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S32212, A NOVEL 5-HT_{2C} RECEPTOR INVERSE AGONIST/ α_2-ADRENOCEPTOR ANTAGONIST AND POTENTIAL ANTIDEPRESSANT: II. A BEHAVIORAL, NEUROCHEMICAL AND ELECTROPHYSIOLOGICAL CHARACTERIZATION

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Abbreviations:

5-HT	Serotonin
ACh	Acetylcholine
AR	Adrenoceptor
BDNF	Brain-Derived Neurotrophic Factor
CMS	Chronic Mild Stress
DA	Dopamine
DRN	Dorsal Raphe Nucleus
EEG	Electroencephalogram
EMG	Electromyogram
FCX	Frontal Cortex
LC	Locus Coeruleus
LRR	Loss of Righting Reflex
NA	Noradrenaline
NOR	Novel Object Recognition
REM	Rapid Eye Movement
SND	Social Novelty Discrimination
SSRI	Selective Serotonin Reuptake Inhibitor
SWS	Slow Wave Sleep
VTA	Ventral Tegmental Area

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ABSTRACT

The present studies characterized the functional profile of S32212, *(N-[*4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-1,2-dihydro-3-***H***-benzo[***e***]indole-3-carboxamide), a combined serotonin $(5\text{-HT})_{2C}$ receptor inverse agonist and α_2-adrenoceptor antagonist that also possesses 5-HT_{2A} antagonist properties (accompanying paper). Upon parenteral and/or oral administration, dose-dependent (0.63-40.0 mg/kg) actions were observed in diverse procedures. Both acute and sub-chronic administration of S32212 reduced immobility time in a forced swim test in rats. Acutely, it also suppressed marble-burying and aggressive behaviour in mice. Long-term administration of S32212 was associated with rapid (1 week) and sustained (5 weeks) normalization of sucrose intake in rats exposed to chronic mild stress, and with elevated levels of mRNA encoding brain-derived neurotrophic factor in hippocampus and amygdala (2 weeks). S32212 accelerated the firing rate of adrenergic perikarya in the locus coeruleus, and elevated dialysis levels of noradrenaline in frontal cortex and hippocampus of freely-moving rats. S32212 also elevated frontocortical levels of dopamine and acetylcholine whereas 5-HT, amino acids and histamine were unaffected. These neurochemical actions were paralleled by "pro-mnemonic" properties: blockade of scopolamine-induced deficits in radial maze performance and social recognition, and reversal of delay-induced impairments in social recognition, social novelty discrimination and novel object recognition. It also showed anxiolytic actions in a Vogel conflict procedure. Further, in an electroencephalographic study of sleep architecture, S32212 enhanced slow-wave and rapid-eye movement sleep, while decreasing waking. Finally, chronic administration of S32212 neither elevated body weight nor perturbed sexual behaviour in male rats. In conclusion, S32212 displays a functional profile consistent with improved mood and cognitive performance, together with satisfactory tolerance.

INTRODUCTION

In the accompanying paper, we describe a novel urea derivative, S32212, that behaves both *in vitro* and *in vivo* as a serotonin $(5\text{-HT})_{2C}$ receptor inverse agonist/α_2-adrenoceptor (AR) antagonist - and that also blocks 5-HT_{2A} receptors. This profile suggests a potentially favourable influence upon depressed mood and, possibly, cognition, which should be expressed in the absence of a deleterious impact upon sleep and sexual function. The studies described herein explored this possibility.

Potential antidepressant properties of S32212 were evaluated in a range of procedures, including the forced swim test in rats, a procedure thought to model "despair" (Kobayashi et al., 2008; Carr and Lucki, 2011). Its effects were also evaluated in marble-burying and isolation-induced aggression tests in mice, two "empirical" procedures responsive to currently-available antidepressants (Dekeyne et al., 2008; Kobayashi et al., 2008). "Psychomotor retardation" can be pharmacologically mimicked by administration of α_2-AR agonists which provokes a loss of righting reflex (LRR) abolished not only by drugs possessing antagonist actions at α_2-ARs, but also by most clinically-active antidepressants (Millan et al., 2000b; Millan et al., 2001; Buyukdura et al., 2011). The prototypical model of chronic mild stress (CMS)-induced reduction in sucrose consumption, considered to reflect "anhedonia", is responsive to antidepressants like imipramine which restore sucrose consumption to non-stressed levels - albeit with variable delays reflecting differences in mechanisms of action (Millan et al., 2001; Willner 2005; Dekeyne et al., 2008). Chronic administration of antidepressants generally enhances gene expression of brain-derived neurotrophic factor (BDNF) - a cellular marker of adaptive plasticity - in the hippocampus and certain other brain structures (Millan, 2006; Schulte-Herbrüggen et al., 2009; Serres et

al., 2011), so the influence of chronic treatment with S32212 upon levels of mRNA encoding BDNF was also examined.

Alpha$_2$-ARs exert a tonic inhibitory influence upon the activity of corticolimbic adrenergic and mesocortical dopaminergic projections. Consequently, their blockade increases extracellular levels of noradrenaline (NA) and dopamine (DA) in the frontal cortex (FCX) and - in the case of NA - other corticolimbic structures (Millan et al., 2000a; Invernizzi and Garattini, 2004; Millan, 2006). GABAergic interneurones inhibitory to ascending monoaminergic pathways bear tonically-active, excitatory 5-HT$_{2C}$ receptors and their blockade likewise facilitates ascending adrenergic and dopaminergic transmission (Millan, 2006; Aloyo et al., 2009; Di Giovanni et al., 2010). Enhancement of frontocortical NA and DA release is related to the positive influence of 5-HT$_{2C}$ and α_2-AR blockade upon depressed mood (Millan, 2005). Accordingly, we examined the influence of S32212 upon the electrical activity of ventral tegmental area (VTA)-localized dopaminergic and locus coeruleus (LC)-localized adrenergic neurons compared to dorsal raphe nucleus (DRN)-localized serotonergic perikarya in anaesthetized rats. In parallel, by use of a dialysis procedure in freely-moving rats, we quantified the impact of S32212 upon extracellular levels of NA and DA *versus* 5-HT in FCX and ventral hippocampus.

Cognitive deficits are increasingly recognized as a core symptom of depression (Castaneda et al., 2008; Marazziti et al., 2010). While certain components of cognitive function are promoted by DA and NA in rats, an acceleration in the release of acetylcholine (ACh) is likewise favourable (El-Ghundi et al., 2007; Robbins and Arnsten, 2009; Millan, 2010; Hasselmo and Sarter, 2011; Klinkenberg et al., 2011). Inasmuch as α_2-ARs are inhibitory to cholinergic projections innervating the FCX, we determined the influence of S32212 upon dialysis levels of ACh as compared to those of histamine and amino acids, likewise

implicated in the control of cognition (Riedel et al., 2003; Passani and Blandina, 2011). In addition, a behavioural evaluation of the impact of S32212 upon cognitive performance was undertaken by the use of several procedures incorporating both visual and olfactory cues, and involving processes of attention, spatial and social cognition (Millan et al., 2010; Winters et al., 2008).

Inasmuch as anxiety is a common and co-morbid symptom of depression, and selective 5-HT_{2C} receptor antagonists possess anxiolytic properties, we examined the potential actions of S32212 in the Vogel conflict test in rats (Millan and Brocco, 2003; Millan, 2005; Dekeyne et al., 2008; Schoevers et al., 2008). Depressive states are accompanied by sexual dysfunction which is exacerbated by agents that elevate extracellular levels of 5-HT, like the selective 5-HT reuptake inhibitor (SSRI), paroxetine (Millan, 2006; Breuer et al., 2008; Serretti and Chiesa, 2009). Conversely, blockade of 5-HT_{2C} receptors and α_2-ARs preserves and may even enhance sexual function (Millan, 2005, 2006; Viitamaa et al., 2006; Kennedy and Rizvi, 2009; De Bodinat et al., 2010). Hence, we studied the potential influence of S32212 in comparison to paroxetine upon sexual function in male rats (Breuer et al., 2008). Finally, depressed states are characterised by perturbed sleep-wake cycle architecture - including insomnia and decreased restorative slow wave sleep (SWS) - and certain antidepressants, like SSRIs, aggravate sleep deficits. While α_2-AR blockade enhances arousal (Ouyang et al., 2004), antagonism of 5-HT_{2C} (and 5-HT_{2A}) receptors promotes SWS (Smith et al., 2002; Landholt and Wehrle, 2009; Descamps et al., 2009). The tetracyclic antidepressant, mirtazapine - which antagonises 5-HT_{2C} receptors - enhances sleep propensity but induces somnolence due to potent histamine H_1 receptor antagonist properties (Mayers and Baldwin, 2005; Millan, 2005; Szegedi and Schwertfeger, 2005). Accordingly, we studied the influence of S32212 in comparison to mirtazapine upon sleep patterns in rats.

METHODS

Animals. Unless otherwise specified below, these studies employed male Wistar rats and NMRI mice supplied by Iffa-Credo (L'Arbresle, France), weighing 200 - 250 g and 22 - 25 g upon arrival, respectively. They were housed in sawdust-lined cages with unrestricted access to standard chow and water. There was a 12 hr/12 hr, light/dark cycle with lights on at 7.30 a.m. Laboratory temperature and humidity were 21 ± 0.5 °C and 60 ± 5 %, respectively. Animals were adapted to laboratory conditions for at least a week prior to testing. All procedures conformed to international European ethical standards for the care and use of laboratory animals (86/609-EEC), together with respective governmental guidelines. They also received local ethical committee approval.

Forced swim test in rats. As previously (Dekeyne et al., 2008), on the first day of the experiment, rats were individually immersed for 15 min in glass cylinders (30 cm h. x 20 cm diam.) filled to a depth of 16 cm with water at 25 °C. The following day, rats were again placed in the water and the duration of immobility was recorded over 5 min. The rat was considered immobile when it remained floating passively in the water, in a upright position, making only the small movements necessary to keep its head above the surface. S32212 or vehicle was administered either acutely, 30 min prior to the test on day 2, or sub-chronically, 24 h, 17 h and 30 min (i.p.) or 60 min (p.o.), before the test on day 2.

Marble-burying behaviour in mice. As previously (Dekeyne et al., 2008), group-housed mice (25 per cage) of 22-26 g were individually placed in transparent polycarbonate cages (30 x 18 x 19 cm) containing a 5 cm layer of sawdust and 24 glass marbles (1.5 cm in diameter) evenly spaced along the cage wall. Thirty min later, animals were removed and the number of marbles at least two-third buried in the sawdust was recorded. Mice were treated 30 min prior to the test with S32212 or vehicle.

Aggression in pre-isolated mice. As previously (Dekeyne et al., 2008), pairs of CD male mice of 22-26 g (Charles River, St. Aubin les Elbeuf, France) were isolated in black cages for 1 month and exposed to each other weekly for 2 months by placement of one mouse ("intruder") in the cage of the other ("resident"). On the test day, the intruder mouse was placed with the resident and the number and duration of fights (emitted by either mouse) monitored for 3 min. Both mice were treated 30 min prior to the test with S32212 or vehicle.

S18616-induced LRR in rats. As previously (Millan et al., 2001; Dekeyne et al., 2008), rats were placed on their backs on a lab surface covered with paper wadding and their ability to right themselves assessed by one ("blinded") observer as follows. Score 0, normal, complete righting reflex; score 1, attempted righting reflex (turn of at least 90 degrees); score 2, attempted righting reflex (turn of less than 90 degrees) and 3, total LRR (no attempt to turn). S32212 or vehicle were administered 30 min prior to the α_2-AR agonist, S18616 (0.63 mg/kg, s.c.), which was administered 30 min prior to scoring of LRR. All rats which received S18616 displayed a score of 3 and the number of rats displaying a score of 2 or less following drug treatment was calculated.

CMS-induced reduction in sucrose consumption in rats. As previously (Dekeyne et al., 2008), this study employed single-housed male Wistar rats of 220 - 250 g (Gorzkowska, Warsaw, Poland). Animals were initially trained to consume a 1 % sucrose solution. Training consisted of ten 1 hr baseline tests (twice weekly) in which sucrose was presented in the home cage following 14 hr food and water deprivation. Sucrose intake was measured by weighing bottles containing the sucrose solution before and at the end of the test. Subsequently, sucrose consumption was monitored under similar conditions (i.e., 1hr access to the sucrose solution after 14 hr of food and water deprivation) and were conducted at weekly intervals throughout the whole experiment. On the basis of their sucrose intakes in the

final baseline test, animals were divided into 2 matched groups. One group was subjected to the CMS procedure for a period of 8 consecutive weeks. Control animals were housed in separate rooms and had no contact with the stressed animals. On the basis of their sucrose intakes, following initial 3 weeks of stress, both stressed and control animals were further divided into matched subgroups, and for the subsequent 5 weeks they received daily injections (i.p.) of S32212, imipramine or vehicle. The drugs were administered at 10.00 a.m. and the weekly sucrose tests were carried out 24 hr following the last drug injection.

Long-term influence upon BDNF expression in the rat hippocampus and amygdala. Male Sprague Dawley rats (Harlan Olac, Bicester, U.K.) weighing 220- 250 g at the start of treatment received twice daily injections of S32212 (10.0 mg/kg, i.p.) or vehicle for 14 days. Two hours following the last injection, mRNA encoding for BDNF was quantified in discrete corticolimbic structures by *in situ* hybridization as described by Serres et al. (2011). In a control study, BDNF expression was measured in animals injected only once (2 hours previously) with S32212 (10.0 mg/kg, i.p.) or vehicle. The influence of drugs was expressed relative to control (vehicle-treated) values (defined as 100 %).

Electrical activity of dopaminergic, noradrenergic and serotonergic cell bodies. The influence of S32212 upon the firing rate of VTA-localized dopaminergic and LC-localized adrenergic cell bodies as compared to DRN-localized serotonergic perikarya was determined as described previously (Millan et al., 2000b). Anesthetized rats were placed in a stereotaxic apparatus and a tungsten microelectrode lowered into the VTA, DRN or LC. Following baseline recording (≥ 5 min), S32212 or vehicle (1/10 ethanol + 4/10 polyethyleneglycol 400+ 5/10 sterile water) were administered i.v. (in a volume of 0.5 ml/kg) in cumulative doses every 2 - 3 min. Drug effects were quantified over the 60 sec bin corresponding to their time of peak action. Spike2 software (CED, Cambridge, England) was employed for data

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acquisition and analysis. Data are expressed as percent change from basal, spontaneous firing rate (0 %).

