S33138 (N-[4-[2-[(3aS,9bR)-8-cyano-1,3a,4,9b-tetrahydro[1]benzopyrano[3,4-c]pyrrol-2(3H)-yl]-ethyl]phenyl-acetamide), a Preferential Dopamine D₃ versus D₂ Receptor Antagonist and Potential Antipsychotic Agent: III. Actions in Models of Therapeutic Activity and Induction of Side Effects

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ABSTRACT

In contrast to clinically available antipsychotics, the novel benzopyranopyrrolidine derivative, S33138 (N-[4-[2-[(3aS,9bR)-8-cyano-1,3a,4,9b-tetrahydro[1]benzopyrano[3,4-c]pyrrol-2(3H)-yl]-ethyl]phenyl-acetamide), behaves as a preferential antagonist of D₃ versus D₂ receptors and does not interact with histamine H₁ and muscarinic receptors. In contrast to haloperidol, clozapine, olanzapine, and risperidone, S33138 (0.16–2.5 mg/kg s.c.) did not disrupt performance in passive-avoidance and five-choice serial reaction time procedures. Furthermore, upon either systemic administration (0.04–2.5 mg/kg s.c.) or introduction into the frontal cortex (0.04–0.63 mg/kg/side), S33138 potently attenuated the perturbation of social recognition by scopolamine or a prolonged intersession delay. Over a comparable and low-dose range, S33138 (0.04–0.63 mg/kg s.c.) elevated dialysis levels of acetylcholine in the frontal cortex of freely moving rats. At higher doses (2.5–10.0 mg/kg s.c.), S33138 also increased frontocortical levels of histamine, whereas monoamines, glutamate, glycine, and GABA were unaffected. By analogy to the other antipsychotics, S33138 (0.16–2.5 mg/kg s.c.) also blocked the reduction of prepulse inhibition elicited by apomorphine. In comparison with the above actions, only “high” doses of S33138 (10.0–40.0 mg/kg s.c.) elicited catalepsy. To summarize, reflecting preferential blockade of D₃ versus D₂ receptors, S33138 preserves and/or enhances cognitive function, increases frontocortical cholinergic transmission, and is active in models of antipsychotic properties at doses well below those inducing catalepsy. In comparison with clinically available agents, S33138 displays, thus, a distinctive and promising profile of potential antipsychotic properties.

We have recently described a novel benzopyranopyrrolidine derivative, S33138 (Millan et al., 2008a,b) that behaves as a preferential antagonist at cloned, human, and native cerebral dopaminergic D₃ versus D₂ receptors. In addition, it displays modest antagonist properties at serotonin (5-HT₂A receptors, 5-HT₇ receptors, and α₂C-adrenoceptors (ARs), blockade of which may also be useful in the treatment of schizophrenia (Meltzer et al., 2003; Svensson, 2003). In contrast, S33138 does not interact with histamine H₁, muscarinic, or α₁-adrenoceptors, antagonism of which provokes cardiovascular-autonomic side effects (Kroeze et al., 2003; Lieberman, 2005). This distinctive receptor-binding profile differentiates S33138 from clinically employed antipsychotics like the neuroleptic and D₂/D₃ receptor antagonist, haloperidol; the “atypical” agent, clozapine; and two further mul-

ABBREVIATIONS: S33138, N-[4-[2-[(3aS,9bR)-8-cyano-1,3a,4,9b-tetrahydro[1]benzopyrano[3,4-c]pyrrol-2(3H)-yl]-ethyl]phenyl-acetamide; 5-HT, serotonin; AR, adrenoceptor; FCX, frontal cortex; 5-CSRT, five-choice serial reaction time; PAV, passive avoidance; ACh, acetylcholine; CAR, conditioned avoidance response; PPI, prepulse inhibition; OCB, open channel blocker; PCP, phencyclidine; PRL, prolactin; HPLC, high-performance liquid chromatography; DA, dopamine; NA, noradrenaline; ANOVA, analysis of variance; L741,826, 3-(4-(4-chlorophenyl-4-hydroxypiperidino)methyl)indole.

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Dopamine D<sub>3</sub> Receptors and Schizophrenia

Schizophrenia is characterized by a disruption of working, social and verbal memory, attention, and executive function (Manoach, 2003; Meltzer, 2004; Glahn et al., 2005; Keefe et al., 2006). Unfortunately, haloperidol does not greatly restore cognitive performance, while the beneficial effects of clozapine and other multireceptorial antipsychotics are unremarkable; furthermore, it is unclear to what extent they represent a specific and primary effect upon mnemonic function (Woodward et al., 2005; Thornton et al., 2006). Experimental models of cognitive function have similarly revealed inconsistent effects of antipsychotics (Terry et al., 2003; Hagan and Jones, 2005; Hou et al., 2006). One reason underlying limited and variable efficacy is their potent antagonism of muscarinic, α<sub>1</sub>-adrenergic, and histamine H<sub>1</sub> receptors, which compromises cognitive function (Bacciotti et al., 2001; Ito, 2004; Sarter et al., 2005). As mentioned above, S33138 possesses negligible affinity for these sites (Millan et al., 2008a). Its distinctive preference for D<sub>3</sub> versus D<sub>2</sub> receptors is also of importance because D<sub>3</sub> receptor blockade improves cognitive function. In contrast, D<sub>2</sub> receptor blockade exerts a negative influence (Laszy et al., 2005; Millan et al., 2007). For example, selective D<sub>3</sub> and D<sub>2</sub> receptor antagonists, respectively, enhance and disrupt social cognition in rats (Millan et al., 2007), a model selected for detailed evaluation of the cognitive actions of S33138. The clinical relevance of “social memory” in rodents is unclear. Nonetheless, this procedure has a marked component of attention and working memory and is of particular interest because social cognition is perturbed in schizophrenic patients, possibly due to a dysfunction of the frontal cortex (FCX) (Beer and Ochsner, 2006; Lee et al., 2006). A disruption of frontocortical function is also implicated in the attentional deficits seen in schizophrenia; accordingly, the actions of S33138 were characterized in a five-choice serial reaction time test (5-CSRT in rats) (Robbins, 2002; Chudasama and Robbins, 2004). Furthermore, we determined its effects in a model of “aversive learning,” the passive avoidance (PAV) paradigm in rats (Hagan and Jones, 2005; Millan et al., 2002). Conditioning of PPI by apomorphine in rats. Both negative-cognitive and positive symptoms of schizophrenia are provoked in healthy subjects by N-methyl-d-aspartate receptor open channel blockers (OCBs) like phencyclidine (PCP) and ketamine (Moore, 1999; Abi-Dargham and Laruelle, 2005; Millan, 2005). In a similar manner, we evaluated the influence of S33138 upon their stimulation of locomotion in rats.

Regarding potential side effects, pharmacological, antisense, and gene knockout studies indicate that preferential blockade of D<sub>3</sub> versus D<sub>2</sub> receptors is associated with a relatively benign effect upon motor function as compared with drugs possessing D<sub>2</sub>/D<sub>3</sub> or principally D<sub>2</sub> antagonist properties (Joyce and Millan, 2005; Sokoloff et al., 2006; Gyertyán and Sághy, 2007). Thus, we examined the ability of S33138 to induce catalepsy in the rat, a well established response predictive of an extrapyramidal motor syndrome in man (Millan et al., 1998; Kapur et al., 2006; Shirzadi and Ghaemi, 2006). Furthermore, although D<sub>3</sub> receptor antagonism is unlikely to modify the effects of blockade D<sub>2</sub> receptors on lactotrophs, the affect of S33138 upon circulating levels of prolactin (PRL) was determined because hyperprolactinemia is a problematic side effect of antipsychotics (Ben-Jonathan and Hnasko, 2001; Turrone et al., 2002; Shirzadi and Ghaemi, 2006).

