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S33138, A PREFERENTIAL DOPAMINE D_3 *VERSUS* D_2 RECEPTOR ANTAGONIST AND POTENTIAL ANTIPSYCHOTIC AGENT. III. ACTIONS IN MODELS OF THERAPEUTIC ACTIVITY AND INDUCTION OF SIDE-EFFECTS

Mark J. Millan, Florence Loiseau, Anne Dekeyne, Alain Gobert, Gunnar Flik, Thomas I. Cremers, Jean-Michel Rivet, Dorothée Sicard, Rodolphe Billiras and Mauricette Brocco

Psychopharmacology Department (M.J.M., F.L., A.D., A.G., J.-M.R., D.S., R.B., M.B.),
Institut de Recherches Servier,
Centre de Recherches de Croissy, 125 Chemin de Ronde,
78290 Croissy-sur-Seine (Paris) France.

Brains-on-Line BV (G.F, T.I.C.)
Antonius Deusinglaan 1,
9713 AV Groningen,
The Netherlands.

Running Title: Dopamine D_3 receptors and schizophrenia

Abbreviations:

ACh	Acetylcholine
AR	Adrenoceptor
CAR	Conditioned avoidance response
5-CSRT	5-choice serial reaction time
DA	Dopamine
ED	Effective dose
FCX	Frontal cortex
GABA	γ-amino-butyric acid
5-HT	Serotonin
ID	Inhibitory dose
NA	Noradrenaline
NMDA	N-methyl-D-aspartate
OCB	Open channel blocker
PAV	Passive avoidance
PCP	Phencyclidine
PPI	Pre-pulse inhibition
PRL	Prolactin

Dr Mark J. Millan, Institut de Recherches Servier, Centre de Recherches de Croissy, Psychopharmacology Department, 125 Chemin de Ronde, 78290 Croissy-sur-Seine, France.
Tel: 33.1.55.72.24.25 - Fax: 33.1.55.72.20.82. mark.millan@fr.netgrs.com

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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 D_3 *vs* D_2 receptors and does not interact with histamine H_1 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 5-choice serial reaction time procedures. Further, upon either systemic administration (0.04-2.5 mg/kg, s.c.) or introduction into the frontal cortex (0.04-0.63 μg/side), S33138 potently attenuated the perturbation of social recognition by scopolamine or a prolonged inter-session 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 (0.63-10.0 mg/kg, s.c.) inhibited conditioned avoidance responses in rats, apomorphine-induced climbing in mice, and hyperlocomotion elicited by amphetamine, cocaine, dizocilpine, ketamine and phencyclidine in rats. S33138 (0.16-2.5 mg/kg, s.c.) also blocked the reduction of pre-pulse inhibition elicited by apomorphine. In comparison to the above actions, only "high" doses of S33138 (10.0-40.0 mg/kg, s.c.) elicited catalepsy. To summarize, reflecting preferential blockade of D_3 *vs* D_2 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 to clinically-available agents, S33138 displays, thus, a distinctive and promising profile of potential antipsychotic properties.

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INTRODUCTION

We have recently described a novel benzopyranopyrrolidine derivative, S33138 (Millan et al., in press, a, b) that behaves as a preferential antagonist at cloned, human and native, cerebral dopaminergic D_3 *vs* D_2 receptors. In addition, it displays modest antagonist properties at serotonin $(5\text{-HT})_{2A}$ receptors, 5-HT_7 receptors and α_{2C}-adrenoceptors (ARs), blockade of which may also be useful in the treatment of schizophrenia (Meltzer et al., 2003; Svensson, 2003). By contrast, S33138 does not interact with histamine H_1, muscarinic or α_1-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 D_2/D_3 receptor antagonist, haloperidol; the "atypical" agent, clozapine; two further multireceptorial antipsychotics, risperidone and olanzapine (Joyce and Millan, 2005; Lieberman, 2005; McEvoy et al., 2006).

Schizophrenia is characterised 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: further, it is unclear to what extent they represent a specific and primary impact 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_1-adrenergic and histamine H_1 receptors which *compromises* cognitive function (Bacciottini et al., 2001; Ito, 2004; Sarter et al., 2005). As mentioned above, S33138 possesses negligible affinity for these sites (Millan et al., in press). Its distinctive preference for D_3 *vs* D_2 receptors is also of importance since D_3 receptor blockade improves cognitive function. Conversely, D_2 receptor blockade exerts a *negative* influence (Laszy et al., 2005; Millan et al., 2007). For example selective D_3 and D_2 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 since 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 5-choice serial reaction time test (5-CSRT in rats) (Robbins, 2002; Chudasama and Robbins, 2004). Further, we determined its effects in a model of

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"aversive learning", the passive avoidance (PAV) paradigm in rats (Hagan and Jones, 2005; El-Ghundi et al., 2007). Inasmuch as D_3 receptor blockade enhances frontocortical release of acetylcholine (ACh) (Lacroix et al., 2006; Millan et al., 2007), the influence of S33138 upon extracellular levels of ACh was determined in the FCX of freely-moving rats. Moreover, we examined its influence upon levels of histamine, monoamines, glutamate and GABA, neurotransmitters likewise implicated in the control of cognition and perturbed in schizophrenia (Bacciottini et al., 2001; Ito, 2004; Millan, 2005; Lewis and Gonzalez-Burgos, 2006; Tanaka, 2006).

Positive symptoms of schizophrenia are related to a hyperactivity and/or hyper-reactivity of mesolimbic dopaminergic pathways, mimicked by administration of the catecholamine releasers, amphetamine and cocaine (Moore, 1999; Geyer and Ellenbroek, 2003; Abi-Dargham and Laruelle, 2005). Therefore, we evaluated the influence of S33138 upon the locomotion elicited by these psychostimulants in rats. Its actions were also evaluated in two further, prototypical models of the control of positive symptoms: suppression of conditioned avoidance responses (CAR) in rats, and inhibition of the induction of climbing by apomorphine in mice (Moore, 1999; Geyer and Ellenbroek, 2003; Kapur et al., 2006). Like other dopaminergic agonists, apomorphine perturbs pre-pulse inhibition (PPI) in rats: this measure of sensory gating reflects the capacity to filter extraneous sensory information and is compromised in psychotic patients (Swerdlow et al., 2006). Thus, we examined the influence of S33138 upon the disruption of PPI by apomorphine in rats. Both negative-cognitive and positive symptoms of schizophrenia are provoked in healthy subjects by N-methyl-D-aspartate (NMDA) receptor open channel blockers (OCBs) like phencyclidine (PCP) and ketamine (Moore et al., 1999; Abi-Dargham and Laruelle, 2005; Millan, 2005). Correspondingly, we evaluated the influence of S33138 upon their stimulation of locomotion in rats.

As regards potential side-effects, pharmacological, antisense and gene knockout studies indicate that preferential blockade of D_3 *vs* D_2 receptors is associated with a relatively benign impact upon motor function as compared to drugs possessing D_2/D_3 or principally D_2 antagonist properties (Joyce and Millan et al., 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). Further, though D_3 receptor antagonism is unlikely to modify the effects of blockade D_2 receptors on lactotrophs, the impact of S33138 upon circulating levels of prolactin (PRL) was determined since hyperprolactinaemia is a problematic side-effect of antipsychotics (Ben-Jonathan and Hnasko, 2001; Turrone et al., 2002; Shirzadi and Ghaemi, 2006).

METHODS

Animals. Unless otherwise specified, male Wistar rats weighing 225-250 g and male NMRI mice weighing 22-25 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 hr/12 hr light-dark cycle, with lights on from 7:30 to 19:30 h. Prior to 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 sec) 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-sec period with the light "on". When the subject did not change compartments, it received the shock. However, when it switched within the 10-sec 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 following control sessions and undertaken once a week. Drugs or vehicle (control session) were administered 30 min prior to testing.

Apomorphine-induced climbing in mice. Climbing behaviour was examined in male CD mice weighing 22-25 g (Charles River, Saint-Aubin-les-Elbeuf, France) 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 0-2 (Millan et al., 1998). The total of two measures made 10 and 20 min following apomorphine (0.75 mg/kg, s.c.) was determined. Drugs or vehicle were administered s.c. 30 min prior to apomorphine.

Amphetamine, cocaine, ketamine, PCP and dizocilpine-induced hyperlocomotion, and spontaneous locomotion, in rats. As previously (Millan et al., 1998), rats were administered with drugs or vehicle (s.c.) and placed for a 30-min habituation period in polycarbonate cages (45 x 30 x 20 cm) in activity chambers (Lablinc System, Coulbourn, Lehigh Valley, PA). Subsequently, animals received vehicle ("spontaneous locomotion"), amphetamine (2.5 mg/kg, i.p.), cocaine (20.0, i.p.), ketamine (40.0, s.c.), PCP (20.0, s.c.) or dizocilpine (0.16, s.c.) and locomotion monitored over 60 min. A "locomotor count" was consecutive interruption of 2 infrared beams 24 cm apart and 4 cm above the cage floor.

Spontaneous locomotion in mice. Thirty min following (s.c.) injection of drugs or vehicle, mice were placed for 10 min in individual, white Plexiglas chambers (27 x 27 x 27 cm) furnished with two rows of 4 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.

Rotarod test in mice. Latency to fall from an accelerating (4-40 rpm over 300 sec) rotarod (Ugo Basile, Varese, Italy) was determined (cut-off of 360 sec). S33138, haloperidol, clozapine, olanzapine and risperidone or vehicle were given (s.c.) 30 min prior testing.

