Neuropsychopharmacology (2009) 34, 1039–1056

www.neuropsychopharmacology.org

Neurokinin$_1$ Antagonists Potentiate Antidepressant Properties of Serotonin Reuptake Inhibitors, Yet Blunt Their Anxiogenic Actions: A Neurochemical, Electrophysiological, and Behavioral Characterization

Alain Gobert[1], Mauricette Brocco[1], Anne Dekeyne[1], Benjamin Di Cara[1], Gaëlle Bouchez[1], Françoise Lejeune[1], Robert L Gannon[2] and Mark J Millan*[,1]

[1]Psychopharmacology Department, Institut de Recherches Servier, Centre de Recherches de Croissy, Paris, France; [2]Department of Biology, Valdosta State University, Valdosta, GA, USA

Though neurokinin$_1$ (NK$_1$) receptor antagonists are active in experimental models of depression, clinical efficacy has proven disappointing. This encourages interest in association of NK$_1$ receptor blockade with inhibition of serotonin (5-HT) reuptake. The selective NK$_1$ antagonist, GR205171, dose-dependently enhanced citalopram-induced elevations of extracellular levels of 5-HT in frontal cortex, an action expressed stereospecifically *vs* its less active distomer, GR226206. Further, increases in 5-HT levels in dorsal hippocampus, basolateral amygdala, nucleus accumbens, and striatum were likewise potentiated, and GR205171 similarly facilitated the influence of fluoxetine upon levels of 5-HT, as well as dopamine and noradrenaline. In parallel electrophysiological studies, the inhibitory influence of citalopram and fluoxetine upon raphe-localized serotonergic neurones was stereospecifically blunted by GR205171. Antidepressant actions of citalopram in a forced-swim test in mice were stereospecifically potentiated by GR205171, and it also enhanced attenuation by citalopram of stress-related ultrasonic vocalizations in rats. Further, GR205171 and citalopram additively abrogated the advance in circadian rhythms provoked by exposure to light in hamsters. By contrast, GR205171 stereospecifically blocked anxiogenic actions of citalopram in social interaction procedures in rats and gerbils, and stereospecifically abolished facilitation of fear-induced foot tapping by fluoxetine in gerbils. By analogy to GR205171, a further NK$_1$ antagonist, RP67580, enhanced the influence of citalopram upon frontocortical levels of 5-HT and potentiated its actions in the forced swim test. In conclusion, NK$_1$receptor blockade differentially modulates functional actions of SSRIs: antidepressant properties are reinforced, whereas anxiogenic effects are attenuated. Combined NK$_1$ receptor antagonism/5-HT reuptake inhibition may offer advantages in the management of depressed and anxious states. *Neuropsychopharmacology* (2009) **34,** 1039–1056; doi:10.1038/npp.2008.176; published online 1 October 2008

Keywords: substance P; neurokinin receptor; dialysis; SSRI; fluoxetine

INTRODUCTION

In view of the inadequacies of current treatment of depression, considerable efforts are being made to find improved drugs (Millan, 2004, 2006; Morilak and Frazer, 2004). In this regard, much attention has been devoted to NK$_1$ receptors which transduce the actions of substance P (Rupniak *et al*, 2001; Holmes *et al*, 2003). Several preclinical and clinical observations support the utility of NK$_1$ receptor blockade in the control of depressed—and anxious—states. First, long-term treatment with various classes of antidepressant modifies central tissue levels of substance P, an effect observed in the absence of an alteration in NK$_1$ receptor expression (Shirayama *et al*, 1996; Sartori *et al*, 2004). Second, NK$_1$ receptors are localized in: the dorsal raphe nucleus (DRN), the origin of ascending serotonergic projections (Froger *et al*, 2001; Santarelli *et al*, 2001; Commons and Valentino, 2002; Ma and Bleasdale, 2002; Lacoste *et al*, 2006); the locus coeruleus, the source of adrenergic input to corticolimbic regions (Santarelli *et al*, 2001; Ma and Bleasdale, 2002); and the ventrotegmental area (VTA), from which dopaminergic pathways project to the frontal cortex (FCX) and limbic regions (Lessard and Pickel, 2005). Third, NK$_1$ receptors are broadly distributed in limbic structures implicated in the control of mood, such as the FCX, hippocampus, lateral septum, nucleus accumbens, basolateral amygdala (BLA), and hypothalamus (Liu *et al*, 2002; Saffroy *et al*, 2003). Fourth, substance P exerts

*Correspondence: Dr MJ Millan, Neuropharmacology Department, Institut de Recherches Servier, Centre de Recherches de Croissy, 125, Chemin de Ronde, Croissy-sur-Seine 78290, Paris, France,
Tel: +33 1 55 72 24 25, Fax: +33 1 55 72 20 82,
E-mail: mark.millan@fr.netgrs.com
Received 9 July 2008; revised 5 August 2008; accepted 19 August 2008

aversive effects in rodents, whereas mice genetically deprived of NK_1 receptors reveal improved resistance to stress and display an anxiolytic phenotype (Rupniak *et al*, 2001; Santarelli *et al*, 2001). Fifth, selective NK_1 receptor antagonists exert antidepressant and anxiolytic actions in several, though not all, experimental models (Rupniak *et al*, 2001; Varty *et al*, 2002; Dableh *et al*, 2005; Renoldi and Invernizzi, 2006), and they attenuate the behavioral response to stress (Ebner *et al*, 2004).

The above observations prompted clinical evaluation of several selective NK_1 receptor antagonists, including MK869 (aprepitant) and L759274, which revealed beneficial effects in major depression with improved tolerance (less sexual dysfunction) as compared to SSRIs (Kramer *et al*, 1998, 2004; Gerald *et al*, 2006). Nonetheless, therapeutic trials of NK_1 antagonists have not overall yielded convincing evidence of robust and reproducible antidepressant actions, tempering enthusiasm for their further development. This has triggered interest in the concept of combining NK_1 antagonist properties with suppression of 5-HT reuptake, either as drug associations or in a single chemical structure (Ryckmans *et al*, 2002; Guiard *et al*, 2004; Millan, 2006). Such agents would be expected to exert complementary antidepressant properties, potentially with greater efficacy and rapidity of action than SSRIs and selective NK_1 blockers, respectively. Support for this notion has been generated by both neurochemical and electrophysiological studies in rodents. Thus, the influence of SSRIs upon extracellular levels of 5-HT in FCX was reinforced in mice genetically lacking NK_1 receptors, possibly due to desensitization of feedback actions at inhibitory 5-HT_{1A} autoreceptors in the DRN (Froger *et al*, 2001; Santarelli *et al*, 2001; Guiard *et al*, 2005; Gobbi *et al*, 2007). By analogy, Guiard *et al* (2004) demonstrated that the effect of paroxetine on frontocortical 5-HT levels in mice were potentiated by the NK_1 antagonists, GR205171 and L733060.

These elegant studies underpin the notion that NK_1 receptor blockade may enhance the influence of SSRIs upon serotonergic transmission, but several important issues and questions remain to be addressed. First, neurochemical studies of the influence of NK_1 antagonists on the actions of SSRIs have to date been restricted to mice, and it would be desirable to reproduce such findings in other species. Second, the influence of NK_1 receptor blockade upon SSRI-elicited increases in 5-HT levels has been evaluated in the FCX, but it would be interesting to extend such work to other corticolimbic structures implicated in depressed states, like the hippocampus, BLA, nucleus accumbens, and striatum. Third, certain 'SSRIs' also enhance extracellular levels of noradrenaline (NA) and dopamine (DA) in FCX (Millan *et al*, 2000), but it has not been determined whether NK_1 receptor antagonists likewise modify the influence of SSRIs upon dopaminergic and adrenergic transmission. Fourth, subactive doses of SSRIs exert antidepressant actions in the mouse forced swim (FS) test in the presence of GR205171 (Chenu *et al*, 2006). Remarkably, however, no other data on how NK_1 antagonists may modify the behavioral actions of SSRIs are available. In this light, it would be of particular interest to examine whether the acute anxiogenic actions of SSRIs (Dekeyne *et al*, 2000) are modified by blockade of NK_1 receptors.

The present studies addressed these questions employing combined neurochemical, electrophysiological, and behavioral approaches, together with the highly selective NK_1 receptor antagonist, GR205171. In contrast to most agents, GR205171 possesses high affinity at NK_1 receptors across a broad range of species, though its affinity is higher for NK_1 receptors in gerbils and guinea pigs (which closely resemble those in humans) than rats and mice (Rupniak *et al*, 2000; Griffante *et al*, 2006; Engberg *et al*, 2007). GR205171 was also chosen in view of its well-characterized actions *in vivo* (Gardner *et al*, 1996; Millan *et al*, 2001b; Lejeune *et al*, 2002; Guiard *et al*, 2004; Chenu *et al*, 2006), and as clinical studies have shown that it relieves social phobia (Furmark *et al*, 2005; Michelgard *et al*, 2007). To underpin the stereospecificity of the actions of GR205171, its effects were compared to those of its less active distomer, GR226206 (op. cit.).

MATERIALS AND METHODS

Animals

Unless specified below, these studies employed male Wistar rats (225–250 g body weight upon arrival) and male CD mice (22–26 g upon arrival) supplied by Charles River (L'Arbresle and Saint Aubin les Elbeuf, France, respectively). Male Mongolian gerbils (50–70 g upon arrival) were acquired from CERJ (Le Genest, St-Isle, France). Male Syrian hamsters (70–80 g upon arrival) were obtained from Charles River Laboratories (Kingston, NY, USA). Rats, mice, and gerbils were housed in standard Macrolon, sawdust-lined cages and hamsters were housed in polycarbonate cages lined with sterilized pine chips and unrestricted access to food and water. Except for hamsters (see below), there was a light/dark cycle with lights on from 07 : 30 to 19 : 30 hours. All animal use procedures conformed to international European ethical standards (86/609-EEC) and the French National Committee (décret 87/848) for the care of laboratory animals. Hamster use was approved by the Institutional Animal Care and Use Committee of Valdosta State University and complied with regulations outlined in the US Animal Welfare Act.

Cerebral Microdialysis and Chromatographic Procedures

The protocol used for quantification of levels of 5-HT, DA, and NA in dialysate samples was detailed elsewhere (Millan *et al*, 2001b). Briefly, a guide-cannula was implanted under pentobarbital anesthesia (60 mg/kg, i.p.) into the FCX, nucleus accumbens, striatum, or dorsal hippocampus of rats as previously described (Millan *et al*, 2001b). For the BLA, coordinates were AP, 2.8; ML, $\pm$ 4.9; and DV, 6.4 from dura. Experiments were performed 5 days later after placement of the guide cannula following placement of a cuprophane CMA/11 probe: 0.24 mm in diameter, 4 mm in length for FCX and striatum, and 2 mm for the dorsal hippocampus, BLA and nucleus accumbens. Samples were taken every 20 min over 1 h, then GR205171 or vehicle was injected i.p. followed, 20 min later, by s.c. administration of citalopram, fluoxetine, 8-OH-DPAT or S15535. In further experiments, injection of citalopram was preceded by treatment with GR226206 or RP67580. Sampling was

pursued for a further 3 h. Monoamine levels were quantified as described previously (above citations) by HPLC and electrochemical detection.

Electrophysiological Procedure

As detailed previously (Lejeune *et al*, 2002), rats were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and, after cannulation of the femoral vein, placed in a stereotaxic apparatus. A tungsten microelectrode was slowly lowered into the DRN (from bregma and the sinus surface, AP, 7.2; ML, ±0.0; and DV, 5.5/6.5). After amplification (CP511; Grass Technology, USA) and A/D conversion (micro1401-mkII; CED, Cambridge, UK) of electrical activity, data were recorded using Spike2 software (CED). Serotonergic neurones were identified by their waveform and spontaneous firing patterns ($\approx$1.2 Hz). One cell was recorded per animal. The spontaneous firing rate was recorded for 5 min before i.v. administration of vehicle, GR205171, or GR226206. After 3 min, the influence of citalopram, fluoxetine, 8-OH-DPAT, S15535, (−)-pindolol, or buspirone, injected i.v. in volumes of 0.5 ml/kg, was evaluated upon administration in cumulative doses at intervals of 2–3 min. Drug effects were characterized over 60 s periods at the time of peak action.

Forced Swim Test in Mice

As previously described (Brocco *et al*, 2006), mice were placed in individual glass cylinders (24 cm h × 12 cm diameter) containing 6 cm of water at 25°C for 6 min. Immobility (s) was measured during the last 4 min of the test. In dose-range studies of citalopram (s.c.) and GR205171 (i.p.), drug or vehicle was administered 30 min before testing. In antagonist studies, GR205171, GR226206, RP67580, or vehicle was administered (i.p.) 30 min before treatment with citalopram (2.5 mg/kg, s.c.) or vehicle, and testing undertaken 30 min later.

Influence Upon Locomotor Activity in Mice

As described (Brocco *et al*, 2002), locomotor activity in mice was evaluated using white Plexiglass cages (27 × 27 × 27 cm) equipped with two rows of four photocells 2 cm above the floor and 6 cm apart connected through an interface to a microcomputer (Hesperid, Loiron, France). GR205171 or vehicle (i.p.), then citalopram or vehicle (s.c.), was administered 60 and 30 min before placing mice in individual activity chambers for 10 min. Each interruption of an infrared beam was counted as a movement.

Ultrasonic Vocalization Test in Rats

As previously (Millan *et al*, 2001a), rats were initially placed in a chamber equipped with a grid floor and were exposed to six randomly distributed, electric shocks (800 μA, 8 s) over a 7 min period. After 24 h, they were placed in the chamber for 2 min and received a single shock. They were returned to the chamber 30 min later and the total duration of ultrasonic vocalizations (USVs), defined as vocalizations with frequencies higher than 20 kHz, were recorded as previously (Millan *et al*, 1997a) over 10-min by use of a microphone linked to an ultrasound recording system (Ultravox; Noldus, Wageningen, The Netherlands). Rats emitting USVs for less than 90 s were not examined further. After 24 h, the procedure was replicated following drug administration. GR205171, GR226206, citalopram or vehicle was administered immediately after the 2 min session and, in potentiation studies, animals received GR205171, GR226206 or vehicle, followed by citalopram or vehicle, immediately after the 2 min session.