Materials and Methods

Animals. Unless otherwise specified, male Wistar rats weighing 225 to 250 g and male NMRI mice weighing 22 to 25 g were used (Charles River, Saint-Aubin-les-Elbeuf, France). They were maintained in sawdust-lined cages with unrestricted access to food and water. Laboratory temperature was 21 ± 1°C, and humidity was 60 ± 5%. There was a 12-/12-h light/dark cycle, with lights on from 7:30 AM to 7:30 PM. Before experimentation, all animals were adapted for at least 1 week to laboratory conditions. All animals use procedures conforming to international European ethical standards (86/609-EEC) and the French National Committee (décret 87/848) for the care and use of laboratory animals.

Conditioned Avoidance Responses in Rats. Rats were trained to avoid an electric shock (560 μA, 5 s) by switching compartments of a shuttle box (Letica, Barcelona, Spain) upon the appearance of a light (Millan et al., 1998). Each trial (10 per session) consisted of a 10-s period with the light “on.” When the subject did not change compartments, it received the shock. However, when it switched within the 10-s period with the light on (CAR), it did not. Upon changing compartments, the trial was considered as complete. The total number of CARs shown in the presence of light (maximum = 10 per session) was determined. Test sessions were performed the day after control sessions and undertaken once a week. Drugs or vehicle (control session) were administered 30 min before testing.

Apomorphine-Induced Climbing in Mice. Climbing behavior was examined in male CD mice weighing 22 to 25 g (Charles River) placed in steel cylinders (14-cm diameter) possessing walls (14 cm high) of vertical bars (1 cm apart and 2-mm diameter). Climbing was assessed according to a rating scale of 0 to 2 (Millan et al., 1998). The total of two measures made 10 and 20 min after apomorphine (0.75 mg/kg s.c.) was determined. Drugs or vehicle were administered s.c. 30 min before apomorphine.

Amphetamine-, Cocaine-, Ketamine-, PCP-, and Dizocilpine-Induced Hyperlocomotion, and Spontaneous Locomotion, in Rats. As previously reported (Millan et al., 1998), rats were adminis-
etered with drugs or vehicle (s.c.) and placed for a 30-min habituation period in polycarbonate cages (45 × 30 × 20 cm) in activity chambers (Labinlife System, Coulbourn, Lehigh Valley, PA). Subsequently, animals received vehicle ("spontaneous locomotion"), amphetamine (2.5 mg/kg i.p.), cocaine (20.0 mg/kg i.p.), ketamine (40.0 mg/kg s.c.), PCP (20.0 mg/kg s.c.), or dizocilpine (0.16 mg/kg s.c.), and locomotion was monitored over 60 min. A "locomotor count" was consecutive interruption of two infrared beams 24 cm apart and 4 cm above the cage floor.

**Spontaneous Locomotion in Mice.** Thirty minutes after s.c. injection of drugs or vehicle, mice were placed for 10 min in individual, white Plexiglas chambers (27 × 27 × 27 cm) furnished with two rows of four photocells, located 2 cm above the floor and 6 cm apart. Photocells were connected to a computer employing software written by Osys/Orga System (Changé, France). Interruption of two adjacent beams corresponded to a locomotion count.

**Rotordor Test in Mice.** Latency to fall from an accelerating (4–40 rpm over 300 s) rotordor (Ugo Basile, Varese, Italy) was determined (cut-off of 360 s). S33138, haloperidol, clozapine, olanzapine, and risperidone or vehicle were given (s.c.) 30 min before testing.

**Disruption of Prepulse Inhibition by Apomorphine in Rats.** The procedure was used essentially that used elsewhere (Geyer et al., 2001). Male Sprague-Dawley rats (300–400 g) (Janvier, Le Genest-Saint-Isle, France) were administered S33138 or vehicle 30 min (s.c.) before the injection of apomorphine (0.5 mg/kg s.c.) or vehicle. They were then placed individually in the startle chambers connected via an interface to a computer that controlled auditory stimuli and monitored startle responses (SR-Startle System; San Diego Instruments, San Diego, CA). Each test chamber consisted of a sound-attenuated cabinet that held a cylindrical, Plexiglas stabilimeter. Acoustic tones were presented by a loudspeaker mounted 24 cm above the animal. Movements of the rat in the cylinder were detected and transduced by a piezoelectric cartridge. After a 5-min acclimation period with a 70-db background noise, the animal was submitted to a series of startle trials consisting of several conditions: 1) a 118-db, 40-ms noise burst presented alone or 2) the same 118-db, 40-ms noise burst preceded at a 100-ms interval by prepulses (20-ms noise) 12 db above background. A variable intertrial interval averaged 15 s. PPI was defined as the percentage of reduction in startle amplitude in the presence of prepulse versus absence of prepulse (100 × amplitude on prepulse trial/amplitude on the presented alone trial).

**Passive Avoidance Procedure in Rats.** Male Sprague-Dawley rats weighing 250 to 275 g were used. The procedure consisted of two sessions performed over 2 consecutive days. On day 1 ("training" session), rats were administered drugs or vehicle and placed individually in polycarbonate home cages. Thirty min later, they were placed in the large light compartment (31 × 31 × 24 cm) of a two-chamber box (Pav apparatus, model LE 870; PANLAB, Cornella de Liobregat, Spain) and allowed 2 min of exploration without access to the small dark compartment (18 × 11 × 13 cm). At the end of the exploration period, the sliding door separating the light compartment from the smaller dark compartment was lifted, and the animal was allowed 5 min (cut-off latency) to enter the small, dark compartment. Once the rat had entered, the door was closed, and 2 s later, an inescapable, constant current, scrambled shock (0.40 mA) was delivered for 5 s. The rat was then removed and placed in its home cage. On day 2 ("retention" session), 24 h later, the animal was again placed in the light compartment for 5 min ("cut-off") with free access to the small, dark compartment. Entry was not followed by shock. The two-chamber box was connected through an interface to a computer that controlled door opening and shock delivery (Shuttle 8; PANLAB). Data collected were latency to enter the dark compartment from bregma: FCX, AP, 6.0; DV, 1.2; L, 3.5. PPI was determined by calculating the percentage of reduction in startle response in the illuminated hole (correct responses) was rewarded by the delivery of a food pellet (45 mg; Noyes, Lancaster, NH). A response in a hole that had not been illuminated (incorrect response), a failure to respond within the prescribed time limit of 5 s (omission), or responses made before the onset of the stimulus during the intertrial interval (anticipatory response) caused a brief period of darkness (time out with house lights off, 5 s). After a pretraining period, individual performances were stabilized (between 60 and 90% correct responses and no more than 20% omissions and anticipatory responses), and test sessions, twice a week, were initiated. Drugs or vehicle were administered 30 min before testing, each rat receiving once only a defined dose. Data were collected by computer (Schedule Manager; Med Associates).