Disruption of pre-pulse inhibition by apomorphine in rats. The procedure used was essentially that used elsewhere (Geyer et al., 2001). Male Sprague-Dawley rats (300-400g) (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[A] background noise, the animal was submitted to a series of startle trials consisting of several conditions: 1) a 118 dB[A] 40 msec noise burst presented alone (P-ALONE), or 2) the same 118 dB[A] 40 msec noise burst preceded at a 100 msec interval by prepulses (20 msec noise) 12 dB[A] above background. A variable intertrial interval averaged 15 sec. PPI was defined as the % reduction in startle amplitude in the presence of prepulse *vs* absence of prepulse (100 x amplitude on prepulse trial/ amplitude on P-ALONE trial).

Passive avoidance procedure in rats. Male Sprague-Dawley rats weighing 250-275g were used. The procedure consisted of two sessions performed over two 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 x 31 x 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 x 11 x 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 allowed 5 min (cut-off latency) to enter the small, dark compartment. Once the rat had entered, the door was closed and two seconds later, an inescapable, constant current, scrambled shock (0.40 mA) was delivered for 5 sec. The rat was then removed and placed in its home cage. On Day 2 (“retention” session), 24 hours 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 on Day 1 (training) and on Day 2 (retention).

Five-choice serial reaction time test in rats. The procedure was adapted from Robbins (2002). Single-housed and food-restricted rats were trained daily in operant chambers (Med Associates, Georgia, VT) to detect a brief light stimulus (150 Lux, 0.5 sec) presented randomly in one of five apertures of a spatial array. Each daily session consisted of 100 trials. For each trial, a nose poke 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 sec (omission), or responses made prior to the onset of the stimulus during the inter-trial interval ("ITI") (anticipatory response) caused a brief period of darkness (time out with house-lights off, 5 sec). After a pre-training 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, 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, St. Albans, VT).

Social recognition test in rats. As described (Millan et al., 2007), adult Wistar rats weighing 240-260 g and juvenile Wistar rats (25-30 days old) (Janvier, Le Genest-Saint-Isle, France) 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 habituation, a juvenile was placed into the home cage for a 5 min observation session and time spent in active social investigation ("T1") evaluated: that is, the time devoted by the adult rat to sniffing, following, biting, jumping and crawling over or under the juvenile. Following 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 one week before testing. Following recovery, they were handled to minimize stress associated with infusion. On the day test, rats were gently restrained while 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 dialysates 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 cuprophan CMA/11 probe (4 mm in length, other 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 $CaCl_2$ (2.3 mM) at pH, 7.3. (For determination of ACh levels, 0.1 µM of 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 prior to s.c. administration of drugs or vehicle and dialysis continued for a further 3 hours. In 3 separate sets of experiments, levels of ACh, monoamines *or* amino acids were quantified. For quantification of ACh, dialysates were collected on 10 µl acetic acid (0.01 %) and analysed by HPLC. The mobile phase was Na_2HPO_4 (25 mM) and Proclin (BAS, West Lafayette, IN) (0.5 %), adjusted to pH 8.2 with H_3PO_4. The stationary phase comprised a cation ion exchanger (Sepstik, 530 x 1 mm, particle size, 10 µm) (BAS) and a "post-column" (post-immobilised enzyme reactor, 50 x 1 mm) of choline oxydase/AChE (BAS) maintained at 35° C. An amperometric detector

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(Decade, Antec-Leyden, Netherlands) was employed for quantification. The electrode was set at + 100 mV *vs* Ag/AgCl. The glassy carbon electrode (MF2098, BAS) was coated with a peroxydase-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 DA, noradrenaline (NA) and 5-HT were simultaneously quantified by HPLC followed by amperometric detection. Dopamine, NA and 5-HT were separated on a reverse-phase column (MD150 x 2 mm, particle size, 3 µm (ESA Inc, Chelmsford, MA) maintained at 28° C. The mobile phase consisted of NaH_2PO_4 (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 *vs* 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 naphtalene dicarboxaldehyde as a fluorophore. The derivatives were fluorimetrically detected (FP2020plus, Jasco, Bouguenais, France) (Ex: 420 nm; Em: 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 C_{18}, 250 x 2.0 mm, particle size, 5 µm) (Thermo Electron corporation, Courtaboeuf, 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 tetrahydrofurane (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 dialysates of freely-moving rats. Male Wistar rats weighing 280-350 g (Harlan, Zeist, Netherlands) were anesthetized with isoflurane (2 %, 400 ml/min N_2O, 400 ml/min O_2) and an I-shaped guide probe (AN 69 membrane, 4 mm exposed surface) (Hospal, Bologna, Italy) inserted into the FCX: AP: + 3.4, DV: - 5.0; L: ± 0.8. Experiments were performed 24-48 hours later employing perfusion of artificial CSF containing NaCl (147 mM), KCl (3.0 mM), $CaCl_2$ (1.2 mM) and $MgCl_2$ (1.2 mM) at a flow rate of 1.5 µl/min. Dialysate samples were collected every 20 min online in an HPLC loop and injected automatically onto a reversed-phase column (C_{18} Hypersil, 100 x 2.0 mm, 3 µm particle size (Bester, Amstelveen, Netherlands). The mobile phase (KH_2PO_4, 160mM; methanol, 1%; 1-octanesulfonic acid, 0.4mM; EDTA, 0.1mM and ± 0.33 ml/l kathon, pH 4.5) was delivered at a flow-rate of 0.5 ml/min. After separation, histamine was derivatised post-column 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 derivatisation 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 x 10 cm). The flow rate was 0.5 ml/min. The derivatisation reaction was performed at ambient temperature. Histamine was quantified by fluorescence (Shimadzu RF-10A, BB'S Hertogen Bosch, Netherlands) (Ex: 350 nm and Em: 450 nm). Assay limit of sensitivity was 1 fmol per 20 µl sample.

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Induction of catalepsy in rats. As described previously (Millan 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 sec. Three independent measures were made, separated by 1 min intervals. Drugs or vehicle were administered s.c. 30 min prior to testing.

Circulating levels of prolactin in rats. PRL levels were determined as previously (Millan et al., 1998) in plasma 30 min following 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 Health Care, Buckingham, England).

Drug evaluation, salts and sources. Full dose-response relationships were evaluated for S33138 and, in most procedures, haloperidol, 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 adjusted to neutrality (> 5.0). They were injected in a volume of 1 ml/kg (rats) or 10 ml/kg (mice). 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 d-amphetamine sulfate (Calais Chimie, Calais, France); cocaine HCl (Coopérative Pharmaceutique Française, Melun, France); clozapine, dizocilpine maleate, apomorphine HCl, haloperidol, ketamine HCl, phencyclidine HCl and scopolamine HCl (Sigma, St Quentin-Fallavier, France). 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) HCl was synthesised by G. Lavielle (Servier), and olanzapine and risperidone by J.-L. Péglion (Servier).

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RESULTS

Inhibition by S33138 of conditioned avoidance responses in rats (Fig. 1A and 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 and Table 1). Haloperidol was potently active in this procedure and clozapine, olanzapine and risperidone also displayed dose-dependent, though less potent, actions (Table 1).

Inhibition by S33138 of the locomotion provoked by "pro-psychotic agents" in rats (Figs. 1B, 1C and 1D, and Table 1). The increase in locomotor activity elicited by amphetamine was dose-dependently blocked by either s.c. or p.o. administration of S33138 (0.63-10.0 mg/kg, in each case), (Fig. 1B and Table 1). S33138 (s.c.) also dose-dependently antagonized the induction of locomotion by cocaine (Table 1). Likewise, haloperidol, 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 the least potent (Table 1). Dizocilpine, PCP and ketamine also elicited a marked locomotor 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.) (Figs. 1C and 1D and Table 1). Haloperidol, clozapine, olanzapine and risperidone all suppressed the induction of locomotion by PCP and ketamine, though the latter three drugs were less potent against dizocilpine (Table 1).

Inhibition by S33138 of the climbing behaviour elicited by apomorphine in mice (Fig. 1E and Table 1). The D_2/D_3 receptor agonist, apomorphine, elicited climbing behaviour in mice. This response was dose-dependently (0.04-2.5 mg/kg, s.c.) blocked by S33138 (Fig. 1E and 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 pre-pulse inhibition by apomorphine in rats (Fig. 1F). PPI was disrupted by apomophine: that is, as measured by the startle reflex, apomorphine interfered with adaptation of rats to an auditive stimulus (118 dB) induced by pre-exposure to the same tone. While 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.) prior to the training session on day 1 did not modify performance (latency to enter the dark compartment). Further, on the following day, during the retention session, performance was

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not modified. In distinction, haloperidol elevated the latency to respond during training and dose-dependently perturbed performance during retention. Clozapine did not modify behaviour during training but disrupted retention. Similarly, a reduction of retention was seen with olanzapine which biphasically elicited a non-significant 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 5-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 though the highest dose increased omissions (Table 2). Haloperidol (0.01-0.16 mg/kg) also did not affect percentage correct responses, although there was a tendency towards a decrease at the highest dose. It elicited a biphasic increase in anticipatory responses at intermediate doses, and markedly increased omissions. Clozapine significantly reduced response accuracy and, though 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 biphasic increase in anticipatory responses. It dose-dependently increased omissions.