Fear-Induced Foot Tapping in Gerbils

As previously (Brocco *et al*, 2008), the procedure involved two sessions: (1) acquisition of fear conditioning followed, 4 h later, by (2) a test session. The apparatus was a chamber with a floor comprised of four metal plates (10 × 4 cm) connected to a shocker (Apelex, Massy, France). In the acquisition session, naive gerbils were placed for 2 min habituation in the chamber, then a shock was (1.75 mA, 0.5 s) delivered manually each time the animal crossed from one plate to another. Each animal received 10 shocks, separated by at least 10 s intervals without shock. For testing, the animal was placed again in the four-plate chamber for 3 min and the duration (s) of foot-tapping bouts recorded. No shock was delivered during the test session. GR205171, GR226206, or vehicle was administered i.p. together with either vehicle or fluoxetine (40.0 mg/kg, i.p.), 30 min before the test session.

Social Interaction Tests in Rats and Gerbils

As previously (Millan *et al*, 2001a; Brocco *et al*, 2008), male Sprague–Dawley rats of 240–260 g (Charles River, Saint-Aubin-les-Elbeuf, France) or gerbils were maintained under a 12/12 h low light (3 lux)/dark cycle for 5 days before testing, and individually housed 5 days (rats) or 3 h (gerbils) before testing. On the test day, they were placed in weight-matched pairs (±5 g for rats and ±3 g for gerbils) in opposite corners of a highly illuminated (300 lux) open-topped arena for a 10 (rats) or 5 min (gerbils) observation session. Data were the duration of active SI: ie, the time spent in grooming, following, sniffing, biting, jumping, or crawling over or under the other animal. If animals remained adjacent to each other without any movement for more than 10 s, scoring was discontinued until active SI resumed. Both animals of the same pair receive the same drug treatment. GR205171, citalopram, or vehicle was administered 30 min before testing. In interaction studies, GR205171, GR226206, or vehicle was administered 45 min before testing, and citalopram or vehicle 30 min before testing.

Light-Induced Phase-Advances in Circadian Wheel Running Rhythms in Hamsters

As previously (Gannon and Millan, 2007), Syrian hamsters were maintained in a 14 : 10 h light : dark schedule for several weeks before being transferred to conditions of constant darkness (DD) where they had access to small running wheels of 19 cm in diameter. Wheel running was recorded in 10 min bins using Actimetrics ClockLab hardware (Evanstown, IL, USA). Food and water was provided *ad libitum* at all times. Onset of wheel running in DD is defined as circadian time (CT 12). Data were

recorded for approximately 10 days in DD and then hamsters were removed from their home cages under dim red light (<1 lux) at circadian time 18.25, weighed, and injected with either drug or vehicle and returned to their home cage. After 45 min at CT, 19 hamsters were again removed from their home cage and exposed to a 10-min pulse of white light (20 lux) and then returned to their home cage for another 10 days. Hamsters were returned to the 14 : 10 h lighting schedule at the conclusion of the experiment. Light pulses delivered at CT 19 to hamsters in DD phase advance the time of onset of wheel running. The magnitude of the phase advance was determined by fitting a line through the activity onset for several days before the light pulse and again for several days after the light pulse once the rhythm has stabilized, normally days 5–10 after light. The difference in time between the two fitted lines on the day of the experiment indicates the magnitude of the light-induced phase advance.

Drugs and Sources

Drug doses are expressed in terms of the base. Drugs were dissolved in distilled water and administered s.c., i.p., or i.v. (electrophysiology). For i.p. administration, drugs were administered as suspensions in water with a few drops of Tween 80. GR205171 (2-methoxy-5-(5-trifluoromethyltetrazol-1-yl)-benzyl-([2*S*,3*S*]-2-phenylpiperidinyl)-amine) diHCl; GR226206 (2-methoxy-5-(5-trifluoromethyltetrazol-1-yl)-benzyl-([2*R*,3*R*]-2-phenylpiperidinyl)-amine) diHCl; citalopram HBr, RP67580 ((3a*R*,7a*R*)-imino-1(methoxy-2-phenyl)-2-diphenyl-7,7-perhydro isoindolone-4), and S15535 (1-(1,4-benzodioxan-5-yl)-4-(indan-2-yl) piperazine mesylate) were synthetized by Servier chemists. 8-OH-DPAT (8-dihydroxy-2(di-*n*-propylamino)tetralin) HBr, (−)-pindolol, WAY-100,635 (*N*-(2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl)-*N*-(2-pyridyl)cyclohexane carboxamide) maleate and buspirone were purchased from Sigma-RBI (Natick, USA). Fluoxetine HCl was purchased from Interchim (Montluçon, France).

RESULTS

Enhancement by GR205171 of the Influence of Citalopram and Fluoxetine Upon Dialysis Levels of 5-HT in Frontal Cortex of Freely Moving Rats

At a dose of 0.63 mg/kg, s.c., citalopram markedly increased levels of 5-HT in the FCX of freely moving rats (Figure 1; Table 1). In contrast, levels of DA and NA were not modified. In an opposite fashion, the selective NK$_1$ antagonist, GR205171 (10.0–40.0 mg/kg, s.c.), had no effect on frontocortical levels of 5-HT, whereas it elevated levels of DA and NA. This effect of GR205171 was expressed stereospecifically in that its less active distomer, GR226206 (40.0 mg/kg, s.c.), did not significantly influence levels of DA, NA, or 5-HT ($p > 0.05$, not shown). GR205171 (10.0–40.0 mg/kg, i.p.) dose-dependently potentiated the increase in extracellular levels of 5-HT induced by citalopram (0.63). This effect was exerted

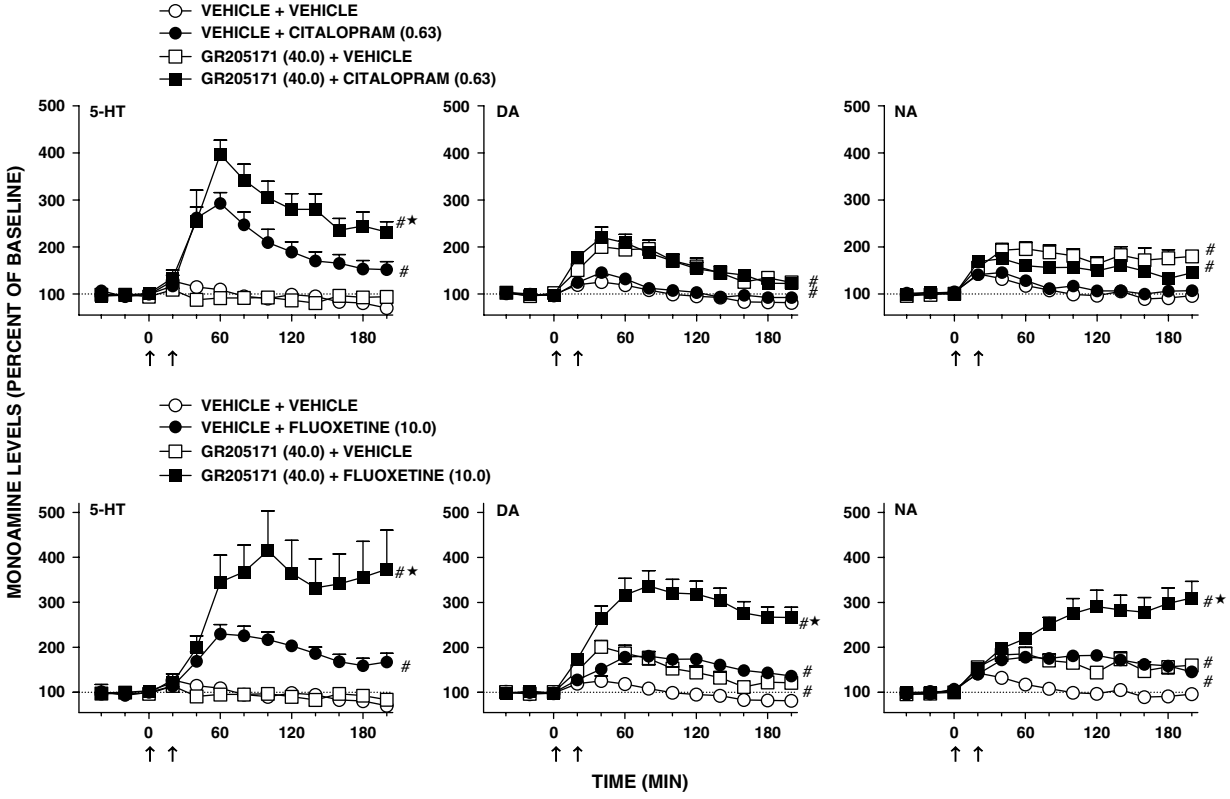

Figure 1 Enhancement by the neurokinin (NK)$_1$ receptor antagonist, GR205171, of the influence of citalopram upon dialysis levels of serotonin in frontal cortex of freely-moving rats. Serotonin (5-HT), dopamine (DA), and noradrenaline (NA) levels are expressed relative to basal values defined as 100%. These were 0.96 ± 0.06, 1.12 ± 0.07, and 1.84 ± 0.08 pg per 20 μl for 5-HT, DA, and NA respectively. Data are means ± SEMs; $N = 5$–9 per value. Arrows denote the injection of drugs. For ANOVA with dose as between factor, see legend to Table 1.

Table 1 Enhancement by NK$_1$ Receptor Antagonists of the Influence of Citalopram and Fluoxetine Upon Dialysis Levels of Serotonin in Frontal Cortex

Drug	Serotonin	Dopamine	Noradrenaline
VEH+VEH	93.1 ± 2.5	98.7 ± 2.7	104.1 ± 3.0
GR (10.0)+VEH	102.2 ± 4.1	112.9 ± 3.6	138.6 ± 3.2$^{\#}$
GR (20.0)+VEH	98.1 ± 4.3	155.1 ± 4.6$^{\#}$	131.4 ± 3.1$^{\#}$
GR (40.0)+VEH	90.7 ± 2.5	161.3 ± 5.6$^{\#}$	181.6 ± 5.3$^{\#}$
VEH+CITAL (0.63)	204.8 ± 10.7$^{\#}$	108.6 ± 3.3	114.4 ± 3.6
GR (10.0)+CITAL (0.63)	183.0 ± 9.5$^{\#}$	132.7 ± 4.5$^{\#,*}$	134.8 ± 5.8$^{\#}$
GR (20.0)+CITAL (0.63)	245.1 ± 14.5$^{\#,*}$	154.4 ± 6.3$^{\#,*}$	139.9 ± 4.1$^{\#}$
GR (40.0)+CITAL (0.63)	285.8 ± 11.0$^{\#,*}$	163.7 ± 5.9$^{\#,*}$	154.0 ± 3.2$^{\#,*}$
VEH+FLUOX (10.0)	191.9 ± 6.3$^{\#}$	161.0 ± 5.2$^{\#}$	170.0 ± 3.3$^{\#}$
GR (10.0)+FLUOX (10.0)	218.2 ± 13.5$^{\#}$	186.8 ± 4.4$^{\#}$	195.1 ± 4.5$^{\#}$
GR (20.0)+FLUOX (10.0)	296.5 ± 9.0$^{\#,*}$	192.5 ± 12.1$^{\#}$	198.2 ± 5.3$^{\#}$
GR (40.0)+FLUOX (10.0)	343.8 ± 22.5$^{\#,*}$	297.0 ± 9.5$^{\#,*}$	267.2 ± 9.9$^{\#,*}$
RP (40.0)+VEH	95.3 ± 2.3	166.6 ± 6.9$^{\#}$	160.5 ± 3.54$^{\#}$
RP (40.0)+CITAL (0.63)	306.3 ± 15.9$^{\#,*}$	139.2 ± 6.7$^{\#}$	122.9 ± 3.1