**Social Recognition Test in Rats.** As described previously (Millan et al., 2007), adult Wistar rats weighing 240 to 260 g and juvenile Wistar rats (25–30 days old) (Janvier) were used. Adult rats were individually housed for 2 days before testing. On the test day, they were placed in their home cages on the observation table. After 5 min of habituation, a juvenile was placed into the home cage for a 5-min observation session, and time spent in active social investigation ("T1") was evaluated; that is, the time devoted by the adult rat to sniffing, following, biting, jumping, and crawling over or under the juvenile. After this first session, the duration of investigation was also monitored during a second 5-min session ("T2") that followed either immediately or 120 min after the first sessions. In one set of experiments, to evaluate a potential disruption of social recognition by adult rats of juvenile conspecifics, drugs or vehicle were administered s.c. 30 min before testing without a delay between the sessions. A significant increase in T2 in the absence of a significant alteration in T1 was considered as a loss of social recognition. In another set of experiments, the second 5-min session was performed 120 min after the first session (spontaneous loss of recognition) either with the same juvenile (for evaluation of potential "promnesic" actions) or with a different one (control for specificity of drug action). Drugs were administered s.c. 1 min after the first 5-min session. The difference, "T2 – T1," was calculated. In further experiments, rats received s.c. injections of drugs or vehicle 45 min before testing followed by scopolamine (1.25 mg/kg s.c.) or vehicle 30 min before testing. The second session was performed just after the first one.

**Influence of FCX Microinjection of S33138 upon Delay-Induced Loss of Social Recognition.** Adult rats were implanted with bilateral stainless steel guide cannulae above the FCX or the striatum. Each guide cannula (Plastics One, Roanoke, VA) consisted of two 22-gauge metal tubes (inner diameter, 0.39 mm). They were either located 1.5 mm apart, projecting 3 mm from the plastic square pedestal for the FCX, or 5 mm apart, projecting 5 mm for the striatum. For implantation of cannulae, rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). The guide cannula was mounted on the arm of the frame and then lowered to the following coordinates from bregma: FCX, AP, +3.0; DV, −2.3, L, ±0.7; and striatum, AP, +0.5; DV, −4.0, L, ±2.5. The cannula was then fastened with dental cement and stainless steel screws. Dummy stylets (Plastics One) were introduced into the guide cannulae to prevent occlusion. Animals were housed individually and allowed to recover for at least 1 week before testing. After recovery, they were handled to minimize stress associated with infusion. On the day of the test, rats were gently restrained, whereas the dummy stylets were removed and replaced with a 28-gauge (inner diameter, 0.18 mm; outer diameter, 0.36 mm) stainless steel double injector extending 1.0 mm beyond the tip of the guide cannulae (Plastics One). The injectors were connected to two 10-μl precision syringes mounted in a infusion pump (Harvard Apparatus, Holliston, MA). S33138 or vehicle were infused bilaterally in a volume of 1.0 μl over 2 min, 1 min after the first 5-min session, which was followed 120 min later by the second session. The cannulae were left in place for a further 2 min before being removed.
Extracellular Levels of ACh, Monoamines, and Amino Acids in FCX Dialyses of Freely Moving Rats. Dialysis experiments employed methods essentially as described previously (Millan et al., 2000, 2007; Di Cara et al., 2007). Rats were implanted in the FCX (AP, +2.2; DV, −0.2; L, ±0.6) with a guide cannula. They were then single-housed and permitted to recover for 5 days. For dialysis, a cuprophane CMA/11 probe (4 mm in length; outer diameter, 0.24 mm) was lowered into position. It was perfused at 1 µl/min with a phosphate-buffered solution of NaCl (147.2 mM), KCl (4 mM), and CaCl₂ (2.3 mM) at pH 7.3 (for determination of ACh levels, 0.1 µM neostigmine was added to this solution). Two hours after implantation, the collection of 20-µl dialysate samples (every 20 min) was initiated. Three basal samples (defined as 100%) were taken before s.c. administration of drugs or vehicle and dialysis continued for a further 3 h. In three separate sets of experiments, levels of ACh, monoamines, or amino acids were quantified. For quantification of ACh, dialysates were collected on 10 µl of acetic acid (0.01%) and analyzed by HPLC. The mobile phase was Na₂HPO₄ (25 mM) and Proclin (BAS, West Lafayette, IN) (0.5%), adjusted to pH 8.2 with H₂PO₄. The stationary phase comprised a cation ion exchanger (Seph- stik, 530 × 1 mm, particle size, 10 µm) (BAS) and a “postcolumn” (postimmobilized enzyme reactor, 50 × 1 mm) of choline oxidase/ AChE (BAS) maintained at 35°C. An amperometric detector (De- cade, Antec-Leyden, The Netherlands) was employed for quantification. The electrode was set at +100 mV versus Ag/AgCl. The glassy carbon electrode (MP2098, BAS) was coated with a peroxidase-redox polymer. The mobile phase was delivered at 0.14 ml/min. Assay limit of sensitivity for ACh was 5.5 fmol in a 20-µl sample. Dialysate levels of dopamine (DA), noradrenaline (NA), and 5-HT were simulta- neously quantified by HPLC followed by amperometric detection. Dopamine, NA, and 5-HT were separated on a reverse-phase column (MD150, ×2 mm; particle size, 3 µm; ESA Inc., Chelmsford, MA) maintained at 28°C. The mobile phase consisted of Na₂HPO₄ (75 mM), EDTA (20 µM), sodium decanesulphonate (1.1 mM), methanol (17%), and triethylamine (0.01%) at pH 5.70 and delivered at a flow rate of 0.2 ml/min. Electrochemical detection (Coulochem II; ESA) was performed by use of a glassy carbon electrode (5041 cell, +270 mV versus a palladium reference electrode). Assay limit of sensitivity was 0.6 fmol per 20-µl sample for DA, NA, and 5-HT in each case. Glutamate, glycine, and GABA were derivatized using naphthalene dicarboxaldehyde as a fluorophore. The derivatives were fluoro- metrically detected (FP2020plus; Jasco, Bouguenais, France) (exci- tation, 420 nm; emission, 490 nm) after separation by linear gradient chromatography (100% A/0% B to 60% A/40% B over 40 min) using a reverse-phase column (Hypersil BDS C18, 250 × 2.0 mm; particle size, 5 µm) (Thermo Electron Corporation, Courtabeuf, France) maintained at 40°C with a flow rate of 0.35 ml/min. Mobile phases were: A, ammonium acetate (50 mM, pH 6.8) plus tetrahydrofuran (3%); and B, ammonium acetate (50 mM, pH 6.8) plus acetonitrile (60%). Assay limit of sensitivity was 1 fmol per 20-µl sample.

Extracellular Levels of Histamine in FCX Dialyses of Freely Moving Rats. Male Wistar rats weighing 280 to 350 g (Harlan, Zeus, The Netherlands) were anesthetized with isoflurane (2%, 400 ml/min N₂O, 400 ml/min O₂), and an I-shaped guide probe (AN 69 membrane, 4-mm exposed surface) (Hospal, Bologna, Italy) was inserted into the FCX: AP, +3.4; DV, −5.0; L, ±0.8. Experi- ments were performed 24 to 48 h later employing perfusion of arti- ficial CSF containing NaCl (147 mM), KCl (3.0 mM), CaCl₂ (1.2 mM), and MgCl₂ (1.2 mM) at a flow rate of 1.5 l/min. Dialysate samples were collected every 20 min online in an HPLC loop and injected automatically onto a reversed-phase column (C₁₈ Hypersil, 100 × 2.0 mm, 3-µm particle size; Bester, Amstelveen, The Netherlands). The mobile phase (KH₂PO₄, 160 mM; methanol, 1%; 1- octanesulfonic acid, 0.4 mM; EDTA, 0.1 mM; and 0.53 mM kathon, pH 4.5) was delivered at a flow rate of 0.5 ml/min. After separation, histamine was derivatized postcolumn by mixing the mobile phase with a 0.002% m/v solution of o-phtaldialdehyde in NaOH (0.15 M). The flows of mobile phase and derivatization reagent were combined by a T-piece leading to a metal mixing coil (inner diameter, 0.55 mm; outer diameter, 1.1 mm; length, 1 m; dimensions, 3 × 10 cm). The flow rate was 0.5 ml/min. The derivatization reaction was performed at ambient temperature. Histamine was quantified by fluorescence (Shimadzu RF-10A, BB’s Hertogen Bosch, The Netherlands) (exci- tation, 350 nm; emission, 450 nm). Assay limit of sensitivity was 1 fmol per 20-µl sample.