Lack of disruption by S33138 of social recognition in rats. Following vehicle, and in the absence of an inter-session interval, there was a marked reduction in the duration of social interaction during the second (T2) as compared to the first session (T1): values of 112 ± 6 and 42 ± 5 sec, respectively ($P < 0.01$). At doses of 0.16-2.5 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 significantly increase T2 values: vehicle = 42 ± 5 sec, S33138 (0.16) = 57 ± 6 sec, S33138 (0.63) = 45 ± 6 sec and S33138 (2.5) = 25 ± 7 sec; $F(3,28) = 4.6$, $P < 0.05$, no difference of S33138 to vehicle in Dunnett's test. Haloperidol likewise did not increase T2 values at doses of 0.0025 - 0.08 mg/kg, though higher doses could not be tested owing to motor effects which reduced both T2 and T1 values (not shown). By 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 sec and T2 = 44 ± 5 sec and clozapine, T1 = 111 ± 8 sec and T2 = 80 ± 11 sec, 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 *vs* vehicle for T2 (but not T1) was significant in Newman-Keuls's 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 (not shown). Further, olanzapine (0.16 - 1.25 mg/kg) dose-dependently reduced both T2 and T1 values (not shown), reflecting perturbation of motor function. Finally, at doses of 0.01 - 0.16 mg/kg, s.c., risperidone did

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not affect T2, but doses of 0.04 and 0.16 reduced T1 due to motor actions (not shown).

Specific enhancement of social recognition by S33138 in a procedure with a 120 min inter-session delay (Fig. 3). When the inter-session interval was prolonged to 2 hrs, 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 sec, respectively ($P > 0.05$ paired 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, though haloperidol reduced T2-T1, this action was non-specific inasmuch as it provoked an identical reduction in exploration with a novel juvenile. Though 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 *vs* clozapine/same juvenile values. Similarly, 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 seconds, vehicle/same juvenile = 9.7 ± 2.3 sec (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: influence of juvenile: $F(1,20) = 12.8$, $P < 0.01$; influence of S33138, $F(1,20) = 26.1$, $P < 0.01$ and interaction, $F(1,20) = 27.2$, $P < 0.01$. S33138/same juvenile values significantly different from vehicle/same and from S33138/different juvenile values in Newman-Keuls test ($P < 0.01$).

Specific improvement of social recognition upon microinfusion of S33138 into the FCX (Fig. 4). Upon administration of vehicle into the FCX, there was no significant difference between T1 (98.7 ± 5.7 sec) and T2 (100.4 ± 10.3 sec) values ($P > 0.05$, paired t-test), indicating a spontaneous deficit in recognition with a 120 min inter-session delay. Bilateral microinjection of S33138 into the FCX dose-dependently (0.04-2.5 μg/side) reduced the duration of exploration in the second (but not first) session (Fig. 6A). This action was specific in that, microinjected at the most effective dose (2.5 μg/side), it did not affect exploration of a novel juvenile (no differences in T1, T2 or T2-T1). Further, bilateral injection of a maximally-effective dose (2.5 μg/side) of S33138 into the striatum did *not* significantly modify time of exploration of a familiar juvenile (Fig 4B).

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Blockade by S33138 of the disruption of social recognition provoked by 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 inter-session interval and clozapine itself reduced recognition (see above), their evaluation against scopolamine had to be restricted to modest doses. At doses which 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. Conversely, over a comparable dose-range (s.c.), no elevation in ACh levels was provoked in dorsal hippocampus (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) 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 (2.5-10.0 mg/kg, s.c.) higher than those which increased levels of ACh. The influence of D_3 *vs* D_2 receptor antagonists upon histamine levels in FCX has not, to date, been documented. Thus, we undertook a further experiment with the selective D_3 antagonist, S33084, and the preferential D_2 antagonist, L741,626, at doses exerting maximally-effective actions at D_3 and D_2 sites, respectively (Millan et al., 2000). Area under the curve analysis over 180 min: 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 *vs* vehicle, $P < 0.01$ in Dunnett's test. By 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 rotarod procedure, haloperidol, olanzapine and risperidone were

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highly active and S33138 was, together with clozapine, the least potent agent.

Induction of catalepsy by S33138 (Fig. 7A). Even the highest tested dose (40.0), S33138 elicited only “sub-maximal" catalepsy relative to the "cut off" of 30 sec. 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 prolactin 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 to that of S33138. Olanzapine increased PRL levels over a dose-range similar to S33138 but with a more pronounced maximal effect. Clozapine exerted little influence upon PRL levels, except at the dose of 20.0 mg/kg.

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DISCUSSION

Actions of S33138 in models of potential antipsychotic properties. Inasmuch as selective D_2 but *not* D_3 receptor antagonists block CARs in rats and apomorphine-induced climbing in mice, D_2 receptor blockade likely underlies their inhibition of S33138, though its modest antagonist properties at 5-HT_{2A} receptors and α_{2C}-ARs may fulfill facilitatory roles (Millan et al., 2000; Wadenberg et al., 2001, 2007; Meltzer et al., 2003; Svensson et al., 2003; Kapur et al., 2006). Psychostimulant-elicited locomotion is also mediated by D_2 *vs* D_3 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_2 antagonist properties. In certain studies, genetic deletion of D_3 receptors enhanced the motor actions of amphetamine and cocaine reflecting: 1), loss of postsynaptic D_3 sites inhibitory to motor function and/or 2), inactivation of D_3 autoreceptors, by analogy to the enhancement of amphetamine-induced DA release by D_2/D_3 antagonists ((Bahi et al., 2005; Chen et al., 2005). Nonetheless, D_3 receptor gene deletion does not invariably enhance the effects of psychostimulants (Karasinska et al., 2005) and, by analogy to selective D_3 receptor antagonists (Millan et al., 2000; Reavill et al., 2000), S33138 did *not* potentiate cocaine or amphetamine-induced locomotion. Further, mimicking D_3 receptor antagonists (Heidbreder et al., 2005), S33138 reduces cocaine-seeking behaviour in rats (Ashby C.R. Jr, unpub. obs.) and D_3 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 NMDA 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 et al., 1999; Geyer and Ellenbroek, 2003; Svensson et al., 2003; Millan, 2005). Herein, suppression of OCB-induced locomotion by S33138 likely involves antagonism of D_2 rather than D_3 sites inasmuch as a similar attenuation is seen with haloperidol whereas selective D_3 receptor antagonists are ineffective (Millan et al., 2000; Reavill et al., 2000). Further, preferential D_3 receptor *agonists* attenuated dizocilpine-induced locomotion in rats, possibly reflecting the inhibitory influence of presynaptic and postsynaptic D_3 receptors upon DA release and locomotion, respectively (Clements and Greenshaw, 2005). Nonetheless, a possible role of postsynaptic D_3 receptor blockade justifies further study inasmuch as the induction of hyperlocomotion by dizocilpine in mice was attenuated by genetic deletion of D_3 receptors (Leriche et al., 2003). The modest antagonist properties of S33138 at 5-HT_{2A} receptors likely also contribute to its inhibition of PCP-induced locomotion since, under the present conditions, this response is dependent upon mesolimbic 5-HT_{2A} 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,

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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, 2003). Though 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 (Svensson, 2003; Meltzer, 2003).

To summarise, 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 causal of, clinic efficacy. Further, the influence of S33138 upon limbic c-fos expression and the spontaneous activity of mesolimbic dopaminergic pathways is mediated by *D_3* receptor blockade (Millan et al., in press). Thus, despite compelling arguments that D_2 receptor blockade controls positive symptoms (Kapur et al., 2006; Seeman et al., 2006), the genuine significance of D_3 *vs* 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_3 receptors, yet absent in conspecifics deprived of D_2 receptors (Ralph et al., 1999). Thus, the effect of S33138 against apomorphine likely 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 likely 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 inter-session delay. Selective antagonists at 5-HT_{2A} receptors, 5-HT_7 receptors and α_2-ARs are inactive in this procedure and, in view of identical effects of selective D_3 receptor antagonists (Millan et al., 2007), these potent actions of S33138 presumably reflect D_3 receptor blockade. Indeed, preferential D_2 antagonists *reduce* social recognition and *interfere* with its enhancement by D_3 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 D_2/D_3 antagonists have proven variable in other models, this finding suggests that instances of pro-cognitive actions reflect blockade of D_3 rather than D_2 receptors (Passetti et al., 2001; Chudusama 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 D_3 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, though the latter's disruptive influence likely reflects blockade of H_1 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 Drago et al. (1997) reported a disruption in *rats*, a finding resembling the present observations. Though motor perturbation complicates interpretation of its effects, a deleterious influence of haloperidol upon PAV likely reflects blockade of D_2 receptors since: 1), selective antagonists at D_3 receptors do not affect PAV procedures and 2), the inhibitory influence of the D_2/D_3 agonist, quinpirole, upon scopolamine-induced disruption of PAV is mediated by D_3 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) likely involve antagonism of muscarinic receptors in view of similar impairments with scopolamine and cholinergic lesions (Mirza and Stolerman, 2000; Robbins, 2002). H_1 antagonist properties may also be implicated in the deficits evoked by clozapine, olanzapine *and* risperidone inasmuch as cortical histaminergic pathways modulate attentional processes in the 5-CSRT paradigm (Bacciottoni et al., 2001; Day et al., 2007). Interestingly, 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 inter-trial intervals are used (Robbins, 2002). Moreover, introduction of the D_2/D_3 antagonist, sulpiride, into the FCX did not compromise accuracy (Granon et al., 2000). In line with these findings, and by analogy to selective D_3 antagonists (Loiseau. F., unpub. obs.), 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., 2001).

