S32212, a Novel Serotonin Type 2C Receptor Inverse Agonist/α₂-Adrenoceptor Antagonist and Potential Antidepressant: II. A Behavioral, Neurochemical, and Electrophysiological Characterization

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Abstract

The present studies characterized the functional profile of N-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-1,2-dihydro-3-H-benzo[e]indole-3-carboxamide (S32212), a combined serotonin (5-HT)₂C receptor inverse agonist and α₂ adrenoceptor antagonist that also possesses 5-HT₂A antagonist properties (J Pharmacol Exp Ther 340: 750–764, 2012). Upon parenteral and/or oral administration, dose-dependent (0.63–40.0 mg/kg) actions were observed in diverse procedures. Both acute and subchronic administration of S32212 reduced immobility time in a forced-swim test in rats. Acutely, it also suppressed marble burying and aggressive behavior 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 the frontal cortex and hippocampus of freely moving rats. S32212 also elevated the frontocortical levels of dopamine and acetylcholine, whereas 5-HT, amino acids, and histamine were unaffected. These neurochemical actions were paralleled by “promnemonic” 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. Furthermore, 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 behavior in male rats. In conclusion, S32212 displays a functional profile consistent with improved mood and cognitive performance, together with satisfactory tolerance.

Introduction

In Millan et al. (2012b), we describe a novel urea derivative, N-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-1,2-dihydro-3-H-benzo[e]indole-3-carboxamide (S32212), that behaves both in vitro and in vivo as a serotonin (5-HT)₂C receptor inverse agonist/α₂ adrenoceptor (AR) antagonist and also blocks 5-HT₂A receptors. This profile suggests a potentially favorable influence on depressed mood and, possibly, cognition, which should be expressed in the absence of a deleterious impact on sleep and sexual function. The studies described herein explored this possibility.

ABBREVIATIONS: S32212, N-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-1,2-dihydro-3-H-benzo[e]indole-3-carboxamide; 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; MED, minimal effective dose; 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; ANOVA, analysis of variance; AP, anteroposterior; ML, mediolateral; DV, dorsoventral; HPLC, high-performance liquid chromatography; T1, first 5-min session; T2, second 5-min session; P1, first 5-min period; P2, second 5-min period; AUC, area under the curve; S18616, (S)-spiro[1-oxa-2-amino-3-azacyclopent-2-ene]-4-(1’2’,3’,4’-tetrahydroxynaphthalene)].
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 the administration of α2-AR agonists, which provokes a loss of righting reflex (LRR) abolished not only by drugs possessing antagonist actions at α2-ARs, but also by most clinically active antidepressants (Millan et al., 2000b, 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 such as imipramine, which restore sucrose consumption to nonstressed 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 on levels of mRNA encoding BDNF was also examined.

α2-ARs exert a tonic inhibitory influence on 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 interneurons inhibit to ascending monoaminergic pathways bear tonically active, excitatory 5-HT2C 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-HT2C and α2-AR blockade on depressed mood (Millan, 2005). Accordingly, we examined the influence of S32212 on the electrical activity of ventral tegmental area (VTA)-localized dopaminergic and locus coeruleus (LC)-localized adrenergic neurons compared with dorsal raphe nucleus (DRN)-localized serotonergic perikarya in anesthetized rats. In parallel, by use of a dialysis procedure in freely moving rats, we quantified the impact of S32212 on the extracellular levels of NA and DA versus 5-HT in the FCX and ventral hippocampus.

Cognitive deficits are increasingly recognized as a core symptom of depression (Castaneda et al., 2008; Marazziti et al., 2010; Millan et al., 2012a). Although certain components of cognitive function are promoted by DA and NA in rats, an acceleration in the release of acetylcholine (ACh) is likewise favorable (El-Ghundi et al., 2007; Robbins and Arnsen, 2009; Millan, 2010; Hasselmo and Sarter, 2011; Klinkenberg et al., 2011). Inasmuch as α2-ARs are inhibitory to cholinergic projections innervating the FCX, we determined the influence of S32212 on the dialysis levels of ACh compared with those of histamine and amino acids, which are likewise implicated in the control of cognition (Riedel et al., 2003; Passani and Blandina, 2011). In addition, a behavioral evaluation of the impact of S32212 on 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 (Winters et al., 2008; Millan et al., 2010).

Inasmuch as anxiety is a common and comorbid symptom of depression, and selective 5-HT2C 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 that is exacerbated by agents that elevate extracellular levels of 5-HT, such as the selective 5-HT reuptake inhibitor (SSRI) paroxetine (Millan, 2006; Breuer et al., 2008; Serretti and Chiesa, 2009). Conversely, blockade of 5-HT2C receptors and α2-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 with paroxetine on sexual function in male rats (Breuer et al., 2008). Finally, depressed states are characterized by perturbed sleep-wake cycle architecture, including insomnia and decreased restorative slow-wave sleep (SWS), and certain antidepressants, such as SSRIs, aggravate sleep deficits. Whereas α2-AR blockade enhances arousal (Ouyang et al., 2004), antagonism of 5-HT2C (and 5-HT2A) receptors promotes SWS (Smith et al., 2002; Descamps et al., 2009; Landholt and Wehrle, 2009).

The tetracyclic antidepressant mirtazapine, which antagonizes 5-HT2C receptors, enhances sleep propensity but induces somnolence caused by potent histamine H1 receptor antagonist properties (Mayers and Baldwin, 2005; Millan, 2005; Szegedi and Schwertfeger, 2005). Accordingly, we studied the influence of S32212 in comparison with mirtazapine on sleep patterns in rats.

### Materials and Methods

**Animals.** Unless otherwise specified below, these studies used male Wistar rats and NMRI mice supplied by Iffa-Credo (L’Arbresle, France), weighing 200 to 250 and 22 to 25 g, respectively, upon arrival. They were housed in sawdust-lined cages with unrestricted access to standard chow and water. There was a 12-h light/dark cycle with lights on at 7:30 AM Laboratory temperature and humidity were 21 ± 0.5°C and 60 ± 5%, respectively. Animals were adapted to laboratory conditions for at least 1 week before testing. All procedures conformed to international European ethical standards for the care and use of laboratory animals (86/609-EEC) and governmental guidelines. They also received local ethical committee approval.