VEH, vehicle; GR, GR205171; CITAL, citalopram; FLUOX, fluoxetine; RP, RP67580.
Data (means ± SEMs) represent 'area under the curve' analysis expressed relative to basal values (100%). For basal values, see legend of Figure 1. $N = 5$–10 per value. ANOVA with dose as between factor as follow. 5-HT: cital, $F(1, 14) = 24.1$, $P < 0.01$; GR (10.0), $F(1, 11) = 0.1$, $P > 0.05$ and interaction, $F(1, 11) = 0.5$, $P > 0.05$; GR (20.0), $F(1, 12) = 0.5$, $P > 0.05$ and interaction, $F(1, 12) = 0.9$, $P > 0.05$; GR (40.0), $F(1, 15) = 0.2$, $P > 0.05$ and interaction, $F(1, 15) = 5.5$, $P < 0.05$. DA: cital, $F(1, 15) = 2.2$, $P > 0.05$; GR (10.0), $F(1, 12) = 4.6$, $P > 0.05$ and interaction, $F(1, 12) = 5.5$, $P < 0.05$; GR (20.0), $F(1, 13) = 45.4$, $P < 0.05$ and interaction, $F(1, 12) = 10.6$, $P < 0.05$; GR (40.0), $F(1, 16) = 31.4$, $P < 0.01$ and interaction, $F(1, 14) = 17.3$, $P < 0.01$. NA: cital, $F(1, 15) = 0.3$, $P > 0.05$; GR (10.0), $F(1, 12) = 39.7$, $P < 0.05$ and interaction, $F(1, 12) = 1.7$, $P > 0.05$; GR (20.0), $F(1, 13) = 22.8$, $P < 0.05$ and interaction, $F(1, 12) = 4.4$, $P > 0.05$; GR (40.0), $F(1, 16) = 29.3$, $P < 0.01$ and interaction, $F(1, 14) = 15.2$, $P < 0.01$. 5-HT: fluox, $F(1, 16) = 43.8$, $P < 0.01$; GR (10.0), $F(1, 11) = 0.1$, $P > 0.05$ and interaction, $F(1, 13) = 0.7$, $P > 0.05$; GR (20.0), $F(1, 12) = 0.5$, $P > 0.05$ and interaction, $F(1, 15) = 23.9$, $P < 0.05$; GR (40.0), $F(1, 15) = 0.2$, $P > 0.05$ and interaction, $F(1, 14) = 10.2$, $P < 0.01$. DA: fluox, $F(1, 17) = 16.0$, $P < 0.01$; GR (10.0), $F(1, 15) = 1.7$, $P > 0.05$ and interaction, $F(1, 12) = 5.5$, $P < 0.05$; GR (20.0), $F(1, 13) = 45.4$, $P < 0.05$ and interaction, $F(1, 15) = 0.9$, $P > 0.05$; GR (40.0), $F(1, 16) = 31.4$, $P < 0.01$ and interaction, $F(1, 16) = 26.7$, $P < 0.01$. NA: fluox, $F(1, 17) = 58.8$, $P < 0.01$; GR (10.0), $F(1, 12) = 39.7$, $P < 0.05$ and interaction, $F(1, 15) = 3.8$, $P > 0.05$; GR (20.0), $F(1, 13) = 22.8$, $P < 0.05$ and interaction, $F(1, 15) = 3.5$, $P > 0.05$; GR (40.0), $F(1, 16) = 29.3$, $P < 0.01$ and interaction, $F(1, 16) = 17.7$, $P < 0.01$. 5-HT: RP, $F(1, 11) = 0.2$, $P > 0.05$ and interaction, $F(1, 11) = 6.9$, $P < 0.05$. DA: RP, $F(1, 12) = 18.0$, $P < 0.01$ and interaction, $F(1, 11) = 4.4$, $P > 0.05$. NA: RP, $F(1, 12) = 37.7$, $P < 0.01$ and interaction, $F(1, 11) = 0.5$, $P > 0.05$.
$^{\#}$Significance ($P < 0.05$) of drug-treated *vs* vehicle-treated groups; *significance ($P < 0.05$) of drug/CITAL-treated groups *vs* VEH/CITAL-treated group or GR/FLUOX-treated groups *vs* VEH/FLUOX-treated group.

stereospecifically in that GR226206 (40.0) did not significantly modify the citalopram-induced increase in 5-HT levels. Area under the curve analyses (%, expressed relative to basal values, defined as 100%) is as follows: vehicle/citalopram (0.63) = 204.8 ± 10.7 *vs* GR226206 (40)/citalopram (0.63) = 207.3 ± 11.3, $P > 0.05$. The combination of GR205171 with citalopram did not differentially influence levels of DA and NA as compared to GR205171 alone. There was no significant influence of GR226206 in combination with citalopram upon levels of DA and NA ($P > 0.05$, not shown). GR205171 (10.0–40.0 mg/kg, i.p.) also dose-dependently potentiated the increase in extracellular levels of 5-HT induced by fluoxetine in FCX. This effect was expressed stereospecifically in that GR226206 (40.0) was inactive. Area under the curve analyses is as follows: vehicle/fluoxetine (10.0) *vs* GR226 206/fluoxetine (10.0), 5-HT = 191.9 ± 6.3 *vs* 215.5 ± 7.7, $P > 0.05$. In addition, at a dose of 10.0 mg/kg, s.c., fluoxetine increased levels of DA and NA. In the presence of increasing doses of GR205171 (10.0–40.0 mg/kg, i.p.), fluoxetine enhanced levels of DA and NA. GR226206 did not modify the influence of fluoxetine upon DA and NA levels. Area under the curve analysis: vehicle/fluoxetine (10.0) *vs* GR226206 (40.0)/fluoxetine (10.0), DA = 161.0 ± 5.2 *vs* 172.9 ± 4.7, $P > 0.05$ and NA = 170.0 ± 3.3 *vs* 184.1 ± 3.6, $P > 0.05$.

Enhancement by GR205171 of the Influence of Citalopram Upon Dialysis Levels of 5-HT in Diverse Cerebral Structures of Freely Moving Rats

In the dorsal hippocampus, BLA, nucleus accumbens, and striatum, at a dose of 0.63 mg/kg, s.c., citalopram increased levels of 5-HT (Figure 2; Table 2). It also slightly elevated the levels of DA and NA in the BLA. By contrast, it did not affect NA (dorsal hippocampus) or DA (nucleus accumbens and striatum). GR205171 (40.0 mg/kg, s.c.), did not affect 5-HT levels in any structure. It did not modify DA levels in nucleus accumbens, and slightly decreased DA levels in striatum. However, it enhanced levels of DA in the BLA and those of NA in BLA and dorsal hippocampus. In the presence of GR205171, the influence of citalopram upon 5-HT was potentiated, but changes in DA and NA were not modified.

Attenuation by GR205171 of the Inhibitory Influence of Citalopram and Fluoxetine Upon the Firing Rate of Serotonergic Neurones

Citalopram suppressed the electrical activity of DRN-localized serotonergic neurones (Figure 3). Administered

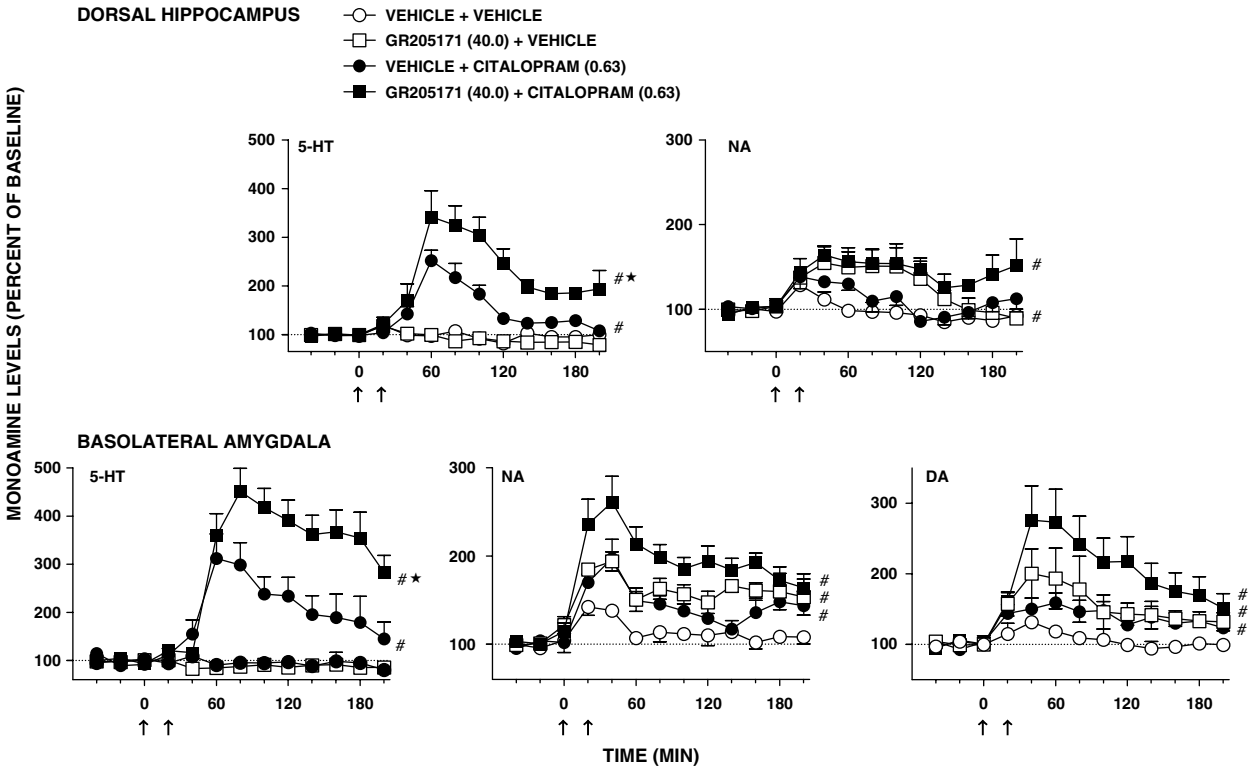

Figure 2 Enhancement by GR205171 of the influence of citalopram upon dialysis levels of serotonin in dorsal hippocampus and basolateral amygdala of freely moving rats. Serotonin (5-HT), dopamine (DA) and noradrenaline (NA) levels are expressed relative to basal values defined as 100%. These were 1.42 ± 0.10 and 0.79 ± 0.14 pg per 20 μl for NA and 5-HT in the dorsal hippocampus, respectively, and 0.91 ± 0.05, 0.47 ± 0.05, and 0.62 ± 0.10 pg per 20 μl for NA, DA, and 5-HT in the BLA, respectively. Data are means ± SEM; $N = 5$–8 per value. Arrows denote the injection of drugs. ANOVA as follows. Dorsal hippocampus, 5-HT: citalopram, $F(1, 11) = 33.3$, $P < 0.01$; GR205171, $F(1, 13) = 2.1$, $P > 0.05$ and interaction, $F(1, 8) = 7.5$, $P < 0.05$ and NA: citalopram, $F(1, 11) = 4.5$, $P > 0.05$; GR205171, $F(1, 13) = 7.6$, $P < 0.05$ and interaction, $F(1, 8) = 5.1$, $P > 0.05$. BLA, 5-HT: citalopram, $F(1, 9) = 7.1$, $P < 0.05$; GR205171, $F(1, 9) = 0.1$, $P > 0.05$ and interaction, $F(1, 11) = 5.6$, $P < 0.05$; DA: citalopram, $F(1, 9) = 10.5$, $P < 0.05$; GR205171, $F(1, 8) = 7.5$, $P < 0.05$ and interaction, $F(1, 11) = 4.1$, $P > 0.05$ and NA: citalopram, $F(1, 9) = 7.2$, $P < 0.05$; GR205171, $F(1, 9) = 16.2$, $P < 0.01$ and interaction, $F(1, 11) = 3.5$, $P > 0.05$. Significance ($P < 0.05$) of drug-treated groups *vs* vehicle-treated groups is indicated by $^{\#}$, and significance ($P < 0.05$) of GR205171/citalopram-treated groups *vs* vehicle/citalopram-treated groups is indicated by asterisks.

Table 2 Enhancement by GR205171 of the Influence of Citalopram Upon Dialysis Levels of Serotonin in Striatum and Nucleus Accumbens

	Striatum		Nucleus Accumbens	
Drug	**Serotonin**	**Dopamine**	**Serotonin**	**Dopamine**
VEH+VEH	96.0 ± 2.4	96.5 ± 2.0	103.3 ± 2.0	98.9 ± 1.9
GR (40.0)+VEH	97.7 ± 3.1	83.2 ± 1.4$^{\#}$	98.2 ± 3.8	91.8 ± 3.1
VEH+CITAL (0.63)	200.6 ± 7.9$^{\#}$	99.2 ± 1.8	202.8 ± 9.9$^{\#}$	103.5 ± 3.5
GR (40.0)+CITAL (0.63)	302.7 ± 10.4$^{\#,*}$	89.6 ± 2.0	328.5 ± 13.4$^{\#,*}$	85.8 ± 1.8

VEH, vehicle; GR, GR205171; CITAL, citalopram.
Data are means ± SEMs and represent 'area under the curve' analysis expressed relative to basal values (100%). Basal values were 12.9 ± 1.6 and 0.46 ± 0.05 pg per 20 μl for DA and 5-HT in the striatum, respectively, and 3.8 ± 0.4 and 0.39 ± 0.03 pg per 20 μl for DA and 5-HT in the nucleus accumbens, respectively. $N = 6$–9 per value. ANOVA as follows. Striatum, serotonin: citalopram, $F(1, 12) = 24.0$, $P < 0.01$; GR205171, $F(1, 14) = 0.1$, $P > 0.05$ and interaction, $F(1, 12) = 12.5$, $P < 0.01$. Dopamine: citalopram, $F(1, 12) = 0.2$, $P > 0.05$; GR205171, $F(1, 14) = 4.7$, $P < 0.05$ and interaction, $F(1, 12) = 2.0$, $P > 0.05$. Nucleus accumbens, serotonin: citalopram, $F(1, 13) = 30.7$, $P < 0.01$; GR205171, $F(1, 15) = 1.3$, $P > 0.05$ and interaction, $F(1, 10) = 10.8$, $P < 0.01$. Dopamine: citalopram, $F(1, 13) = 0.2$, $P > 0.05$; GR205171, $F(1, 15) = 0.6$, $P > 0.05$ and interaction, $F(1, 10) = 2.4$, $P > 0.05$.
$^{\#}$Significance ($P < 0.05$) of drug-treated *vs* vehicle-treated groups; *significance ($P < 0.05$) of GR205171/citalopram-treated *vs* vehicle/citalopram-treated groups.

alone, GR205171 failed to modify firing rate. However, it dose-dependently (1.0–4.0 mg/kg, i.v.) and significantly displaced the dose–response curve for inhibition of serotonergic neurones by citalopram to the right. The effects of citalopram alone and of citalopram in the presence of GR205171 were both blocked by the selective 5-HT$_{1A}$ receptor antagonist, WAY100635 (0.1 mg/kg, i.v.) (Newman-Tancredi *et al*, 1998). In distinction to GR205171,