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Influence of S33138 upon of neurotransmitters modulating cognitive function in FCX. The above findings coincide with evidence that preferential blockade of D_3 *vs* D_2 receptors *preserves* and even enhances cognitive performance (Laszy et al., 2005; Micale et al., 2006; Millan et al., 2007). Cholinergic pathways in the FCX, subject to tonic inhibition by D_3 receptors, are a potential substrate for this beneficial influence of D_3 antagonists upon cognition (Sarter et al., 2005; Lacroix et al., 2006; Millan et al., 2007). Accordingly, S33138 elevated extracellular levels of ACh in FCX at low doses similar to those active in the social recognition procedure and other models of D_3 receptor-mediated activity (Millan et al., in press). Interestingly, this role of D_3 receptor blockade in the induction of frontocortical ACh release by S33138 differs to receptor mechanisms implicated in elevations provoked by atypical antipsychotics: M_2 antagonism for clozapine and olanzapine (Johnson et al., 2005a); M_1 agonism (*via* the desmethylated metabolite) for clozapine (Li et al., 2005); 5-HT_{1A} partial agonism for risperidone (Sato et al., 2006) and indirect D_1 agonism for all (Ichikawa et al., 2002; Di Cara et al., 2007). Glutamatergic, GABAergic and monoaminergic mechanisms also control cognitive function (Robbins, 2002; Meltzer et al., 2003; Lewis and Gonzalez-Burgos, 2006) but, like selective D_3 antagonists (Millan et al., 2000; unpub. obs.), 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 (Bacciottini et al., 2001; Ito, 2004; Horner et al., 2006). D_3 receptor blockade is unlikely to be involved since doses of S33138 were much higher than those affecting ACh. Moreover, mice lacking D_3 receptors show no increase in basal histaminergic activity (Morisset et al., 2002) and the selective D_3 antagonist, S33084, did *not* increase levels of histamine. By contrast, the preferential D_2 receptor antagonist, L741,626, enhanced histamine levels (Results), suggesting that blockade of D_2 sites by high doses of S33138 may stimulate histamine release. One additional possibility would be the modest antagonist properties of S33138 at 5-HT_{2A} receptors, blockade of which by clozapine increases cerebral histamine turnover (Morisset et al., 1999).

Potential induction of side-effects by S33138. Selective D_3 receptor blockade does not evoke catalepsy in rodents, and moderates its induction by D_2 antagonists (Millan et al., 2000, 2004; Reavill et al., 2000; Sokoloff et al., 2006; Gyertyan and Saghy, 2007). Accordingly, though S33138 elicited catalepsy, its maximal effect was less pronounced than haloperidol and risperidone and the dose-window to antipsychotic actions more marked. This limited extrapyramidal potential of S33138 is consistent with its preferential induction of c-fos in limbic *vs* striatal structures, *facilitation* of motor function in parkinsonian primates, and a more potent influence upon ventrotegmental *vs* substantia nigra dopaminergic neurone firing (Millan et al., in press). Antagonism of α_{2C}-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-HT_{2A} receptor antagonist

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properties are of lesser importance than for clozapine, olanzapine and risperidone (Millan et al., 1998; Meltzer, 2003). Thus, while S33138 shares the low extrapyramidal potential of atypical agents, the underlying mechanism (preferential D_3 *vs* D_2 receptor blockade) differs.

The reason for the less marked effect of S33138 than haloperidol upon PRL levels is unclear since D_3 receptor antagonism should not moderate the induction of lactotrophic secretion of PRL by D_2 receptor blockade (Millan et al., 2000; Ben-Jonathan and Hnasko, 2001). However, haloperidol behaves as an inverse agonist at constitutively-active D_2 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 D_3 as compared to D_2 receptors, and by its negligible affinity for histamine H_1, muscarinic and α_1-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 "D_3 receptor" doses of S33138 upon mnemonic function is supported by ongoing studies in primate models of attention, working memory and executive performance (Millan, M.J. et al., unpub. obs.). S33138 is also active in rodent procedures predictive of antipsychotic properties, principally *via* antagonism of D_2 receptors. In contrast to haloperidol and risperidone, only high doses of S33138 provoke catalepsy, likely reflecting its preferential affinity for D_3 sites, blockade of which attenuates the induction of extrapyramidal motor effects by D_2 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 (Millan, M.J. et al., unpub obs). S33138 is, thus, an innovative, well-tolerated and promising candidate for the improved treatment of schizophrenia, especially cognitive symptoms. However, only therapeutic evaluation of S33138 (Phase IIb) and similar agents can clarify the genuine significance of preferential D_3 *vs* D_2 receptor blockade to the treatment of psychotic states and other CNS disorders.

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LEGENDS FOR FIGURES

Figure 1. Actions of S33138 in diverse models of potential antipsychotic activity.
Panel A, Inhibition by S33138 of conditioned avoidance response in rats; Panel B, Inhibition by S33138 of the locomotor activity elicited by amphetamine (2.5 mg/kg, i.p.) in rats. Panel C, Inhibition by S33138 of the locomotor activity elicited by ketamine (40.0 mg/kg, s.c.) in rats; Panel D, Inhibition by S33138 of the locomotor activity elicited by phencyclidine (PCP, 20.0 mg/kg, s.c.) and dizocilpine (0.16 mg/kg, s.c.) in rats; Panel E, Inhibition by S33138 of apomorphine (0.75 mg/kg, s.c.)-induced climbing in mice and Panel F, Inhibition by S33138 of apomorphine-induced disruption of pre-pulse inhibition in rats. Locomotion measurements were made over 60 min except for ketamine (20 min). VEH = vehicle. Data are means ± SEMs. N = 5-10 per value. ANOVA as follows. Panel B, S33138 (s.c.) *vs* amphetamine, $F(3,41) = 8.79$, $P < 0.01$ and S33138 (p.o.) *vs* amphetamine, $F(3,20) = 13.4$, $P < 0.01$; Panel C, S33138 *vs* ketamine, $F(3,30) = 2.93$, $P < 0.05$ and Panel D, S33138 *vs* PCP, $F(4,37) = 4.62$, $P < 0.05$, and S33138 *vs* dizocilpine, $F(3,23) = 4.59$, $P < 0.01$. Asterisks indicate significance of differences to vehicle values in a paired Wilcoxon test (Panel A), in a Fisher Exact Probability test (Panel B) and in Dunnett's test following ANOVA (Panels B-D). Panel 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 $F(4,47) = 6.6$, $P < 0.01$. The open asterisk indicates a significant difference of vehicle/apomorphine to vehicle/vehicle values, and the closed asterisk a significant difference of S33138/apomorphine to vehicle/apomorphine values in Newman Keuls test. * $P < 0.05$.

Figure 2. Lack of disruption by S33138 of performance in a passive avoidance procedure in rats: a comparison to haloperidol, clozapine, olanzapine and risperidone.
Panel A, S33138; Panel B, haloperidol; Panel C, clozapine; Panel D, olanzapine and Panel E, risperidone. VEH = vehicle. Data are means ± SEMs. N = 6-8 per value. ANOVA as follows. Training session: S33138, $F(6,42) = 1.1$, $P > 0.05$; haloperidol, $F(5,48) = 6.7$, $P < 0.01$; clozapine, $F(4,38) = 1.1$, $P > 0.05$; olanzapine, $F(4,36) = 2.2$, $P > 0.05$ and risperidone, $F(4,35) = 15.1$, $P < 0.01$. Test session: S33138, $F(6,42) = 1.6$, $P > 0.05$; haloperidol, $F(5,48) = 4.5$, $P < 0.01$; clozapine, $F(4,38) = 9.2$, $P < 0.01$; olanzapine, $F(4,36) = 8.2$, $P < 0.01$ and risperidone, $F(4,35) = 10.2$ $P < 0.01$. Asterisks indicate significance of differences to vehicle values in Dunnett's test. * $P < 0.05$.

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Figure 3. Improvement by S33138 of social recognition in a procedure with a 120 min delay: a comparison to haloperidol, clozapine, olanzapine and risperidone.

Panel A, S33138, s.c.; Panel B, S33138, p.o.; Panel C, Haloperidol; Panel D, Clozapine; Panel E, Olanzapine and Panel F, Risperidone. VEH = vehicle. Data are means ± SEMs. N = 5-12 per value. For dose-response curves, one-way ANOVA as follow: S33138, s.c., $F(3,36) = 3.3$, $P < 0.05$; S33138, p.o., $F(3,28) = 9.6$, $P < 0.01$; haloperidol, $F(3,29) = 11.5$, $P < 0.01$; clozapine, $F(3,35) = 9.5$, $P < 0.01$; olanzapine, $F(3,23) = 3.3$, $P < 0.05$ and risperidone, $F(3,28) = 9.0$, $P < 0.01$. Closed asterisks indicate significance of differences between drugs and vehicle values in Dunnett's test (* $P < 0.05$). For the specificity of drug actions, two-way ANOVA as follows: S33138, s.c.; influence of juvenile, $F(1,33) = 11.3$, $P < 0.01$; influence of drug, $F(1,33) = 12.4$, $P < 0.01$ and interaction, $F(1,33) = 8.4$, $P < 0.01$. S33138, p.o.; influence of juvenile, $F(1,29) = 21.3$, $P < 0.01$; influence of drug, $F(1,29) = 30.3$, $P < 0.01$ and interaction, $F(1,29) = 15.1$, $P < 0.01$. Haloperidol; juvenile, $F(1,38) = 3.2$, $P > 0.05$; drug, $F(2,38) = 24.1$, $P < 0.01$ and interaction, $F(2,38) = 0.1$, $P > 0.05$. Clozapine; juvenile, $F(1,46) = 10.6$, $P < 0.01$; drug, $F(2,46) = 22.0$, $P < 0.01$ and interaction, $F(2,46) = 1.1$, $P > 0.05$. Olanzapine; juvenile, $F(1,23) = 11.8$, $P < 0.01$; influence of drug, $F(1,23) = 0.8$, $P > 0.05$ and interaction, $F(1,23) = 0.4$, $P > 0.05$. Risperidone; juvenile, $F(1,23) = 0.2$, $P > 0.05$; drug, $F(1,23) = 29.5$, $P < 0.01$ and interaction, $F(1,23) = 0.1$, $P > 0.05$. Open asterisks indicate significance of differences in Newman-Keuls test between values for a different *vs* the same juvenile, * $P < 0.05$.