**Forced-Swim Test in Rats.** As described previously (Dekeyne et al., 2008), on the first day of the experiment, rats were individually immersed for 15 min in glass cylinders (30-cm height × 20-cm diameter) filled to a depth of 16 cm with water at 25°C. The next 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 an upright position, making only the small movements necessary to keep its head above the surface. S32212 or vehicle was administered either acutely, 30 min before the test on day 2, or subchronically, 24 h, 17 h, and 30 min (intraperitoneally) or 60 min (orally) before the test on day 2.

**Marble-Burying Behavior in Mice.** As described previously (Dekeyne et al., 2008), group-housed mice (25 per cage) of 22 to 26 g were individually placed in transparent polycarbonate cages (30 × 18 × 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 minutes later, the animals were removed, and the number of marbles...
with at least two-thirds buried in the sawdust was recorded. Mice were treated 30 min before the test with S32212 or vehicle.

Aggression in Preisolated Mice. As described previously (Dekeyne et al., 2008), pairs of CD male mice of 22 to 25 g (Charles River Laboratories, 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) were monitored for 3 min. Both mice were treated 30 min before the test with S32212 or vehicle.

(S)-Spiro(1-Oxa-2-Amino-3-Azacyclopent-2-Ene)-4,2'- actyl-3,4'-Tetrahydronaphthalene)-Induced LRR in Rats. As described previously (Millan et al., 2001; Dekeyne et al., 2008), rats were placed on their backs on a surface covered with paper wadding. Their ability to right themselves was assessed by one (blinded) observer as follows: score 0, normal, complete righting reflex; score 1, attempted righting reflex (turn of at least 90°); score 2, attempted righting reflex (turn of less than 90°) and score 3, total LRR (no attempt to turn). S32212 or vehicle was administered 30 min before the α2-AR agonist, (S)-spiro(1-oxa-2-amino-3-azacyclopent-2-ene)-4,2'-actyl-3,4'-tetrahydronaphthalene) (S18616) (0.63 mg/kg s.c.), which was administered 30 min before scoring of LRR. All rats that received S18616 displayed a score of 3, and the number of rats displaying a score of 2 or less after drug treatment was calculated.

CMS-Induced Reduction in Sucrose Consumption in Rats. As described previously (Dekeyne et al., 2008), this study used single-housed male Wistar rats of 220 to 250 g (Gorzkowska, Warsaw, Poland). Animals were initially trained to consume a 1% sucrose solution. Training consisted of 10 1-h baseline tests (twice weekly) in which sucrose was presented in the home cage after 14 h of 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., 1-h access to the sucrose solution after 14 h of food and water deprivation) and at weekly intervals throughout the whole experiment. On the basis of their sucrose intake in the final baseline test, the animals were divided into two matched groups. One group was subjected to the CMS procedure for 8 consecutive weeks. Control animals were housed in separate rooms and had no contact with the stressed animals. On the basis of their sucrose intake, after initial 3 weeks of stress, both stressed and control animals were divided further into matched subgroups, and for the subsequent 5 weeks they received daily injections (intraperitoneally) of S32212, imipramine, or vehicle. The drugs were administered at 10:00 AM, and the weekly sucrose tests were carried out 24 h after the last drug injection.

Long-Term Influence on BDNF Expression in the Rat Hippocampus and Amygdala. Male Sprague-Dawley rats (Harlan Olac, Bicester, U.K.), weighing 220 to 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 after 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 h 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 on the firing rate of VTA-localized dopaminergic and LC-localized adrenergic cell bodies compared with 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 was lowered into the VTA, DRN, or LC. After baseline recording (≥5 min), S32212 or vehicle (1/10 ethanol + 4/10 polyethylene glycol 400 + 5/10 sterile water) was administered intravenously (in a volume of 0.5 ml/kg) in cumulative doses every 2 to 3 min. Drug effects were quantified over the 60-s bin corresponding to their time of peak action. Spike2 software (CED, Cambridge, UK) was used for data acquisition and analysis. Data are expressed as percentage of change from the basal, spontaneous firing rate (0%).

Dialysate Levels of Monoamines, ACh, and Amine Acids in Freely Moving Rats. As described elsewhere (Millan et al., 2000b; Gobert et al., 2003, 2011), male rats (200–250 g) were implanted: 1) in the FCX (AP, +2.2 from bregma; ML, +0.6 or −0.6; 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; 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; DV, −4.5 from dura) and the striatum (AP, +0.5 from bregma; ML, −2.8; 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 CaCl2 (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 before intraperitoneal or oral administration of S32212 or vehicle, and dialysis continued for 3 h. Monoamine and ACh levels were quantified as described previously (Gobert et al., 2003; Dekeyne et al., 2008) by HPLC and electrochemical detection. Glutamate, glycine, and GABA were precolumn-derivatized by using naphthalene dicarboxaldehyde as a fluorophore and quantified by HPLC coupled to fluorometric detection.

Dialysate Levels of Histamine in FCX Dialysates of Freely Moving Rats. As described previously (Dekeyne et al., 2008), single-housed male Wistar rats weighing 280 to 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 because 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 to 48 h after implantation by using perfusion of artificial cerebrospinal fluid 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), CaCl2 (1.2 mM), and MgCl2 (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 postcolumn-derivatized by using o-phthalaldeyde as fluorophore and quantified by fluorometry.