Dialysate levels of monoamines, ACh and amino acids in freely-moving rats. As described elsewhere (Millan et al., 2000b; Gobert et al., 2003 and 2011), male rats (200-250g) were implanted: 1), in the FCX (AP: + 2.2 from bregma, ML: + 0.6 or –0.6 and DV: -0.2 from dura) with a guide cannula for quantification of monoamines, ACh or amino acids; 2), in the ventral hippocampus (AP: - 5.3, ML: + 5.0 or –5.0 and DV: - 3.2 from dura) with a guide cannula for quantification of monoamines or 3), in the nucleus accumbens (AP: + 0.8 from bregma, ML: + 0.6, and DV: -4.5 from dura) and the striatum (AP: + 0.5 from bregma, ML: -2.8, and DV: -3.0 from dura) with two guide cannulae for quantification of DA and 5-HT. Animals were then single-housed and permitted to recover for 5 days. For dialysis, a cuprophan CMA/11 probe (4 mm in length for the FCX and the striatum, 2 mm for the nucleus accumbens; 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. Two hours after implantation, collection of 20 µl dialysate samples (every 20 min) was initiated. Three basal samples (100 %) were taken prior to i.p. or p.o. administration of S32212 or vehicle and dialysis continued for 3 hours. Monoamine and ACh levels were quantified as previously (Gobert et al., 2003; Dekeyne et al., 2008) by HPLC and electrochemical detection. Glutamate, glycine and GABA were pre-column derivatized using naphtalene dicarboxaldehyde as a fluorophore and quantified by HPLC coupled to fluorimetric detection.

Dialysate levels of histamine in FCX dialysates of freely-moving rats. As previously (Dekeyne et al., 2008), single-housed male Wistar rats weighing 280-350 g (Harlan, Zeist, Netherlands) were implanted with an I-shaped guide probe (AN 69 membrane, 4 mm exposed

surface) (Hospal, Bologna, Italy) into the FCX at the following coordinates: AP = + 3.4 from bregma, ML = +0.8 or –0.8 and DV = - 1.0. These coordinates differ slightly to those indicated above for the other transmitters since the weight of the rats used to measure histamine was somewhat greater. Nonetheless, according to Paxinos and Watson (1998), the area targeted was the same (cingular, prelimbic and infralimbic territories of frontal cortex). Experiments were performed 24-48 hours after implantation employing perfusion of artificial CSF designed, by analogy to studies of monoamines and other transmitters, to maintain stable and "physiological" levels of resting histamine: 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. After separation, histamine was post column derivatized using *o*-phtaldialdehyde as fluorophore and quantified by fluorimetry.

Scopolamine-induced amnesia in the radial maze test in rats. The influence of chronic administration of S32212 (once daily, 7 days) was evaluated upon blockade of scopolamine-induced spatial working memory deficits in the radial maze test in rats. The experiment was conducted with male Wistar rats (Elevage Janvier, Le Genest-Saint-Isle, France) weighing 210-250 g, housed by 5 with restricted access to food (15 g/day/rat). The apparatus was constructed in black Plexiglas and consisted of a central platform (30 cm in diameter) with 8 open branches (68 x 10 cm), elevated 80 cm above the floor. The animals were submitted to 3 training sessions (one per day) and 5 days later, to 3 test sessions (one per day). A session consists in placing the rats individually in the center of the maze, baited with a single food pellet (45 mg) at the end of each branch, and allowing them to make 8 choices in less than 5 min. The number of errors (branches revisited) was recorded. S32212 or vehicle was administered i.p. once daily for 4 days prior to testing and then, 45 min before each test session. Scopolamine (0.5 mg/kg, i.p.) was administered 15 min before each test session.

Social recognition in rats. As previously (Loiseau et al., 2008), the experiments were conducted with adult Wistar rats weighing 240-260 g, individually housed for 2 days before testing, and juvenile, group-housed Wistar rats (25-30 days old) (Elevage Janvier, Le Genest-Saint-Isle, France). On the test day, a juvenile was placed into the home cage of an adult rat for a first 5-min session (T1) and the time spent by the adult rat exploring the juvenile was recorded. The duration of investigation was also monitored during a second (T2) 5-min session. As previously shown (Loiseau et al., 2008), in the absence of delay between T1 and T2, control (vehicle-treated) rats display shorter T2 than T1, and, reflecting amnesic properties, the muscarinic antagonist, scopolamine (1.25 mg/kg, s.c.), significantly increased T2 in the absence of a significant alteration in T1. In a first set of experiments, T2 was performed just after T1 and rats received scopolamine (1.25 mg/kg, s.c.) or vehicle 30 min before T1, preceding by S32212 or vehicle (45 min, s.c. and 60 min, p.o., before T1). In the second set of experiments, a "spontaneous" deficit of recognition was induced by a 120-min delay between the two sessions. T2 was conducted either with the same juvenile (for evaluation of potential "promnesic" actions of drugs) or with a different juvenile (to control for the specificity of drug actions), and S32212 or vehicle was administered 1 min after T1.

Delay-induced deficit in novel object recognition (NOR) in rats. Twelve adult male Lister Hooded rats (Charles River, UK) weighing 170g upon arrival were housed in groups of 4 for one week before testing. As previously described (Millan et al., 2010), NOR was assessed in unfamiliar Perspex arena (39 x 23.5 x 24.5 cm). Twenty-four hours before the test day, each rat was habituated to its arena for 1 hour. On a test day, rats were re-habituated to their arena for 3 minutes before being returned to the home cage for 1 minute. During the "familiarisation trial", two identical white bottles were placed in the arena and the rat allowed to explore the objects for 3 minutes. During the second "choice" trial, one object was

replaced with a novel unfamiliar object (white with black horizontal stripes) and the rat allowed to explore the objects again for 3 minutes. During each trial, the exploration of each object was recorded. A "D2" discrimination ratio (exploration of the novel object - exploration of familiar object/ total object exploration) was calculated from raw data of choice trial. A high value of this ratio can be obtained with a short inter-trial delay (2 hours) and, reflecting natural forgetting, has been shown to decrease with an inter-trial interval extended to 4 hours. In order to reproduce this spontaneous deficit, this study employed a 4-hours delay. Test days were repeated once a week for 4 weeks, each rat receiving every treatment (vehicle or S32212 0.63, 2.5 or 10 mg/kg, s.c.) in a pseudorandom order, 30 min before the familiarization trial.

Social novelty discrimination (SND) in rats. The procedure was conducted as previously (Millan et al., 2010). Animals and housing conditions were as for the social recognition. During a first 5-min period (P1), a juvenile was placed into the home cage (43 x 28 x 20 cm) of an adult and the time spent by the adult in investigation of the juvenile was recorded. Thirty minutes later, this juvenile (familiar) was reintroduced for a second 5-min period (P2), together with a novel unfamiliar juvenile. During P2, the times of investigation of each juvenile (P2 novel and P2 familiar) were recorded and the ratio P2 novel/P2 familiar was calculated. S32212 or vehicle was administered s.c., 30 min before P1.

Motor function in mice and rats. As previously (Dekeyne et al., 2008), the latency of mice to fall from an accelerating (4 to 40 rpm over 300 sec; then constant speed from 300 to 360 sec) rotarod (Ugo Basile, Varese, Italy) was determined. There was a cut-off of 360 sec. S32212 or vehicle was administered 30 min prior to the test. Spontaneous locomotion in mice and rats was also determined as previously (Dekeyne et al., 2008). Mice activity chambers were white Plexiglass cages (27 x 27 x 27 cm) equipped with two rows of 4 photocells 2 cm

above the floor and 6 cm apart connected through an interface (Hesperid, France) to a microcomputer. In mice, S32212 or vehicle was given 30 min before placing the animals for 10 min in individual chambers. Rat activity chambers were transparent polycarbonate cages (35 x 24 x 19 cm) equipped with two infrared beams 4 cm above the floor and 22 cm apart, connected through Lablinc System interface (Coulbourn, PA, USA) to a microcomputer. Rats were placed in the activity chambers just after drug or vehicle injection and locomotor activity was recorded 30 min later for 1 hour. In both procedures, data were locomotion counts, with one count corresponding to the consecutive interruption of 2 infrared beams.

Vogel conflict test in rats. As previously (Millan and Brocco, 2003; Dekeyne et al., 2008), the test was conducted in polycarbonate cages (32 x 25 x 30 cm) possessing a grid floor with the spout of a water bottle located 6 cm above the floor. Both the grid and the spout were connected to an Anxiometer (Columbus Instruments, Ohio, USA) used to record licks and deliver electrical shocks. During the 3 days preceding testing, rats were housed in groups of four and were restricted to 1-h-per-day access to tap water (from 9:00 to 10:00 a.m.). On day 4, just after water delivery, they were isolated in cages with a grid-floor. Testing took place on day 5. Rats were administered with S32212 or vehicle 30 min prior being placed in the cages. The session was initiated after the animal had made 20 licks and received a first, mild shock (a single, 0.5-sec constant current pulse of 0.3-mA intensity) through the spout. Thereafter, a shock was delivered to the animal every 20th lick during a period of 3 min and the number of licks emitted during this 3-min session was recorded.

Long-term influence upon sexual behaviour in male rats. As previously described (Breuer et al., 2008), 120 male and 120 female Wistar rats (Harlan, Zeist, The Netherlands) of approximately eight weeks of age were group-housed under a reversed day-night schedule (lights off at 6.00 a.m.). After one week of habituation, males were paired (training tests)

during 30 min, once weekly for 4 consecutive weeks, with an oestrus female in an observation cage (30 x 40 x 60 cm). Females were brought into oestrus by s.c. injection of 50 µg estradiol benzoate (in 0.1 ml of sesame oil saturated with lecithin), 36 h prior to testing. In the present study, 36 males with an average of 2 to 3 ejaculations during the last training test (normal performers) were selected for drug testing. They were subsequently submitted to 4 additional tests (once a week) at days 0, 7, 14 and 21. Animals received daily injection of S32212 (2.5 mg/kg, i.p.), paroxetine (10.0 mg/kg, i.p.) or vehicle (N = 12 per group) from day 0 to day 14. On day 0 (acute treatment), 7 (sub-chronic) and 14 (chronic), animals were injected 30 min before testing. The test at day 21 was conducted to assess the putative rebound or after-effects of treatments. Data analyzed were the number of ejaculations and the latency to the first ejaculation.

Sleep-wake cycle architecture in the rat. As previously (Descamps et al., 2009), male rats were equipped with polygraphic electrodes under chloral hydrate (300 mg/kg, i.p.) anesthesia. After 2 weeks of recovery and habituation, recording of electroencephalogram (EEG) and electromyogram (EMG) was initiated and continued until stable baselines of sleep-wake states were obtained. Then, the animals were divided in two groups (N = 11 in each), one receiving treatments at the beginning of the dark period, the other at the beginning of the light phase. In both cases, animals were treated with vehicle then, 48 h later, with S32212 (10 mg/kg, s.c.) or mirtazapine (10 mg/kg, s.c.). Then, after a 3-days washout period, they received vehicle and, 48 h later, mirtazapine or S32212. Visual scoring of digitized EEG and EMG traces (EEG filtering, 0.5-49.9 Hz and EMG, 15-49.9 Hz) was performed over 10 sec epochs throughout 2 x 24 h following each drug or vehicle administration. Quantified data were the duration of sleep-wake episodes.

Data analyses. Unless otherwise specified below, dose-effects were analyzed by one-way ANOVA followed by Dunnett's test. Significance of inhibition of S18616-induced LRR were evaluated by the Fisher Exact Probability test. In the CMS procedure, data were analyzed by multiple ANOVA with 3 between-subjects factors (stress/control, drug treatment and successive sucrose tests) followed by paired-t tests to evaluate significance of differences *versus* pre-treatment (week 0) values. Data for BDNF gene expression were analyzed by unpaired t-tests. In dialysis studies, data were analyzed by ANOVA with dose as the between factor and sampling time as the repeated within-subject factor. In the study of scopolamine-induced amnesia in the radial maze, data were analyzed with unpaired t tests. In the procedure of scopolamine-induced deficit in social recognition, the difference "T2-T1" was calculated and analyzed by two-way ANOVA with scopolamine and drug as between factors, followed, if significant, by one-way ANOVA; comparisons of drug/vehicle to vehicle/vehicle and of drug/scopolamine to vehicle/scopolamine values were made by the use of Dunnett's test. For delay-induced deficits in social recognition, the difference "T2-T1" was calculated and dose-response curves were analyzed by one-way ANOVA followed by Dunnett's test; the specificity of drug effects was analyzed by two-way ANOVA followed by Newman-Keuls test. In NOR and SND procedures, raw data were analysed by ANOVA with the exploration of novel and familiar object/juvenile as the repeated within-subject factor and the treatment as the between-subject factor; "D2" or "P2 novel/P2 Familiar" ratios were analyzed by one-way ANOVA followed by Dunnett's test. The influence of drugs upon waking state, SWS and rapid eye movement (REM) sleep was analyzed by two-way ANOVA followed by Newman-Keuls test.

Drug doses, administration and structures. For essentially all procedure, a broad range of doses was tested to well cover the putative active dose range (see Results). The route

employed was not identical in view of the very broad palette of procedures employed, but essentially followed well-established, standard procedures being identical to those used in our previous exploitation of these procedures (Dekeyne et al., 2008; Millan et al., 2000b; 2001). For example i.v. administration was necessarily used for electrophysiological work. In several procedures, like dialyses levels of monoamines, forced test, and social recognition procedure, we confirmed data acquired by the systemic (s.c.) route using oral administration. For chronic administration, the i.p. rather than s.c. route was preferred to avoid any potential complications of poor cutaneous tolerance.

Drug doses are in terms of the base. When administered s.c., drugs were dissolved in sterile water, if necessary plus a few drops of lactic acid and pH readjusted with NaOH to neutrality. When administered by the i.p. or p.o. routes, they were prepared as suspensions in distilled water added with a few drops of Tween 80. In rats, the volume of injection was 1 ml/kg, i.p. or s.c., and 10 ml/kg, p.o.. In mice, it was 10 ml/kg whatever the route of administration. Drug salts and sources were as follows: mirtazapine, paroxetine HCl, S32212 {***N***-[4-methoxy-3-(4-methyl piperazin-1-yl)phenyl]-1,2-dihydro-3-***H***-benzo[***e***]indole-3-carboxamide} HCl, and S18616 {(S)-spiro[(1-oxa-2-amino-3-azacyclopent-2-ene)-4,2'-(1',2',3',4'-tetrahydronaphthalene)]} HCl, were synthetized by Servier. Imipramine HCl and scopolamine HCl were obtained from Sigma (St Quentin-Fallavier, France).