Figure 4. Improvement by S33138 of social recognition in rats upon microinjection into the FCX.

VEH = vehicle. Data are means ± SEMs. N = 6-8 value. For dose-response curve, one-way ANOVA as follows: $F(3,31) = 3.3$, $P < 0.05$. The closed asterisk indicates significance of the difference between S33138 and vehicle values in Dunnett's test. For the specificity of action, two-way ANOVA as follows: juvenile, $F(1,28) = 4.7$, $P < 0.05$; S33138, $F(1,28) = 4.1$, $P < 0.05$ and interaction, $F(1,28) = 3.8$, $P < 0.05$. The open asterisk indicates the significance of the difference in Newman-Keuls test between values for the different juvenile/S33138 *vs* same juvenile/S33138. * $P < 0.05$.

Figure 5. Blockade by S33138 of the disruption of social recognition in rats by scopolamine: a comparison to haloperidol, clozapine, olanzapine and risperidone.

Panel A, S33138, s.c.; Panel B, S33138, p.o.; Panel C, Haloperidol; Panel D, Clozapine; Panel E, Olanzapine and Panel F, Risperidone. VEH = vehicle. Data are means ± SEMs. N = 5-12 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, $F(3,51) = 9.7$, $P < 0.01$. S 33138, 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 indicate significance of differences between vehicle/scopolamine and vehicle/vehicle values: closed asterisks indicate significance of differences between values for antipsychotic/scopolamine *vs* vehicle/scopolamine in Newman-Keuls test, * P < 0.05.

Figure 6. Elevation by S33138 of extracellular levels of acetylcholine (ACh) and histamine, but not monoamines or amino acids, in frontal cortex of freely-moving rats.
Panel A, Elevation of ACh levels by S33138, s.c.; Panel B, S33138, area under the curve analysis; Panel C, Increase of histamine levels by S33138, s.c.; Panel D, Lack of influence of S33138 (10 mg/kg, s.c.) upon dopamine (DA), serotonin (5-HT) and noradrenaline (NA) and Panel E, Lack of influence of S33138 (10 mg/kg, s.c.) upon GABA, glutamate and glycine levels. In Panels D and E, open symbols represent vehicle, and closed symbols S33138. Dialysate levels are expressed as a percentage of basal, pre-injection values (100 %). These were 20.6 ± 2.2 nM, 497 ± 49 pM, 373 ± 20 pM, 441 ± 15 pM, 224 ± 17 pM, 60 ± 3 nM, 0.74 ± 0.10 µM and 9.7 ± 0.6 µM/dialysate sample for ACh, histamine, DA, NA, 5-HT, GABA, glutamate and glycine, respectively. Data are means ± SEMs. N = 59. ANOVA as follows: Panel A, S33138 (0.01), F (1,9) = 1.5, P > 0.05; S33138 (0.04), F (1,9) = 9.8, P < 0.05; S33138 (0.16), F (1,10) = 7.6, P < 0.05 and S33138 (0.63), F (1,10) = 12.7, P < 0.01. Panel C, S33138 (2.5), F (1,8) = 0.6, P > 0.05; S33138 (5.0), F (1,8) = 11.8, P < 0.05 and S33138 (10.0), F (1,9) = 55.2, P < 0.01. Panel D, DA, F (1,9) = 4.2, P > 0.05; NA, F (1,9) = 1.7, P > 0.05 and 5-HT, F (1,9) = 1.2, P > 0.05. Panel E: GABA, F (1,13) = 2.5, P > 0.05; glutamate, F (1,10) = 0.3, P > 0.05 and glycine, F (1,11) = 1.6, P > 0.05. Asterisks indicate significance of S33138-treated *vs* vehicle-treated values. * P < 0.05.

Figure 7. Induction of catalepsy and influence upon circulating levels of prolactin in rats: a comparison of S33138 to haloperidol, clozapine, olanzapine and risperidone.
Drugs were administered s.c. Panel A, Catalepsy. Data are means ± SEMs. N = 4-19. S33138, F (3,16) = 10.1, P < 0.01; Haloperidol, F (3,16) = 24.3, P < 0.01; Clozapine, F (3,20) = 2.36, P > 0.05; Olanzapine, F (4,29) = 14.8, P < 0.01 and risperidone, F (4,21) = 26.9, P < 0.01. Panel B, prolactin levels. Data are means ± SEMs. N = 6-8. S33138, F (5,66) = 16.9 P < 0.01; haloperidol, F (7,83) = 26.4, P < 0.01; clozapine, F (6,86) = 6.2, P < 0.01; olanzapine, F (7,77) = 15.8, P < 0.01 and risperidone, F (6,48) = 3.61, P < 0.01. Asterisks indicate significance of differences to vehicle in Dunnett's test. * P < 0.05.

Table 1. Summary of the actions of S33138 as compared to haloperidol, clozapine, olanzapine and risperidone in models of potential antipsychotic properties as compared to induction of extrapyramidal side-effects.

Drug	CAR	APO	A-LOC	C-LOC	P-LOC	D-LOC	CATAL	PRL
S33138	3.8	0.32	1.4	1.1	0.73	4.9	10.0	0.16
	100 (20.0)	100 (2.5)	100 (10.0)	100 (10.0)	100 (10.0)	86 (10.0)	61 (40.0)	75 (2.5)
Haloperidol	0.08 *	0.02	0.04	0.02	0.08	0.1	0.16	0.04
	97 (0.63)	100 (0.16)	100 (0.16)	100 (0.16)	100 (0.63)	99 (0.63)	100 (0.63)	205 (0.63)
Clozapine	4.4 *	2.3	7.2	2.6	0.08	1.7	>40.0	20.0
	94 (20.0)	100 (5.0)	100 (40.0)	100 (40.0)	100 (0.63)	96 (10.0)	2 (40.0)	30 (20)
Olanzapine	0.60	0.08	0.30	1.6	0.002	0.44	5.0	0.63
	90 (2.5)	100 (0.63)	100 (10.0)	94 (10.0)	100 (0.04)	84 (2.5)	81 (20.0)	170 (10.0)
Risperidone	0.49	0.002	0.20	0.04	0.002	0.32	0.63	0.01
	97 (2.5)	100 (0.16)	100 (2.5)	100 (2.5)	100 (0.04)	100 (2.5)	100 (5.0)	83 (0.63)

All antipsychotics were administered s.c. The first line gives the Effective $Dose_{50}$ (CAR and APO), Inhibitory $Dose_{50}$ (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 percent) followed by the maximally-effective dose in brackets except for Prolactin where maximal absolute levels are given in ng/ml. CAR = inhibition of conditioned avoidance responses in rat; APO = inhibition of climbing behaviour elicited by apomorphine (0.75 mg/kg, s.c.) in mice; LOC = Inhibition of locomotion elicited in rats by amphetamine ("A", 2.5 mg/kg, i.p.), cocaine ("C", 20.0 mg/kg, i.p.), phencyclidine ("P", 20.0 mg/kg, s.c.) and dizocilpine ("D", 0.16 mg/kg, s.c.). * Data from Millan et al., 1998. As can be deduced from the maximal effects, each drug (3-5 doses tested, N = 5-6 per dose) exerted highly significant effects in all models ($P < 0.05$ in ANOVA in every case), so "F" values are omitted for reasons of space. For cocaine (effect of S33138 not shown in Figure 1), $F(4,22) = 6.67$, $p < 0.01$.

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Table 2. Influence of S33138 in comparison to haloperidol, clozapine, olanzapine and risperidone upon performance in the 5-choice serial reaction time test in rats.

Drug	Dose (mg/kg, s.c.)	% Correct responses	Anticipatory responses	Omissions
S33138	0	62.2 ± 2.2	13.4 ± 1.5	9.4 ± 2.0
	0.04	62.9 ± 5.1	16.6 ± 3.7	5.5 ± 1.1
	0.63	60.3 ± 4.0	13.1 ± 3.8	9.0 ± 2.2
	2.5	57.0 ± 3.7	9.3 ± 2.1	35.1 ± 12.1 *
Haloperidol	0	67.7 ± 2.5	6.4 ± 1.4	6.9 ± 1.1
	0.01	69.5 ± 2.7	7.1 ± 1.1	5.9 ± 1.9
	0.04	61.5 ± 3.5	13.1 ± 2.2 *	39.4 ± 9.2 *
	0.16	53.0 ± 11.8	1.3 ± 0.4	88.7 ± 3.2 *
Clozapine	0	67.5 ± 2.7	11.4 ± 2.7	9.6 ± 2.8
	0.16	68.7 ± 3.1	10.5 ± 2.5	8.6 ± 1.5
	0.63	58.8 ± 3.4	13.2 ± 1.9	10.3 ± 3.3
	2.5	48.7 ± 4.6 *	16.2 ± 3.7	48.9 ± 10.6 *
Olanzapine	0	69.8 ± 3.0	7.2 ± 2.1	8.6 ± 1.8
	0.04	67.4 ± 2.2	12.5 ± 3.4	7.6 ± 2.8
	0.16	67.1 ± 2.9	7.7 ± 1.8	11.6 ± 3.4
	0.63	55.9 ± 2.0 *	13.0 ± 2.9	39.8 ± 6.4 *
Risperidone	0	67.4 ± 3.8	5.9 ± 0.6	5.6 ± 1.2
	0.04	67.5 ± 3.0	8.4 ± 1.2	10.3 ± 1.6
	0.16	59.4 ± 3.2	17.2 ± 3.2 *	28.0 ± 4.4 *
	0.63	54.1 ± 3.9 *	10.4 ± 1.8	48.5 ± 6.8 *

Data are means ± SEMs. N = 7-11 per value. ANOVA as follows. Percentage correct responses: S33138, $F(3,39) = 0.5$, $P > 0.05$; haloperidol, $F(3,34) = 1.5$, $P > 0.05$; clozapine, $F(3,32) = 6.8$, $P < 0.01$; olanzapine, $F(3,32) = 6.4$, $P < 0.01$ and risperidone, $F(3,38) = 3.6$, $P < 0.05$. Anticipatory responses: S33138, $F(3,39) = 1.1$, $P > 0.05$; haloperidol, $F(3,34) = 10.4$, $P < 0.01$; clozapine, $F(3,32) = 0.8$, $P > 0.05$; olanzapine, $F(3,32) = 1.3$, $P > 0.05$ and risperidone, $F(3,38) = 5.6$, $P < 0.01$. Omissions: S 33138, $F(3,39) = 6.7$, $P < 0.01$; haloperidol, $F(3,34) = 56.0$, $P < 0.01$; clozapine, $F(3,32) = 11.6$, $P < 0.01$); olanzapine, $F(3,32) = 14.0$, $P < 0.01$ and risperidone, $F(3,38) = 18.4$, $P < 0.01$. Asterisks indicate significance of differences to vehicle values in Dunnett's test following ANOVA. * $P < 0.05$.