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-Ise, France), weighing 210 to 250 g, housed in groups of 5 with restricted access to food (15 g/day/rat). The apparatus was constructed of black Plexiglas and consisted of a central platform (30 cm in diameter) with eight open branches (68 × 10 cm) elevated 80 cm above the floor. The animals were submitted to three training sessions (one per day) and 5 days later to three test sessions (one per day). A session consisted of 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 eight choices in less than 5 min. The number of errors (branches revisited) was recorded. S32212 or vehicle was administered intraperitoneally once daily for 4 days before 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 described previously (Loiseau et al., 2008), the experiments were conducted with adult Wistar rats,
were transparent polycarbonate cages (35 × 23 × 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, Columbus, OH) used to record licks and deliver electrical shocks. During the 3 days before testing, rats were housed in groups of four and restricted to access to tap water for 1 h per day (from 9:00 to 10:00 AM). 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 S32212 or vehicle 30 min before 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-s constant current pulse of 0.3-mA intensity) through the spout. Thereafter, a shock was delivered to the animal every 20th lick for 3 min, and the number of licks emitted during this 3-min session was recorded.

Long-Term Influence on Sexual Behavior in Male Rats. As described previously (Breuer et al., 2008), 120 male and 120 female Wistar rats (Harlan, Zeist, The Netherlands) of approximately 8 weeks of age were group-housed under a reversed day-night schedule (lights off at 6:00 AM). After 1 week of habituation, males were paired (training tests) for 30 min, once weekly for 4 consecutive weeks, with an oestrus female in an observation cage (30 × 40 × 60 cm). Females were brought into oestrus by subcutaneous injection of 50 μg of estradiol benzoate (in 0.1 ml of sesame oil saturated with lecinth) 36 h before testing. In the present study, 36 males with an average of two to three ejaculations during the last training test (normal performers) were selected for drug testing. They were subsequently submitted to four additional tests (once a week) on 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 days 0 to 14. On days 0 (acute treatment), 7 (subchronic), 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 described 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 each), one receiving treatment 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-day 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; EMG, 15–49.9 Hz) was performed over 10-s epochs throughout 2 × 24 h after 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 was evaluated by the Fisher’s exact probability test. In the CMS procedure, data were analyzed by multiple ANOVA with three between-subjects factors (stress/control, drug treatment, and successive sucrose tests) followed by paired t tests to evaluate the significance of differences versus pretreatment (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 drug/scopolamine to vehicle/scopolamine values were made by the use of Dunnnett’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 analyzed 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 used was not identical in view of the very broad palette of procedures used, but essentially followed well established, standard procedures, being identical to those used in our previous exploitation of these procedures (Millan et al., 2000b, 2001; Dekeyne et al., 2008). For example, intravenous administration was necessarily used for electrophysiological work. In several procedures, such as dialysis levels of monoamines, forced test, and social recognition procedure, we confirmed data acquired by the systemic (subcutaneous) route by using oral administration. For chronic administration, the intraperitoneal rather than subcutaneous route was preferred to avoid any potential complications of poor cutaneous tolerance.

Drug doses are in terms of the base. When administered subcutaneously, drugs were dissolved in sterile water, and if necessary plus a few drops of lactic acid, and pH was readjusted with NaOH to neutrality. When administered by the intraperitoneal or oral 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, and S18616 HCl were synthesized by Servier (Neuilly-sur-Seine, France). Imipramine HCl and scopolamine HCl were obtained from Sigma (St. Quentin-Fallavier, France).

Results

Reduction of Immobility in a Forced-Swim Test in Rats. When administered subchronically (24 h, 17 h, and 30 min before testing), S32212 significantly decreased immobility time in rats both upon intraperitoneal and oral administrations: minimal effective dose (MED) was 2.5 and 10.0 mg/kg, respectively (Fig. 1A). Upon acute injection (intraperitoneally) 30 min before testing, S32212 also induced a dose-dependent and significant decrease of immobility (MED, 5.0 mg/kg).

Decrease of Marble-Burying Behavior in Mice. Mice placed into cages containing marbles displayed spontaneous burying behavior (Fig. 1B). Within the 30-min observation session, 18 ± 2 marbles were buried by vehicle-treated subjects. S32212 (0.63–40.0 mg/kg i.p.) dose-dependently reduced marble-burying behavior.

Decrease of Aggressive Behavior in Mice. In pairs of presocialized, familiar mice, placement of the intruder into the cage of the resident elicited aggressive behavior (Fig. 1C). S32212 (0.63–40.0 mg/kg i.p.) dose-dependently attenuated aggressive behavior as revealed by a reduction in the number and the duration (data not shown) of attacks.

Blockade of the LRR Induced by S18616 in Rats. The potent, high efficacy $\alpha_2$-AR agonist S18616 (0.63 mg/kg s.c.) exerted a marked depressive influence on motor activity in rats, which was expressed as a LRR. S32212, which did not itself affect the righting reflex (data not shown), dose-dependently and fully blocked this action of S18616 (Fig. 1D).

Suppression of CMS-Induced Anhedonia in Rats. After exposure to CMS for 3 weeks, rats displayed a marked reduction in sucrose intake relative to nonstressed rats over the 5 weeks of testing: $F_{1,84} = 16.4; P < 0.01$ (Fig. 2). 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 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
nonstressed 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 (data not shown).

**Induction of BDNF Expression in Hippocampus and Amygdala of Rats.** 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 (Fig. 3). 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 on the Electrical Activity of Adrenergic, Dopaminergic, and Serotonergic Cell Bodies.** S32212 dose-dependently and significantly increased the electrical activity of LC-localized noradrenergic perikarya (Fig. 4). 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 (0.125–2 mg/kg i.v.), S32212 exerted no significant influence on the firing rate of dopaminergic cells of the VTA. It similarly had no significant effect on the firing rate of serotonergic neurons of the DRN.