RESULTS

Reduction of immobility in a forced swim test in rats (Figure 1A). When administered sub-chronically (24 h, 17 h and 30 min before testing), S32212 significantly decreased immobility time in rats both upon i.p. and oral administrations: minimal effective dose (MED), 2.5 and 10.0 mg/kg, respectively. Upon acute injection (i.p.) 30 min before testing, S32212 also induced a dose-dependent and significant decrease of immobility (MED, 5.0 mg/kg).

Decrease of marble-burying behaviour in mice (Figure 1B). Mice placed into cages containing marbles displayed spontaneous burying behaviour. Within the 30 min observation session, 18 ± 2 marbles were buried by vehicle-treated subjects. S32212 (0.63-40.0 mg/kg, s.c.) dose-dependently reduced marble-burying behaviour.

Decrease of aggressive behaviour in mice (Figure 1C). In pairs of pre-isolated, familiar mice, placement of the "intruder" into the cage of the "resident" elicited aggressive behaviour. S32212 dose-dependently (0.63-40.0 mg/kg, i.p.) attenuated aggressive behaviour as revealed by a reduction in the number - and the duration (not shown) - of attacks.

Blockade of the LRR induced by S18616 in rats (Figure 1D). The potent, high efficacy α_2-AR agonist, S18616 (0.63 mg/kg, s.c.), exerted a marked depressive influence upon motor activity in rats, which was expressed as a LRR. S32212, which did not itself affect the righting reflex (not shown), dose-dependently and fully blocked this action of S18616.

Suppression of CMS-induced "anhedonia" in rats (Figure 2). Following exposure to CMS for 3 weeks, rats displayed a marked reduction in sucrose intake relative to non-stressed rats over the 5 weeks of testing 5: $F(1,84) = 16.4$, $P<0.01$. In stressed subjects, the prototypical antidepressant and internal reference, imipramine (10.0 mg/kg, i.p), enhanced sucrose consumption from week 4. Daily administration of S32212 at doses of 0.63 and 2.5 mg/kg,

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i.p. also significantly and time-dependently augmented sucrose consumption in stressed rats: for both doses, a significant effect was apparent throughout weeks 1 to 5. A lower dose (0.16 mg/kg, i.p.) did not significantly restore sucrose consumption. In control, non-stressed animals, neither S32212 nor imipramine modified sucrose consumption. Chronic treatment with S32212 did not modify body weight *versus* vehicle-treated animals at any time or dose (not shown).

Induction of BDNF expression in hippocampus and amygdala of rats (Figure 3). Chronic treatment with S32212 (10.0 mg/kg, i.p., 14 days, twice daily) caused a significant increase in the abundance of mRNA encoding BDNF in the amygdala, CA1 region of the hippocampus and the hippocampal dentate gyrus. Acute administration of S32212 (10.0 mg/kg, i.p, N = 5) did not, in contrast, increase BDNF expression in the amygdala (90 ± 4 % *versus* control values, defined as 100%), CA1 region of the hippocampus (90 ± 5) or dentate gyrus (79 ± 5).

Influence of S32212 upon the electrical activity of adrenergic, dopaminergic and serotonergic cell bodies (Figure 4). S32212 dose-dependently and significantly increased the electrical activity of LC-localized noradrenergic perikarya. This effect attained a maximal increase of +52 % *versus* baseline values at the highest dose tested (2 mg/kg, i.v.). Over the same cumulative dose range (0.125 to 2 mg/kg, i.v.), S32212 exerted no significant influence upon the firing rate of dopaminergic cells of the VTA. It similarly had no significant effect upon the firing rate of serotonergic neurons of the DRN.

Influence of S32212 upon extracellular levels of NA, DA and 5-HT in FCX and ventral hippocampus of freely-moving rats (Figure 5). S32212 (0.63-40.0 mg/kg, s.c.) elicited a pronounced, sustained and dose-dependent increase in dialysis levels of NA and DA in the FCX, whereas 5-HT levels were not significantly modified (Fig. 5, upper panels). Upon oral

administration, in FCX, a similar pattern of effects was obtained: area under the curve (AUC) analysis expressed relative to basal values (100%) as follows: NA, vehicle = 114.9 ± 3.1 *versus* S32212 (2.5), 113.0 ± 3.0, $P > 0.05$; S32212 (5.0) = 131.7 ± 4.0, $F(1,14) = 7.3$, $P < 0.05$; S32212 (10.0) = 140.8 ± 4.4, $F(1,13) = 9.0$, $P < 0.01$ and S32212 (40.0) = 160.2 ± 5.6, $F(1,16) = 11.6$, $P < 0.01$. DA, vehicle = 106.0 ± 2.1 *versus* S32212 (2.5), 121.2 ± 5.9, $P > 0.05$; S32212 (5.0) = 128.6 ± 3.9, $F(1,14) = 11.7$, $P < 0.01$; S32212 (10.0) = 128.6 ± 4.0, $F(1,13) = 12.0$, $P < 0.01$ and S32212 (40.0) = 155.9 ± 5.0, $F(1,15) = 22.2$, $P < 0.01$. 5-HT, vehicle = 99.2 ± 2.4 *versus* S32212 (2.5), 87.8 ± 4.1, $P > 0.05$; S32212 (5.0) = 94.9 ± 3.5, $P > 0.05$; S32212 (10.0) = 96.1 ± 4.9, $P > 0.05$ and S32212 (40.0) = 89.1 ± 5.0, $P > 0.05$. Administered at a dose of 40 mg/kg, s.c. that strongly increased DA levels in FCX (AUC, vehicle = 101.3 ± 2.7 *versus* S32212 = 148.3 ± 4.9, $F(1,8) = 12.1$, $P < 0.01$), S32212 did not modify extracellular levels of DA in the nucleus accumbens (AUC, vehicle = 95.1 ± 1.7 *versus* S32212 = 93.4 ± 2.5, $P > 0.05$) or striatum (AUC, vehicle = 100.5 ± 2.1 *versus* S32212 = 101.0 ± 1.3, $P > 0.05$). Further, S32212 did not affect 5-HT levels in these structures (not shown). At a maximally effective dose (40.0 mg/kg, s.c.) in FCX, S32212 also increased NA and DA levels in the ventral hippocampus whereas 5-HT levels were not modified (Fig. 5, lower panels).

Influence of S32212 upon extracellular levels of ACh *versus* histamine, glutamate, glycine and GABA in FCX dialysates of freely-moving rats (Figure 6). S32212 (0.63-10.0 mg/kg, s.c.) elicited a sustained and dose-dependent increase in dialysis levels of ACh in the FCX, whereas it did not significantly modify histamine levels. At the highest dose evaluated (10.0 mg/kg, s.c.), FCX levels of GABA and glutamate were not significantly modified.

Reversal by S32212 of a deficit induced by scopolamine in the radial maze test in rats (Table 1). Scopolamine (0.5 mg/kg, i.p.) administered before each test session provoked an increase in mean (3 sessions) number of revisited arms. This deficit was significantly reduced by daily (4 days preceding testing and 3 days of testing) injection of S32212 at the dose of 10.0 mg/kg, i.p. The lower dose of 2.5 mg/kg was ineffective.

Reversal by S32212 of scopolamine- and delay-induced deficits in social recognition in rats (Figure 7). In the absence of a delay between T1 and T2, adult rats treated s.c. or p.o. with vehicle displayed significant ($P < 0.05$, paired t-tests) shorter durations of investigation of the juvenile during T2 as compared to T1 (s.c., T2 = 58 ± 7 sec and T1 = 106 ± 8 sec and p.o., T2 = 48 ± 8 sec and T1 = 96 ± 5 sec). Contrariwise, adults treated with scopolamine failed to recognize the juvenile, as revealed by T2 values ($P > 0.05$, paired t-tests) similar to T1 values (s.c., T2 = 70 ± 4 sec and T1 = 78 ± 4 sec and p.o., T2 = 64 ± 4 sec and T1 = 79 ± 3 sec). Accordingly, as depicted in Figs 7A and 7B, the difference T2-T1 was significantly decreased by scopolamine. Administered both s.c. and p.o., S32212 abrogated the deficit induced by scopolamine with similar MEDs (0.04 mg/kg). This influence of S32212 reflected a specific decrease of T2 rather than increase of T1 values. Thus, at the highest doses tested (0.63 mg/kg, s.c. and 0.16 mg/kg, p.o.), S32212 + scopolamine values were as follows: s.c., T2 = 35 ± 9 sec and T1 = 80 ± 10 sec and p.o., T2 = 33 ± 5 sec and T1 = 75 ± 5 sec. With a 2-hours delay between T1 and T2, vehicle-treated rats displayed an equivalent ($P > 0.05$) duration of investigation of the same juvenile across sessions, reflecting a failure of recognition (s.c., T2 = 90 ± 8 sec and T1 = 90 ± 8 sec and p.o., T2 = 89 ± 8 sec and T1 = 84 ± 7 sec). Hence, T2-T1 differences were mimimal (Figs 7C and 7D). S32212 abrogated this delay-induced deficit, as revealed by high negative T2-T1 values. At the most effective doses (2.5 mg/kg, s.c. and 10 mg/kg, p.o.), S32212 did not provoke negative T2-T1 values when T2

was performed with a juvenile different from T1. This suggests a specific influence upon cognitive processes.

Reversal by S32212 of a delay-dependent deficit in NOR in rats (Figure 8). After a 4 h delay, control (vehicle) rats spent equivalent time exploring the familiar *versus* novel object during the choice trial. As shown by the D2 index score, S32212 dose-dependently and significantly abolished this deficit. Further analysis revealed that, at doses of 0.63 and 2.5 mg/kg, S32212 increased time spent at the novel object rather than decreasing time spent at the familiar object. At the highest dose (10.0 mg/kg, s.c.), it decreased interest for the familiar object while interest for the novel object slightly increased. Suggesting that S32212 did not exert non-specific effects or impair locomotor function, it had no significant effect on total exploration of objects during either the familiarization or choice trials. ANOVAs as follows. Familiarization: vehicle, 12.7 ± 0.1 sec; S32212, 0.63 mg/kg, 13.5 ± 0.1; 2.5 mg/kg, 13.2 ± 0.1 and 10.0 mg/kg, 11.1 ± 0.1, $F(3,44) = 0.5$, $P > 0.05$. Choice: vehicle, 9.3 ± 1.6; S32212, 0.63 mg/kg, 10.4 ± 2.6; 2.5 mg/kg, 12.0 ± 2.5 and 10.0 mg/kg, 7.8 ± 1.5, $F(3,44) = 0.7$, $P > 0.05$.

Facilitation by S32212 of SND in rats (Figure 9). With a short period P1 (5 min), control (vehicle) animals spent equivalent time investigating the familiar *versus* novel juvenile during P2 (Fig. 9A). S32212 increased time spent investigating the novel juvenile without modifying time spent with the familiar (Fig. 9A): the increase of P2 novel/P2 familiar ratio was statistically significant at the intermediate doses of 0.16 and 0.63 mg/kg (Fig. 9B). Suggesting that it did not have a non-specific influence, S32212 exerted no effect on total investigation of juveniles during either P1 or P2. ANOVAs as follows. P1: vehicle, 77.9 ± 4.5 sec; S32212, 0.04 mg/kg, 87.8 ± 11.0; 0.08 mg/kg, 86.1 ± 9.3; 0.16 mg/kg, 90.0 ± 7.6; 0.63 mg/kg, 95.1 ± 4.8 and 2.5 mg/kg, 100.5 ± 7.1, $F(5,34) = 0.9$, $P > 0.05$. P2: vehicle, 75.3 ±

6.1; S32212, 0.04 mg/kg, 67.2 ± 16.7; 0.08 mg/kg, 66.9 ± 9.6; 0.16 mg/kg, 83.1 ± 15.7; 0.63 mg/kg, 79.2 ± 9.6 and 2.5 mg/kg, 100.0 ± 11.6, $F(5,34) = 0.8$, $P > 0.05$.

Influence upon motor function (Table 2). In the rotarod procedure, S32212 (0.63-40.0 mg/kg, s.c.) significantly modified the latency to fall only at the highest dose tested (40.0). In mice non-habituated to the activity chamber, S32212 (0.63-40.0 mg/kg, s.c.) significantly decreased locomotor activity at doses of 10.0 and 40.0 mg/kg. In habituated rats, S32212 (0.63-10.0 mg/kg, s.c.) did not significantly affect locomotor activity.

Anxiolytic properties in the Vogel conflict test in rats (Table 3). S32212 (0.63-40.0 mg/kg, i.p.) elicited a dose-dependent and robust increase in punished responses in the Vogel conflict test.

Lack of influence upon sexual behaviour of male rats (Table 4). After acute treatment (Day 0) of S32212 (2.5 mg/kg, i.p.) or paroxetine (10.0 mg/kg, i.p.), there were no significant effects upon either the number of ejaculations or the latency to the first ejaculation as compared to vehicle. After sub-chronic (7 days) and chronic (14 days), S32212 still lacked a deleterious effect upon sexual behaviour, whereas paroxetine displayed a marked inhibitory influence. During a test session performed on day 21, that is, one week after cessation of S32212 administrations, no “after-effect” was observed, and paroxetine-treated rats had completely recovered from their low sexual performances relative to vehicle-treated rats. S32212 did not affect body weight as compared to vehicle, whereas paroxetine significantly reduced body weight after 14 days of treatment and even 7 days after withdrawal, body weight had not returned to normal.