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Table 3. Influence of S33138 in comparison to haloperidol, clozapine, olanzapine and risperidone upon motor behaviour in rats and mice.

		Spontaneous Locomotion		Rotarod
		Rats	Mice	Mice
Drug	Dose	(Counts)	(Counts)	(Latency to fall, sec)
S33138	0	54.6 ± 7.5 (7)	323 ± 25 (8)	263 ± 38 (6)
	0.63	43 ± 10.2 (4)	274 ± 23 (5)	257 ± 39 (5)
	2.5	8.4 ± 2.7 (5)*	108 ± 21 (7)*	120 ± 14 (5)*
	10.0	1.6 ± 0.7 (5)*	53 ± 12 (5)*	65 ± 15 (5)*
Haloperidol	0	58.9 ± 8.4 (15)	320 ± 15 (20)	259 ± 17 (22)
	0.01	49.4 ± 7.3 (7)	-	-
	0.02	35.6 ± 6.9 (7)*	-	-
	0.04	22.4 ± 6.5 (7)*	268 ± 24 (9)	252 ± 41 (6)
	0.08	-	-	100 ± 25 (7)*
	0.16	5.9 ± 1.7*	163 ± 14 (9)*	103 ± 19 (7)*
	0.63	-	91 ± 12 (13)*	87 ± 23 (8)*
	2.5	-	-	37 ± 7 (7)*
Clozapine	0	54 ± 4.8 (19)	302 ± 19 (8)	282 ± 24 (11)
	0.16	-	284 ± 30 (5)	253 ± 51 (6)
	0.63	-	177 ± 31 (5)*	201 ± 54 (7)
	2.5	41.4 ± 7.3 (8)	59.8 ± 15.9 (6)*	155 ± 50 (7)*
	5.0	17.2 ± 6.4 (10)*	-	23 ± 10 (6)*
	10.0	14.5 ± 1.8 (8)*	26.5 ± (6)*	3 ± 1 (6)*
	40.0	10.5 ± 1.5 (8)*	-	-
	80.0	5.8 ± 2.3 (5)*	-	-
Olanzapine	0	54.2 ± 5.5 (13)	370 ± 27 (7)	267 ± 21 (9)
	0.01	50 ± 9.0 (5)	-	-
	0.04	48.5 ± 6.5 (6)	332 ± 32 (6)	260 ± 41 (6)
	0.16	38.5 ± 4.2 (6)*	261 ± 40 (6)*	159 ± 26 (12)*
	0.63	15.8 ± 3.2 (6)*	102 ± 12 (6)*	150 ± 33 (12)*
	2.5	6.7 ± 1.5 (6)*	35 ± 12 (6)*	92 ± 38 (5)*
	10.0	2 ± 0.9 (4)*	16 ± 10 (5)*	4 ± 1 (5)*
Risperidone	0	53.9 ± 5.0 (14)	289 ± 23.8 (6)	266 ± 32 (9)
	0.04	32.2 ± 6.7 (5)*	237 ± 33 (5)	195 ± 33 (5)
	0.16	21.8 ± 5.6 (5)*	46.4 ± 17.6 (5)*	132 ± 25 (8)*
	0.63	10.2 ± 2.5 (5)*	18.4 ± 8 (5)*	88 ± 36 (6)*
	2.5	1.9 ± 1.0 (8)*	-	38 ± 10 (9)*
	10.0	-	-	3 ± 1 (5)*

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Doses are in mg/kg, s.c. Data are means ± SEMs. N is given in brackets. ANOVA as follows. Influence upon spontaneous locomotor activity in rats, S33138, $F(3,20) = 16.8$, $P < 0.01$; haloperidol, $F(4,25) = 8.1$, $P < 0.01$; clozapine, $F(5,57) = 15.6$, $P < 0.01$; olanzapine, $F(6,45) = 14.2$ $P < 0.01$ and risperidone, $F(4,36) = 21.6$, $P < 0.01$. Influence upon spontaneous locomotor activity in mice: S33138, $F(3,24) = 33.3$, $P < 0.01$; haloperidol, $F(3,50) = 63.0$, $P < 0.01$; clozapine, $F(4,29) = 37.7$, $P < 0.01$; olanzapine, $F(5,35) = 36.0$, $P < 0.01$ and risperidone, $F(3,20) = 36.1$, $P < 0.01$. Rotarod procedure in mice: S33138, $F(3,20) = 10.7$, $P < 0.01$; haloperidol, $F(5,56) = 18.2$, $P < 0.01$; clozapine, $F(5,42) = 9.6$, $P < 0.01$; olanzapine, $F(5,48) = 7.7$, $P < 0.01$ and risperidone, $F(5,41) = 14.7$, $P < 0.01$. Asterisks indicate significance of differences to vehicle values in Dunnett's test following ANOVA. * $P < 0.05$.