**Influence of S32212 on Extracellular Levels of NA, DA, and 5-HT in FCX and Ventral Hippocampus of Freely Moving Rats.** S32212 (0.63–40.0 mg/kg s.c.) elicited a pronounced, sustained, and dose-dependent increase in the dialysis levels of NA and DA in the FCX, whereas 5-HT levels were not significantly modified (Fig. 5, top). Upon oral administration, in FCX, a similar pattern of effects was obtained. Area under the curve (AUC) analysis expressed relative to basal values (100%) produced the following results. For NA, vehicle = 114.9 ± 3.1 versus S32212 (2.5 mg/kg), 113.0 ± 3.0, P > 0.05; S32212 (5.0 mg/kg) = 131.7 ± 4.0, F,1,14 = 7.3, P < 0.05; S32212 (10.0 mg/kg) = 140.8 ± 4.4, F,1,13 = 9.0, P < 0.01; and S32212 (40.0 mg/kg) = 160.2 ± 5.6, F,1,13 = 11.6, P < 0.01. For DA, vehicle = 106.0 ± 2.1 versus S32212 (2.5 mg/kg), 121.2 ± 5.9, P > 0.05; S32212 (5.0 mg/kg) = 128.6 ± 3.9, F,1,14 = 11.7, P < 0.01; S32212 (10.0 mg/kg) = 128.6 ± 4.0, F,1,13 = 12.0, P < 0.01; and S32212 (40.0 mg/kg) = 155.9 ± 5.0, F,1,14 = 22.2, P < 0.01. For 5-HT, vehicle = 99.2 ± 2.4 versus S32212 (2.5 mg/kg), 87.8 ± 4.1, P > 0.05; S32212 (5.0 mg/kg) = 94.9 ± 3.5, P > 0.05; S32212 (10.0 mg/kg) = 96.1 ± 4.9, P > 0.05; and S32212 (40.0 mg/kg) = 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). Furthermore, S32212 did not affect 5-HT levels in these structures (data 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, bottom).

**Influence of S32212 on Extracellular Levels of ACh versus Histamine, Glutamate, Glycine, and GABA in FCX Dialysates of Freely Moving Rats.** S32212 (0.63–10.0 mg/kg s.c.) elicited a sustained and dose-dependent increase in the 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.), F3X levels of GABA and glutamate were not significantly modified (Fig. 6).

**Reversal by S32212 of a Deficit Induced by Scopolamine in the Radial Maze Test in Rats.** Scopolamine (0.5 mg/kg i.p.) administered before each test session provoked an increase in mean (three sessions) number of revisited arms (Table 1). This deficit was significantly reduced by daily (4 days before testing and 3 days of testing) injection of S32212 at a dose of 10.0 mg/kg i.p. The lower dose of 2.5 mg/kg was ineffective.

**Reversal by S32212 of a Delay-Dependent Deficit in NOR in Rats.** After a 4-h delay, control (vehicle) rats spent an equivalent time exploring the familiar versus novel object...
during the choice trial (Fig. 8). 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, whereas interest for the novel object slightly increased. Suggesting that S32212 did not exert nonspecific effects or impair locomotor function, it had no significant effect on total exploration of objects during either the familiarization or choice trials. ANOVA produced no significant effect on total exploration of objects during P1 or P2. ANOVA results were as follows: for P1, vehicle, 95.1 ± 9.3, S32212 0.08 mg/kg, 100.5 ± 7.1, F_{5,6} = 0.9; P > 0.05; for P2, vehicle, 75.3 ± 6.1, S32212, 0.04 mg/kg, 66.9 ± 9.6, S32212 0.16 mg/kg, 83.1 ± 15.7, S32212 0.63 mg/kg, 79.2 ± 9.6, and S32212 2.5 mg/kg, 100.0 ± 11.6; F_{5,34} = 0.8; P > 0.05.

Facilitation by S32212 of SND in Rats. With a short period P1 (5 min), control (vehicle) animals spent an equivalent time investigating the familiar versus novel juvenile during P2 (Fig. 9A). S32212 increased the time spent investigating the novel juvenile without modifying the 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 nonspecific influence, S32212 exerted no effect on total investigation of juveniles during either P1 or P2. ANOVA results were as follows: for P1, vehicle, 77.9 ± 4.5 s; S32212, 0.04 mg/kg, 87.8 ± 11.0, S32212 0.08 mg/kg, 86.1 ± 9.3, S32212 0.16 mg/kg, 90.0 ± 7.6, S32212 0.63 mg/kg, 95.1 ± 4.8, and S32212 2.5 mg/kg, 100.5 ± 7.1; F_{5,34} = 0.9; P > 0.05; for P2, vehicle, 75.3 ± 6.1; S32212, 0.04 mg/kg, 67.2 ± 16.7, S32212 0.08 mg/kg, 66.9 ± 9.6, S32212 0.16 mg/kg, 83.1 ± 15.7, S32212 0.63 mg/kg, 79.2 ± 9.6, and S32212 2.5 mg/kg, 100.0 ± 11.6; F_{5,34} = 0.8; P > 0.05.

Influence on Motor Function. 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) (Table 2). In mice nonhabituated 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.
polamine, in unpaired
whereas paroxetine significantly reduced body weight after
S32212 did not affect body weight compared with vehicle,
low sexual performances relative to vehicle-treated rats.
paroxetine-treated rats had completely recovered from their
S32212 administrations, no “after-effect” was observed, and
displayed a marked inhibitory influence. During a test ses-
a deleterious effect on sexual behavior, whereas paroxetine
subchronic (7 days) and chronic (14 days), S32212 still lacked
the first ejaculation compared with vehicle (Table 4). After
effects on either the number of ejaculations or the latency to
After acute treatment (day 0) of S32212 (2.5 mg/kg i.p.)
Rats.
conflict test (Table 3).
S32212 (0.63–40.0 mg/kg i.p.) elicited a dose-depen-
dent and robust increase in punished responses in the Vogel
Conflict Test in rats. Treatment 1 is once daily, 4 days before testing and 3 days of testing (45 min before
each test session). Treatment 2 is once daily, 30 min before each test session. Data are means ± S.E.M. (n = 10 per value).

