 (8.9)
	0.63 mg/kg (6)		103.2 (6.8)	125.8 (10.6)
L741,626	Vehicle (8)	30min	114.0 (5.1)	89.3 (4.5)
	0.63mg/kg (5)		120.2 (8.3)	81.3 (4.3)
	2.5 mg/kg (6)		92.4 (8.7)	71.0 (1.0)
	5.0 mg/kg (5)		79.1 (6.4)*	46.7 (7.3)*
Vehicle/ Vehicle (8)	30min	105.0 (5.4)	146.2 (9.2)	
L741,626/ Vehicle (9)		96.0 (5.8)	121.9 (4.7)*	
Vehicle/ S33084 (9)		113.3 (5.4)	144.2 (6.8)	
L741,626/ S33084 (9)		111.4 (9.8)	126.6 (10.9)	
S33084	Vehicle (7)	No delay	111.6 (8.0)	107.8 (9.4)
	0.04 mg/kg (7)		101.1 (9.7)	104.7 (8.2)
	0.16 mg/kg (7)		109.1 (11.0)	118.8 (9.5)
	0.63 mg/kg (7)		110.5 (10.3)	105.5 (9.6)
L741,626	Vehicle (8)	No delay	108.7 (7.4)	112.4 (12.9)
	0.63mg/kg (5)		103.0 (6.1)	90.4 (14.7)
	2.5 mg/kg (6)		130.9 (2.7)*	118.0 (11.3)
	5.0 mg/kg (5)		105.7 (5.4)	101.3 (7.1)
PD128,907	Vehicle (9)	No delay	100.2 (6.7)	62.3 (9.5)
	0.63µg/kg (5)		123.0 (10.2)	84.3 (13.6)
	2.5µg/kg (5)		109.1 (13.4)	71.3 (10.8)
	10.0µg/kg (9)		97.6 (6.4)	59.6 (8.7)
	40.0 µg/kg (6)		84.7 (13.5)	44.5 (3.8)
S33084 (microinjection into PFC)	Vehicle (5)	30min	132.6 (5.0)	131.0 (16.8)
	2.5µg/side (6)		137.4 (8.7)	129.2 (12.1)
S33084 (microinjection into striatum)	Vehicle (6)	30min	116.9 (11.8)	131.0 (16.8)
	2.5µg/side (6)		109.8 (9.1)	129.2 (12.1)
WT (mice, 6)	30min	132.4 (13.3)	121.3(14.9)	
DRD3 ^{-/-} (mice, 6)		133.4 (9.1)	153.3 (12.0)	
WT/ Vehicle (mice, 6)	30min	169.8 (18.3)	169.8 (25.8)	
DRD3 ^{-/-} / vehicle (mice, 6)		160.7 (13.1)	127.2 (20.3)	
WT/ S33084 (mice, 6)		144.3 (12.9)	104.8 (4.9) *	
DRD3 ^{-/-} / S33084 (mice, 6)		142.9 (12.2)	144.8 (17.5)	

Table 2 Novel Object Recognition

Drug/ Dose		Inter-trial Interval	T1 Total Object Exploration (s)	T2 Total Object Exploration (s)
S33084	Vehicle	4h	14.3 (2.0)	9.3 (1.7)
	0.01 mg/kg		14.1 (1.8)	6.7 (1.2)
	0.04 mg/kg		14.1 (2.0)	10.8 (2.2)
	0.16 mg/kg		15.3 (1.9)	12.3 (1.7)
L741,626	Vehicle	4h	13.2 (1.6)	7.7 (1.7)
	0.16 mg/kg		15.1 (2.4)	10.0 (2.4)
	0.63 mg/kg		13.0 (1.6)	6.8 (1.6)
	2.5 mg/kg		9.3 (1.6)	7.5 (1.7)
Vehicle / Vehicle		4h	13.8 (2.1)	18.7 (2.7)
L741,626/ Vehicle			15.0 (1.6)	10.7 (1.5)
Vehicle/ S33084			14.5 (1.6)	15.5 (1.7)
L741,626/ S33084			15.3 (1.7)	12.5 (2.3)
L741,626	Vehicle	2min	9.5 (1.87)	8.7 (1.6)
	0.16 mg/kg		11.0 (1.6)	9.2 (1.4)
	0.63 mg/kg		12.2 (1.9)	9.1 (1.7)
	2.5mg/kg		7.8 (1.1)	4.6 (1.2)
PD128,907	Vehicle	2min	15.4 (1.7)	11.4 (1.2)
	0.63µg/kg		16.5 (1.3)	10.1 (1.1)
	2.5µg/kg		15.5 (1.5)	10.3 (1.8)
	10.0µg/kg		12.7 (1.2)	12.4 (1.8)
Vehicle/ Vehicle		2min	13.9 (1.7)	11.8 (0.9)
S33084/ Vehicle			17.3 (0.9)	14.2 (1.3)
Vehicle/ PD128,907			14.0 (1.2)	12.5 (1.2)
S33084/ PD128,907			15.3 (2.5)	13.2 (1.5)
S33084 (microinjection into PFC)	Vehicle	4h	33.9 (3.9)	15.8 (2.3)
	0.63µg/side		32.6 (3.0)	15.4 (1.9)
	2.5µg/side		35.3 (3.7)	17.3 (2.1)
L741,626 (microinjection into PFC)	Vehicle	2min	19.1 (2.6)	13.1 (1.6)
	0.63µg/side		17.6 (2.4)	14.9 (2.3)
	2.5µg/side		18.7 (1.5)	14.8 (2.6)
	5.0µg/side		17.9 (2.0)	14.1 (2.0)
S33084 (microinjection into striatum)	Vehicle (10)	4h	21.3 (3.3)	15.9 (2.0)
	2.5µg/side (10)		24.0 (2.7)	20.6 (2.4)
L741,626 (microinjection into striatum)	Vehicle (9)	2min	24.4 (1.5)	15.5 (1.1)
	5.0µg/side (9)		19.6 (1.5)	17.1 (1.4)