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FIGURE 1

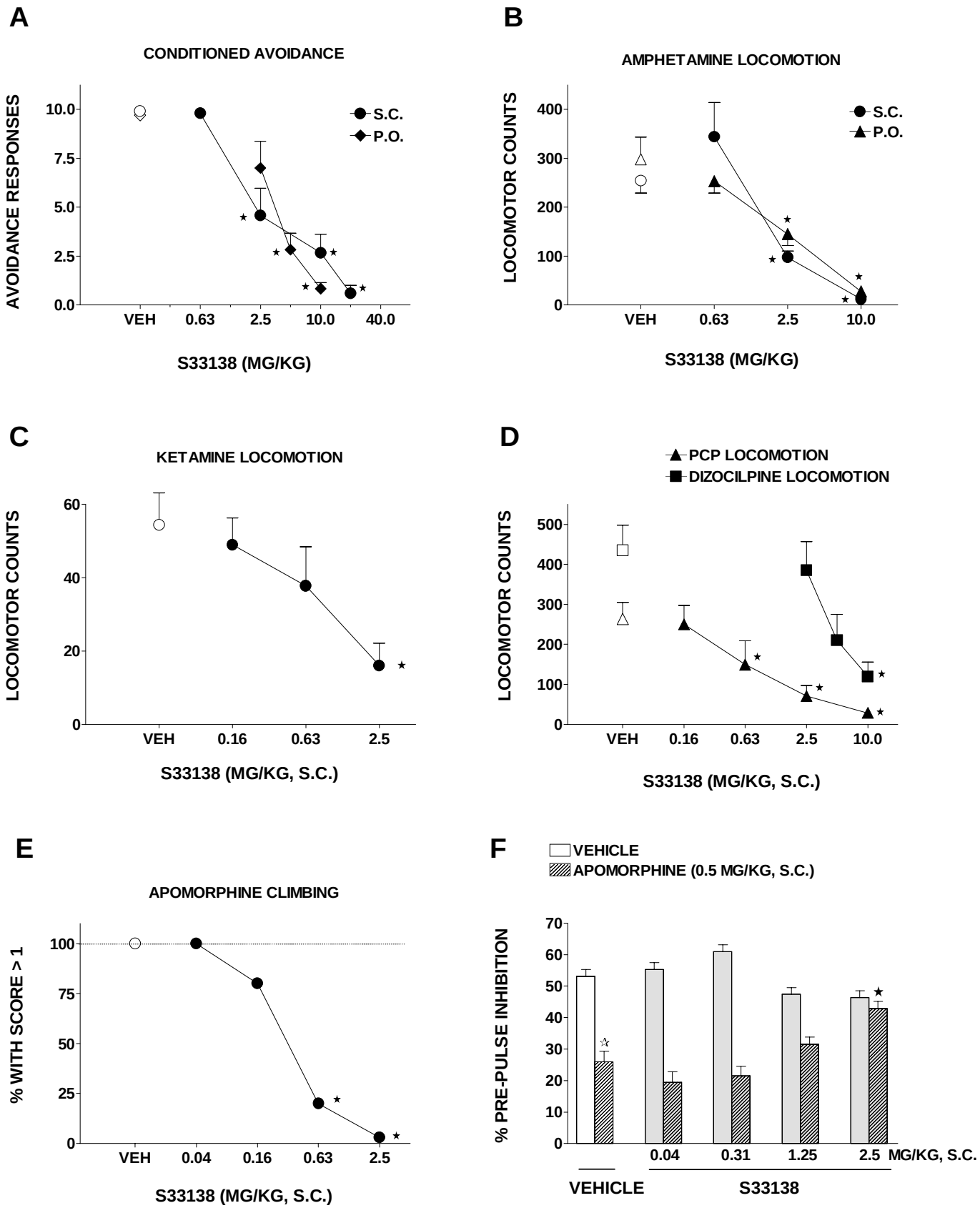


FIGURE 2

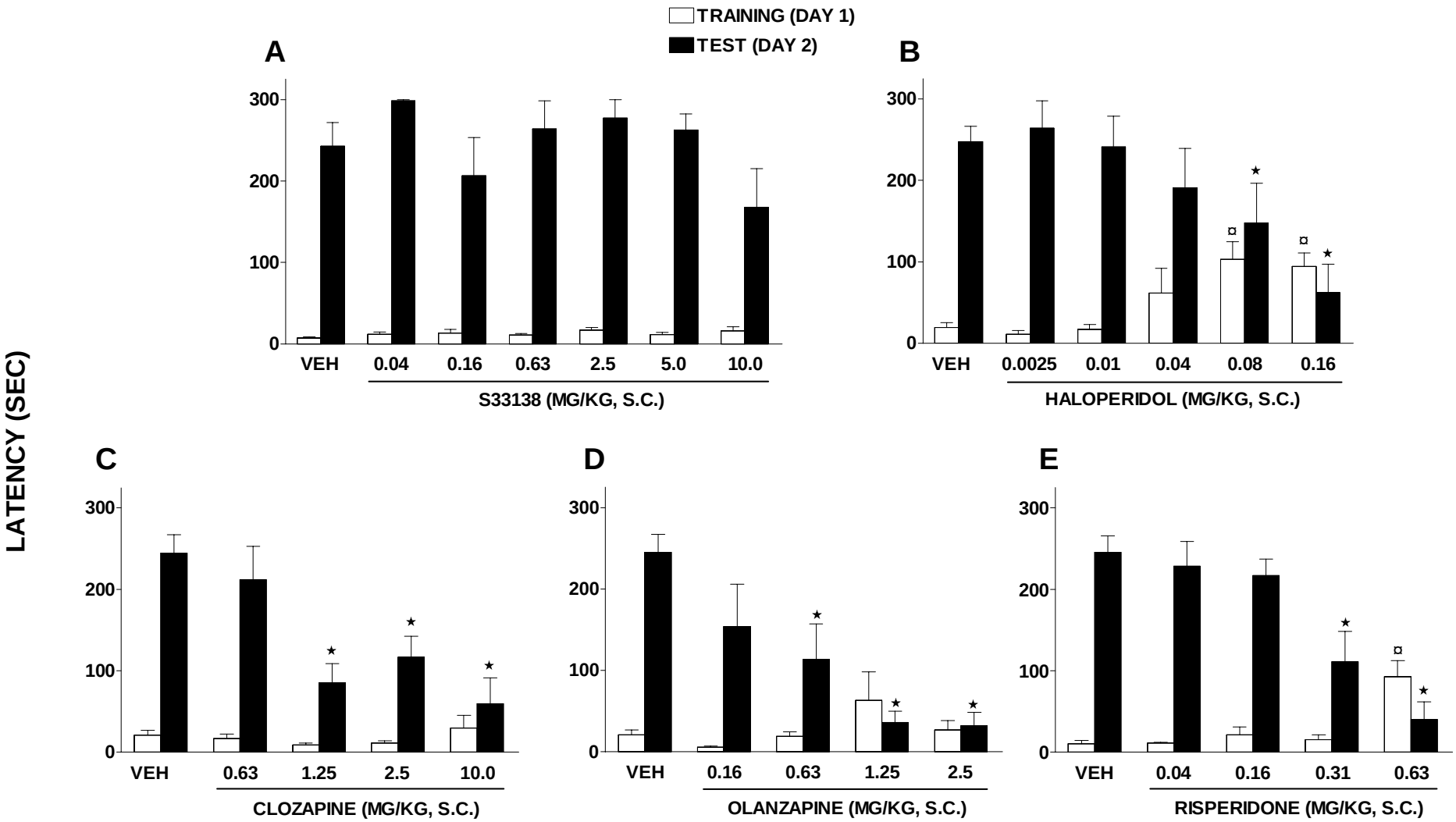
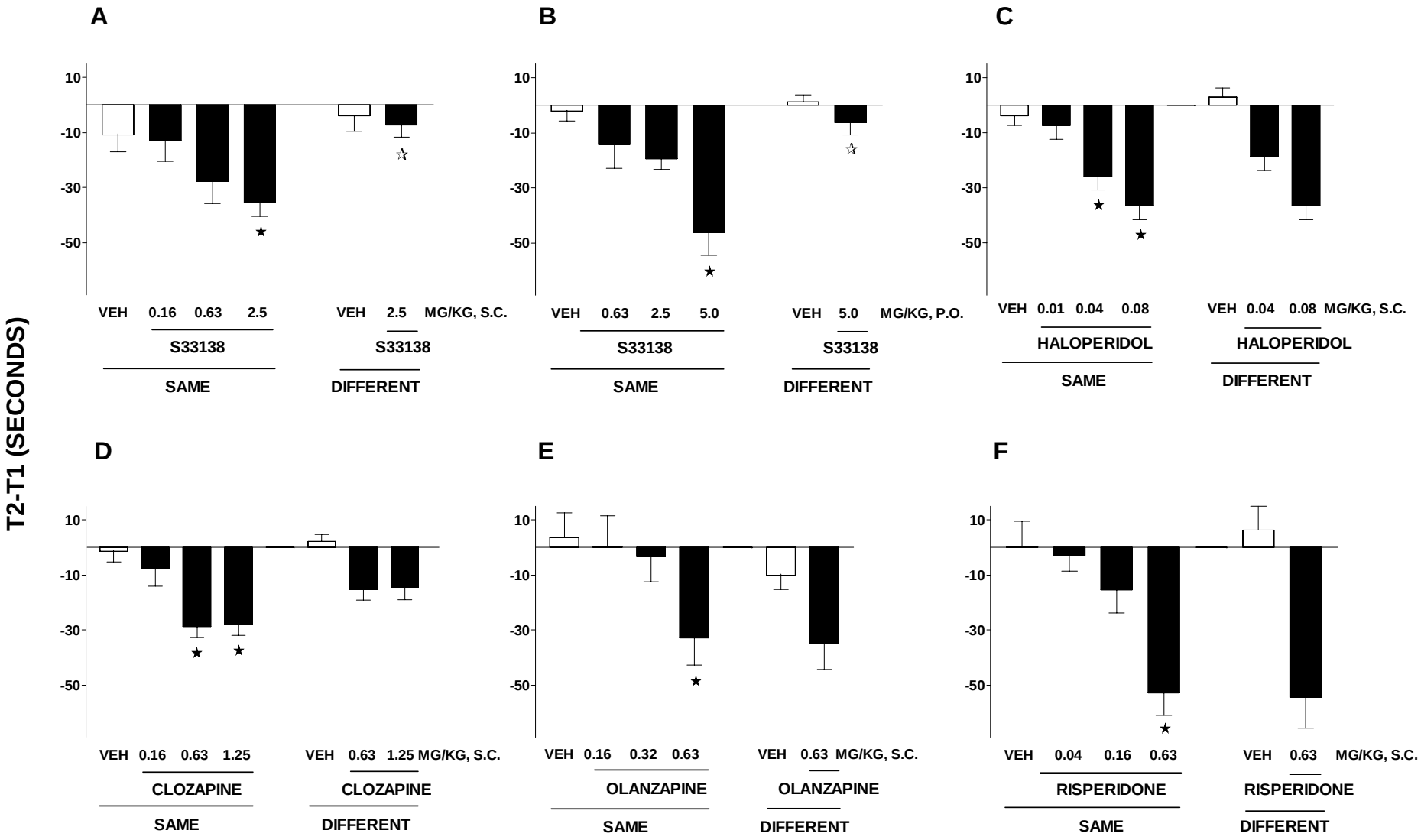