<table>
<thead>
<tr>
<th>Treatment 1 (Dose)</th>
<th>Treatment 2 (Dose)</th>
<th>Number of Errors, Mean Three Test Sessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>Vehicle</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Vehicle</td>
<td>Scopolamine (0.5 mg/kg i.p.)</td>
<td>2.3 ± 0.2*</td>
</tr>
<tr>
<td>S32212 (2.5 mg/kg i.p.)</td>
<td>Scopolamine (0.5 mg/kg i.p.)</td>
<td>2.1 ± 0.2*</td>
</tr>
<tr>
<td>S32212 (10.0 mg/kg i.p.)</td>
<td>Scopolamine (0.5 mg/kg i.p.)</td>
<td>1.9 ± 0.2*</td>
</tr>
</tbody>
</table>

# indicates significance of differences versus vehicle + vehicle. 
* indicates significance of differences between S32212 + scopolamine and vehicle + scopo-
| Values were 21.7, 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, F1,9 = 1.7, P > 0.05; S32212 2.5 mg/kg, F1,10 = 5.7, P < 0.05; and S32212 10.0 mg/kg, F1,9 = 7.2, P < 0.05. No F values were significant for histamine, GABA, and glut.

**Anxiolytic Properties in the Vogel Conflict Test in Rats.** S32212 (0.63–40.0 mg/kg i.p.) elicited a dose-dependent and robust increase in punished responses in the Vogel conflict test (Table 3).

**Lack of Influence on the Sexual Behavior of Male Rats.** 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 on either the number of ejaculations or the latency to the first ejaculation compared with vehicle (Table 4). After subchronic (7 days) and chronic (14 days), S32212 still lacked a deleterious effect on sexual behavior, whereas paroxetine displayed a marked inhibitory influence. During a test session performed on day 21, that is, 1 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 compared with 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 on Sleep-Wake Cycle Architecture in Rats.** As shown in Fig. 10 left, 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, although significance was seen only 2 h after the onset of its impact on waking and SWS. During the subsequent light period, no significant influence of S32212 was observed. Furthermore, there was no significant influence of S32212 throughout the subsequent 24-h period (data not shown). In rats treated at the onset of the light phase (data not shown), S32212 induced similar, although less marked, quantitative changes: a decrease in waking (3–6 h postinjection), an increase of SWS (4–5 h), and a delayed increase in REM sleep (8–9 h). As shown in Fig. 10, right, 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 (3–6 versus 3–9 h postinjection). However, in contrast to S32212, mirtazapine rapidly reduced REM sleep (1–4 versus 6–9 h postinjection). Mirtazapine did not provoke further quantitative changes, either during the subsequent light period or the second 24-h period (data not shown). In animals receiving mirtazapine at the onset of light phase (data not shown), quantitative changes were similar: a decrease in waking, an increase of SWS (3–5 h postinjection), and a rapid decrease in REM sleep (1–4 h).

**Discussion**

**Actions in Models of Potential Antidepressant Properties.** Anti-immobility actions in the forced-swim test are
**Fig. 7.** Reversal by S32212 of deficits in social recognition in rats, provoked either by scopolamine or delay. A and B, improvement by S32212, subcutaneously (A) and orally (B) of the disruption of social recognition provoked by scopolamine (1.25 mg/kg s.c.). Values are means ± S.E.M. \((n = 5–13 \text{ per value})\). Two-way ANOVA results were as follows: S32212 subcutaneously, scopolamine, \(F_{1,60} = 29.1, P < 0.001\); S32212, \(F_{4,60} = 7.3, P < 0.001\) and interaction, \(F_{4,45} = 10.9, P < 0.001\); S32212 orally, scopolamine, \(F_{1,45} = 28.5, P < 0.001\); S32212, \(F_{3,45} = 4.2, P < 0.05\) and interaction, \(F_{3,45} = 8.7, P < 0.001\). ★ indicates significance of differences between vehicle/scopolamine and vehicle/vehicle values; ☆ indicates significance of differences between S32212/scopolamine and vehicle scopolamine values in Newman-Keuls test. C and D, improvement by S32212, subcutaneously (C) and orally (D) of the disruption of social recognition provoked by a delay (2 h). Values are means ± S.E.M. \((n = 7–10 \text{ per value})\). For dose-response curves, one-way ANOVA results were as follows: S32212, subcutaneously, \(F_{4,36} = 11.4, P < 0.05\) and S32212, orally, \(F_{4,31} = 10.8, P < 0.001\). ★ indicates significance of differences between S32212 and vehicle values in Dunnett’s test. For the specificity of drug actions, two-way ANOVA results were as follows: S32212, subcutaneously, influence of juvenile, \(F_{1,23} = 17.6, P < 0.01\); influence of drug, \(F_{3,23} = 28.7, P < 0.001\), and interaction, \(F_{3,23} = 3.5, P > 0.05\). S32212, orally, influence of juvenile, \(F_{1,20} = 5.3, P < 0.05\); influence of drug, \(F_{1,20} = 19.3, P < 0.001\), and interaction, \(F_{1,20} = 5.3, P < 0.05\). ☆ indicates the significance of differences in Newman-Keuls test between values for a different juvenile versus the same juvenile. \(P < 0.05\).

**Fig. 8.** Reversal by S32212 of a delay-dependent deficit in novel object recognition in rats. A, exploration of novel and familiar objects during choice trial after a 4-h intertrial interval. B, dose-dependent improvement by S32212 of the D2 index score. Values are means ± S.E.M. \((n = 12 \text{ per value})\). The two-way ANOVA performed on exploration of novel and familiar objects during choice trial showed a significant object × treatment interaction: \(F_{4,44} = 3.3, P < 0.05\). The one-way ANOVA performed on D2 scores was as follows: \(F_{3,44} = 3.1, P < 0.05\). ★ indicates significance of differences to vehicle values in Dunnett’s test, \(P < 0.05\).
generally attributed to antidepressant properties and were expressed by S32212 both subchronically and, in contrast to certain antidepressants such as 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: although not of any obvious construct validity for depressed states, suppression of marble burying may be related to the influence of SSRIs and tricyclics on compulsive behaviors (Millan et al., 2001; Kobayashi et al., 2008; Thomas et al., 2009). By analogy to marble burying, suppression of aggressive behavior 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; Malkesman et al., 2009). In view of its reduction by S32212, it would be interesting to determine whether S32212 specifically affects agonistic behavior under other conditions (Mitchell 2005) and whether it affects behavior in models of social defeat (Razzoli et al., 2009; Carr and Lucki, 2011).