Influence upon sleep-wake cycle architecture in rats (Figure 10). As shown in the left panels of Fig. 10, S32212 (10.0 mg/kg, s.c.) administered at the beginning of the dark period decreased the duration of waking, and in parallel, significantly increased SWS. S32212 also

increased REM sleep, though significance was seen only 2 hours after the onset of its impact on waking and SWS. During the subsequent light period, no significant influence of S32212 was observed. Further, there was no significant influence of S32212 throughout the subsequent 24h period (not shown). In rats treated at the onset of light phase (not shown), S32212 induced similar though less marked quantitative changes: decrease in waking (3 to 6 hours post-injection), increase of SWS (4 to 5 hours) and a delayed increase in REM sleep (8 to 9 hours). As shown in the right panels of Fig. 10, mirtazapine (10 mg/kg, s.c.) given at the onset of the dark period slightly decreased waking and increased SWS. These effects were similar to those with S32212, although less pronounced and shorter (3 to 6 *versus* 3 to 9 hours post-injection). However, in contrast to S32212, mirtazapine rapidly *reduced* REM sleep (1 to 4 *versus* 6 to 9 hours post-injection). Mirtazapine did not provoke further quantitative changes, neither during the subsequent light period nor the second 24 hr period (not shown). In animals receiving mirtazapine at the onset of light phase (not shown), quantitative changes were similar: decrease in waking, increase of SWS (3 to 5 hours post-injection) and a rapid decrease in REM sleep (1 to 4 hours).

DISCUSSION

Actions in models of potential antidepressant properties. Anti-immobility actions in the forced swim test are generally attributed to antidepressant properties and were expressed by S32212 both sub-chronically and, in contrast to certain antidepressants like SSRIs, acutely (Millan et al., 2001; Cryan et al., 2005; Millan, 2006, Kobayashi et al., 2008; Carr and Lucki, 2011). S32212 inhibited spontaneous marble-burying in mice over a similar dose-range: though not of any obvious construct validity for depressed states, suppression of marble-burying may be related to the influence of SSRIs and tricyclics upon compulsive behaviours (Millan et al., 2001; Kobayashi et al., 2008; Thomas et al., 2009). By analogy to marble-burying, suppression of aggressive behaviour in isolated mice is an empirical screen not easily related to core deficits of depression, despite the high incidence of aggression and irritability in patients (Millan et al., 2001; Millan, 2006; Dekeyne et al., 2008; Malkesmann et al., 2009). In view of its reduction by S32212, it would be interesting to determine whether S32212 specifically impacts agonistic behaviour under other conditions (Mitchell 2005), and whether it affects behaviour in models of social defeat (Razzoli et al., 2009; Carr and Lucki, 2011). Reversal by S32212 of the motor-depressant action (LRR) of S18616 mimics numerous antidepressants, and has been putatively related to the relief of psychomotor retardation (Millan et al., 2001; Dekeyne et al., 2008). Selective 5-HT_{2C} antagonists are effective in *all* the above procedures, whereas 5-HT_{2A} antagonists express variable and modest actions (Millan et al., 2000b; Millan, 2005, 2006; Dekeyne et al., 2008; Landholt and Wehrle, 2009; Pandey et al., 2010; Carr and Lucki, 2011). Immobility in the forced swim procedure is reduced by α_2-AR antagonists and genetic deletion of α_{2C}-ARs (Sallinen et al., 2007; Millan, 2010). Alpha$_2$-ARs antagonists also reduce aggressive behaviour (Haller et al., 1996; Brocco, M. et al., unpub. obs.), suppress marble-burying (Lähdesmäki et al 2002) and

block S18616-induced LRR (Millan et al., 2000b). Thus, the precise contribution of 5-HT_{2C} and/or α_2-AR properties to these "antidepressant-like" actions of S32212 will require additional study.

The reversal of CMS-induced sucrose consumption by chronic administration of S32212 suggests "anti-anhedonic" action and mirrors actions of clinically-efficacious antidepressants like imipramine (Willner, 2005), though S32212 displayed a more rapid onset of action (week 1). Selective α_2-AR antagonists have not been studied in the CMS procedure, but they accelerate the onset of action of monoamine reuptake inhibitors in other experimental paradigms and in patients, mainly by blocking presynaptic α_2-ARs inhibitory to monoaminergic pathways (Millan 2006; Dhir and Kulkarni, 2007; Yanpallewar et al., 2010; Serres et al., 2011). Inasmuch as 5-HT_{2C} antagonists are active in the CMS procedure, and enhance extracellular levels of monoamines (Millan, 2005; Dekeyne et al., 2008; Carr and Lucki, 2011), the complementary α_2-AR properties of S32212 may well speed up its antidepressant actions.

By analogy to many antidepressants, chronic administration of S32212 increased BDNF gene expression in the hippocampal dentate gyrus/CA1 region and amygdala of rats (Millan, 2006; De Bodinat et al., 2010; Hashimoto, 2010; Serres et al., 2011). BDNF favours processes underpinning neuronal proliferation and survival, synaptic plasticity, cognition and mood, and reductions in BDNF levels are seen both in patients and in animal models of depression (Schulte-Herbrüggen et al., 2009; Hashimoto, 2010). Hence, it would be instructive to determine the speed of onset of BDNF induction by S32212, and to extend these observations to neurogenesis in normal and "depressed" animals (Pittenger and Duman, 2008; Schulte-Herbrüggen et al., 2009; De Bodinat et al., 2010). While chronic 5-HT_{2C} receptor blockade enhances BDNF gene expression (Dekeyne et al., 2008; Soumier et al., 2009) and is likely

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implicated in the effects of S32212, a role for α_2-AR antagonism, mirroring the induction of other effector genes like Arc (Yanpallewar et al., 2010; Serres et al., 2011), should not be excluded.

Influence on extracellular levels of NA, DA and ACh in freely-moving rats. Reflecting blockade of (constitutively-active) 5-HT_{2C} receptors and α_2-ARs inhibitory to VTA- and LC-derived dopaminergic and adrenergic projections, respectively (Millan et al., 2000a; Millan, 2006; Dekeyne et al., 2008; Ayolo et al., 2009; Di Giovani et al., 2010), S32212 elevated extracellular levels of DA and NA in FCX and hippocampus of freely-moving rats. The acceleration of LC firing rate reflects blockade of tonically-active α_2-AR autoreceptors on adrenergic perikarya - principally the α_{2A}-AR subtype with a subsidiary role for α_{2C}-ARs (Millan et al., 2000a; Millan, 2010; Dwyer et al., 2010) - and both are antagonised by S32212 (accompanying paper). Blockade of α_{2A}-ARs on dopaminergic *terminals* likely underlies the S32212-induced increase in DA levels in FCX, since it did not affect dopaminergic cell bodies (Millan et al., 2000a; Millan, 2010; Dwyer et al., 2010). Indeed, α_2-ARs do not control resting activity of VTA-localized dopaminergic perikarya, yet like LC-adrenergic neurons, they are tonically inhibited by (constitutively-active) 5-HT_{2C} receptors acting *via* GABAergic interneurones (Millan et al., 2000a; Dekeyne et al., 2008; Ayolo et al., 2009; Di Giovani et al., 2010). Thus, it remains to be elucidated why S32212 did not excite VTA-dopaminergic perikarya, as well as the precise contribution of 5-HT_{2C} receptor and α_2-AR blockade to its facilitation of adrenergic and dopaminergic transmission.

Apart from a favourable impact on depressed mood, a reinforcement of DA and NA release by S32212 favours cognitive function, which should be further strengthened by its elevation of ACh release in FCX at doses of 2.5-10.0 mg/kg (Millan, 2010; Hasselmo and Sarter, 2011; Klinkenberg et al., 2011). This action reflects blockade of α_{2A}-ARs exerting a tonic,

inhibitory influence on cholinergic pathways projecting to FCX (Gobert et al., 2003; Nair and Gudelsky, 2004). It should be mentioned that the transient increase in levels of ACh just following injection is a characteristic "arousal" response to the "mild stress" of handling and injection - as discussed in previous studies (Millan et al., 2000a; Gobert et al., 2003). Stimulation of α_2-ARs *in vitro* inhibits the release of glutamate and histamine, neurotransmitters which also modulate cognitive processes, but the influence of α_2-AR (or 5-HT_{2C}) blockade upon glutamatergic and histaminergic transmission *in vivo* remains unclear (Riedel et al., 2003; Millan, 2010; Passani and Blandina, 2010). Indeed, S32212 did not affect extracellular levels of histamine, glutamate or other amino acids in FCX, underscoring the specificity of its influence upon levels of NA, DA and ACh.

Actions in models of potential pro-cognitive properties. S32212 improved performance in several tests of cognitive function. Though the issue of underlying neural substrates lies outside the scope of the present work, spatial tasks like the radial maze involve the dorsal hippocampus, and the hippocampal formation (including the perirhinal cortex) is also important for integration of NOR (Winters et al., 2008; Van Strien et al., 2009). In addition, performance in tests of visual memory is subject to "top-down" modulation from the FCX and parietal cortex, which promotes investment of the requisite attentional resources (Noudoost et al., 2010). Interestingly, social recognition may also be enhanced by strengthening FCX-integrated top-down attentional control (Loiseau et al., 2008). Hence, the improvement by S32212 of social recognition may reflect such a cortical mechanism rather than (or as well as) actions in structures controlling social cognition like the septum and amygdala (Adolphs, 2009). S32212 also facilitated SND, another cortically-modulated procedure requiring a particularly high level of attention to successfully differentiate a novel from a familiar juvenile (Terranova et al., 2005; Millan et al., 2010). Other than enhanced

attention, it remains to be ascertained which dimensions of cognitive processing are modulated by S32212, though efficacy upon administration *after* the familiarization trial in the social recognition procedure is consistent with an influence on consolidation and/or retrieval.

As regards the enhancement of attention by S32212, this is likely related to an α_2-AR blockade-mediated acceleration of corticolimbic release of ACh (Hasselmo and Sarter, 2011; Klinkenberg et al., 2011). Correspondingly, α_2-AR antagonists similarly increase ACh levels in FCX and mimic the positive actions of S32212 in tests of SND, social recognition and NOR (Gobert et al., 2003; Millan, 2010; A. Dekeyne and K. Fone, unpub. obs.). Inasmuch as α_2-AR antagonists also enhance FCX-integrated cognitive flexibility in behaviourally-depressed rats (Lapiz and Morilak, 2006), similar work with S32212 would be of interest. Nonetheless, the role of α_2-ARs in cognition is *not* unitary in that recruitment of α_{2A}-ARs on pyramidal cells in the FCX promotes working memory (Millan, 2010; Robbins and Arnsten, 2010), so the overall impact on cognition of S32212 warrant more extensive study. In addition, a role for 5-HT_{2C} receptor antagonism in the influence of S32212 upon cognition should not be excluded since, together with α_2-AR blockade, it reinforces corticolimbic liberation of DA and NA, which also favour cognitive processing (*vide supra*; Robbins and Arnsten, 2009). Indeed, blockade of 5-HT_{2C} receptors countered a delay-induced deficit in NOR (Pitsikas and Sakellaridis, 2005) and has been reported to improve reversal learning (Boulougouris and Tsaltas, 2009). Conversely, social recognition and SND are unaffected by 5-HT_{2C} antagonists (A. Dekeyne et al., unpub. obs.). Finally, despite reports that 5-HT_{2A} antagonists improve visuospatial attention and enhance working memory in primates, they are ineffective in social recognition, SND and NOR models (Terry et al., 2005; Boulougouris and

Tsaltas, 2009; Landholt and Wehrle, 2009; A. Dekeyne et al., unpub. obs.). Hence, 5-HT_{2A} blockade is unlikely to participate in the cognitive effects of S32212.

Potential anxiolytic properties. Inasmuch as blockade of 5-HT_{2C} receptors in amygdala and dorsal hippocampus is associated with anxiolytic effects (Millan and Brocco, 2003; Millan, 2005; Dekeyne et al., 2008; Heisler et al., 2007), the actions of S32212 in the Vogel conflict test can be principally ascribed to 5-HT_{2C} receptor blockade - though a contribution of 5-HT_{2A} antagonism cannot be excluded (Millan and Brocco, 2003). α_2-AR antagonists are inactive in the Vogel conflict test, and reflecting over-activation of adrenergic projections, they may even display anxiogenic and panicogenic properties (Crespi, 2009). Indeed, they block the anxiolytic effects of 5-HT_{2C} antagonists in the social interaction procedure, probably explaining the inactivity of S32212 in this test of anxiolytic properties (A. Dekeyne, unpub. obs).

Influence on male sexual behavior. Sexual dysfunction, including reduced ejaculation and libido, is a common side-effect of SSRIs (Millan, 2006; Kennedy and Rizvi, 2009). Their actions can be reproduced in a recently-described model whereby chronic paroxetine decreases sexual performance in male rats paired with receptive females, observations confirmed herein (Breuer et al., 2008). Conversely, S32212 did not impair sexual behavior, consistent with its lack of increase in extracellular levels of 5-HT which decreases sexual arousal and ejaculation by *recruitment* of hypothalamic and mesolimbic 5-HT_{2C} receptors (Millan, 2006; Kennedy and Rizvi, 2009). In line with these observations, antidepressants possessing 5-HT_{2C} receptor antagonist properties, like agomelatine and mirtazapine, elicit less sexual side-effects than SSRIs (Millan, 2006; Kennedy and Rizvi, 2009; De Bodinat et al., 2010). By analogy to 5-HT_{2C} receptor antagonism, blockade of α_2-ARs promotes sexual motivation by enhancing corticolimbic release of DA (Millan 2006; Viitamaa et al., 2006;

Hull and Dominguez, 2007). Accordingly, the possibility that S32212 restores abnormally low sexual function warrants evaluation, for example in "behaviorally depressed" rats, or animals treated with SSRIs.