Induction of Catalysis in Rats. As described previously (Mil- lan et al., 1998), the left and right hind paws of rats were placed over the corresponding forepaws, and the duration of this position was determined, with a maximum possible duration of 30 s. Three inde- pendent measures were made, separated by 1-min intervals. Drugs or vehicle were administered s.c. 30 min before testing.

Circulating Levels of Prolactin in Rats. PRL levels were determined as previously (Millan et al., 1998) in plasma 30 min after s.c. application of drugs or vehicle. Levels of PRL were determined by radioimmunoassay employing a highly selective antibody against rat PRL that displayed <0.1% cross-reactivity to all other hormones tested (RPA553; GE HealthCare, Little Chal- font, Buckinghamshire, UK).

Drug Evaluation, Salts, and Sources. Full dose-response relations were evaluated for S33138 and, in most procedures, haloperi- dol, clozapine, olanzapine, and risperidone. All drug doses are in terms of the base. For s.c. administration, drugs were dissolved in sterile water, to which a few drops of lactic acid were added, and the pH was adjusted to neutrality (~5.0). They were injected in a volume of 1 (rats) or 10 (mice) ml/kg. For p.o. administration, drugs were suspended in distilled water plus a few drops of Tween 80 and administered at 10 ml/kg. Drug structures, sources, and salts were 2-aminophenetidine sulfate (Calais Chimie, Calais, France); cocaine HCl (Coopérative Pharmaceutique Française, Melun, France); and cloza- pine, dizocilpine maleate, apomorphine HCl, haloperidol, ketamine HCl, phencyclidine HCl, and scopolamine HCl (Sigma-Aldrich, St. Quentin-Fallavier, France). S33138 HCl was synthesized by G. La- vielle (Servier, Croissy-sur-Seine, France), and olanzapine and risperidone were synthesized by J.-L. Pégillon (Servier).

Results

Inhibition by S33138 of Conditioned Avoidance Responses in Rats (Fig. 1A; Table 1). Upon both s.c. and p.o. administration and across comparable dose-response ranges (0.63–20.0 and 2.5–10.0, respectively), S33138 dose-dependently decreased CARs in rats (Fig. 1A; Table 1). Haloperidol was potently active in this procedure, and clozapine, olanza- pine, and risperidone also displayed dose-dependent, al- though less potent, actions (Table 1).

Inhibition by S33138 of the Locomotion Provoked by “Propsychotic Agents” in Rats (Fig. 1, B–D; Table 1). The increase in locomotor activity elicited by amphetamine was dose-dependently blocked by either s.c. or p.o. adminis- tration of S33138 (0.63–10.0 mg/kg in each case) (Fig. 1B; Table 1). S33138 (s.c.) also dose-dependently antagonized the induction of locomotion by cocaine (Table 1). Likewise, haloperi- dol, clozapine, olanzapine, and risperidone blocked the induction of locomotion by amphetamine and, at similar doses, cocaine. Haloperidol and risperidone were the most potent agents, and clozapine was the least potent (Table 1). Dizocilpine, PCP, and ketamine also elicited a marked loco- motor response that was dose-dependently suppressed by S33138; its actions against dizocilpine (2.5–10.0 mg/kg s.c.) were less potently expressed than against PCP (0.16–10.0 mg/kg s.c.) and ketamine (0.16–2.5 mg/kg s.c.) (Fig. 1C and D; Table 1). Haloperidol, clozapine, olanzapine, and risperi- done all suppressed the induction of locomotion by PCP and...
Fig. 1. Actions of S33138 in diverse models of potential antipsychotic activity. A, inhibition by S33138 of conditioned avoidance response in rats. B, inhibition by S33138 of the locomotor activity elicited by amphetamine (2.5 mg/kg i.p.) in rats. C, inhibition by S33138 of the locomotor activity elicited by ketamine (40.0 mg/kg s.c.) in rats. D, inhibition by S33138 of the locomotor activity elicited by PCP (20.0 mg/kg s.c.) and dizocilpine (0.16 mg/kg s.c.) in rats. E, inhibition by S33138 of apomorphine (0.75 mg/kg s.c.)-induced climbing in mice. F, inhibition by S33138 of apomorphine-induced disruption of prepulse inhibition in rats. Locomotion measurements were made over 60 min except for ketamine (20 min). VEH, vehicle. Data are means ± S.E.M. n = 5 to 10 per value. ANOVA as follows. B, S33138 (s.c.) versus amphetamine, F(3,41) = 8.79, P < 0.01 and S33138 (p.o.) versus amphetamine, F(3,20) = 13.4, P < 0.01. C, S33138 versus ketamine, F(3,30) = 2.93, P < 0.05. D, S33138 versus PCP, F(4,37) = 4.62, P < 0.05 and S33138 versus dizocilpine, F(3,23) = 4.59, P < 0.01. E, significance of differences from vehicle values in a paired Wilcoxon test (A), in a Fisher’s exact probability test (B), and in Dunnett’s test following ANOVA (B–D). F, two-way ANOVA as follows: apomorphine (0.75 mg/kg s.c.), F(1,47) = 92.4, P < 0.01; S33138, F(4,47) = 3.1, P < 0.05; and interaction, F(4,47) = 6.6, P < 0.01. Open asterisk, significant difference of vehicle/apomorphine to vehicle/vehicle values; closed asterisk, significant difference of S33138/apomorphine to vehicle/apomorphine values in Newman-Keuls test. * P < 0.05.
ketamine, although the latter three drugs were less potent against dizocilpine (Table 1).

Inhibition by S33138 of the Climbing Behavior Elicited by Apomorphine in Mice (Fig. 1E; Table 1). The D2/D3 receptor agonist, apomorphine, elicited climbing behavior in mice. This response was dose-dependently (0.04–2.5 mg/kg s.c.) blocked by S33138 (Fig. 1E; Table 1). The induction of climbing was also abrogated by haloperidol, olanzapine, risperidone, and, less potently, by clozapine (Table 1).

Prevention by S33138 of the Perturbation of Prepulse Inhibition by Apomorphine in Rats (Fig. 1F). PPI was disrupted by apomorphine; that is, as measured by the startle reflex, apomorphine interfered with adaptation of rats to an auditory stimulus (118 db) induced by pre-exposure to the same tone. Although S33138 did not itself significantly modify PPI, it dose-dependently (0.04–2.5 mg/kg s.c.) abolished the perturbation of PPI by apomorphine. Comparable findings have been documented under these conditions for haloperidol, clozapine, olanzapine, and risperidone (Geyer et al., 2001).