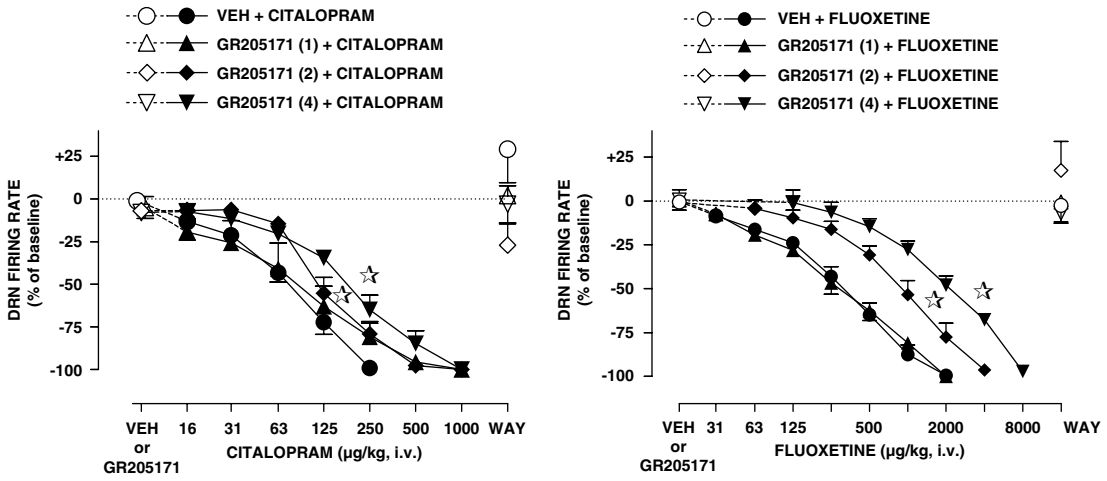

Figure 3 Attenuation by GR205171 of the inhibitory influence of citalopram and fluoxetine upon the firing rate of serotonergic neurones in anesthetized rats. VEH = vehicle; WAY = WAY100635. The influence of drugs is expressed as the percentage change from baseline defined as 0%. Baseline firing rate, 1.2 ± 0.2 Hz. Data are means ± SEMs; $N = 5$–9 per value. Two-way ANOVA as follows. VEH + citalopram *vs* GR205171 (1) + citalopram, $F(8, 112) = 1.8$, $P > 0.05$; VEH + citalopram *vs* GR205171 (2) + citalopram, $F(8, 96) = 10.5$, $P < 0.01$; VEH + citalopram *vs* GR205171 (4) + citalopram, $F(8, 112) = 8.7$, $P < 0.01$; VEH + fluoxetine *vs* GR205171 (1) + fluoxetine, $F(8, 72) = 0.3$, $P > 0.05$; VEH + fluoxetine *vs* GR205171 (2) + fluoxetine, $F(10, 100) = 4.5$, $P < 0.01$; VEH + fluoxetine *vs* GR205171 (4) + fluoxetine, $F(10, 100) = 10.9$, $P < 0.01$. Asterisks indicate significance ($P < 0.05$) of GR205171-treated groups *vs* vehicle-treated group.

its less active distomer, GR226206 (4.0 mg/kg, i.v.), failed to affect the influence of citalopram upon DRN firing: vehicle + citalopram, inhibitory dose$_{50}$ (ID_{50}) in µg/kg, i.v = 66.4 ± 8.0 *vs* GR226206 + citalopram, $ID_{50} = 71.0 \pm 9.2$, $P > 0.05$. By analogy to citalopram, fluoxetine blocked the activity of serotonergic perikarya. GR205171 dose-dependently (1.0–4.0 mg/kg, i.v.) displaced the dose–response curve of fluoxetine to the right. The effects of fluoxetine alone and of fluoxetine plus GR205171 were blocked by WAY100635. Further, GR226206 (4.0 mg/kg, i.v.) failed to affect the influence of fluoxetine: vehicle + fluoxetine, ID_{50} in µg/kg, i.v = 280.5 ± 45.6 *vs* GR226206 + fluoxetine, $ID_{50} = 216.7 \pm 25.6$, $P > 0.05$.

Influence of GR205171 Upon Inhibition of Serotonergic Neurones by 5-HT$_{1A}$ Agonists

The 5-HT$_{1A}$ partial agonist, S15535 (Gobert *et al*, 1995; Millan *et al*, 1997a, b; Lejeune and Millan, 2000), dose dependently reduced the firing rate of DRN neurones (Figure 4). Its dose–response curve was shifted to the right by GR205171 (4.0 mg/kg, i.v.). This effect was expressed stereospecifically in that GR226206 (4.0 mg/kg, i.v.) failed to influence the decrease in DRN firing rate induced by S15535. In contrast, GR205171 (4.0) did not modify the inhibitory influence of the weak 5-HT$_{1A}$ partial agonist, (−)-pindolol (Newman-Tancredi *et al*, 1998), nor of the 5-HT$_{1A}$ agonists, 8-OH-DPAT and buspirone (Gobert *et al*, 1995; Lejeune *et al*, 1997; Newman-Tancredi *et al*, 1998), upon DRN firing rate. Actions of all 5-HT$_{1A}$ agents were antagonized by WAY100635 (0.1 mg/kg, i.v.).

Influence of GR205171 Upon the Modulation of FCX Levels of Monoamines By 5-HT$_{1A}$ Agonists

S15535 decreased levels of 5-HT in the FCX of freely-moving rats, and increased levels of DA and NA (Figure 5). The inhibitory influence of S15535 upon 5-HT levels was not affected by GR205171. Interestingly, coadministration of GR205171 and S15535 was accompanied by a more pronounced elevation in levels of DA and NA than for either drug alone. 8-OH-DPAT also decreased 5-HT levels in FCX while increasing the levels of DA and NA. In the presence of GR205171, the reduction in 5-HT levels provoked by 8-OH-DPAT was unaffected, whereas the elevation in DA and NA was facilitated.

Enhancement by GR205171 of the Antidepressant Action of Citalopram in the Forced Swim Procedure in Mice

In the mice FS test, citalopram decreased the duration of immobility, whereas GR205171 had no effect up to 40.0 mg/kg, i.p. (Figure 6). In the presence of GR205171 (40.0), however, a subactive dose of citalopram (2.5) significantly decreased immobility. This effect was not observed with GR226206 (40.0), which was inactive alone. Lower doses (2.5 and 10.0) of GR205171 did not influence the effect of citalopram (2.5) upon immobility (s): vehicle + citalopram, 154.7 ± 11.1 ($N = 20$) *vs* GR205171 (2.5) + citalopram (2.5), 145.0 ± 19.7 ($N = 8$), $P > 0.05$ and *vs* GR205171 (10.0) + citalopram (2.5), 128.6 ± 18.2 ($N = 12$), $P > 0.05$.

Lack of Enhancement by GR205171 of the Facilitatory Influence of Citalopram Upon Locomotor Activity in Mice

Citalopram (10.0 mg/kg, s.c.) increased locomotor activity in mice (Table 3). This action was dose-dependently (2.5–40.0 mg/kg, i.p.) attenuated by GR205171. Locomotor activity was not significantly modified by GR205171 alone.

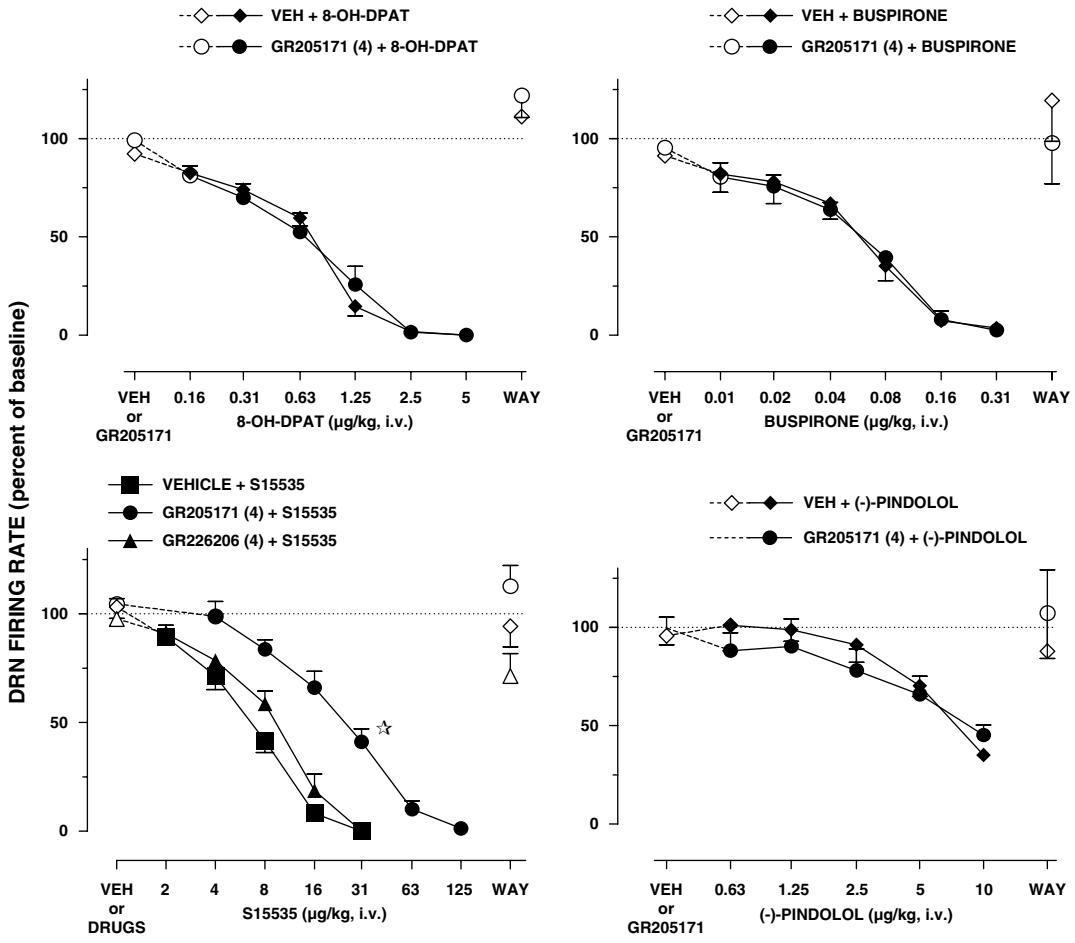

Figure 4 Influence of GR205171 on the inhibition of serotonergic neurones by the 5-HT$_{1A}$ agonists, S15535, (−)-pindolol, 8-OH-DPAT and buspirone, in anesthetized rats. VEH, vehicle; WAY, WAY100635. Data are means ± SEMs; $N = 5$–7 per value. Two-way ANOVA as follows. Vehicle + S15535 *vs* GR205171 (4) + S15535, $F(8, 104) = 8.6$, $P < 0.01$; vehicle + S15535 *vs* GR226206 (4) + S15535, $F(8, 72) = 1.0$, $P > 0.05$; vehicle + (−)-pindolol *vs* GR205171 (4) + (−)-pindolol, $F(6, 42) = 0.6$, $P > 0.05$; vehicle + 8-OH-DPAT *vs* GR205171 (4) + 8-OH-DPAT, $F(6, 72) = 1.3$, $P > 0.05$; vehicle + buspirone *vs* GR205171 (4) + buspirone, $F(7, 63) = 0.4$, $P > 0.05$. Asterisk indicates significance ($P < 0.05$) of GR205171-treated groups *vs* vehicle-treated groups.

Enhancement by GR205171 of the Actions of Citalopram in the Ultrasonic Vocalization Procedure in Rats

Citalopram dose-dependently decreased the duration of fear-induced USV in rats, though significance was obtained only for the highest dose tested (10.0 mg/kg, s.c.) (Figure 7). A similar profile was obtained with GR205171 (40.0 mg/kg, i.p.). GR226206 was inactive up to 40.0 mg/kg, i.p. In the presence of GR205 171 (10.0, i.p.), a subactive dose of citalopram (5.0, s.c.) significantly decreased USV, an effect not observed in the presence of GR226206 (40.0, i.p.).

Additive Inhibition by GR205171 and Citalopram of Light-Induced Phase Advances in Circadian Wheel Running Rhythms in Hamsters

A 10-min light pulse advanced circadian rhythms by 1.6 ± 0.1 h following vehicle (Table 4). Citalopram (5 mg/kg, i.p.) and GR205171 (5.0 mg/kg, i.p) alone did not influence light-induced phase advances. In contrast, combination of these doses significantly inhibited the effect of light (−47%). Further, citalopram (10.0 mg/kg) and GR205171 (20.0 mg/kg) significantly inhibited the effect of light by −40 and −70%, respectively. When injected simultaneously, the combination inhibited the effect of light by 60%.

Blockade by GR205171 of the Facilitation by Fluoxetine of Fear-Induced Foot-Tapping in Gerbils

In animals without fear conditioning (ie not submitted to shocks during the acquisition session), fluoxetine (40.0 mg/kg, i.p.) did not induce foot tapping (Figure 8). In contrast, in fear-conditioned gerbils (submitted to shocks), fluoxetine dose-dependently induced foot tapping at doses of 20.0 and 40.0 mg/kg, i.p. The dose of 40.0 mg/kg, i.p. was selected for antagonist studies. GR205171 (0.16 mg/kg, i.p.) blocked the effect of fluoxetine (40.0 mg/kg, i.p.) upon fear-conditioned foot tapping whereas GR226206 was inactive.

Blockade by GR205171 of the Anxiogenic Actions of Citalopram in the Social Interaction Test in Rats and Gerbils

Citalopram reduced the duration of SI between unfamiliar rats and gerbils exposed to a novel environment (Figure 9).