FIGURE 3



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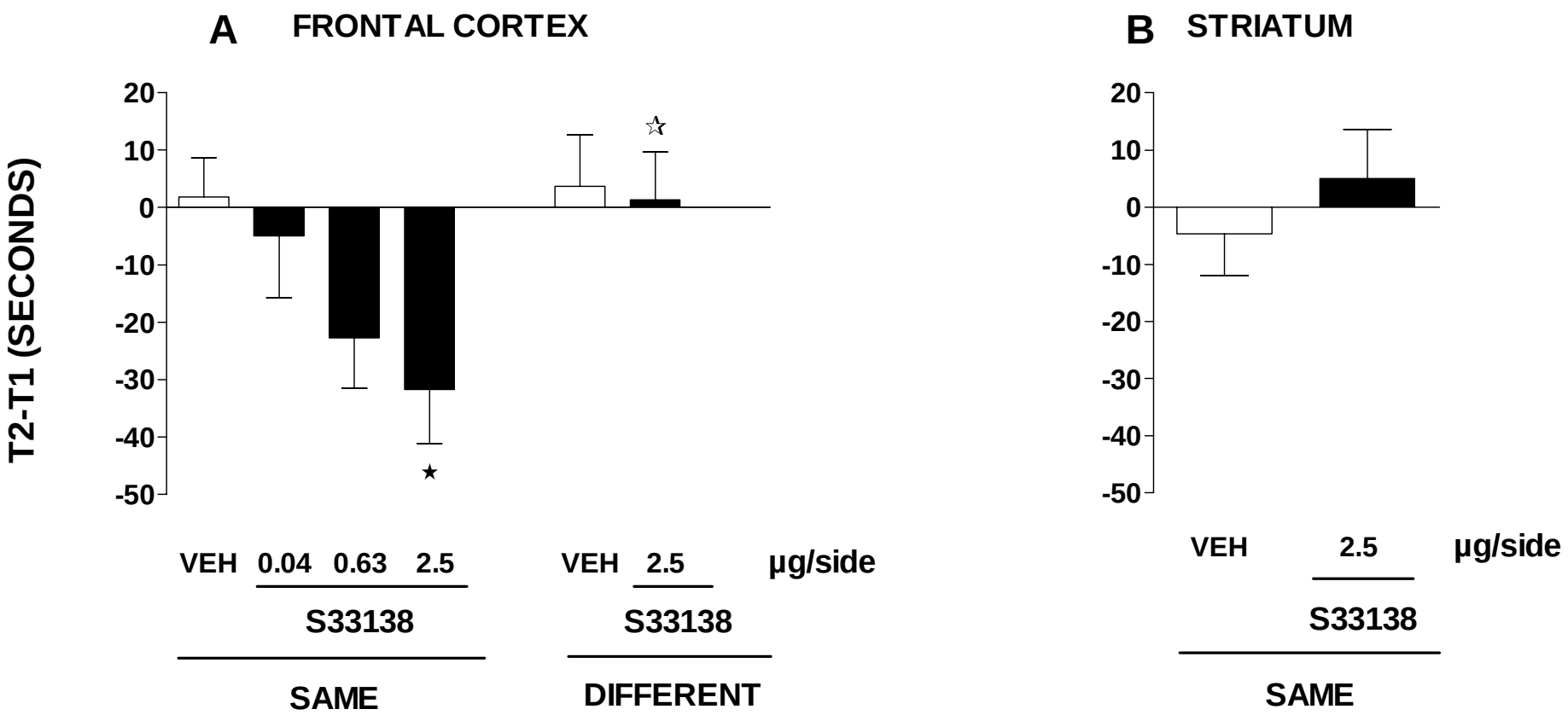

FIGURE 5

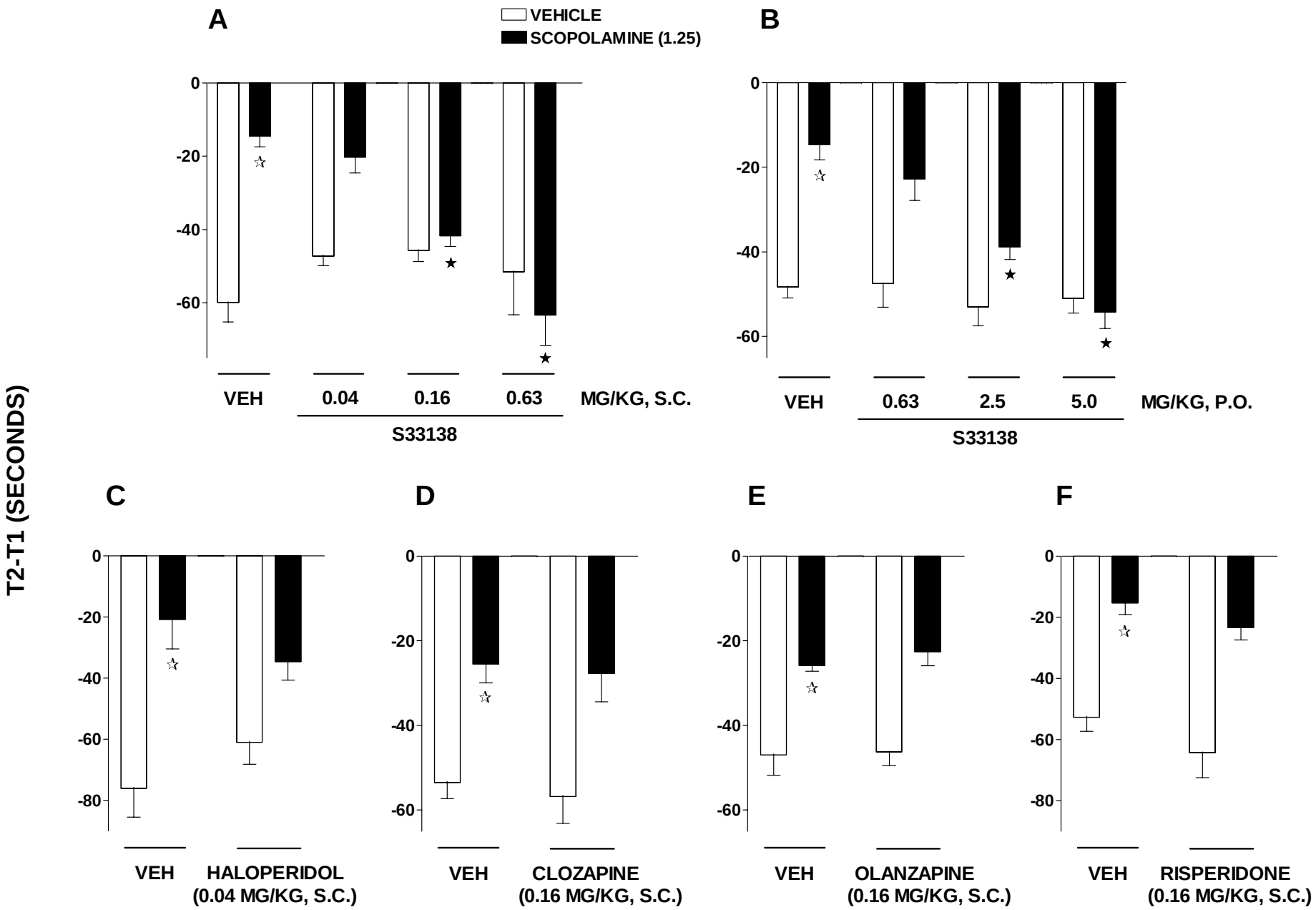

FIGURE 6

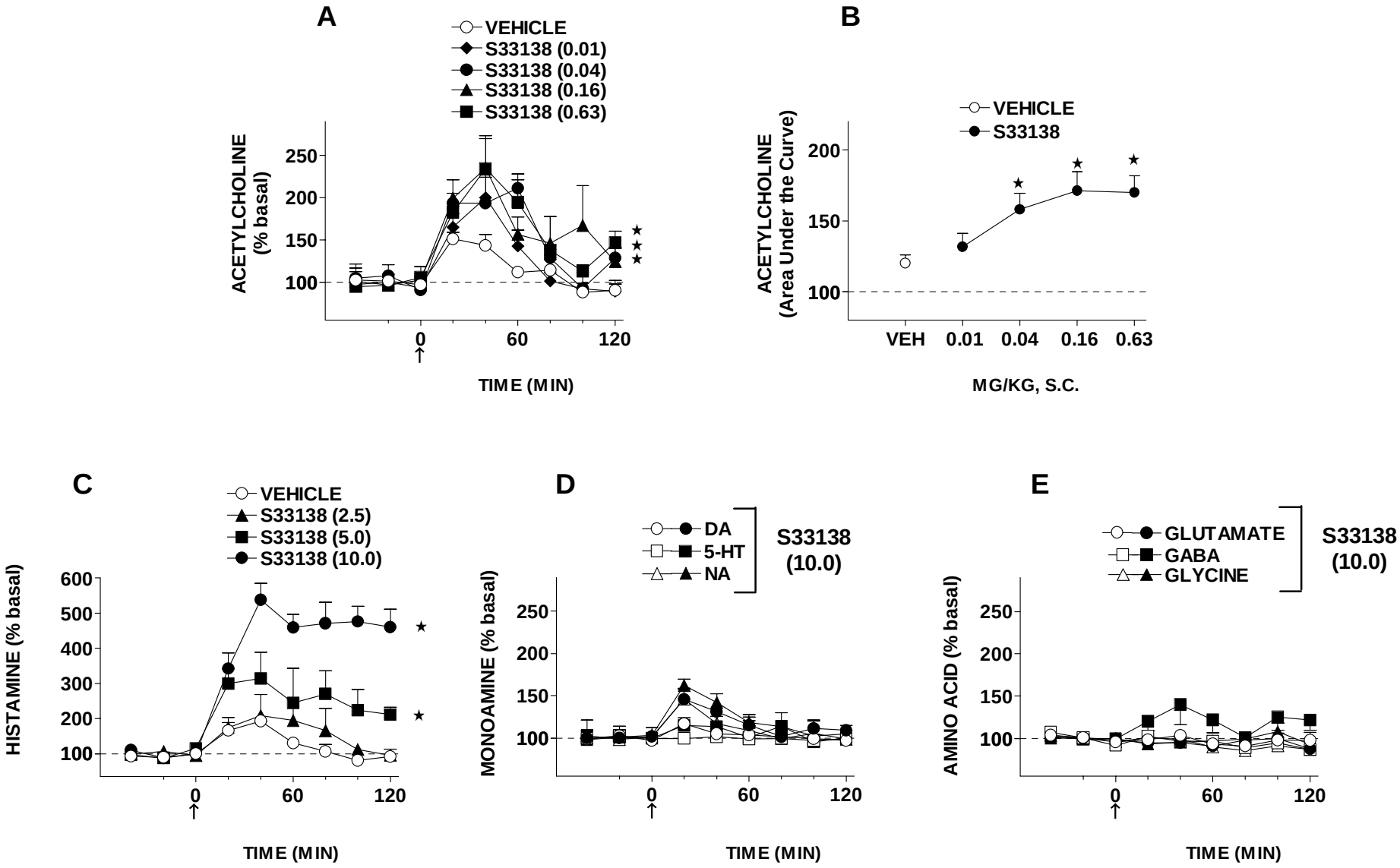

FIGURE 7