**Lack of Disruption by S33138 of Performance in a Passive Avoidance Procedure in Rats (Fig. 2).** In a PAV test in rats, administration of S33138 (0.04–10.0 mg/kg s.c.) before the training session on day 1 did not modify performance (latency to enter the dark compartment). Furthermore, on the following day, during the retention session, performance was not modified. In distinction, haloperidol elevated the latency to respond during training and dose-dependently perturbed performance during retention. Clozapine did not modify behavior during training but disrupted retention. Likewise, a reduction of retention was seen with olanzapine that biphasically elicited a nonsignificant increase in latency to enter the dark compartment during training. Only the highest dose of risperidone increased latencies during training, but it disrupted retention in the second session.

**Lack of Disruption by S33138 of Performance in the Five-Choice Serial Reaction Time Test in Rats (Table 2).** At doses of 0.04 to 2.5 mg/kg, S33138 did not interfere with correct responses. It also did not modify the number of anticipatory responses, although the highest dose increased omissions (Table 2). Haloperidol (0.01–0.16 mg/kg) also did not affect percent correct responses, although there was a tendency toward a decrease at the highest dose. It elicited a bifasic increase in anticipatory responses at intermediate doses and markedly increased omissions. Clozapine significantly reduced response accuracy, and, although it did not affect anticipatory responses, it increased omissions. Olanzapine likewise reduced correct responses without affecting anticipatory responses, and it increased omissions at the highest dose. Risperidone reduced accuracy and, like haloperidol, showed a bifasic increase in anticipatory responses. It dose-dependently increased omissions.

**Lack of Disruption by S33138 of Social Recognition in Rats.** After vehicle, and in the absence of an intersession interval, there was a marked reduction in the duration of social interaction during the second (T2) as compared with the first (T1) session; values of 112 ± 6 and 42 ± 5 s, respectively (P < 0.01). At doses of 0.16 to 2.5 mg/kg, S33138 did not markedly modify values of T1, T2, and T2 – T1. In contrast to scopolamine (see below), demonstrating that there was no deficit in recognition, S33138 did not signifi-

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**TABLE 1**

Summary of the actions of S33138 compared to haloperidol, clozapine, olanzapine, and risperidone in models of potential antipsychotic properties. All antipsychotics were administered s.c. The first line gives the EC50 (CAR and APO), IC50 (A-LOC, C-LOC, P-LOC, and D-LOC), or minimal effective dose (catalepsy (CATAL) and prolactin (PRL)). The second line shows the maximal observed effect (in percentage) followed by the maximally effective dose in brackets, with the exception of prolactin where maximal absolute levels are given in nanograms/milliliter. As can be deduced from the maximal n = 5–6 per dose) exerted highly significant effects in all models (P < 0.05 in ANOVA in every case), so values are omitted for reasons of space. For cocaine (effect of S33138 not shown).

<table>
<thead>
<tr>
<th>Drug</th>
<th>CAR A-LOC</th>
<th>APO C-LOC</th>
<th>LOC P-LOC</th>
<th>D-LOC CATAL</th>
<th>PRL</th>
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<td>S33138</td>
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<td>0.32 (0.25)</td>
<td>0.02 (0.01)</td>
<td>0.08 (0.01)</td>
<td>0.04 (0.01)</td>
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<td>0.02 (0.01)</td>
<td>0.08 (0.01)</td>
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<td>0.08 (0.01)</td>
<td>0.04 (0.01)</td>
<td>0.08 (0.01)</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>0.08 (0.01)</td>
<td>0.02 (0.01)</td>
<td>0.04 (0.01)</td>
<td>0.02 (0.01)</td>
<td>0.04 (0.01)</td>
</tr>
<tr>
<td>Risperidone</td>
<td>0.04 (0.01)</td>
<td>0.02 (0.01)</td>
<td>0.04 (0.01)</td>
<td>0.02 (0.01)</td>
<td>0.04 (0.01)</td>
</tr>
</tbody>
</table>

CAR, inhibition of conditioned avoidance responses in rat; APO, inhibition of climbing behavior elicited by apomorphine (0.75 mg/kg s.c.) in mice; LOC, inhibition of locomotion elicited in rats by amphetamine (2.5 mg/kg i.p.), cocaine (20.0 mg/kg i.p.), phencyclidine (20.0 mg/kg s.c.), and dizocilpine (0.16 mg/kg s.c.).
cantly increase T2 values; vehicle, 42 ± 5 s; S33138 (0.16), 57 ± 6 s; S33138 (0.63), 45 ± 6 s; and S33138 (2.5), 25 ± 7 s; F(3,28) = 4.6, P < 0.05, no difference of S33138 from vehicle in Dunnnett's test. Haloperidol likewise did not increase T2 values at doses of 0.0025 to 0.08 mg/kg, although higher doses could not be tested because of motor effects that reduced both T2 and T1 values (data not shown). In contrast, clozapine (0.16–2.5 mg/kg) augmented T2 values without affecting T1 values at a dose of 0.63, leading to a marked reduction in T2 – T1; that is, clozapine reduced recognition: vehicle, T1 = 122 ± 10 s and T2 = 44 ± 5 s and clozapine, T1 = 111 ± 8 s and T2 = 80 ± 11 s, influence of session, F(1,27) = 181.3, P < 0.01; influence of clozapine, F(1,27) = 1.5, P > 0.05 and interaction, F(1,27) = 14.5, P < 0.01. The difference of clozapine versus vehicle for T2 (but not T1) was significant in Newman-Keuls test (P < 0.01). A further increase in the dose of clozapine to 1.25 mg/kg s.c. markedly decreased T2 values due to sedation (data not shown). Furthermore, olanzapine (0.16–1.25 mg/kg) dose-dependently reduced both T2 and T1 values (data not shown), reflecting perturbation of motor function. Finally, at doses of 0.01 to 0.16 mg/kg s.c., risperidone did not affect T2, but doses of 0.04 and 0.16 reduced T1 due to motor actions (data not shown).

Specific Enhancement of Social Recognition by S33138 in a Procedure with a 120-Min Intersession Delay (Fig. 3). When the intersession interval was prolonged to 2 h, adults "spontaneously" failed to recognize the juvenile, and the difference in time of active interaction between the two sessions (T1 – T2) was low. In a typical experiment, in the presence of vehicle, T1 and T2 values were 94 ± 9 and 91 ± 8 s, respectively (P > 0.05, paired Student's t test). S33138 dose-dependently (0.16–2.5 mg/kg s.c.) diminished the duration of exploration during the second session (negative values of T2 – T1), indicating improved recognition. Even at the most effective dose (2.5 mg/kg s.c.), S33138 did not affect the time of exploration of a novel juvenile rat, underpinning the specificity of its influence upon cognitive processes. A similar pattern of enhanced recognition was seen upon oral administration of S33138 (0.63–5.0 mg/kg p.o.). In distinction, although haloperidol reduced T2 – T1, this action was nonspecific inasmuch as it provoked an identical reduction in exploration with a novel juvenile. Although clozapine reduced the duration of exploration of a familiar juvenile at doses of 0.63 and 1.25 mg/kg s.c., it also reduced the exploration of a novel juvenile: no significant difference of clozapine/different juvenile versus clozapine/same juvenile values. Likewise, neither olanzapine nor risperidone specifically improved recognition in this procedure. When administered s.c. (once a day for 5 days) at a dose of 2.5 mg/kg s.c., S33138 specifically enhanced recognition with effects similar to those observed after acute administration, T2 – T1 s, vehicle/same juvenile = 9.7 ± 2.3 s (n = 6); S33138/same = −37 ± 4.9 (6), vehicle/different juvenile = 2.3 ± 6.3 (6); and S33138/different juvenile = 2.8 ± 3.6 (6). Two-way ANOVA values were as follows: influence of juvenile, F(1,20) = 12.8, P < 0.01; influence of S33138, F(1,20) = 26.1, P < 0.01; and interaction, F(1,20) =
risperidone, dose-dependently (0.04–2.5 mg/kg/s.c.). Microinfusion of S33138 into the FCX (Fig. 4).