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-HT2c antagonists are effective in all of the above procedures, whereas 5-HT2a 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 α2-AR antagonists and genetic deletion of α2c-ARs (Sallinen et al., 2007; Millan, 2010). α2-AR antagonists also reduce aggressive behavior (Haller et al., 1996; M. Brocco, unpublished observation), suppress marble burying (Lähdesmäki et al., 2002), and block S18616-induced LRR (Millan et al., 2000b). Thus, the precise contribution of 5-HT2c and/or α2-AR properties to these antidepressant-like actions of S32212 will require additional study.

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### Table 2

Influence of S32212 on motor behavior in rodents

<table>
<thead>
<tr>
<th>Dose</th>
<th>Rotarod, Mice</th>
<th>Spontaneous Locomotion, Mice</th>
<th>Spontaneous Locomotion, Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>236 ± 38</td>
<td>407 ± 41</td>
<td>49 ± 12</td>
</tr>
<tr>
<td>S32212 0.63 mg/kg s.c.</td>
<td>173 ± 46</td>
<td>348 ± 37</td>
<td>35 ± 8</td>
</tr>
<tr>
<td>S32212 2.5 mg/kg s.c.</td>
<td>192 ± 66</td>
<td>241 ± 16*</td>
<td>34 ± 7</td>
</tr>
<tr>
<td>S32212 40.0 mg/kg s.c.</td>
<td>67 ± 13*</td>
<td>117 ± 8*</td>
<td></td>
</tr>
</tbody>
</table>

* indicates significance of differences to vehicle values in Dunnett’s test after ANOVA; P < 0.05.

### Table 3

Anxiolytic properties of S32212 in the Vogel conflict test in rats

<table>
<thead>
<tr>
<th>Rats</th>
<th>Dose</th>
<th>Number of Licks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpunished</td>
<td>Vehicle</td>
<td>672 ± 75</td>
</tr>
<tr>
<td>Punished</td>
<td>Vehicle</td>
<td>79 ± 17*</td>
</tr>
<tr>
<td>S32212 0.63 mg/kg i.p.</td>
<td>79 ± 17*</td>
<td></td>
</tr>
<tr>
<td>S32212 2.5 mg/kg i.p.</td>
<td>193 ± 55</td>
<td></td>
</tr>
<tr>
<td>S32212 10.0 mg/kg i.p.</td>
<td>433 ± 106*</td>
<td></td>
</tr>
<tr>
<td>S32212 40.0 mg/kg i.p.</td>
<td>525 ± 110*</td>
<td></td>
</tr>
</tbody>
</table>

* indicates significance of differences to vehicle values in Dunnett’s test; P < 0.05.

### Table 4

Influence of chronic (14 days) administration of S32212 compared with paroxetine on sexual behavior and body weight in male rats

<table>
<thead>
<tr>
<th>Day</th>
<th>Vehicle</th>
<th>S32212 (2.5 mg/kg i.p.)</th>
<th>Paroxetine (10.0 mg/kg i.p.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td></td>
<td>1.5 ± 0.3</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Day 7</td>
<td>7</td>
<td>2.3 ± 0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Day 14</td>
<td>14</td>
<td>1.9 ± 0.2</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Day 21</td>
<td>21</td>
<td>1.8 ± 0.3</td>
<td>1.8 ± 0.4</td>
</tr>
</tbody>
</table>

* indicates significance of differences to vehicle values in Dunnett’s test after ANOVA; P < 0.05.

For body weight, day 0, F2,33 = 4.0, P = 0.05; day 14, F2,33 = 4.0, P = 0.05; and day 21, F2,33 = 4.0, P = 0.05. For latency, day 0, F2,33 = 9.6, P = 0.001; day 14, F2,33 = 6.5, P = 0.01; and day 21, F2,33 = 1.9, P = 0.05.

### Fig. 9

Improvement by S32212 of social novelty discrimination in rats. 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. Two-way ANOVA performed on exploration of novelty versus familiar juveniles during P2 showed a significant effect of factor juvenile: F1,34 = 17.7; P < 0.001. B, improvement by S32212 of the ratio P2 novel/P2 familiar. Values are means ± S.E.M. (n = 4–8 per value). Results of one-way ANOVA performed on ratios P2 novel/P2 familiar were: F1,34 = 2.6; P = 0.11. * indicates significance of differences to vehicle values in Dunnett’s test; P < 0.05.
The reversal of CMS-induced sucrose consumption by chronic administration of S32212 suggests antianhedonic action and mirrors actions of clinically efficacious antidepressants such as imipramine (Willner, 2005), although S32212 displayed a more rapid onset of action (week 1). Selective α2-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 α2-ARs inhibitory to monoaminergic pathways (Millan 2006; Dhir and Kulkarni, 2007; Yanpallewar et al., 2010; Serres et al., 2011). Inasmuch as 5-HT2C antagonists are active in the CMS procedure and enhancing extracellular levels of monoamines (Millan, 2005; Dekeyne et al., 2008; Carr and Lucki, 2011), the complementary α2-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 favors processes underpinning neuronal proliferation and survival, synaptic plasticity, cognition, and mood, and reductions in BDNF levels are seen in both patients and 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 extend these observations to neurogenesis in normal and “depressed” animals (Pittenger and Duman, 2008; Schulte-Herbrüggen et al., 2009; de Bodinat et al., 2010). Although chronic 5-HT2C receptor blockade enhances BDNF gene expression (Dekeyne et al., 2008; Soumier et al., 2009) and is probably implicated in the effects of S32212, a role for α2-AR antagonism, mirroring the induction of other effector genes such as Arc (Yanpallewar et al., 2010; Serres et al., 2011), should not be excluded.