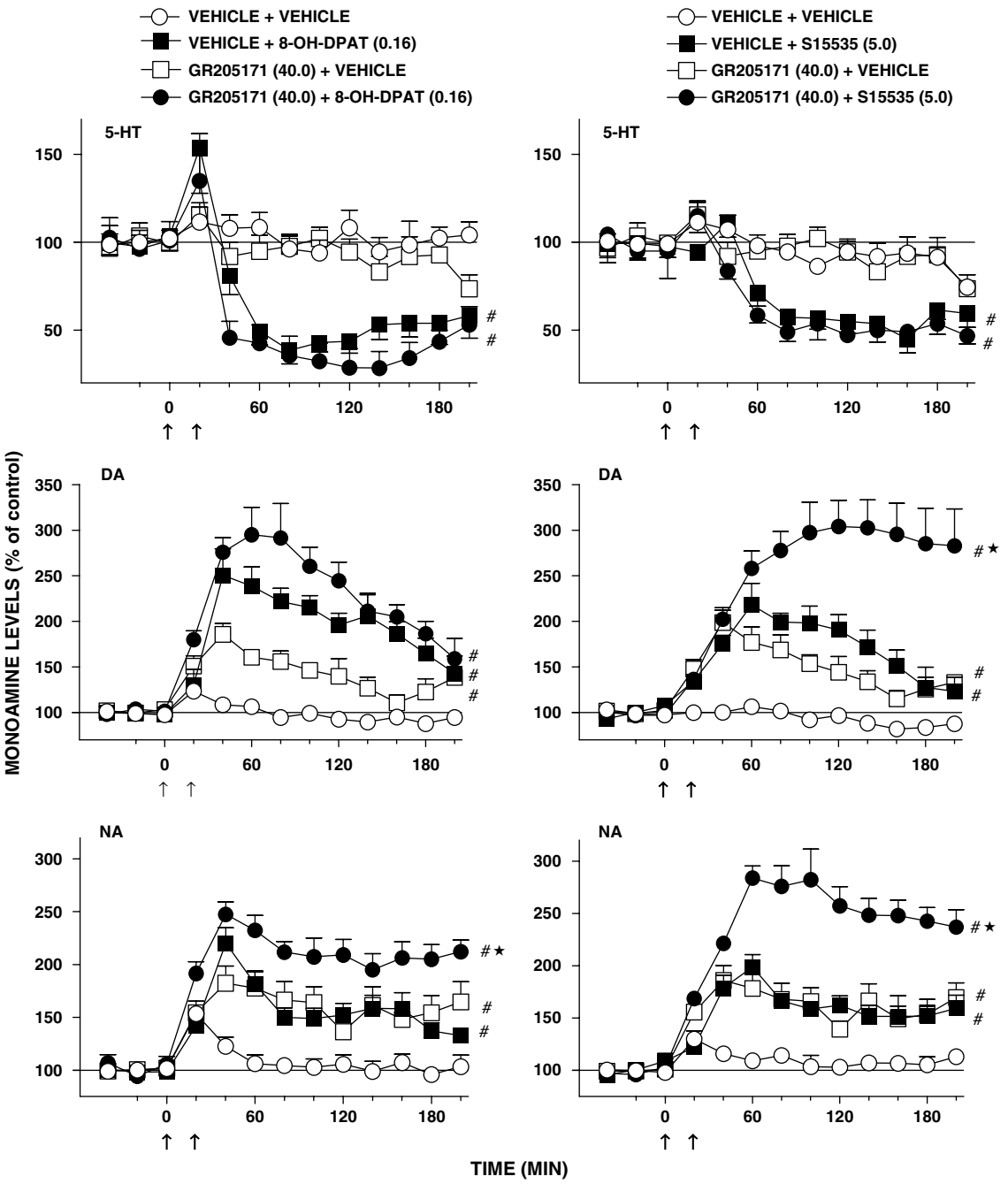

Figure 5 Influence of GR205171 upon the modulation of frontocortical levels of monoamines by the 5-HT$_{1A}$ agonists, S15535 and 8-OH-DPAT, in freely moving rats. Serotonin (5-HT), dopamine (DA), and noradrenaline (NA) levels are expressed relative to basal values defined as 100% (see legend of Figure 1). Data are means ± SEMs; $N = 5$–9 per value. Arrows denote the injection of drugs. ANOVA as follows. S15535, 5-HT: S15535, $F(1, 11) = 50.8$, $P < 0.01$; GR205171, $F(1, 15) = 0.2$, $P > 0.05$ and interaction, $F(1, 8) = 3.0$, $P > 0.05$; DA: S15535, $F(1, 12) = 49.3$, $P < 0.01$; GR205171, $(1, 16) = 31.3$, $P < 0.01$ and interaction, $F(1, 8) = 12.7$, $P < 0.01$ and NA: S15535, $F(1, 12) = 59.8$, $P < 0.01$; GR205171, $(1, 16) = 29.3$, $P < 0.01$ and interaction, $F(1, 8) = 30.5$, $P < 0.01$. 8-OH-DPAT, 5-HT: 8-OH-DPAT, $F(1, 12) = 54.5$, $P < 0.01$, GR205171, $F(1, 15) = 0.2$, $P > 0.05$ and interaction, $F(1, 10) = 2.2$, $P > 0.05$; DA: 8-OH-DPAT, $F(1, 13) = 89.2$, $P < 0.01$; GR205171, $(1, 16) = 31.3$, $P < 0.01$ and interaction, $F(1, 11) = 2.6$, $P > 0.05$ and NA: 8-OH-DPAT, $F(1, 13) = 46.3$, $P < 0.01$; GR205171, $(1, 16) = 29.3$, $P < 0.01$ and interaction, $F(1, 11) = 14.8$, $P < 0.01$. Significance ($P < 0.05$) of drug-treated groups *vs* vehicle-treated groups is indicated by $^{\#}$, and the significance ($P < 0.05$) of GR205171/8-OH-DPAT or GR205171/S15535-treated groups *vs* vehicle/8-OH-DPAT- or vehicle/S15535-treated groups is indicated by asterisks.

In contrast, GR205171 alone did not modify the duration of SI in either species. However, GR205171 (10.0 mg/kg, i.p. in rat, and 0.63 in gerbils) antagonized the anxiogenic effect of citalopram. Conversely, GR226206 was inactive.

Influence of RP67580 Upon Neurochemical and Antidepressant Actions of Citalopram

The NK$_1$ antagonist, RP67580 (40.0 mg/kg, i.p.), potentiated citalopram-induced increases in 5-HT levels in FCX. It also

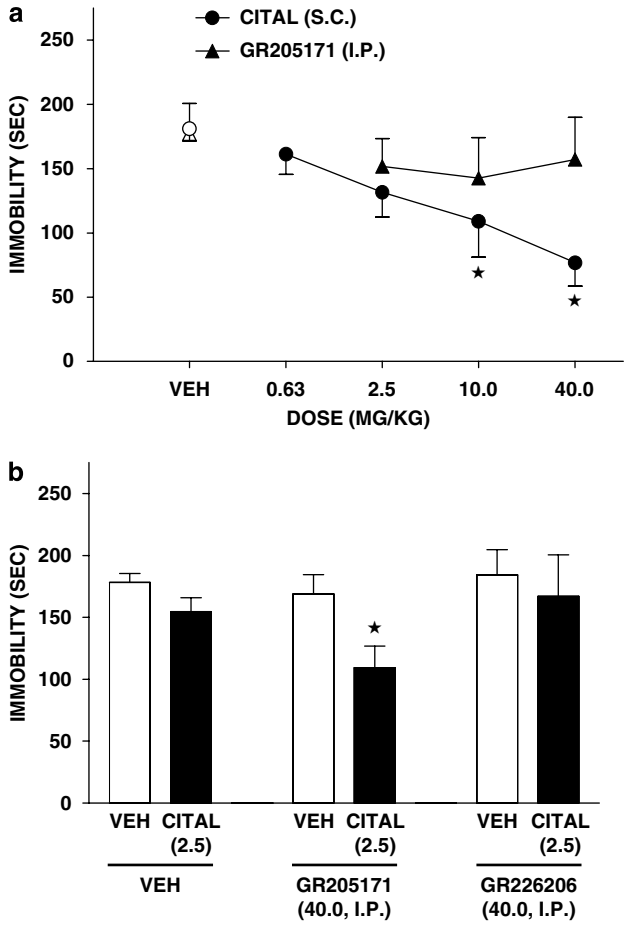

Figure 6 Enhancement by GR205171 of the antidepressant action of citalopram in the forced swim procedure in mice. VEH, vehicle and CITAL, citalopram. (a) Dose-dependent reduction of immobility by citalopram as compared to GR205171; (b) stereospecific enhancement by GR205171 as compared to its active distomer, GR226206, of the action of citalopram. Data are means ± SEMs ($N = 7$–20). (a) One-way ANOVA as follows: citalopram, $F(4, 30) = 5.5$, $P < 0.01$; GR205171, $F(3, 20) = 0.4$, $P > 0.05$. Asterisks indicate significance of differences to respective vehicle values in Dunnett's test. (b) Two-way ANOVA as follows: citalopram, $F(1, 75) = 5.1$, $P < 0.05$; GR205171, $F(1, 75) = 11.6$, $P < 0.01$ and interaction, $F(1, 75) = 2.2$, $P > 0.05$; GR226206, $F(1, 55) = 0.5$, $P > 0.05$ and interaction, $F(1, 55) = 0.2$, $P > 0.05$. The asterisk indicates significance of differences in Dunnett's test *vs* vehicle/vehicle values. *$P < 0.05$.

mimicked GR205171 in elevating levels of DA and NA alone, but there was no difference between RP67580/citalopram *vs* RP67580/vehicle and vehicle/citalopram values (Table 1). In the FS test, the action of RP67580 (20.0 mg/kg, i.p.) and citalopram (2.5 mg/kg, s.c.) together was greater ($P < 0.05$) than RP67580 or citalopram alone: vehicle/vehicle (s), 178.7 ± 10.6; citalopram/vehicle, 168.3 ± 9.0; vehicle/ RP67580, 168.3 ± 9.0 and citalopram/ RP67580, 122.4 ± 17.0.

DISCUSSION

Potentiation by GR205171 of the Influence of Citalopram Upon 5-HT Levels

Several arguments underpin the specificity of the facilitation by GR205171 of increases in levels of 5-HT elicited by citalopram in FCX. First, GR205171 is a highly selective antagonist of NK_1 receptors and expressed its actions across a dose range identical to that exerting other cerebral actions in rodents (Gardner *et al*, 1996; Millan *et al*, 2001b; Rupniak *et al*, 2001; Lejeune *et al*, 2002; Brocco *et al*, 2008). Further, its actions were stereospecific in as much as its less active isomer, GR226206, was ineffective. Second, the influence of GR205171 was reproduced with another selective NK_1 antagonist, RP67580 (Garret *et al*, 1991; Gobbi *et al*, 2007), and GR205171 also stereospecifically augmented the influence of fluoxetine upon 5-HT levels in FCX. Third, GR205171 potentiates paroxetine-induced increases in extracellular levels of 5-HT in the FCX of mice (Guiard *et al*, 2004). Fourth, herein, GR205171 also enhanced the action of citalopram in four further structures receiving serotonergic input from the DRN and controlling affect (Millan, 2003, 2006): hippocampus, BLA, nucleus accumbens, and striatum. Fifth, the enhancement of SSRI-induced increases in FCX levels of 5-HT by GR205171 was paralleled by its suppression of their inhibition of DRN-localized serotonergic neurones. Finally, pharmacokinetic interactions are unlikely to be involved as: (1) two different NK_1 antagonists facilitated the neurochemical effects of two different SSRIs; (2) compatible findings were acquired in electrophysiological studies using the i.v. route, a route which avoids first pass elimination in the liver; (3) certain responses to SSRIs were blocked by NK_1 antagonists; (4) similar results were seen in mice using paroxetine (Chenu *et al*, 2006), and (5) genetic deletion of NK_1 receptors likewise enhances the influence of SSRIs upon levels of 5-HT (Guiard *et al*, 2004, 2005).

Table 3 Influence of GR205171 Upon the Induction of Locomotion in Mice by Citalopram

	Locomotor counts
Vehicle+vehicle	400.0 ± 28.7
GR205171 (2.5)+vehicle	404.2 ± 33.8
GR205171 (10.0)+vehicle	405.7 ± 24.7
GR205171 (40.0)+vehicle	298.8 ± 35.1
Vehicle+citalopram (0.63)	494.5 ± 32.1
GR205171 (2.5)+citalopram (0.63)	439.0 ± 30.6
GR205171 (10.0)+citalopram (0.63)	429.5 ± 49.5
GR205171 (40.0)+citalopram (0.63)	320.8 ± 30.2*
Vehicle+citalopram (10.0)	521.0 ± 43.0$^{\#}$
GR205171 (2.5)+citalopram (10.0)	584.3 ± 32.0
GR205171 (10.0)+citalopram (10.0)	562.5 ± 49.3
GR205171 (40.0)+citalopram (10.0)	422.3 ± 32.4

Doses in mg/kg. Data are means ± SEMs and represent locomotor counts during 10 min. $N = 6$–7 per value. ANOVA as follows. Citalopram (0.63), $F(1, 10) = 4.6$, $P > 0.05$; influence of GR205171, $F(3, 22) = 2.8$, $P > 0.05$ and interaction, $F(3, 18) = 4.9$, $P < 0.05$. Citalopram (10.0), $F(1, 11) = 5.8$, $P < 0.05$; influence of GR205171, $F(3, 22) = 2.8$, $P > 0.05$ and interaction, $F(3, 21) = 3.3$, $P < 0.05$.
$^{\#}$Significance ($P < 0.05$) of citalopram-treated group *vs* vehicle-treated group; *significance ($P < 0.05$) of GR205171/citalopram-treated group *vs* vehicle/ citalopram-treated group.