Influence upon sleep-wake cycle architecture. Principally reflecting suppression of 5-HT reuptake, antidepressants disrupt sleep patterns, and interfere with restorative SWS (Mayers and Baldwin, 2005; Millan, 2006; Papakostas et al., 2008; De Bodinat et al., 2010). Conversely, by analogy to mirtazapine - which does *not* elicit insomnia (Mayers and Baldwin, 2005; Papakostas et al., 2008) - S32212 promoted SWS. There is no evidence that α_2-AR blockade increases SWS (Ouyang et al., 2004; Mallick et al., 2005), so this action is best attributed to antagonism of 5-HT_{2C} and/or 5-HT_{2A} receptors, with the latter perhaps fulfilling a predominant role (Smith et al., 2002; Landholt and Wehrle, 2009; De Bodinat et al., 2010). Enhanced SWS is of significance in view of its role in the consolidation of memory and other cognitive processes (Walker and Stickgold, 2006). Suppression of REM sleep is characteristic of monoamine reuptake inhibitors, and has been related to relief of depressed mood (Millan, 2006; Mayers and Baldwin, 2005). However, this relationship is contentious since the clinically-active antidepressants, mirtazapine and agomelatine, do *not* suppress REM sleep in *patients* (Mayers and Baldwin, 2005; De Bodinat et al., 2010), whereas H_1 antagonists suppress REM sleep yet are not effective antidepressants (Rojas-Zamorano et al., 2009). In the present work, mirtazapine rapidly reduced REM sleep, presumably reflecting its powerful H_1 antagonist properties (Figure 10, Mayers and Baldwin, 2005) and the absence of H_1 receptor blockade with S32212 (accompanying paper) is consistent with its lack of REM suppression. On the other hand, it was surprising that S32212 promoted a (slightly delayed *versus* SWS) increase in REM sleep since both 5-HT_{2C} and α_2-AR antagonists inhibit REM sleep (Ouyang et al., 2004; Mallick et al., 2005;

Descamps et al., 2009; Landholt and Wehrle, 2009). The most parsimonious explanation for the increased REM sleep with S32212 is 5-HT_{2A} receptor blockade in light of the REM sleep-promoting and suppressing actions of 5-HT_{2A} antagonists and agonists, respectively (Landholt and Wehrle, 2009). The reduction of waking by mirtazapine can be attributed to antagonism of histamine H_1 receptors and, in view of its sedative properties, mirtazapine is taken in the evenings (Mayers and Baldwin, 2005; Szegedi and Schwertfeger, 2005). Since S32212 has no affinity for H_1 sites (accompanying paper), blockade of α_2-ARs is arousing, and 5-HT_{2C} receptor blockade does not suppress waking (Millan et al., 2000b; Ouyang et al., 2004; Descamps et al., 2009; Landholt and Wehrle, 2009), the reduction of waking by S32212 may be secondary to increased SWS and REM sleep.

Conclusions. As summarized in Table 5, S32212 was active in several procedures of potential antidepressant and pro-cognitive properties: it also countered anxiety, enhanced SWS, preserved sexual function, and was essentially devoid of sedative properties and respects body weight. This distinctive functional profile reflects dual inverse agonist properties at 5-HT_{2C} receptors and antagonist actions at α_2-ARs, with a possible role for 5-HT_{2A} receptor antagonism, in particular as regards its influence on sleep. Though additional studies are required to underpin these observations, S32212 exemplifies the promise of multi-target strategies for the improved treatment of depressed states.

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FIGURE LEGENDS

Figure 1. Potential antidepressant properties of S32212 as evaluated in rodent behavioural models. Panel A, decrease of the duration of immobility in the forced-swim test in rats after sub-chronic or acute treatment; Panel B, inhibition of marble-burying behaviour in mice; Panel C, inhibition of aggressive behaviour in isolated mice and Panel D, blockade of S18616 (0.63 mg/kg, s.c.)-induced loss of righting reflex in rats. Panels A, B and C: values are means ± SEMs. N = 4-8 per value. ANOVA as follows. Forced-swim test, sub-chronic, i.p., $F(3,23) = 3.6$, $P < 0.05$; p.o., $F(3,24) = 7.3$, $P < 0.01$ and acute, $F(4,27) = 5.4$, $P < 0.01$. Marble-burying behaviour, $F(4,30) = 17.6$, $P < 0.001$ and aggressive behaviour, $F(3,18) = 17.7$, $P < 0.001$. Asterisks indicate significance of differences to vehicle values in Dunnett's test following ANOVA. Panel D: data are percentages of rats not showing a loss of righting reflex (score < 3). N = 4-8 per value. The asterisk indicates significance *versus* vehicle values (0 %) in the Fisher Exact Probability test. * $P < 0.05$.

Figure 2. Influence of S32212 upon the reduction of sucrose consumption elicited by chronic mild stress (CMS) in rats. Panel A, influence of imipramine and Panels B-D, influence of S32212 in animals submitted to CMS compared to non stressed rats. Data are means ± SEMs. N = 8 per value. ANOVA as follows. For stressed groups: imipramine (10.0 mg/kg) *versus* vehicle, $F(1,84) = 8.8$, $P < 0.01$; S32212 (0.16) *versus* vehicle, $F(1,84) = 0.36$, $P > 0.05$; S32212 (0.63) *versus* vehicle, $F(1,84) = 26.8$, $P < 0.001$ and S32212 (2.5) *versus* vehicle, $F(1,84) = 33.6$, $P < 0.001$. For non-stressed groups: no F values were significant for imipramine or S32212. Asterisks indicate significance of differences to pre-treatment (week 0) values in paired-t tests. * $P < 0.05$.

Figure 3. Induction by chronic administration of S32212 of mRNA encoding brain-derived neurotrophic factor (BDNF) in rat brain. Panel A, influence of chronic S32212 (14 days, 10.0 mg/kg, i.p., twice daily) upon BDNF expression. Data are means ± SEMs in % *versus* control values (defined as 100 %). N = 6 per value. CA1 and CA3 = CA1 and CA3 regions of hippocampus and DG = dentate gyrus. Asterisks indicate significance of drug *versus* vehicle values in unpaired t-tests. * $P < 0.05$. Panel B, representative autoradiograms showing the localization of BDNF mRNA at the level of the hippocampus, in rats treated chronically with S32212 or vehicle.

Figure 4. Influence of S32212 upon the electrical activity of adrenergic, dopaminergic and serotonergic cell bodies in anesthetized rats. Data are means ± SEMs. N = 4 per value. ANOVA as follows. Locus coeruleus (LC), $F(5,18) = 34.9$, $P < 0.001$; ventral tegmental area (VTA), $F(6,21) = 2.6$, $P > 0.05$ and dorsal raphe nucleus (DRN), $F(5,12) = 0.5$, $P > 0.05$. Asterisks indicate significance of drug differences to vehicle values in Dunnett's test. * $P < 0.05$.

Figure 5. Influence of S32212 upon dialysate levels of monoamines in the frontal cortex (FCX) and ventral hippocampus of freely-moving rats. Data are means ± SEMs of dopamine (DA), noradrenaline (NA) and serotonin (5-HT) levels expressed relative to basal, pre-treatment values (defined as 100 %). In vehicle-treated groups, basal values were: FCX, 0.92 ± 0.07; 1.64 ± 0.08 and 0.75 ± 0.06 and ventral hippocampus, 0.63 ± 0.06; 1.71 ± 0.09 and 0.87 ± 0.09 pg per sample for DA, NA and 5-HT, respectively. N = 5-8 per value. ANOVA as follows. FCX, DA, S32212, 0.63, $F(1,9) = 0.3$, $P > 0.05$; 2.5, $F(1,8) = 6.2$, $P < 0.05$; 10.0, $F(1,10) = 8.8$, $P < 0.05$ and 40.0, $F(1,8) = 12.1$, $P < 0.01$. NA, S32212, 0.63, $F(1,9) = 1.3$, $P > 0.05$; 2.5, $F(1,8) = 5.6$, $P < 0.05$; 10.0, $F(1,10) = 16.8$, $P < 0.01$ and 40.0, $F(1,10) = 31.5$, $P < 0.01$. 5-HT, S32212, 0.63, $F(1,8) = 1.9$, $P > 0.05$; 2.5, $F(1,8) = 0.1$, $P >$

0.05; 10.0, F (1,8) = 0.1, P > 0.05 and 40.0, F (1,10) = 1.4, P > 0.05. Ventral hippocampus, S32212, 40.0, DA, F (1,13) = 21.2, P < 0.01; NA, F (1,13) = 24.5, P < 0.01 and 5-HT, F (1,12) = 0.6, P > 0.05. Asterisks indicate significance of drug-treated groups *versus* vehicle-treated group.* P < 0.05.

Figure 6. Influence of S32212 upon dialysate levels of acetylcholine (ACh), histamine, GABA and glutamate in the frontal cortex (FCX) of freely-moving rats. Data are means ± SEMs of extracellular levels expressed relative to basal, pre-treatment values (defined as 100 %). In vehicle-treated groups, basal value of ACh was 20.2 ± 2.0 nM per sample. Basal values of histamine, GABA and glutamate were 21.7 ± 0.7, 31.0 ± 4.2 and 1.0 ± 0.1 fmol per sample, respectively. N = 5-10 per value. Drug effects were as follows. ACh, S32212 0.63 mg/kg, F (1,9) = 1.7, P > 0.05; 2.5, F (1,10) = 5.7, P < 0.05 and 10.0, F (1,9) = 7.2, P < 0.05. No F values were significant for histamine, GABA and glutamate. Asterisks indicate significance of drug-treated groups *versus* vehicle-treated group.* P < 0.05.

Figure 7. Reversal by S32212 of deficits in social recognition in rats, either provoked by scopolamine or by delay. Panels A and B, improvement by S32212, s.c. and p.o., respectively, of the disruption of social recognition provoked by scopolamine (1.25 mg/kg, s.c.). Values are means ± SEMs. N = 5-13 per value. Two way ANOVA as follows. S32212 s.c.: scopolamine, F (1,60) = 29.1, P < 0.001; S32212, F (4,60) = 7.3, P < 0.001 and interaction., F (4,60) = 10.9, P < 0.001. S32212 p.o.: scopolamine, F (1,45) = 28.5, P < 0.001; S32212, F (3,45) = 4.2, P < 0.05 and interaction, F (3,45) = 8.7, P < 0.001. Closed asterisks indicate significance of differences between vehicle/scopolamine and vehicle/vehicle values; open asterisks indicate significance of differences between S32212/scopolamine and vehicle scopolamine values in Newman-Keuls test. Panels C and D, improvement by S32212, s.c. and p.o., respectively, of the disruption of social recognition

provoked by a delay (2 hours).Values are means ± SEMs. N = 7-10 per value. For dose-response curves, one-way ANOVA as follow: S32212, s.c., $F(4,36) = 11.4$, $P < 0.05$ and S32212, p.o., $F(4,31) = 10.8$, $P < 0.001$. Closed asterisks indicate significance of differences between S32212 and vehicle values in Dunnett's test. For the specificity of drug actions, two-way ANOVA as follows: S32212, s.c.; influence of juvenile, $F(1,23) = 17.6$, $P < 0.01$; influence of drug, $F(1,23) = 28.7$, $P < 0.001$ and interaction, $F(1,23) = 3.5$, $P > 0.05$. S32212, p.o.; influence of juvenile, $F(1,20) = 5.3$, $P < 0.05$; influence of drug, $F(1,20) = 19.3$, $P < 0.001$ and interaction, $F(1,20) = 5.3$, $P < 0.05$. Open asterisks indicate the significance of differences in Newman-Keuls test between values for a different juvenile *versus* the same juvenile. * $P < 0.05$.

Figure 8. Reversal by S32212 of a delay-dependent deficit in novel object recognition in rats. Panel A, exploration of novel and familiar objects during choice trial after a 4-hours intertrial interval and Panel B, dose-dependent improvement by S32212 of the D2 index score. Values are means ± SEMs. N = 12 per value. The two-way ANOVA performed on exploration of novel and familiar objects during choice trial showed a significant object X treatment interaction: $F(3,44) = 3.3$, $P < 0.05$. The one-way ANOVA performed on D2 scores was as follows: $F(3,44) = 3.1$, $P < 0.05$. The asterisk indicates significance of differences to vehicle values in Dunnett's test. * $P < 0.05$.

Figure 9. Improvement by S32212 of social novelty discrimination in rats. Panel A, exploration of novel *versus* familiar juveniles during a second 5-min period (P2), performed 30 min after a first 5-min period where only one of these juveniles was introduced to the adult. Panel B, improvement by S32212 of the ratio P2 novel/P2 familiar. Values are means ± SEMs. N = 4-8 per value. Panel A, two-way ANOVA performed on exploration of novel *versus* familiar juveniles during P2 showed a significant effect of factor “juvenile”: $F(1,34) =$

17.7, $P < 0.001$. Panel B, one-way ANOVA performed on ratios P2 novel/P2 familiar as follows: $F(5,34) = 2.6$, $P < 0.05$. Asterisks indicate significance of differences to vehicle values in Dunnett's test. * $P < 0.05$.

Figure 10. Influence of S32212 upon sleep-wake architecture, as evaluated by EEG recording. Left panels, influence of S32212 and right panels, influence of mirtazapine, administered at the onset of the dark phase (shaded areas) upon the duration of waking state, slow wave sleep (SWS) and rapid eye movement (REM) sleep. Data are means ± SEMs. N = 11 per value. Drug effect after ANOVA as follows. S32212, dark phase: waking state, $F(1,11) = 17.4$, $P < 0.001$; SWS, $F(1,11) = 20.2$, $P < 0.001$ and REM sleep, $F(1,11) = 3.9$, $P < 0.05$. S32212, light phase, waking state, $F(1,11) = 0.04$, $P > 0.05$; SWS, $F(1,11) = 0.02$, $P > 0.05$ and REM sleep, $F(1,11) = 1.3$, $P > 0.05$. Mirtazapine, dark phase: waking state, $F(1,11) = 4.1$, $P < 0.05$; SWS, $F(1,11) = 10.1$, $P < 0.01$ and REM sleep, $F(1,11) = 7.5$, $P < 0.01$. Mirtazapine, light phase, waking state, $F(1,11) = 1.4$, $P > 0.05$; SWS, $F(1,11) = 0.3$, $P > 0.05$ and REM sleep, $F(1,11) = 3.5$, $P > 0.05$. Asterisks indicate significance of differences to vehicle values in Newman-Keuls test. * $P < 0.05$.

Table 1. Reversal by chronic (7 days) administration of S32212 of the deficit induced by scopolamine in the radial maze test in rats.

Treatment 1 (dose)	Treatment 2 (dose)	Number of errors (mean 3 test sessions)
Vehicle	Vehicle	1.5 ± 0.1
Vehicle	Scopolamine (0.5)	2.3 ± 0.2 #
S32212 (2.5)	Scopolamine (0.5)	2.1 ± 0.2 #
S32212 (10.0)	Scopolamine (0.5)	1.9 ± 0.2 *

Doses are in mg/kg, i.p.. Treatment 1 = once daily, 4 days before testing and 3 days of testing (45 min before each test session). Treatment 2 = once daily, 30 min before each test session. Data are means ± SEMs. N = 10 per value. #, significance of differences *versus* Vehicle + Vehicle and *, significance of differences between S32212 + scopolamine and vehicle + scopolamine, in unpaired t-tests. * $P < 0.05$.