Fig. 10. Influence of S32212 on sleep-wake architecture, as evaluated by EEG recording. Influence of S32212 (left) and influence of mirtazapine (right) administered at the onset of the dark phase (shaded areas) on the duration of waking state, SWS, and REM sleep. Data are means ± S.E.M. (n = 11 per value). Drug effect after ANOVA was 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, \( F_{1,11} = 3.5, P > 0.05. \) * indicates significance of differences to vehicle values in Newman-Keuls test; \( P < 0.05. \)
Influence on Extracellular Levels of NA, DA, and ACh in Freely Moving Rats. Reflecting blockade of (constitutively active) 5-HT<sub>2C</sub> receptors and α<sub>2-AR</sub> antagonists inhibitory to VTAs- and LC-derived dopaminergic and adrenergic projections, respectively (Millan et al., 2000a; Millan, 2006; Dekeyne et al., 2008; Aloyo et al., 2009; Di Giovanni et al., 2010), S32212 elevated extracellular levels of DA and NA in the FCX and hippocampus of freely moving rats. The acceleration of LC firing rate reflects blockade of tonically active α<sub>2A</sub>-AR autoreceptors on adrenergic perikarya, principally the α<sub>2A</sub>-AR subtype with a subsidiary role for α<sub>2C</sub>-ARs (Millan et al., 2000a; Dwyer et al., 2010; Millan, 2010), and both are antagonized by S32212 (Millan et al., 2012b). Blockade of α<sub>2A</sub>-ARs on dopaminergic terminals probably underlies the S32212-induced increase in DA levels in the FCX, because it did not affect dopaminergic cell bodies (Millan et al., 2000a; Dwyer et al., 2010; Millan, 2010). Indeed, α<sub>2A</sub>-ARs do not control the resting activity of VTA-localized dopaminergic perikarya, yet like LC-adrenergic neurons, they are tonically inhibited by (constitutively active) 5-HT<sub>2C</sub> receptors acting via GABAergic interneurons (Millan et al., 2000a; Dekeyne et al., 2008; Aloyo et al., 2009; Di Giovanni et al., 2010). Thus, it remains to be elucidated why S32212 did not excite VTA-dopaminergic perikarya and what is the precise contribution of 5-HT<sub>2C</sub> receptor and α<sub>2A</sub>-AR blockade to its facilitation of adrenergic and dopaminergic transmission.

Apart from a favorable impact on depressed mood, a reinforcement of DA and NA release by S32212 favors cognitive function, which should be further strengthened by its elevation of ACh release in the FCX at doses of 2.5 to 10.0 mg/kg (Millan, 2010; Hasselmo and Sarter, 2011; Klinkenberg et al., 2011). Correspondingly, α<sub>2A</sub>-AR antagonists similarly increase ACh levels in the 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. C. F. Fone, unpublished observation). Inasmuch as α<sub>2A</sub>-AR antagonists also enhance FCX-integrated cognitive flexibility in behaviorally depressed rats (Lapiz and Morilik, 2006), similar work with S32212 would be of interest. Nonetheless, the role of α<sub>2</sub>-ARs in cognition is not unitary in that recruitment of α<sub>2A</sub>-ARs on pyramidal cells in the FCX promotes working memory (Robbins and Arnsten, 2009; Millan, 2010), so the overall impact on cognition of S32212 warrant more extensive study. In addition, a role for 5-HT<sub>2C</sub> receptor antagonism in the influence of S32212 on cognition should not be excluded because, together with α<sub>2A</sub>-AR blockade, it reinforces corticolumbic liberation of DA and NA, which also favor cognitive processing (see below; Robbins and Arnsten, 2009). Indeed, blockade of 5-HT<sub>2C</sub> receptors countered a delay-induced deficit in NOR (Pitsikas and Sakellardis, 2005) and has been reported to improve reversal learning (Boulougouris and Tsaltas, 2008). Conversely, social recognition and SND are unaffected by 5-HT<sub>2C</sub> antagonists (A. Dekeyne and F. Loiseau, unpublished observation). Finally, despite reports that 5-HT<sub>2A</sub> 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, 2008; Landholt and Wehrle, 2009; A. Dekeyne and F. Loiseau, unpublished observation). Hence, 5-HT<sub>2A</sub> blockade is unlikely to participate in the cognitive effects of S32212.

Potential Anxiolytic Properties. Inasmuch as blockade of 5-HT<sub>2C</sub> receptors in amygdala and dorsal hippocampus is associated with anxiolytic effects (Millan and Brocco, 2003; Millan, 2005; Heisler et al., 2007; Dekeyne et al., 2008), the actions of S32212 in the Vogel conflict test can be principally ascribed to 5-HT<sub>2C</sub> receptor blockade, although a contribution of 5-HT<sub>2A</sub> antagonism cannot be excluded (Millan and Brocco, 2003). α<sub>2A</sub>-AR antagonists are inactive in the Vogel conflict test, and reflecting overactivation of adrenergic projections, they may even display anxiogenic and panicogenic properties (Crespi, 2009). Indeed, they block the anxiolytic effects of 5-HT<sub>2C</sub> antagonists in the social interaction procedure, probably explaining the inactivity of S32212 in this test of anxiolytic properties (A. Dekeyne, unpublished observation).