Integration at the Level of the DRN: Significance of 5-HT$_{1A}$ Autoreceptors

NK_1 antagonists are unlikely to directly affect actions of SSRIs as GR205171 and RP67580 have negligible affinities

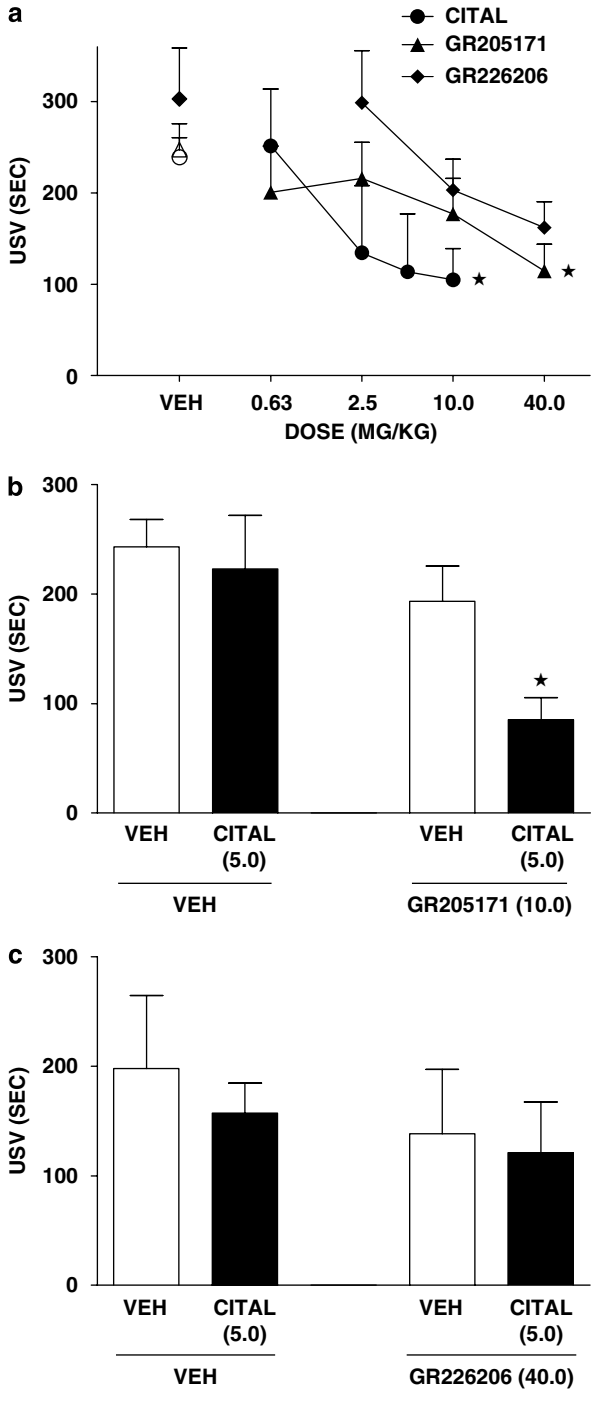

Figure 7 Enhancement by GR205171 of the actions of citalopram in the ultrasonic vocalization procedure in rats. VEH = vehicle and CITAL = citalopram. (a) Dose-dependent reduction of USV by CITAL and GR205171 as compared with its less active distomer, GR226206. (b, c) Stereospecific enhancement by GR205171 of the action of CITAL. Data are means ± SEMs; $N = 5$–15 per value. (a) ANOVA as follows: CITAL, $F(4, 51) = 3.0$, $P < 0.05$; GR205171, $F(4, 47) = 1.8$, $P > 0.05$ and GR226206, $F(3, 21) = 2.3$, $P > 0.05$. Asterisks indicate significance of differences to respective VEH values in Dunnett's test. (b, c) ANOVA as follows. (b) CITAL, $F(1, 29) = 3.8$, $P > 0.05$; GR205171, $F(1, 29) = 18.9$, $P < 0.01$ and interaction, $F(1, 29) = 1.8$, $P < 0.05$. (c) CITAL, $F(1, 14) = 0.3$, $P < 0.05$, GR226206, $F(1, 14) = 0.8$, $P > 0.05$ and interaction, $F(1, 14) = 0.1$, $P > 0.05$. The asterisk indicates significance of differences in Newman–Keuls test *vs* VEH/VEH values. *$P < 0.05$.

Table 4 Additive Influence of Low Doses of GR205171 and Citalopram Upon the Phase Advances in Circadian Wheel Running Rhythms Elicited by Light in Hamsters

	Phase advance (h)
Vehicle+vehicle	1.32 ± 0.09
GR205171 (5.0)+vehicle	1.13 ± 0.13
Vehicle+citalopram (5.0)	1.05 ± 0.13
GR205171 (10.0)+citalopram (5.0)	0.70 ± 0.10*
Vehicle+vehicle	1.58 ± 0.13
GR205171 (20.0)+vehicle	0.95 ± 0.12*
Vehicle+citalopram (10.0)	0.47 ± 0.14*
GR205171 (20.0)+citalopram (10.0)	0.60 ± 0.12*

Data are means ± SEMs ($N = 5$–8). One-way ANOVA; interaction of citalopram (5.0 mg/kg) with GR205171 (5.0 mg/kg), $F(3, 25) = 6.3$, $P < 0.01$ and interaction of citalopram (10.0 mg/kg) with GR205171 (20.0 mg/kg), $F(3, 21) = 13.5$, $P < 0.01$.
*$P < 0.05$ *vs* vehicle/vehicle values in Student–Newman–Keuls test.

for 5-HT transporters (pK_i values, < 5.0), and do not modify the actions of SSRIs at synaptosomes *in vitro* (Lieb *et al*, 2005; Millan, MJ, unpublished observation). Antagonists at 5-HT$_{1B}$ autoreceptors on serotonergic terminals enhances increases in 5-HT levels elicited by SSRIs (Gobert *et al*, 1997). However, GR205171 and RP67580 have no affinity for 5-HT$_{1B}$ receptors, and this would not explain their abrogation of the inhibitory influence of SSRIs on serotonergic neurones in the DRN (Gardner *et al*, 1996; Millan *et al*, 2000). Rather, inhibitory 5-HT$_{1A}$ autoreceptors are localized on serotonergic perikarya, and 5-HT$_{1A}$ antagonists likewise enhance increases in FCX levels of 5-HT elicited by SSRIs (Gobert *et al*, 1997; Millan *et al*, 2000; Guilloux *et al*, 2006). Though GR205171 and RP67580 do not behave as 5-HT$_{1A}$ antagonists (pK_i values, < 5.0; Gardner *et al*, 1996; Figure 5), it is important to consider the significance of 5-HT$_{1A}$ autoreceptors.

In agreement with most studies of NK$_1$ antagonists (Haddjeri and Blier, 2000; Conley *et al*, 2002; Lejeune *et al*, 2002; Guiard *et al*, 2005; Gobbi *et al*, 2007), GR205171 did not affect the spontaneous activity of DRN-localized serotonergic neurones. However, its reduction of the inhibitory influence of citalopram and fluoxetine upon DRN firing mimics the effects of chronic administration of NK$_1$ antagonists (Haddjeri and Blier, 2001; Guiard *et al*, 2005), and of genetic elimination of NK$_1$ receptors in mice (Froger *et al*, 2001; Santarelli *et al*, 2001; Gobbi *et al*, 2007). A subpopulation (some 30%) of 5-HT neurones in the DRN bear NK$_1$ receptors (Froger *et al*, 2001; Ma and Bleasdale, 2002; Lacoste *et al*, 2006), raising the possibility that NK$_1$ antagonists (*via* signalling crosstalk) disrupt the inhibitory influence of SSRI-engaged 5-HT$_{1A}$ autoreceptors upon DRN firing. Indeed, the inhibitory influence of 5-HT$_{1A}$ agonists on the DRN is blunted by chronic treatment with NK$_1$ antagonists (Haddjeri and Blier, 2001; Guiard *et al*, 2005), and by genetic deletion of NK$_1$ receptors (Froger *et al*, 2001; Santarelli *et al*, 2001). However, acute blockade of NK$_1$ receptors does not generally desensitize 5-HT$_{1A}$ autoreceptors (Blier *et al*, 2004; Guiard *et al*, 2007; Gobbi *et al*, 2007), and GR205171 did not modify the influence of 5-HT$_{1A}$ agonists on DRN firing rate *in vitro* (Guiard *et al*, 2005).

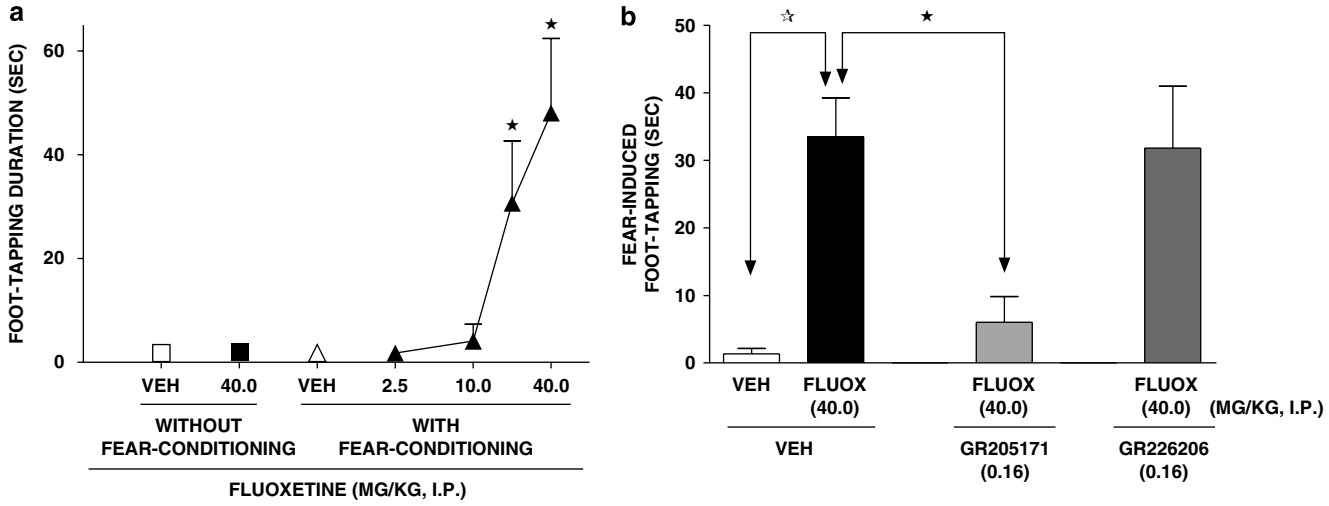

Figure 8 Blockade by GR205171 of the facilitation by fluoxetine of fear-induced foot tapping in gerbils. VEH = vehicle and FLUOX = fluoxetine. (a) Induction of foot tapping by FLUOX and (b) stereospecific blockade by GR205171 of the action of FLUOX. Data are means ± SEMs; $N = 4$–10 per values. ANOVA as follows. (a) FLUOX, $F(3, 15) = 6.6$, $P < 0.05$. Asterisks indicate significance of differences in Dunnett's test between fear-conditioning/FLUOX and fear-conditioning /VEH values. (b) Interaction, $F(2, 35) = 4.4$, $P < 0.05$. The closed asterisk indicates significance of differences in Dunnett's test between VEH/FLUOX and GR205171/FLUOX values and open asterisks, between fluoxetine and vehicle values. $^*P < 0.05$.

Correspondingly, the lack of influence of GR205171 upon the inhibition of DRN firing (Figure 4) by two 5-HT$_{1A}$ agonists, 8-OH-DPAT and buspirone (Lejeune *et al*, 1997), suggests that it does not markedly affect the sensitivity of 5-HT$_{1A}$ autoreceptors. Conversely, GR205171 attenuated the inhibitory influence of a lower efficacy (ca 30–40% *vs* 5-HT) 5-HT$_{1A}$ ligand, S15535 (Millan *et al*, 1997a, b; Newman-Tancredi *et al*, 1998) upon DRN firing. Thus, this partial agonist may reveal a subtle alteration by NK$_1$ receptor blockade of coupling at 5-HT$_{1A}$ autoreceptors. However, changes in firing rate do not invariably translate into alterations in release (Gobert *et al*, 1995; Millan and Gobert, 1999; Millan *et al*, 2000; Artigas *et al*, 2001; Gobbi *et al*, 2007), and the reduction by S15535 (and 8-OH-DPAT) of 5-HT release in FCX was not modified by GR205171. Moreover, GR205171 did not modify the influence on serotonergic neurones of (−)-pindolol, a low efficacy 5-HT$_{1A}$ ligand (ca 20%) which only submaximally decreases firing rate (Newman-Tancredi *et al*, 1998; Millan and Gobert, 1999; Guilloux *et al*, 2006). Recruitment of postsynaptic 5-HT$_{1A}$ receptors in FCX triggers long-loop, inhibitory feedback to serotonergic cell bodies in raphe nuclei (Haddjeri *et al*, 2000; Celada *et al*, 2001; Sharp *et al*, 2007). However, they are unlikely to be involved in the activations of GR205171 as the sensitivity of postsynaptic 5-HT$_{1A}$ receptors is unaffected by NK$_1$ antagonists (Froger *et al*, 2001; Haddjeri and Blier, 2001; Santarelli *et al*, 2001). Further, though microinjection of substance P into the FCX suppressed 5-HT release, this action did not involve frontocortical 5-HT$_{1A}$ receptors (Guiard *et al*, 2007).

Possible Involvement of Gabaergic, Glutatamergic, and Adrenergic Mechanisms

NK$_1$ receptors have been identified on GABAergic neurones surrounding 5-HT cell bodies in the DRN, and studies in the septum and cortex suggest that NK$_1$ receptor antagonists may indirectly excite serotonergic neurones *via* a reduction of inhibitory GABAergic tone (Sloviter *et al*, 2001; Ma and Bleasdale, 2002; Stacey *et al*, 2002; Szeidemann *et al*, 1995; Ebner *et al*, 2008). This would enhance their responsiveness to SSRIs, by analogy to GABA$_B$ antagonists (Millan, 2006; Cremers *et al*, 2007). Glutamatergic terminals targeting serotonergic DRN neurones also bear NK$_1$ receptors (Liu *et al*, 2002; Valentino *et al*, 2003), and their stimulation by intraraphe perfusion of substance P enhances local release of 5-HT *via* recruitment of AMPA receptors (Guiard *et al*, 2007). This results in a (delayed) decrease in cortical 5-HT release due to activation of 5-HT$_{1A}$ autoreceptors. That is, in line with the present observations, NK$_1$ receptors are 'upstream' of 5-HT$_{1A}$ autoreceptors. Interestingly, by analogy to lateral septum (Ebner *et al*, 2008), frontocortical perfusion of substance P suppressed local release of 5-HT in mice, an effect blocked by GR205171 and absent in mice lacking NK$_1$ receptors (Guiard *et al*, 2007). Thus, NK$_1$ antagonists conceivably also enhance SSRI-induced increase in 5-HT levels by actions in the FCX and other structures innervated by the DRN. Finally, Gobbi *et al* (2007) found that functionally intact adrenergic terminals are essential for the facilitatory influence of NK$_1$ receptor antagonists upon DRN firing. This is coherent with the excitatory impact of NK$_1$ receptor blockade upon electrical activity of LC-derived adrenergic neurones (Millan *et al*, 2001b; Gobbi *et al*, 2007).