Table 2. Influence of S32212 on motor behavior in rodents.

Dose	Rotarod (mice)	Spontaneous Locomotion (mice)	Spontaneous Locomotion (rats)
Vehicle	236 ± 38	407 ± 41	49 ± 12
0.63	173 ± 46	348 ± 37	38 ± 8
2.5	—	335 ± 37	51 ± 7
10.0	192 ± 66	241 ± 16 *	34 ± 7
40.0	67 ± 13*	117 ± 8 *	—

Doses of S32212 are in mg/kg, s.c.. Data are means latency (sec) to fall ± SEMs (rotarod) or means locomotion counts ± SEMs (spontaneous locomotion). N = 4-7 per value. ANOVA as follows. Rotarod, $F(3,17) = 2.8$, $P < 0.05$; spontaneous locomotion in non-habituated mice, $F(4,30) = 13.0$, $P < 0.001$ and spontaneous locomotion in habituated rats, $F(3,19) = 0.9$, $P > 0.05$. Asterisks indicate significance of differences to vehicle values in Dunnett's test following ANOVA. * $P < 0.05$.

Table 3. Anxiolytic properties of S32212 in the Vogel conflict test in rats.

	Dose	Number of licks
Non punished	Vehicle	672 ± 75
Punished	Vehicle	79 ± 17 #
	0.63	97 ± 34
	2.5	193 ± 55
	10.0	433 ± 106 *
	40.0	528 ± 110 *

Doses of S32212 are in mg/kg, i.p. Data are means ± SEMs. N = 4-10 per value. ANOVA for punished groups as follows: $F(4,37) = 5.4$, $P < 0.01$. The symbol # indicates significance of differences to vehicle non-punished value in Student's t-test. Closed asterisks indicate the significance of differences to vehicle values in Dunnett's test. * $P < 0.05$.

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Table 4. Influence of chronic (14 days) administration of S32212 compared to paroxetine, upon sexual behaviour and body weight in male rats.

		Drug (dose)		
	Day	Vehicle	S32212 (2.5)	Paroxetine (10.0)
Number of ejaculations	0	1.5 ± 0.3	1.8 ± 0.3	1.9 ±0.3
	7	2.3 ± 0.2	1.8 ± 0.2	0.3 ± 0.1 *
	14	1.9 ± 0.2	1.6 ± 0.2	0.6 ± 0.3 *
	21	1.8 ± 0.3	1.8 ± 0.4	1.1 ± 0.4
Latency (sec) to the first ejaculation	0	1032 ± 179	874 ± 132	858 ± 177
	7	808 ± 112	964 ±107	1507 ± 134 *
	14	743 ± 123	763 ±137	1398 ± 172 *
	21	662 ± 164	851 ± 202	1195 ± 212
Body weight (g)	0	418 ± 10	412 ± 7	425 ± 8
	7	428 ± 10	416 ± 7	417 ± 9
	14	435 ± 11	422 ± 7	402 ± 7 *
	21	449 ± 12	446 ± 7	412 ± 8 *

Doses are in mg/kg, i.p.. Data are means ± SEMs. Number of ejaculations and latency to the first ejaculation were recorded in 30-min sessions performed at day 0, day 7, day 14 and day 21. Drugs or vehicle were administered daily from day 0 (acute treatment) to day 14 (chronic treatment). N = 12 per value. ANOVA as follows. Ejaculations: day 0, $F(2,33) = 0.5$, $P > 0.05$; day 7, $F(2,33) = 36.1$, $P < 0.001$; day 14, $F(2,33) = 8.2$, $P < 0.01$ and day 21, $F(3,23) = 1.2$, $P > 0.05$. Latency: day 0, $F(2,33) = 0.3$, $P > 0.05$; day 7, $F(2,33) = 9.6$, $P < 0.001$; day 14, $F(2,33) = 6.5$, $P < 0.01$ and day 21, $F(2,33) = 1.9$, $P > 0.05$. Body weight: day 0, $F(2,33) = 0.7$, $P > 0.05$; day 7, $F(2,33) = 0.5$, $P > 0.05$; day 14, $F(2,33) = 4.0$, $P < 0.05$ and

day 21, $F(2,33) = 5.2$, $P < 0.05$. Asterisks indicate significance of differences to vehicle values in Dunnett's test following ANOVA. * $P < 0.05$.

Table 5. Summary of overall functional profile of S32212 in procedures related to potential therapeutic properties.

	Model (species)	Active dose-range
Antidepressant properties	Reduction of immobility in a forced swim test (rat)	2.5 mg/kg, i.p., and 10.0-40.0 mg/kg, p.o., sub-chronic 2.5-10.0 mg/kg, i.p., acute
	Blockade of marble burying behaviour (mice)	10.0-40.0 mg/kg, s.c., acute
	Blockade of isolation-induced aggressive behavior (mice)	10.0-40.0 mg/kg, i.p., acute
	Blockade of S18616-induced loss of righting reflex (rat)	80.0 mg/kg, i.p., acute
	Reversal of chronic mild stress-induced reduction of sucrose intake (rat)	0.63-2.5 mg/kg, i.p., chronic (5 weeks) 10.0 mg/kg, i.p., chronic (14 days, twice daily)
	Increase in BDNF expression in hippocampus and amygdale (rat)	0.5-2.0 mg/kg, i.v., acute
	Increase in electrical activity of adrenergic cell bodies (rat)	10.0-40.0 mg/kg, s.c. and 5.0-40.0 mg/kg, p.o., acute
	Increase in DA and NA levels in the frontal cortex (rat)	
Pro-cognitive properties	Increase in ACh levels in the frontal cortex (rat)	2.5-10.0 mg/kg, s.c., acute
	Reversal of scopolamine-induced deficit in the radial maze (rat)	10.0 mg/kg, i.p., acute
	Reversal of scopolamine-induced deficits in social recognition (rat)	0.04-0.63 mg/kg, s.c., and 0.04-0.16 mg/kg, p.o., acute
	Reversal of delay-induced deficits in social recognition (rat)	1.25-2.5 mg/kg, s.c., and 5.0-10.0 mg/kg, p.o., acute
	Reversal a delay-dependent deficit in novel object recognition (rat)	10.0 mg/kg, s.c., acute
	Facilitation of social novelty discrimination (rat)	0.16-0.63 mg/kg, s.c., acute
Anxiolytic properties	Vogel conflict (rat)	10.0-40.0 mg/kg, i.p., acute
Influence on Sexual function	Lack of disruption (male rat)	*10.0 mg/kg, i.p., chronic (14 days), inactive*
Influence on sleep pattens	Increase in SWS and REM sleep, decrease in waking state (rat)	10.0 mg/kg, s.c., acute

Figure 1

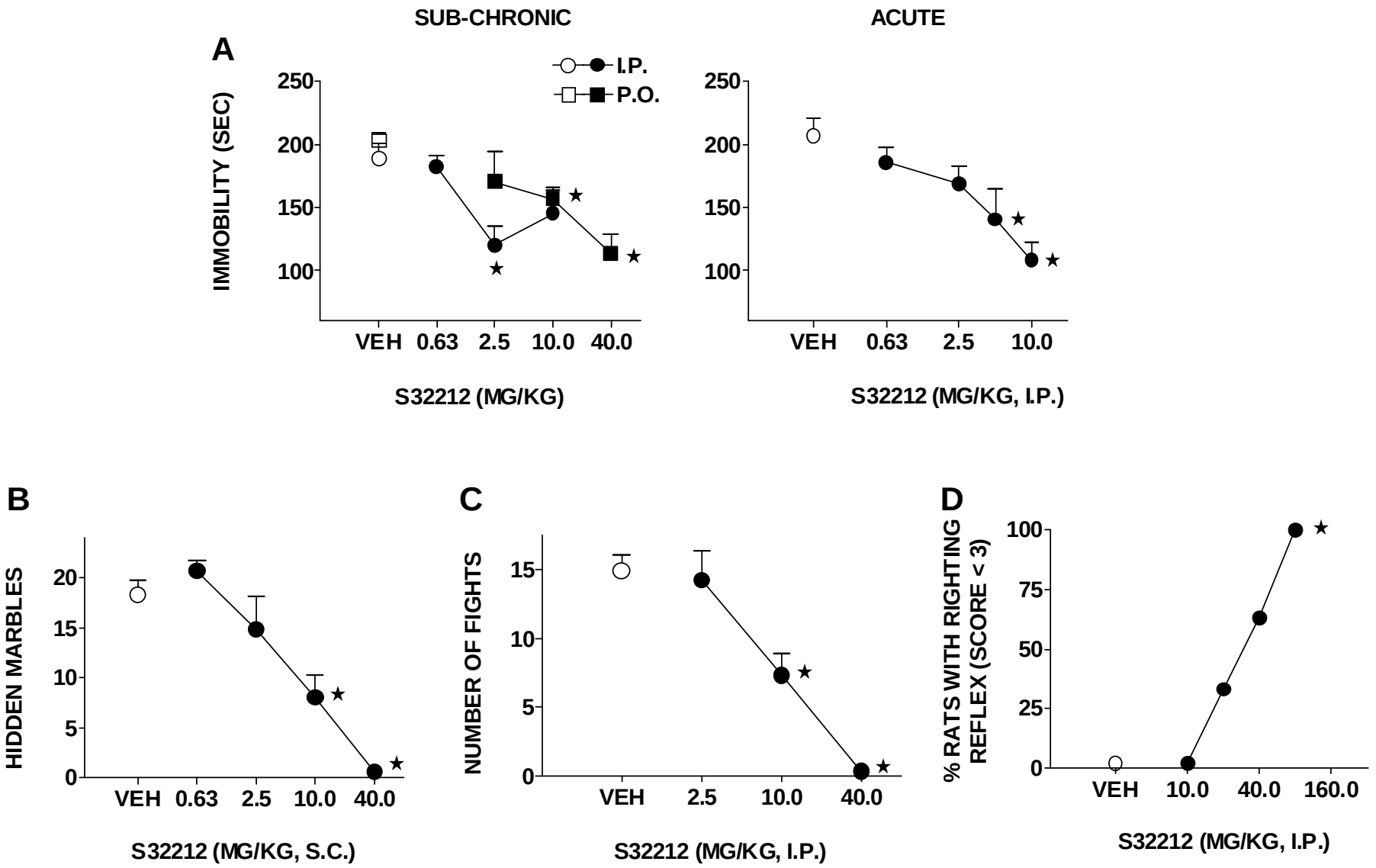

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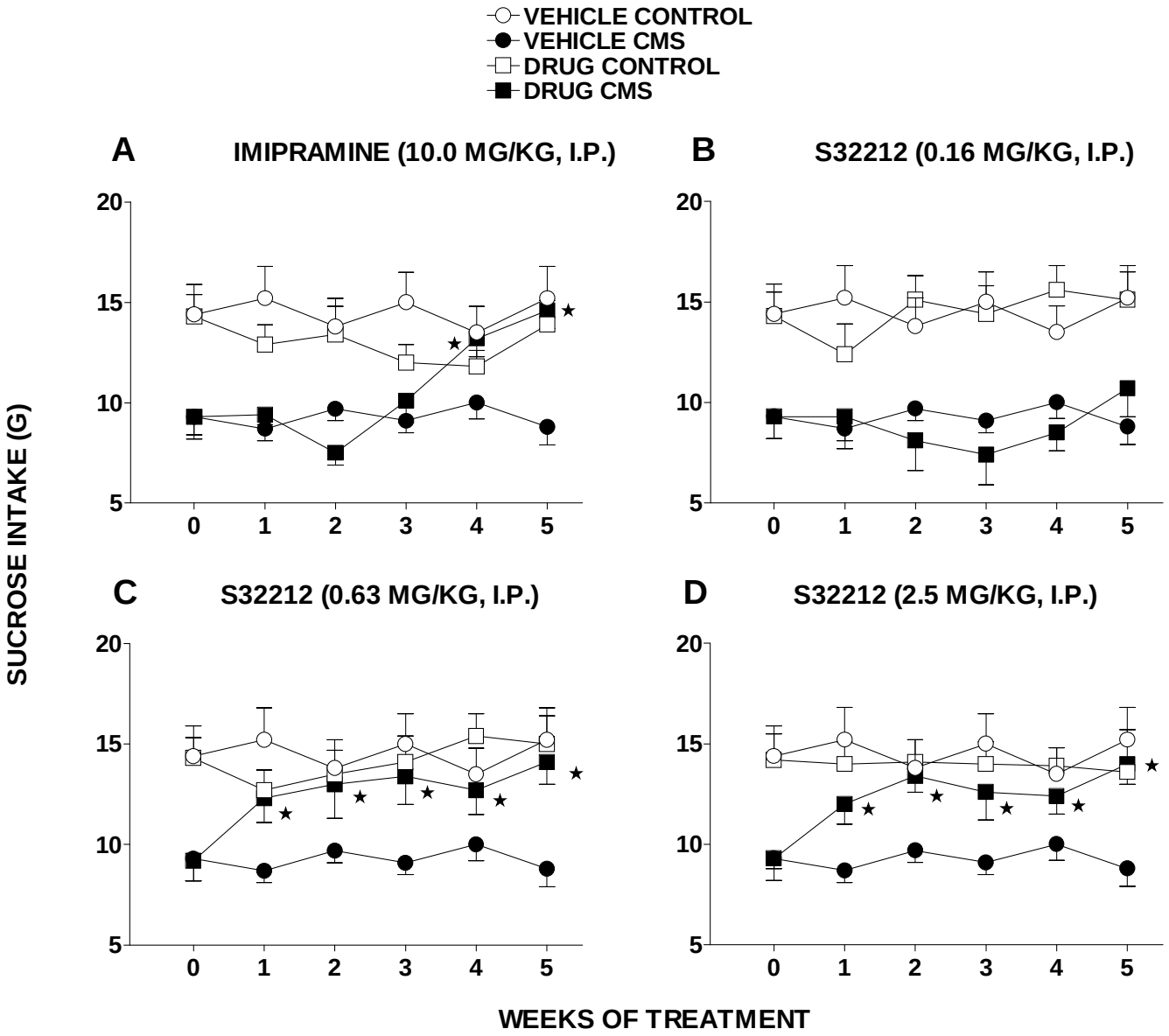
Figure 2