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 model whereby chronic parox-
etine 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-HT2C receptors (Millan, 2006; Kennedy and Rizvi, 2009). In line with these observations, antidepressants possessing 5-HT2C receptor antagonist properties, such as agomelatine and mirtazapine, elicit fewer sexual side effects than SSRIs (Millan, 2006; Kennedy and Rizvi, 2009; de Bodinat et al., 2010). By analogy to 5-HT2C receptor antagonist, blockade of α2-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 on 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; Millan et al., 2012b), S32212 promoted SWS. There is no evidence that α2-AR blockade increases SWS (Ouyang et al., 2004; Mallick et al., 2005), so this action is best attributed to antagonism of 5-HT2C and/or 5-HT2A receptors, with the latter perhaps fulfilling a predominant role (Smith et al., 2009; 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 (Mayers and Baldwin, 2005; Millan, 2006). However, this relationship is contentious because the clinically active antidepressants mirtazapine and agomelatine do not suppress REM sleep in patients (Mayers and Baldwin, 2005; de Bodinat et al., 2010), whereas H1 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 H1 antagonist properties (Fig. 10; Mayers and Baldwin, 2005), and the absence of H1 receptor blockade with S32212 (Millan et al., 2012b) 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 because both 5-HT2C and α2-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-HT2A receptor blockade in light of the REM sleep-promoting and -suppressing actions of 5-HT2A antagonists and agonists, respectively (Landholt and Wehrle, 2009). The reduction of waking by mirtazapine can be attributed to antagonism of histamine H1 receptors and, in view of its sedative properties, mirtazapine taken in the evening (Mayers and Baldwin, 2005; Szegedi and Schwertfeger, 2005). Because S32212 has no affinity for H1 sites (Millan et al., 2012b), blockade of α2-ARs is arousing, and 5-HT2C 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 procognitive properties. It also countered anxiety, enhanced SWS, preserved sexual function, was essentially devoid of sedative properties, and respects body weight. This distinctive func-

### Table 5

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

<table>
<thead>
<tr>
<th>Model (Species)</th>
<th>Active Dose Range</th>
</tr>
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<tbody>
<tr>
<td><strong>Antidepressant properties</strong></td>
<td></td>
</tr>
<tr>
<td>Reduction of immobility in a forced swim test (rat)</td>
<td>2.5 mg/kg i.p., and 10.0–40.0 mg/kg p.o., subchronic</td>
</tr>
<tr>
<td>Blockade of marble-burying behavior (mice)</td>
<td>2.5–10.0 mg/kg i.p., acute</td>
</tr>
<tr>
<td>Blockade of isolation-induced aggressive behavior (mice)</td>
<td>10.0–40.0 mg/kg s.c., acute</td>
</tr>
<tr>
<td>Blockade of S18616-induced loss of righting reflex (rat)</td>
<td>10.0–40.0 mg/kg i.p., acute</td>
</tr>
<tr>
<td>Reversal of chronic mild stress-induced reduction in sucrose intake (rat)</td>
<td>80.0 mg/kg i.p., acute</td>
</tr>
<tr>
<td>Increase in BDNF expression in hippocampus and amygdale (rat)</td>
<td>0.63–2.5 mg/kg i.p., chronic (5 weeks)</td>
</tr>
<tr>
<td>Increase in electrical activity of adrenergic cell bodies (rat)</td>
<td>10.0 mg/kg i.p., chronic (14 days, twice daily)</td>
</tr>
<tr>
<td>Increase in DA and NA levels in the frontal cortex (rat)</td>
<td>0.5–2.0 mg/kg i.v., acute</td>
</tr>
<tr>
<td>Increase in DA and NA levels in the frontal cortex (rat)</td>
<td>10.0–40.0 mg/kg s.c. and 5.0–40.0 mg/kg p.o., acute</td>
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<tr>
<td><strong>Procognitive properties</strong></td>
<td></td>
</tr>
<tr>
<td>Increase in ACh levels in the frontal cortex (rat)</td>
<td>2.5–10.0 mg/kg s.c., acute</td>
</tr>
<tr>
<td>Reversal of scopolamine-induced deficit in the radial maze (rat)</td>
<td>10.0 mg/kg i.p., acute</td>
</tr>
<tr>
<td>Reversal of scopolamine-induced deficits in social recognition (rat)</td>
<td>0.04–0.63 mg/kg s.c. and 0.04–0.16 mg/kg p.o., acute</td>
</tr>
<tr>
<td>Reversal of delay-induced deficits in social recognition (rat)</td>
<td>1.25–2.5 mg/kg s.c. and 5.0–10.0 mg/kg p.o., acute</td>
</tr>
<tr>
<td>Reversal a delay-dependent deficit in novel object recognition (rat)</td>
<td>10.0 mg/kg s.c., acute</td>
</tr>
<tr>
<td>Facilitation of social novelty discrimination (rat)</td>
<td>0.16–0.63 mg/kg s.c., acute</td>
</tr>
<tr>
<td><strong>Anxiolytic properties</strong></td>
<td></td>
</tr>
<tr>
<td>Vogel conflict (rat)</td>
<td>10.0–40.0 mg/kg i.p., acute</td>
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<tr>
<td><strong>Influence on sexual function</strong></td>
<td></td>
</tr>
<tr>
<td>Lack of disruption (male rat)</td>
<td>10.0 mg/kg i.p., chronic (14 days), inactive</td>
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<tr>
<td><strong>Influence on sleep patterns</strong></td>
<td></td>
</tr>
<tr>
<td>Increase in SWS and REM sleep, decrease in waking state (rat)</td>
<td>10.0 mg/kg s.c., acute</td>
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</tbody>
</table>
tional profile reflects reciprocal inverse agonist properties at 5-HT_{2C} receptors and antagonist actions at α_{2A}-ARs, with a possible role for 5-HT_{2A} receptor antagonism, in particular as regards its influence on sleep. Although additional studies are required to substantiate these observations, S32212 exemplifies the promise of multifaceted strategies for the improved treatment of depressed states.

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Authorship Contributions

Conducted experiments: Dekeyne, Brocco, Loiseau, Gobert, Rivet, Di Cara, Cremers, Flik, Fone, Watson, Papp, Sharp, Serres, Cespaglia, Olivier, Chan, and Lavielle.

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