Influence of NK$_1$ Receptor Blockade Upon NA and DA Levels

Elevations in levels of NA in FCX are seen in mice genetically deprived of NK$_1$ receptors and are elicited by NK$_1$ antagonists in parallel with an excitation of the LC (Millan *et al*, 2001b; Maubach *et al*, 2002; Blier *et al*, 2004; Ebner and Singewald, 2007; Gobbi *et al*, 2007). Correspondingly, GR205171 and RP67580 increased extracellular levels

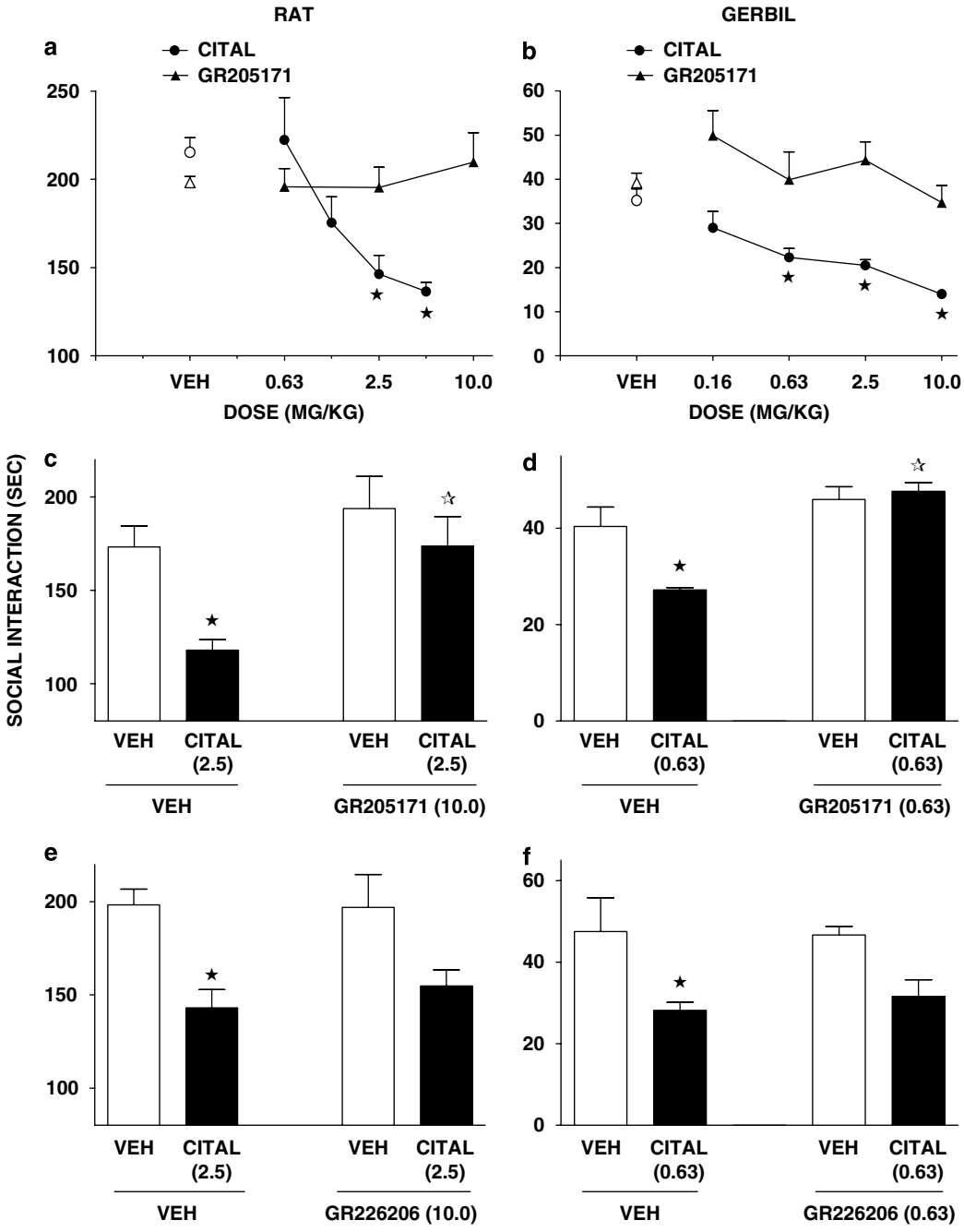

Figure 9 Blockade by GR205171 of the anxiogenic actions of citalopram in the social interaction test in rats and gerbils. VEH = vehicle and CITAL = citalopram. (a, b) Dose-dependent reduction of SI by CITAL as compared with GR205171 in rats and gerbils, respectively, and (c–f) stereospecific blockade by GR205171 of the action of CITAL. Data are means ± SEMs; $N = 5$–18 per value. (a, b) ANOVA as follows: CITAL in rats, $F(4, 38) = 10.6$, $P < 0.01$, GR205171 in rats, $F(3, 22) = 0.4$, $P > 0.05$, CITAL in gerbils, $F(4, 26) = 13.9$, $P < 0.01$ and GR205171 in gerbils, $F(4, 33) = 1.6$, $P > 0.05$. Asterisks indicate significance of differences to respective vehicle values in Dunnett's test. (c–f) ANOVA as follows. (c) CITAL, $F(1, 18) = 7.1$, $P < 0.05$; GR205171, $F(1, 18) = 7.3$, $P < 0.05$ and interaction, $F(1, 18) = 1.6$, $P > 0.05$. (d) CITAL, $F(1, 17) = 5.3$, $P < 0.05$; GR205171, $F(1, 17) = 27.2$, $P < 0.01$ and interaction, $F(1, 17) = 8.9$, $P < 0.01$. (e) CITAL, $F(1, 25) = 15.4$, $P < 0.01$; GR226206, $F(1, 25) = 0.2$, $P > 0.05$ and interaction, $F(1, 25) = 0.3$, $P > 0.05$. (f) CITAL, $F(1, 19) = 13.6$, $P < 0.01$; GR226206, $F(1, 19) = 0.2$, $P > 0.05$ and interaction, $F(1, 19) = 0.4$, $P > 0.05$. Closed asterisks indicate significance of differences in Newman–Keuls test between VEH/CITAL and VEH/VEH values and open asterisks, between NK$_1$ antagonist/CITAL and VEH/CITAL values. $^*P < 0.05$.

of NA in FCX, as well as in the BLA and hippocampus—structures likewise innervated by the LC (Millan *et al*, 2001b). The VTA possess a high density of NK$_1$ sites (Lessard and Pickel, 2005), and electrical activity of VTA-localized dopaminergic neurons is accelerated by blockade of NK$_1$ receptors, an effect accompanied by increases in DA levels in FCX (Lejeune *et al*, 2002). This finding was confirmed herein for GR205171, reproduced with RP67580 and extended to the BLA. Interestingly, neither agent affected DA levels in striatum or nucleus accumbens which are innervated by different subpopulations of dopaminergic cell bodies: that is, substantia nigra pars compacta (nigrostriatal) and paranigral-VTA (mesolimbic) *vs* parabrachial-VTA (mesocortical), respectively (Lejeune *et al*,

1997; Lejeune and Millan, 2000). These observations accord with the differential control of these clusters of dopaminergic neurons (Millan *et al*, 2000; Adell and Artigas, 2004; Alex and Pehek, 2007), including 5-HT$_{1A}$ receptor agonism which disinhibits VTA-derived mesocortical dopaminergic and LC-derived adrenergic pathways *via* GABAergic interneurones (Millan *et al*, 2000; Invernizzi *et al*, 2007). Interestingly, 8-OH-DPAT and S15535-induced elevations in FCX levels of DA and NA (Millan *et al*, 1997b; Gobert *et al*, 1995, 1999), were additive to those of GR205171. This indicates that 5-HT$_{1A}$ agonism and NK$_1$ receptor blockade offer complementary strategies for improvement of affect (Lucki *et al*, 1994; Millan, 2003, 2006; Czeh *et al*, 2006). GR205171 and *fluoxetine* likewise additively augmented dialysate levels of NA and DA in FCX, suggesting complementary mood-elevating properties *independent* of 5-HT (Millan *et al*, 2000). Accordingly, association of NK$_1$ receptor blockade with high doses of SSRIs, with the NA reuptake inhibitor, reboxetine, or with 5-HT/NA reuptake inhibitors like venlafaxine (Morilak and Frazer, 2004; Millan, 2006), may lead to more pronounced elevations in NA levels in FCX and improved antidepressant efficacy.

Enhancement of the Effects of Citalopram in a Forced Swim Procedure

Addition of GR205171 to a subactive dose of citalopram led to a significant antidepressant effect, an action exerted stereoselectively *vs* GR226206. These observations support the finding of a recent study of GR205171 and paroxetine by Chenu *et al* (2006)—though stereospecificity was not demonstrated. Apart from the hippocampus, the septum is implicated in antidepressant actions of SSRIs (Sheehan *et al*, 2004; Millan, 2006), and NK$_1$ antagonists may enhance 5-HT release in this region *via* inhibition of GABAergic interneurons (Szeidemann *et al*, 1995; Millan, 2006; Ebner *et al*, 2008). As GR205171 blunted the increase in locomotor activity elicited by citalopram herein (Table 3), potentiation of the actions of citalopram in the FS procedure is unlikely to reflect stimulation of motor behavior (Redrobe and Bourin, 1998; Brocco *et al*, 2006). Likewise, augmentation of paroxetine-induced decreases in immobility in the FS test occurred at a dose of GR205171 which did not increase its motor actions (Chenu *et al*, 2006).

Enhancement of the Action of Citalopram in the Stress-Related Ultrasonic Vocalization Test

Exposure of rats to stress elicits USVs at a characteristic frequency of 22 kHz (De Vry *et al*, 1993; Sánchez, 2003). This model is sensitive to SSRIs and, accordingly, citalopram reduced USVs (Molewijk *et al*, 1996; Sanchez and Meier, 1997; Millan *et al*, 2001a; Sánchez, 2003). Curiously, actions of NK$_1$ antagonists have not, as yet been documented. In fact, periaqueductal grey administration of substance P reduced USVs elicited by isolation in rats (Bassi *et al*, 2007), but NK$_1$ antagonists suppress isolation-induced (nonultrasonic) vocalizations in juveniles *via* actions in the amygdala (Molewijk *et al*, 1996; Rupniak *et al*, 2000; Brocco *et al*, 2008). This observation is coherent with the present finding that GR205171 attenuates stress-induced USVs. Moreover, though the difference in potency between GR205171 and GR226206 was, for unclear reasons, less marked in this procedure than for other paradigms, only GR205171 potentiated the inhibitory influence of citalopram on USVs. These data suggest that NK$_1$ receptor blockade may enhance the abrogation of stress-elicited behaviors by SSRIs.

Additive Influence on Circadian Rhythms

A normalization of perturbed diurnal scheduling of behavior is implicated in the therapeutic actions of certain antidepressants (Duncan, 1996; Millan, 2006). Light-induced phase shifts of activity rhythms in hamsters are blunted by several classes of antidepressant, including SSRIs (Millan, 2006; Gannon and Millan 2007). They likely act in the suprachiasmatic nucleus, which contains a high density of 5-HT transporters on serotonergic terminals originating in the median raphe nucleus (Legutko and Gannon, 2001). Intriguingly, a similar suppression of light-induced circadian phase-shifts is seen with NK$_1$ antagonists, which act upstream in the raphe to intensify serotonergic input to the suprachiasmatic nucleus (Valentino *et al*, 2003; Gannon and Millan, 2005). Consistent with contrasting mechanisms of action, subthreshold doses of citalopram and GR205171 additively prevented light-triggered phase-shifts. Accordingly, combined NK$_1$ receptor blockade and suppression of 5-HT reuptake may mutually restore circadian rhythmicity in certain depressed patients.

Blockade of the Anxiogenic Effects of Citalopram and Fluoxetine

SSRIs elicits anxiogenic effects in the SI procedure in rats, an observation extended herein to gerbils (Dekeyne *et al*, 2000; Millan, 2003). By contrast, NK$_1$ receptor antagonists either fail to affect, or enhance, SI, probably dependent upon the degree of substance P release (File, 1997; Dekeyne *et al*, 2000; Varty *et al*, 2002; Millan, 2003; Brocco *et al*, 2008; Ebner *et al*, 2008). GR205171 stereospecifically abolished the reduction in SI elicited by citalopram in rats, and similar results were acquired in gerbils, a species in which NK$_1$ receptors are potently blocked by GR205171 (Griffante *et al*, 2006; Engberg *et al*, 2007). Serotonin$_{2C}$ receptors in the hippocampus, amygdala and/or locus coeruleus mediate anxiogenic actions of SSRIs (Dekeyne *et al*, 2000; Millan 2003; Campbell and Merchant, 2003; Salchner and Singewald, 2006). However, GR205171 does not affect the reduction in SI provoked by 5-HT$_{2C}$ agonists (Dekeyne A, unpub. obs.), suggesting an action 'upstream' of 5-HT$_{2C}$ sites. These observations are supported by a further paradigm based on enhancement of fear-conditioned anxiety by SSRIs. Association of a mild aversive stimulus with fluoxetine triggers a characteristic foot-thumping response in gerbils (Millan, 2003; Rupniak *et al*, 2003; Burghardt *et al*, 2007; Brocco *et al*, 2008). By analogy to other NK$_1$ antagonists (op. cit.), this behavior was stereospecifically abrogated by GR205171, supporting the contention that acute anxiogenic effects of SSRIs are tempered by NK$_1$receptor blockade. SSRI-induced nervousness reduces compliance, triggers early abandonment of therapy and is associated with a long delay to efficacy (Millan, 2003, 2006; Morilak and Frazer, 2004). The present observations suggest

that NK_1 receptor antagonists, which show anxiolytic properties in patients with major depression or social phobia (Kramer *et al*, 1998, 2004; Michelgard *et al*, 2007), may alleviate anxiogenic effects of SSRIs at the onset of therapy.