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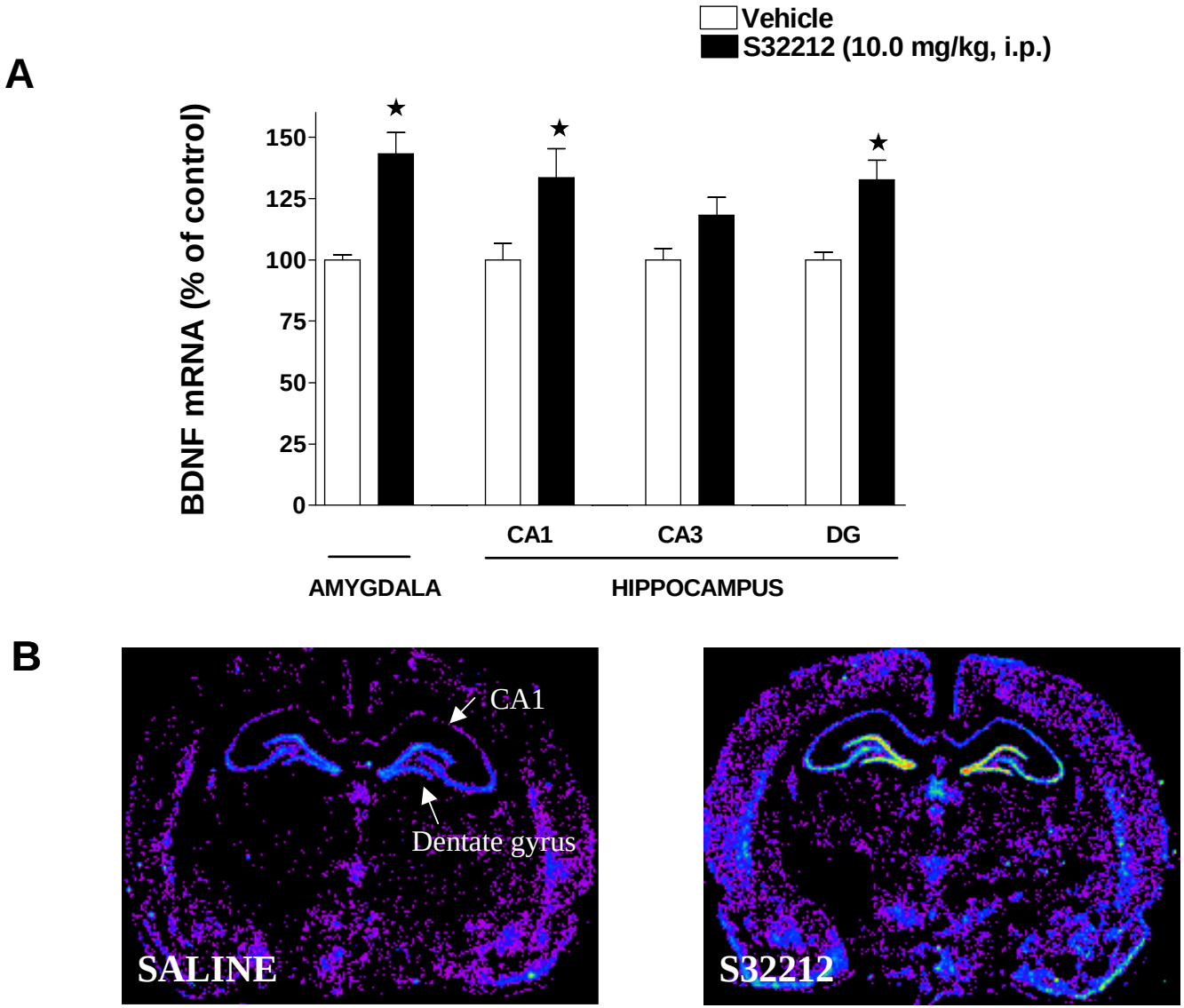

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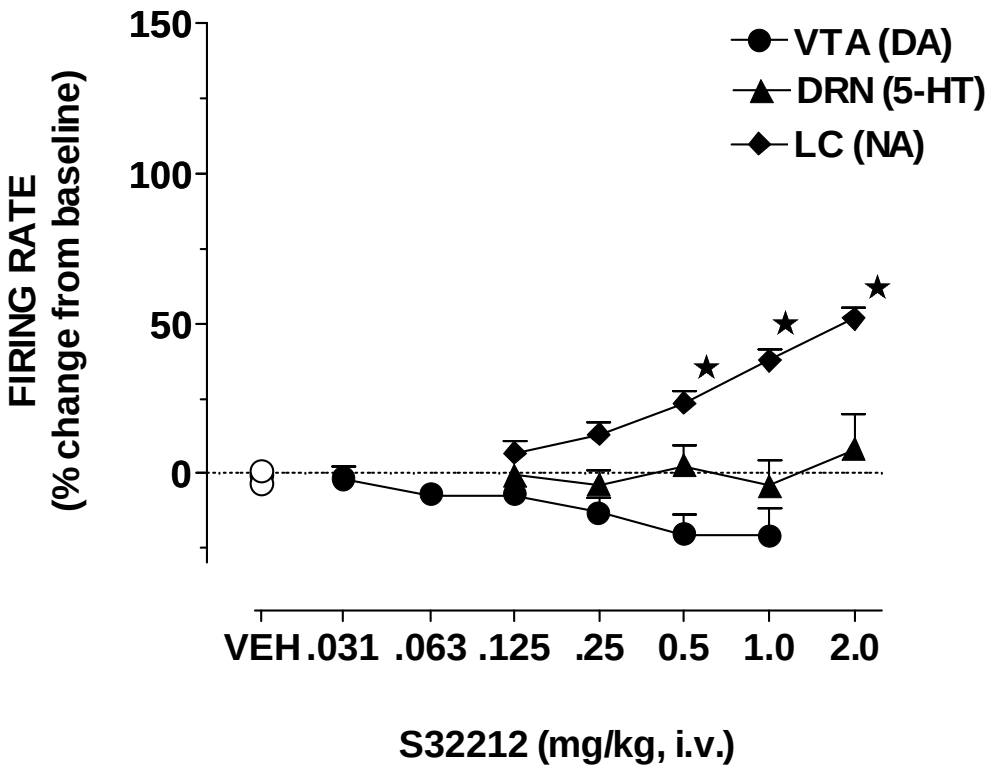

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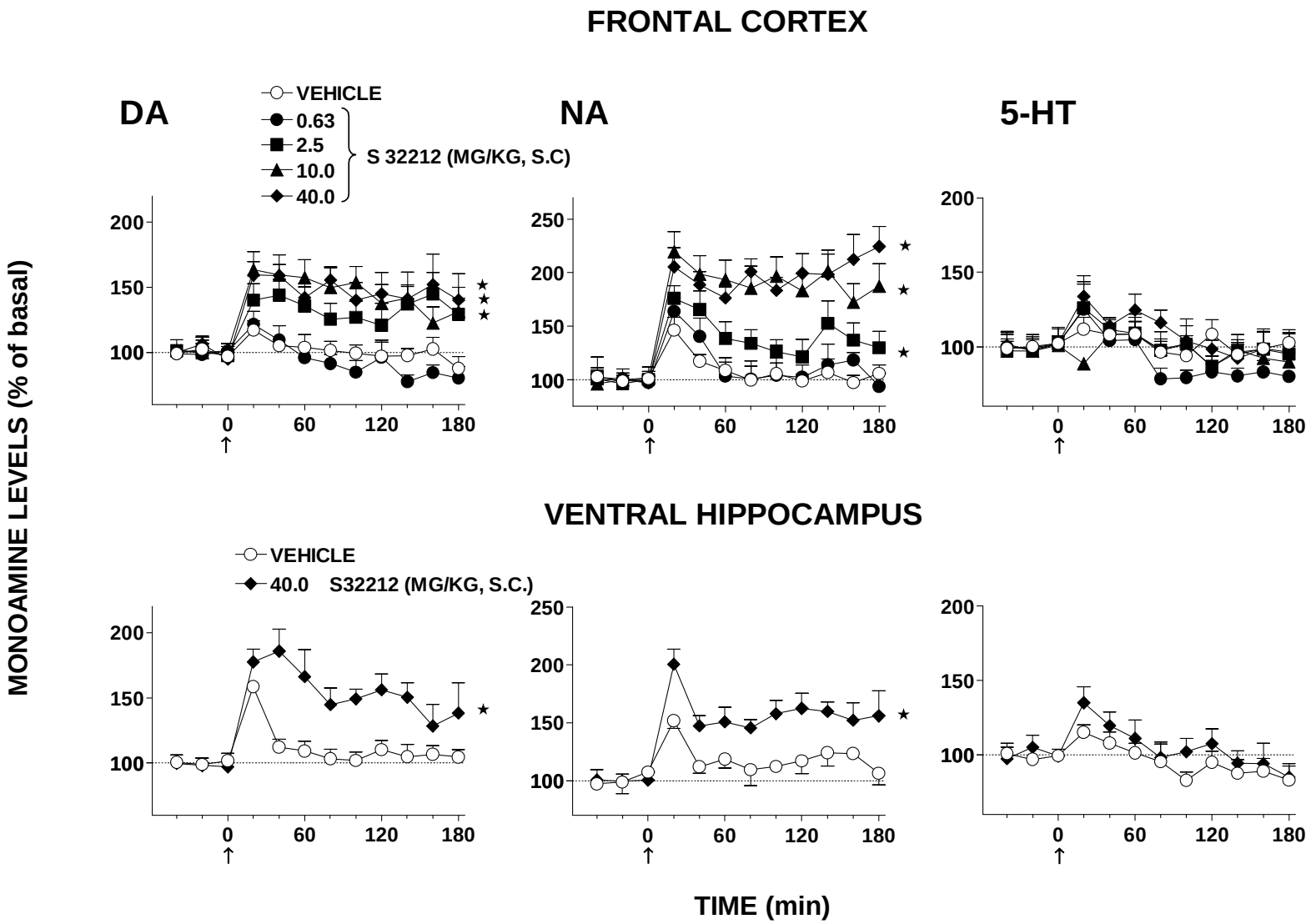

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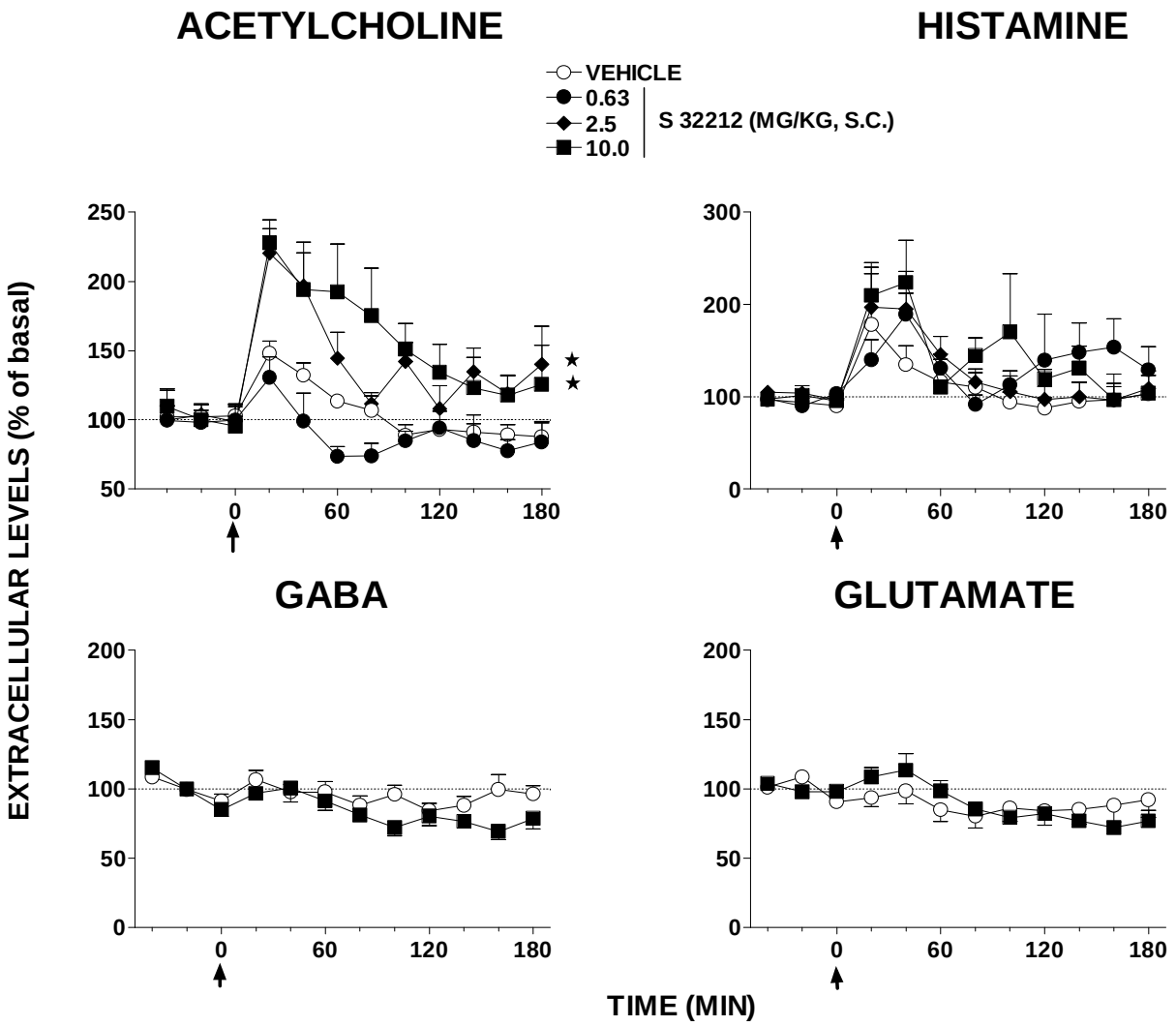
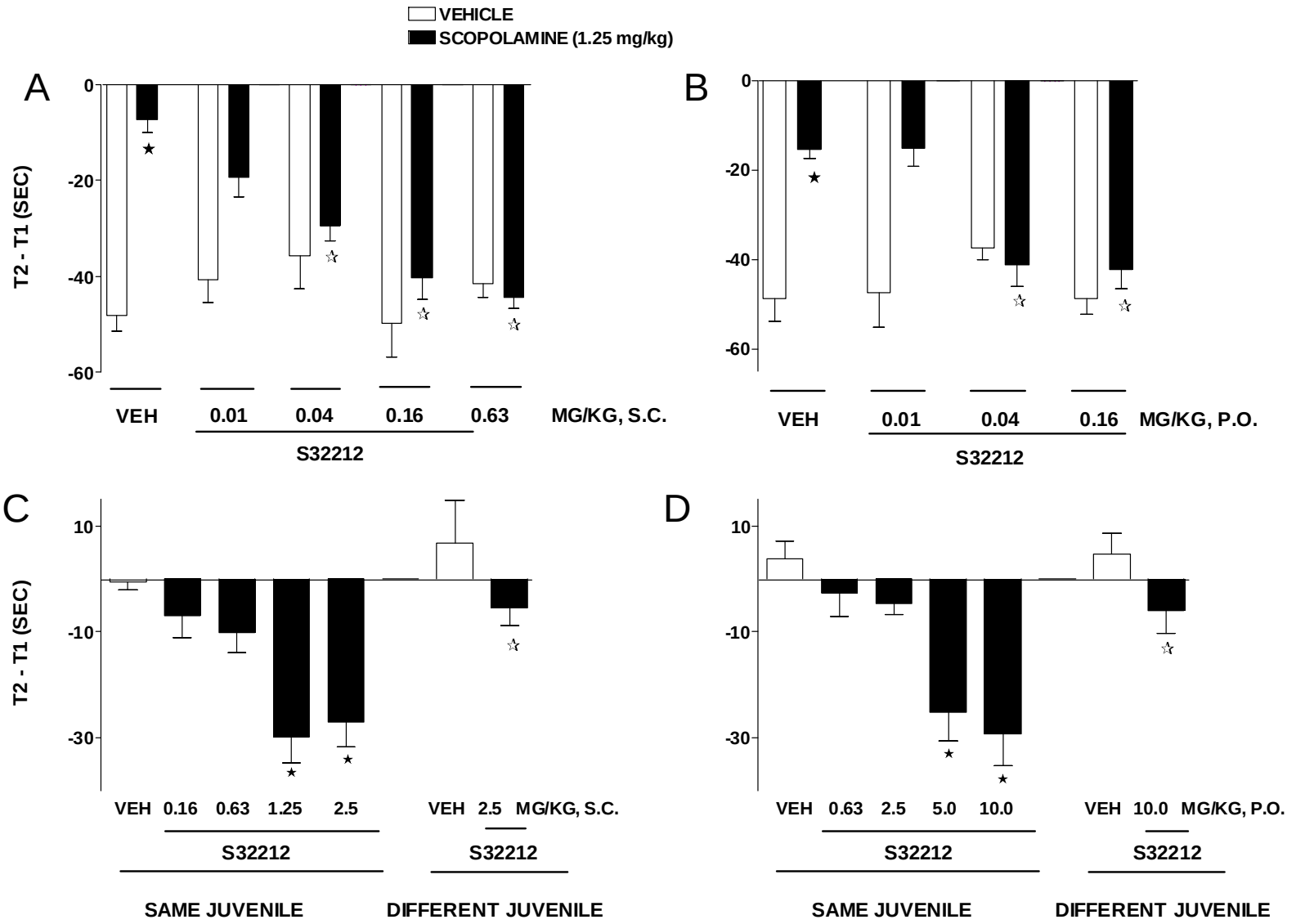