Blockade by S33138 of the Disruption of Social Recognition upon Scopolamine (Fig. 5). The muscarinic antagonist, scopolamine (1.25 mg/kg/s.c.), reduced T2 – T1 values, reflecting a disruption of social recognition. This action was blocked by S33138 upon both s.c. and p.o. administration (0.04–0.63 and 0.63–5.0 mg/kg, respectively). Inasmuch as haloperidol, olanzapine, and risperidone reduced T1 in procedures with no intersession interval, and clozapine itself reduced recognition (see above), their evaluation against scopolamine had to be restricted to modest doses. At doses that could be tested, haloperidol (0.04), clozapine (0.16), olanzapine (0.16), and risperidone (0.16) all failed to block the influence of scopolamine upon social recognition.

Elevation by S33138 of Acetylcholine Levels in the FCX of Freely Moving Rats (Fig. 6). S33138 potently elicited a dose-dependent (0.01–0.63 mg/kg/s.c.) elevation in levels of ACh in the FCX. In contrast, over a comparable dose range (s.c.), no elevation in ACh levels was provoked in dorsal hippocampus (data not shown). In contrast to S33138, haloperidol did not affect ACh levels in the FCX, whereas they were likewise elevated by clozapine, olanzapine, and risperidone (all at 0.63 mg/kg/s.c.). Values (area under the curve analysis) were as follows: vehicle, 120 ± 5.7%; haloperidol, 119.9 ± 8.7%; F(1,10) = 0.1, P < 0.05; clozapine, 162.2 ± 11.5%; F(1,10) = 6.5, P < 0.05; olanzapine, 195.5 ± 17.6%; F(1,9) = 19.8, P < 0.01; and risperidone, 159.4 ± 11.2%; F(1,9) = 15.6, P < 0.01.

Elevation by S33138 of Extracellular Levels of Histamine but Not Monoamines or Amino Acids in FCX (Fig. 6). S33138 dose-dependently elevated levels of histamine in the FCX, albeit at doses (2.5–10 mg/kg s.c.) higher than those that increased levels of ACh. The influence of D2 versus D3 receptor antagonists upon histamine levels in FCX has not, to date, been documented. Thus, we undertook a further experiment with the selective D3 antagonist, S33084, and the preferential D2 antagonist, L741,626, at doses exerting maximal effective actions at D3 and D2 sites, respectively (Milan et al., 2000). Area under the curve analysis over 180 min values were as follows: vehicle, 3.09 ± 0.65 ng/ml; S33084 (0.63 mg/kg s.c.), 3.54 ± 1.6 ng/ml; and L741,626 (10.0 mg/kg s.c.), 11.85 ± 1.71 ng/ml, F(2,13) = 14.7, P < 0.01, L741,626 versus vehicle, P < 0.01 in Dunnett’s test. In contrast to histamine, S33138 did not significantly modify levels of DA, 5-HT, NA, GABA, glutamate, or glycine.

**Influence of S33138 upon Spontaneous Locomotor Activity (Table 3).** In rats habituated to observation chambers for 30 min, that is, under conditions in which the hyperlocomotion elicited by amphetamine and other drugs was examined, all drugs reduced spontaneous locomotor activity. Haloperidol, olanzapine, and risperidone all acted very potently, and, with the exception of clozapine, S33138 was the least potent drug. In mice, all drugs likewise reduced locomotor activity: risperidone and olanzapine were highly active, and S33138 was the least potent drug. In a Rotorod procedure, haloperidol, olanzapine, and risperidone were highly active, and S33138 was, together with clozapine, the least potent agent.

**Induction of Catalepsy by S33138 (Fig. 7A).** Even at the highest tested dose (40.0), S33138 elicited only “submaximal” catalepsy relative to the cut-off of 30 s. In contrast, haloperidol potently elicited a pronounced catalepsy. Risperidone showed a similar profile of marked catalepsy, likewise exerting its actions over a lower dose range than S33138. Induction of catalepsy by olanzapine was seen at doses slightly lower than those of S33138 and with a slightly higher maximal effect. Clozapine did not elicit catalepsy.

**Influence of S33138 upon Circulating Levels of Pro lactin in Rats (Fig. 7B).** S33138 dose-dependently increased circulating levels PRL, with a peak effect at a dose of 2.5. Haloperidol also elevated levels of PRL, exerting its actions more potently than S33138 and with a greater maximal effect. Risperidone potently elevated levels of PRL, albeit with a maximal effect comparable with that of S33138. Olanzapine increased PRL levels over a dose range similar to S33138 but with a more pronounced maximal effect. Cloza-
pine exerted little influence upon PRL levels, except at the dose of 20.0 mg/kg.

Discussion

Actions of S33138 in Models of Potential Antipsychotic Properties. Inasmuch as selective D₂ but not D₃ receptor antagonists block CARs in rats and apomorphine-induced climbing in mice, D₂ receptor blockade probably underlies their inhibition of S33138, although its modest antagonist properties at 5-HT₂A receptors and α₁C-ARs may fulfill facilitatory roles (Millan et al., 2000; Wadenberg et al., 2001, 2007; Meltzer et al., 2003; Svensson, 2003; Kapur et al., 2006). Psychostimulant-elicited locomotion is also mediated...
Dopamine D<sub>3</sub> Receptors and Schizophrenia

Fig. 5. Blockade by S33138 of the disruption of social recognition in rats by scopolamine: a comparison with haloperidol, clozapine, olanzapine, and risperidone. A, S33138 s.c. B, S33138 p.o. C, haloperidol. D, clozapine. E, olanzapine. F, risperidone. VEH, vehicle. Data are means ± S.E.M. n = 5 to 12 per value. Two-way ANOVA as follows. S33138 s.c., scopolamine, F(1,51) = 16.4, P < 0.01; drug, F(3,51) = 7.3, P < 0.01; and interaction, F(3,51) = 9.7, P < 0.01. S33138 p.o., scopolamine, F(1,51) = 26.4, P < 0.01; drug, F(3,51) = 7.3, P < 0.01; and interaction, F(3,51) = 9.7, P < 0.01. Haloperidol, scopolamine, F(1,20) = 24.5, P < 0.01; drug, F(1,20) = 0.1, P > 0.05; and interaction, F(1,20) = 3.0, P > 0.05. Clozapine, scopolamine, F(1,20) = 162.3, P < 0.01; drug, F(1,20) = 1.5, P > 0.05; and interaction, F(1,20) = 0.1, P > 0.05. Olanzapine, scopolamine, F(1,19) = 249.0, P < 0.01; drug, F(1,19) = 1.9, P > 0.05; and interaction, F(1,19) = 0.8, P > 0.05. Risperidone, scopolamine, F(1,19) = 281.5, P < 0.01; drug, F(1,19) = 17.8, P < 0.01; and interaction, F(1,19) = 0.6, P > 0.05. Open asterisks, significance of differences between vehicle/scopolamine versus vehicle/scopolamine values; closed asterisks, significance of differences between values for antipsychotic/scopolamine versus vehicle/scopolamine in Newman-Keuls test; *, P < 0.05.