Figure 7



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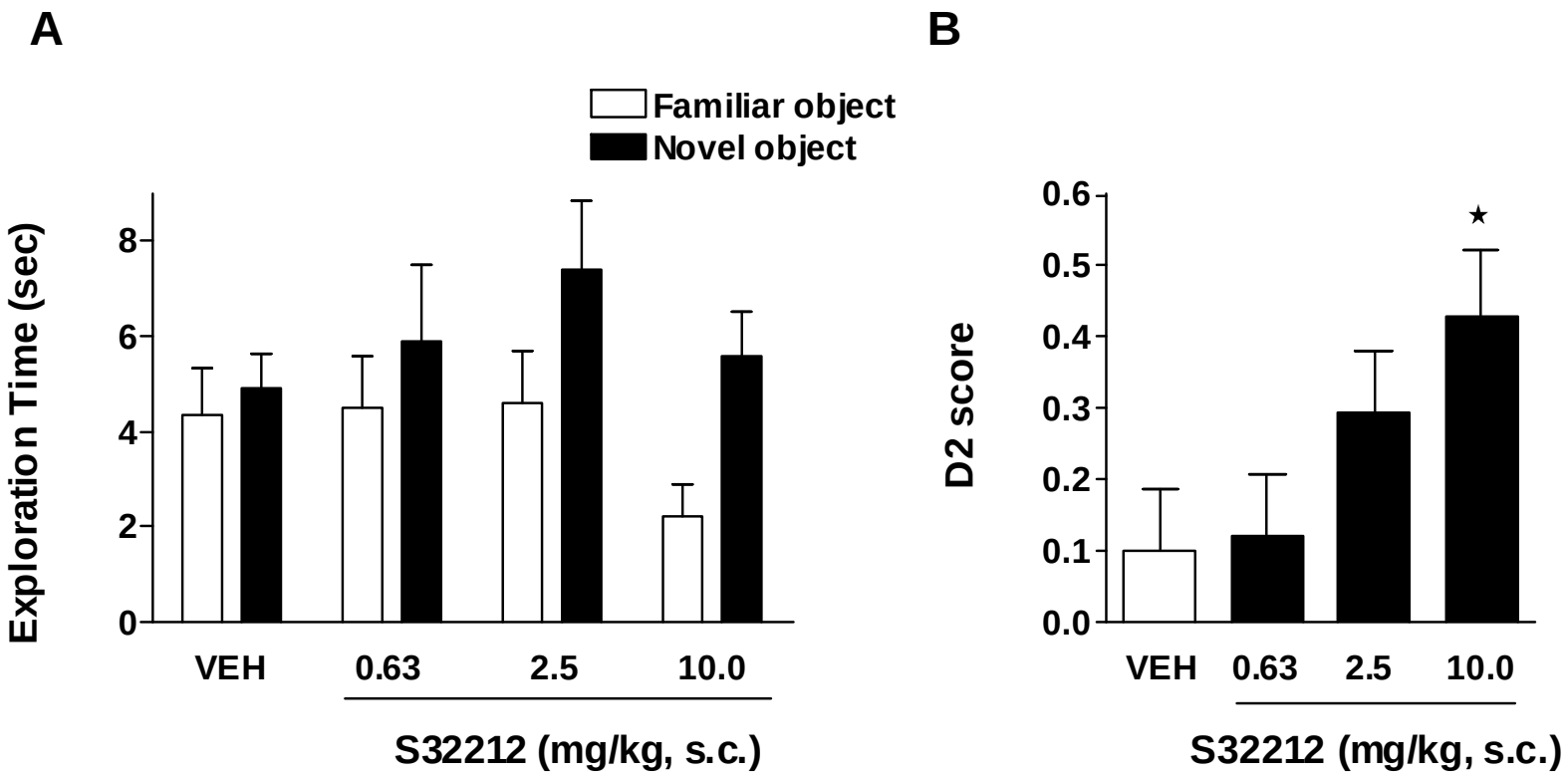

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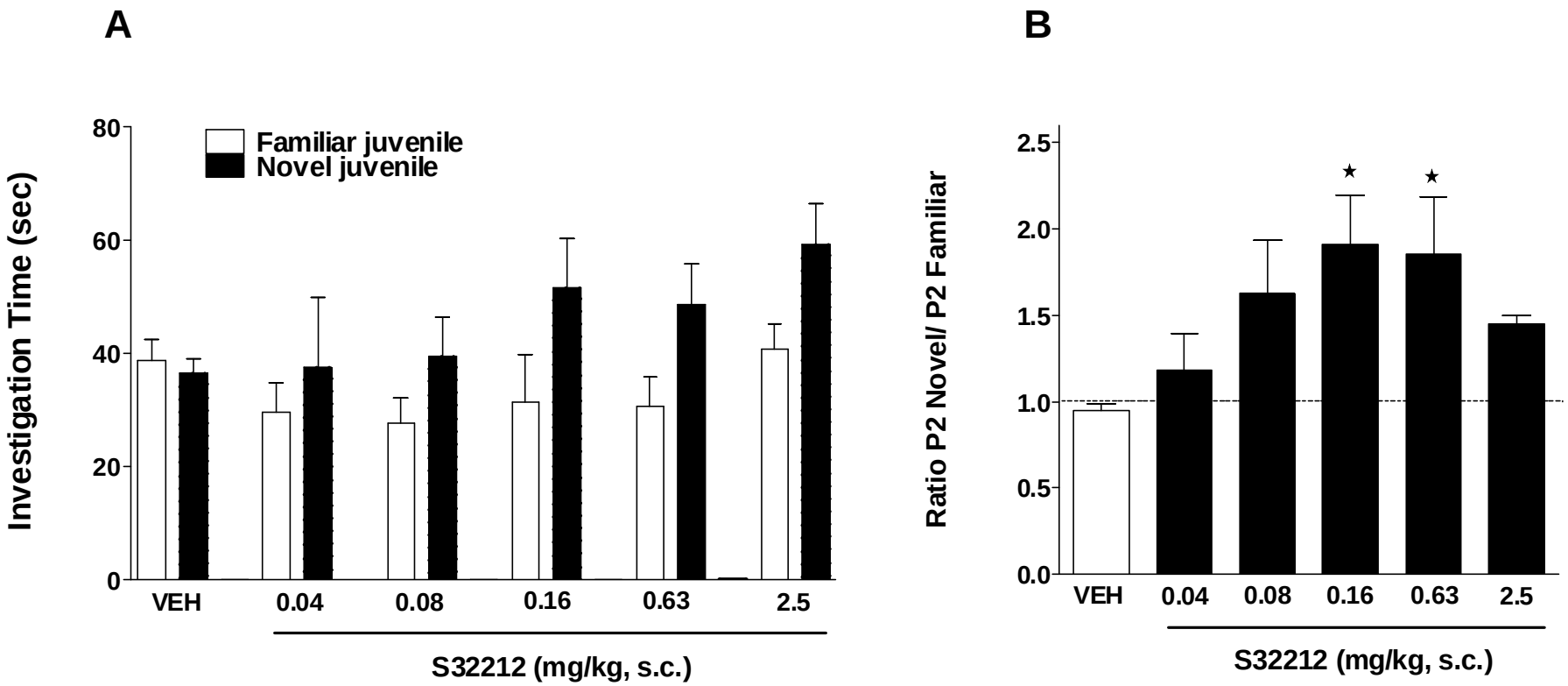

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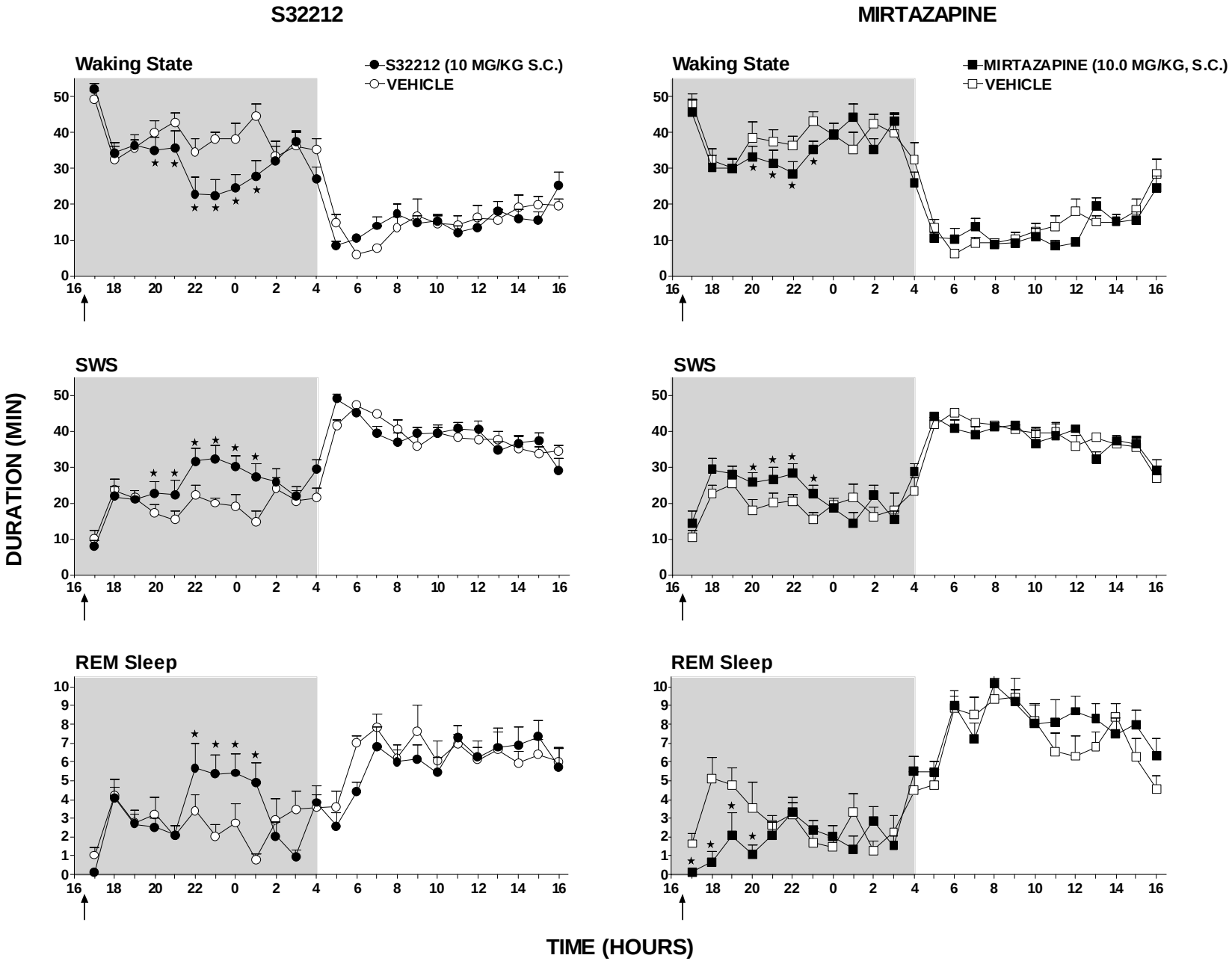

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