by D<sub>3</sub> versus D<sub>2</sub> receptors (Millan et al., 2000; Reavill et al., 2000; Kapur et al., 2006), so the interruption of cocaine and amphetamine-induced hyperactivity by S33138 principally reflects its D<sub>2</sub> antagonist properties. In certain studies, genetic deletion of D<sub>3</sub> receptors enhanced the motor actions of amphetamine and cocaine reflecting the following: 1) loss of postsynaptic D<sub>3</sub> sites inhibitory to motor function and/or 2) inactivation of D<sub>3</sub> autoreceptors, by analogy to the enhancement of amphetamine-induced DA release by D<sub>2</sub>/D<sub>3</sub> antagonists (Bahia et al., 2005; Chen et al., 2005). Nonetheless, D<sub>3</sub> receptor gene deletion does not invariably enhance the effects of psychostimulants (Karaiskou et al., 2005), and, by analogy to selective D<sub>3</sub> receptor antagonists (Millan et al., 2000; Reavill et al., 2000), S33138 did not potentiate cocaine or amphetamine-induced locomotion. Furthermore, mimicking D<sub>3</sub> receptor antagonists (Heidbreder et al., 2005), S33138 reduces cocaine-seeking behavior in rats (C.R. Ashby Jr., unpublished observation), and D<sub>3</sub> receptor blockade counters the sensitization elicited by chronic exposure to psychostimulants, a process related to the genesis of psychotic states (Richtand, 2006).

Neuronal substrates involved in the induction of hyperlocomotion by OCBs at N-methyl-D-aspartate receptors are drug-, dose-, and protocol-dependent, and both adrenergic and serotonergic as well as dopaminergic mechanisms have been implicated (Millan et al., 1999; Moore, 1999; Geyer and Ellenbroek, 2003; Svensson, 2003; Millan, 2005). Herein, suppression of OCB-induced locomotion by S33138 probably involves antagonism of D<sub>2</sub> rather than D<sub>3</sub> sites inasmuch as a similar attenuation is seen with haloperidol, whereas selective D<sub>3</sub> receptor antagonists are ineffective (Millan et al., 2000; Reavill et al., 2000). Furthermore, preferential D<sub>3</sub> receptor agonists attenuated dizocilpine-induced locomotion in rats, possibly reflecting the inhibitory influence of presynaptic and postsynaptic D<sub>3</sub> receptors upon DA release and locomotion, respectively (Clements and Greenshaw, 2005). Nonetheless, a possible role of postsynaptic D<sub>3</sub> receptor blockade justifies further study inasmuch as the induction of hyperlocomotion by dizocilpine in mice was attenuated by genetic deletion of D<sub>3</sub> receptors (Leriche et al., 2003). The modest antagonist properties of S33138 at 5-HT<sub>2</sub>A receptors probably also contribute to its inhibition of PCP-induced locomotion because, under the present conditions, this response is dependent upon mesolimbic 5-HT<sub>2</sub>A sites (Millan et al.,
1999). The greater potencies of clozapine, olanzapine, and risperidone than S33138 against PCP reflect, then, their higher affinities for 5-HT_{2A} receptors (Millan et al., 1999; Meltzer et al., 2003). The participation of 5-HT_{2A} receptor blockade by S33138 to the induction of locomotion by psychostimulants is likely less pronounced than for PCP (McMahon and Cunningham, 2001; Meltzer et al., 2003). Although a complementary (facilitatory) role of α_{2C}-AR and/or 5-HT_{7} receptor blockade cannot be excluded, this is unlikely to be of major importance (Meltzer et al., 2003; Svensson, 2003).

To summarize, the actions of S33138 in the above models support potential efficacy of S33138 against positive symptoms and principally reflect blockade of D_{2} receptors. However, these are empirical models, and D_{2} receptor antagonist properties may be correlated with, rather than causative, clinical efficacy. Furthermore, the influence of S33138 upon limbic c-fos expression and the spontaneous activity of mesolimbic dopaminergic pathways are mediated by D_{2} receptor blockade (Millan et al., 2008b). Thus, despite compelling arguments that D_{2} receptor blockade controls positive symptoms (Kapur et al., 2006), the genuine significance of D_{3} versus D_{2} sites will only become clear upon clinical trials of S33138 and other drugs differentiating D_{3} from D_{2} receptors (Joyce and Millan, 2005; Sokoloff et al., 2006).

**Actions of S33138 in Procedures Related to Cognitive Function.** Restoration of apomorphine-disrupted PPI in rats is a common property of haloperidol, clozapine, olanzapine, and risperidone (Geyer et al., 2001; Swerdlow et al., 2006), and, under essentially identical conditions, S33138 acted similarly. The PPI-interfering effects of apomorphine are resistant to D_{3} receptor antagonists (Reavill et al., 2000), which only weakly oppose spontaneous PPI deficits in DBA/2J mice (Zhang et al., 2005). Moreover, disruption of PPI by amphetamine is unaffected in mice genetically lacking D_{2} receptors, yet absent in conspecifics deprived of D_{2} receptors (Ralph et al., 1999). Thus, the effect of S33138 against apomorphine probably reflects antagonism of D_{2} receptors. Nonetheless, it would be of interest to examine its influence upon the reduction in PPI provoked by isolation rearing, which is reversed by selective antagonists at D_{3} receptors (Reavill et al., 2000; Geyer et al., 2001).

The disruption of juvenile recognition by clozapine probably reflects blockade of muscarinic receptors in light of similar effects of scopolamine (Di Cara et al., 2007; Millan et al., 2007). In contrast, S33138 alleviated the disruption of social recognition provoked by scopolamine and by a prolonged intersession delay. Selective antagonists at 5-HT_{2A} receptors, 5-HT_{7} receptors, and α_{2C}-ARs are inactive in this procedure (Kristian et al., 2005). Thus, antagonists at D_{3} receptors (Millan et al., 2007), these potent actions of S33138 presumably reflect D_{3} receptor blockade. Indeed, preferential D_{3} antagonists reduce social recognition and...
interfere with its enhancement by D₃ antagonists (Millan et al., 2007). Consistent with the role of frontocortical dopaminergic mechanisms in cognitive function (Robbins, 2002; Tanaka, 2006; El-Ghundi et al., 2007), microinjection of S33138 into the FCX improved social recognition. Although effects of frontocortical administration of mixed D₂/D₃ antagonists have proven variable in other models, this finding suggests that instances of proacognitive actions reflect blockade of D₃ rather than D₂ receptors (Passetti et al., 2001; Chudasama and Robbins, 2004; Pakdel and Rashidy-Pour, 2007). The present observations also accord with evidence that a dysfunction of the FCX contributes to disturbed social cognition in schizophrenia (Lee et al., 2006). It remains to be seen whether S33138 also modulates (social) cognition by actions in the D₃ receptor-rich nucleus accumbens, which displays reciprocal interactions with the FCX (Pezze et al., 2007; Zmarowski et al., 2007).

Reflecting antagonism of muscarinic receptors, clozapine and olanzapine interfered with PAV performance in mice, observations extended herein to rats and to risperidone, although the latter’s disruptive influence probably reflects blockade of H₁ receptors (Ninan and Kulkarni, 1996; Hagan and Jones, 2005; Hou et al., 2006). The influence of haloperidol upon PAV in mice is variable (Hagan and Jones, 2005), but Dragó et al. (1997) reported a disruption in rats, a finding resembling the present observations. Although motor perturbation complicates interpretation of its effects, a deleterious influence of haloperidol upon PAV probably reflects blockade of D₂ receptors in view of the following: 1) selective antagonists at D₃ receptors do not affect PAV procedures, and 2) the inhibitory influence of the D₂/D₃ agonist, quinpirole, upon scopolamine-induced disruption of PAV is mediated by D₃ receptors (Sigala et al., 1997). In line with these comments, S33138 did not modify PAV performance.

The deficits in response accuracy provoked by clozapine and olanzapine in a 5-CSRT procedure for monitoring attentional processes (Chudasama and Robbins, 2004) probably involve antagonism of muscarinic receptors in view of similar impairments with scopolamine and cholinergic lesions (Mirza and Stolerman, 2000; Robbins, 2002). H₁ antagonist properties may also be implicated in the deficits evoked by clozapine, olanzapine, and risperidone insomuch as cortical histaminergic pathways modulate attentional processes in the 5-CSRT paradigm (Bacciottini et al., 2001; Day et al., 2007). It is interesting to note that similar reductions in choice accuracy with clozapine and risperidone were found using a visual signal detection task (Rezvani et al., 2006).

Confirming previous work (Robbins, 2002), haloperidol did not significantly reduce accuracy in the 5-CSRT test even at doses provoking substantial increases in omissions. These results accord with the minor influence of dopaminergic lesions upon accuracy when stable intertrial intervals are used (Robbins, 2002). Moreover, introduction of the D₂/D₃ antagonist, sulpiride, into the FCX did not compromise accuracy (Granon et al., 2000). In line with these findings and by analogy to selective D₃ antagonists (F. Loiseau, unpublished observation), S33138 did not undermine accuracy. This observation differentiates S33138 from atypical antipsychotics, and it would be interesting to examine its actions in rats with lesions of the FCX in which sulpiride alleviated the disruption of response accuracy (Passetti et al., 2003).

**Influence of S33138 upon of Neurotransmitters Modulating Cognitive Function in FCX.** The above findings coincide with evidence that preferential blockade of D₃ versus D₂ receptors preserves and even enhances cognitive performance (Laszy et al., 2005; Micale et al., 2006; Millan et al., 2007). Accord-

### TABLE 3

<table>
<thead>
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<th>Dose</th>
<th>Spontaneous Locomotion</th>
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<td></td>
<td>Rats</td>
<td>Mice</td>
</tr>
<tr>
<td></td>
<td></td>
<td>counts</td>
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<td>108 ± 21 (7)*</td>
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<td>53 ± 12 (5)*</td>
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<td>108 ± 21 (7)*</td>
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<td>0.05</td>
<td>54 ± 7.4 (9)*</td>
<td>108 ± 21 (7)*</td>
</tr>
<tr>
<td>Risperidone</td>
<td>0</td>
<td>54 ± 7.4 (9)*</td>
<td>108 ± 21 (7)*</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>58.9 ± 8.4 (15)</td>
<td>320 ± 15 (20)</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>49.4 ± 7.3 (7)</td>
<td>108 ± 21 (7)*</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>54 ± 7.4 (9)*</td>
<td>108 ± 21 (7)*</td>
</tr>
</tbody>
</table>

* P < 0.05, significance of differences to vehicle values in Dunnett’s test following ANOVA.
GABAergic, and monoaminergic mechanisms also control cognitive function (Robbins, 2002; Meltzer et al., 2003; Lewis and Gonzalez-Burgos, 2006), but, like selective D₃ antagonists (M. J. Millan, A. Gobert, and J.-M. Rivet; unpublished observation), S33138 failed to modify levels of amino acids, DA, NA, or 5-HT in FCX. Contrariwise, S33138 elevated frontocortical levels of histamine, which, in interaction with cholinergic mechanisms, modulates cognitive processes (Bacciotini et al., 2001; Ito, 2004; Horner et al., 2006). D₃ receptor blockade is unlikely to be involved because doses of S33138 were much higher than those affecting ACh. Moreover, mice lacking D₃ receptors show no increase in basal histaminergic activity (Morisset et al., 2002), and the selective D₃ antagonist, S33084, did not increase levels of histamine. In contrast, the preferential D₂ receptor antagonist, L741,626, enhanced histamine levels (see Results), suggesting that blockade of D₂ sites by high doses of S33138 may stimulate histamine release. One additional possibility would be the modest antagonist properties of S33138 at 5-HT₂₅ receptors, blockade of which by clozapine increases cerebral histamine turnover (Morisset et al., 1999).

**Potential Induction of Side Effects by S33138.** Selective D₃ receptor blockade does not evoke catalepsy in rodents and moderates its induction by D₂ antagonists (Millan et al., 2000, 2004; Reavill et al., 2000; Sokoloff et al., 2006; Gyertyán and Sághy, 2007). Accordingly, although S33138 elicited catalepsy, its maximal effect was less pronounced than haloperidol and risperidone, and the dose window to antipsychotic actions was more marked. This limited extrapyramidal potential of S33138 is consistent with its preferential induction of c-fos in limbic versus striatal structures, facilitation of motor function in parkinsonian primates, and a more potent influence upon ventrotegmental versus substantia nigra dopaminergic neuron firing (Millan et al., 2008b). Antagonism of α₂C-ARs (enriched in the striatum) may also participate in the low cataleptogenic potential of S33138 (Kalkman and Loetscher, 2003; Svensson, 2003; Wadenberg et al., 2007), but its 5-HT₂₅ receptor antagonist properties are of lesser importance than for clozapine, olanzapine, and risperidone (Millan et al., 1998; Meltzer et al., 2003). Thus, although S33138 shares the low extrapyramidal potential of atypical agents, the underlying mechanism (preferential D₃ versus D₂ receptor blockade) differs.

The reason for the less marked effect of S33138 than haloperidol upon PRL levels is unclear because D₂ receptor antagonism should not moderate the induction of lactotrophic secretion of PRL by D₃ receptor blockade (Millan et al., 2000; Ben-Jonathan and Hnasko, 2001). However, haloperidol behaves as an inverse agonist at constitutively active D₂ receptors controlling PRL release (Nilsson et al., 1996), and the possibility that S33138 is a “neutral” antagonist is under investigation.

**Summary and Conclusions.** S33138 can be distinguished from the comparator antipsychotics tested herein by its preferential blockade of D₃ as compared with D₂ receptors and by its negligible affinity for histamine H₁, muscarinic, and α₁-adrenergic receptors. This distinctive receptor-binding profile is associated with preservation of cognitive function, enhanced social recognition, and reinforced frontocortical cholinergic transmission. A positive influence of low “D₃ receptor” doses of S33138 upon mnemonic function is supported by ongoing studies in primate models of attention, working memory, and executive performance (M. J. Millan, J. Schneider, and J. Buccafusco, unpublished observation). S33138 is also active in rodent procedures predictive of antipsychotic properties, principally via antagonism of D₃ receptors. In contrast to haloperidol and risperidone, only high doses of S33138 provoke catalepsy, probably reflecting its preferential affinity for D₃ sites, blockade of which attenuates the induction of extrapyramidal motor effects by D₂ receptor antagonists (Joyce and Millan, 2005; Sokoloff et al., 2006; Gyertyán and Sághy, 2007). Finally, in experimental and clinical studies, in contrast to olanzapine and clozapine, S33138 elicited neither obesity nor increases in circulating levels of glucose and insulin (M. J. Millan and M. Brocco, unpublished observation). S33138 is, thus, an innovative, well tolerated, and promising candidate for the improved treatment of schizophr-
nia, especially cognitive symptoms. However, only therapeutic evaluation of S33138 (phase Iib) and similar agents can clarify the genuine significance of preferential D3 versus D2 receptor blockade to the treatment of psychotic states or other central nervous system disorders.

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References


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