General Discussion

First, the present data show that the antidepressant efficacy of SSRIs is enhanced by blockade of NK_1 receptors, yet that their acute anxiogenic actions are abrogated. Thus, the present observation support the association of SSRIs and selective NK_1 antagonists and the development of mixed NK_1 antagonists/5-HT reuptake inhibitors for treatment of depression and anxiety (Ryckmans *et al*, 2002; Chenu *et al*, 2006; Millan, 2006; Brocco *et al*, 2008). Second, association of GR205171 with fluoxetine led to additive increases in levels of NA and DA suggesting that interactions between NK_1 antagonists and antidepressants may also involve dopaminergic and adrenergic mechanisms. GR205171 did not potentiate antidepressant actions of the NA reuptake inhibitor, desipramine (Chenu *et al*, 2006), and desipramine did not more markedly increase NA levels in NK_1 knock-out mice (Herpfer *et al*, 2005). Nonetheless, it would be of interest to examine the influence of GR205171 upon actions of mixed 5-HT/NA reuptake inhibitors like venlafaxine, and the atypical agent, mirtazapine (Millan *et al*, 2000; Morilak and Frazer, 2004; Millan, 2006). This argument is underpinned by the marked elevation in FCX levels of DA and NA elicited by GR205171 together with 8-OH-DPAT or S15535, 5-HT_{1A} receptor agonists possessing antidepressant and anxiolytic properties (Lucki *et al*, 1994; Millan *et al*, 1997a, b; Millan, 2006). Third, NK_2 and NK_3 receptors influence mood and modulate monoaminergic pathways, possibly in interaction with NK_1 receptors (Steinberg *et al*, 2001; Bert *et al*, 2002; Léger *et al*, 2002; Spooren *et al*, 2005). Accordingly, it would be interesting to examine how blockade of NK_2 and NK_3 receptors affects the functional actions of antidepressants.

CONCLUSIONS

The present observations strongly suggest that *combining* NK_1 receptor blockade with suppression of 5-HT reuptake may yield benefits in the treatment of anxious and depressed states relative to selective NK_1 antagonists and SSRIs.

ACKNOWLEDGEMENTS

We thank Marianne Soubeyran for secretarial assistance and Rodolphe Billiras, Raphael Boulanger, Laetitia Cistarelli, Loretta Iob, Dorothée Sicard, and Sylvie Veiga for technical assistance.

DISCLOSURE/CONFLICT OF INTEREST

Alain Gobert, Mauricette Brocco, Anne Dekeyne, Benjamin Di Cara, Gaëlle Bouchez, Françoise Lejeune, and Mark J Millan declare that, except for income received from their primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest. Robert L Gannon work's was funded in part by NSF IBN 0549980. Robert L. Gannon has received compensation from Servier and Shaw Science Partners.

REFERENCES

Adell A, Artigas F (2004). The somatodendritic release of dopamine in the ventral tegmental area and its regulation by afferent transmitter systems. *Neurosci Biobehav Rev* **28**: 415–431.

Alex KD, Pehek EA (2007). Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacol Ther* **113**: 296–320.

Artigas F, Celada P, Laruelle M, Adell A (2001). How does pindolol improve antidepressant action? *TIPS* **22**: 224–228.

Bassi GS, Nobre MJ, Carvalho MC, Brandão ML (2007). Substance P injected into the dorsal periaqueductal gray causes anxiogenic effects similar to the long-term isolation as assessed by ultrasound vocalizations measurements. *Behav Brain Res* **182**: 301–307.

Bert L, Rodier D, Bougault I, Allouard N, Le-Fur G, Soubrié P *et al* (2002). Permissive role of neurokinin NK_3 receptors in NK_1 receptor-mediated activation of the locus coeruleus revealed by SR 142801. *Synapse* **43**: 62–69.

Blier P, Gobbi G, Haddjeri N, Santarelli L, Mathew G, Hen R (2004). Impact of substance P receptor antagonism on the serotonin and norepinephrine systems: relevance to the antidepressant/anxiolytic response. *J Psychiatry Neurosci* **29**: 208–218.

Brocco M, Dekeyne A, Mannoury la Cour C, Touzard M, Girardon S, Veiga S *et al* (2008). Cellular and behavioural profile of the novel, selective neurokinin$_1$ receptor antagonist, vestipitant: a comparison to other agents. *Eur Neuropsychopharmacol* **18**: 729–750.

Brocco M, Dekeyne A, Papp M, Millan MJ (2006). Antidepressant-like properties of the anti-Parkinson agent, piribedil, in rodents: mediation by dopamine D_2 receptors. *Behav Pharmacol* **17**: 559–572.

Brocco M, Dekeyne A, Veiga S, Girardon S, Millan MJ (2002). Induction of hyperlocomotion in mice exposed to a novel environment by inhibition of serotonin reuptake a pharmacological characterization of diverse classes of antidepressant agents. *Pharmacol Biochem Behav* **71**: 667–680.

Burghardt NS, Bush DEA, McEwen BS, LeDoux JE (2007). Acute selective serotonin reuptake inhibitors increase conditioned fear expression: blockade with a 5-HT_{2C} receptor antagonist. *Biol Psychiatry* **62**: 1111–1118.

Campbell BM, Merchant KM (2003). Serotonin$_{2C}$ receptors within the basolateral amygdala induce acute fear-like responses in an open-field environment. *Brain Res* **993**: 1–9.

Celada P, Puig MV, Casanovas JM, Guillazo G, Artigas F (2001). Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: involvement of serotonin-1A, GABA(A), and glutamate receptors. *J Neurosci* **21**: 9917–9929.

Chenu F, Guiard BP, Bourin M, Gardier AM (2006). Antidepressant-like activity of selective serotonin reuptake inhibitors combined with a NK_1 receptor antagonist in the mouse forced swimming test. *Behav Brain Res* **172**: 256–263.

Commons KG, Valentino RJ (2002). Cellular basis for the effects of substance P in the periaqueductal gray and dorsal raphe nucleus. *J Comp Neurol* **447**: 82–97.

Conley RK, Cumberbatch MJ, Mason GS, Williamson DJ, Harrison T, Locker K *et al* (2002). Substance P (neurokinin 1) receptor antagonists enhance dorsal raphe neuronal activity. *J Neurosci* **22**: 7730–7736.

Cremers TI, Rea K, Bosker FJ, Wikström HV, Hogg S, Mork A *et al* (2007). Augmentation of SSRI effects on serotonin by 5-HT$_{2C}$ antagonists: mechanistic studies. *Neuropsychopharmacology* **31**: 1550–1557.

Czeh B, Fuchs E, Simon M (2006). NK$_1$ receptor antagonists under investigation for the treatment of affective disorders. *Expert Opin Investig Drugs* **15**: 479–486.

Dableh LJ, Yashpal K, Rochford J, Henry JL (2005). Antidepressant-like effects of neurokinin receptor antagonists in the forced swim test in the rat. *Eur J Pharmacol* **507**: 99–105.

De Vry J, Benz U, Schreiber R, Traber J (1993). Shock-induced ultrasonic vocalization in young adult rats: a model for testing putative anti-anxiety drugs. *Eur J Pharmacol* **249**: 331–339.

Dekeyne A, Denorme B, Monneyron S, Millan MJ (2000). Citalopram reduces social interaction in rats by activation of serotonin (5-HT)$_{2C}$ receptors. *Neuropharmacology* **39**: 114–1117.

Duncan WC (1996). Circadian rhythms and the pharmacology of affective illness. *Pharmacol Ther* **71**: 253–312.

Ebner K, Rupniak NM, Saria A, Singewald N (2004). Substance P in the medial amygdala: emotional stress-sensitive release and modulation of anxiety-related behavior in rats. *Proc Natl Acad Sci USA* **101**: 4280–4285.

Ebner K, Singewald GM (2007). Stress-induced release of substance P in the locus coeruleus modulates cortical noradrenaline release. *Naunyn Schmiedebergs Arch Pharmacol* **376**: 73–82.

Ebner K, Singewald GM, Whittle N, Ferraguti F, Singewald N (2008). Neurokinin$_1$ receptor antagonism promotes active stress coping via enhanced septal 5-HT$_{1A}$ transmission. *Neuropsychopharmacology* **33**: 1929–1941.

Engberg S, Ahlstedt I, Leffler A, Lindström E, Kristensson E, Svensson A *et al* (2007). Molecular cloning, mutations and effects of NK$_1$ receptor antagonists reveal the human-like pharmacology of gerbil NK$_1$ receptors. *Biochem Pharmacol* **73**: 259–269.

File SE (1997). Anxiolytic action of a neurokinin$_1$ receptor antagonist in the social interaction test. *Pharmacol Biochem Behav* **58**: 747–752.

Froger N, Gardier AM, Moratalla R, Alberti I, Lena I, Boni C *et al* (2001). 5-hydroxytryptamine (5-HT)$_{1A}$ autoreceptor adaptive changes in substance P (neurokinin$_1$) receptor knock-out mice mimic antidepressant-induced desensitization. *Neuroscience* **15**: 8188–8197.

Furmark T, Appel L, Michelgård A, Wahlstedt K, Ahs F, Zancan S *et al* (2005). Cerebral flow changes after treatment of social phobia with the neurokinin-1 antagonist GR205171, citalopram or placebo. *Biol Psychiatry* **58**: 132–142.

Gannon RI, Millan MJ (2005). The selective neurokinin$_1$ (NK$_1$) receptor antagonist, GR 205171, stereospecifically inhibits light-induced phase shifts of hamster circadian activity rhythms. *Eur J Pharmacol* **527**: 86–93.

Gannon RL, Millan MJ (2007). Evaluation of serotonin, noradrenaline and dopamine reuptake inhibitors on light-induced phase advances in hamster circadian activity rhythms. *Psychopharmacology* **195**: 325–332.

Gardner CJ, Armour DR, Beattie DT, Gale JD, Hawcock AB, Kilpatrick GJ *et al* (1996). GR205171: a novel antagonist with high affinity for the tachykinin NK$_1$receptor, and potent broad-spectrum anti-emetic activity. *Regul Pept* **65**: 45–53.

Garret C, Carruette A, Fardin V, Moussoui S, Peyronel JF, Blanchard JC *et al* (1991). Pharmacological properties of a potent and selective nonpeptide substance P antagonist. *Proc Natl Acad Sci USA* **88**: 10208–10212.

Gerald CPG, Antonijevic IA, Branchek TA (2006). Novel mechanisms to treat depression and anxiety. Will neuropeptide antagonists succeed? *Drug Discov Today Ther Strateg* **4**: 467–473.

Gobbi G, Cassano T, Radja F, Morgese MG, Cuomo V, Santarelli L *et al* (2007). Neurokinin 1 receptor antagonism requires norepinephrine to increase serotonin function. *Eur Neuropsychopharmacol* **17**: 328–338.

Gobert A, Lejeune F, Rivet J-M, Audinot V, Newman-Tancredi A, Millan MJ (1995). Modulation of the activity of central serotoninergic neurones by novel serotonin$_{1A}$ receptor agonists and antagonists: A comparison to adrenergic and dopaminergic neurones in individual rats. *J Pharmacol Exp Ther* **273**: 1032–1046.

Gobert A, Rivet JM, Cistarelli L, Melon C, Millan MJ (1999). Buspirone modulates basal and fluoxetine-stimulated dialysate levels of dopamine, noradrenaline and serotonin in the frontal cortex of freely moving rats: activation of serotonin$_{1A}$ receptors and blockade of alpha$_2$-adrenergic receptors underlie its actions. *Neuroscience* **93**: 1251–1262.

Gobert A, Rivet J-M, Cistarelli L, Millan MJ (1997). Potentiation of the fluoxetine-induced increase in dialysate levels of serotonin (5-HT) in the frontal cortex of freely-moving rats by combined blockade of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors with WAY100 635 and GR127 935. *J Neurochem* **68**: 1159–1163.

Griffante C, Carletti R, Andreetta F, Corsi M (2006). [^{3}H]GR205171 displays similar NK$_1$receptor binding profile in gerbil and human brain. *Br J Pharmacol* **148**: 39–45.

Guiard BP, Froger N, Hamon M, Gardier AM, Lanfumey L (2005). Sustained pharmacological blockage of NK$_1$ substance P receptors causes functional desensitization of dorsal raphe 5-HT$_{1A}$ autoreceptors in mice. *J Neurochem* **95**: 1713–1723.

Guiard BP, Guilloux JP, Reperant C, Hunt SP, Toth M, Gardier AM (2007). Substance P neurokinin$_1$ receptor activation within the dorsal raphe nucleus controls serotonin release in the mouse frontal cortex. *Mol Pharmacol* **72**: 1411–1418.

Guiard BP, Przybylski C, Guilloux JP, Seif I, Froger N, de Felipe C *et al* (2004). Blockade of substance P (neurokinin$_1$) receptors enhances extracellular serotonin when combined with a selective serotonin reuptake inhibitor: an *in vivo* microdialysis study in mice. *J Neurochem* **89**: 54–63.

Guilloux JP, David DJ, Guiard BP, Chenu F, Repérant C, Toth M *et al* (2006). Blockade of 5-HT$_{1A}$ receptors by (+/−)-pindolol potentiates cortical 5-HT outflow, but not antidepressant-like activity of paroxetine: microdialysis and behavioural approaches in 5-HT$_{1A}$ receptor knockout mice. *Neuropsychopharmacology* **31**: 2162–2172.

Haddjeri N, Blier P (2000). Effect of neurokinin-$_1$ receptor antagonists on the function of 5-HT and noradrenaline neurons. *Neuroreport* **11**: 1323–1327.

Haddjeri N, Blier P (2001). Sustained blockade of neurokinin-$_1$ receptors enhances serotonin neurotransmission. *Biol Psychiatry* **50**: 191–199.

Haddjeri N, Lucas G, Blier P (2000). Role of cholinergic and GABAergic systems in the feedback inhibition of dorsal raphe 5-HT neurons. *Neuroreport* **11**: 3397–3401.

Herpfer L, Hunt SP, Stanford SC (2005). A comparison of neurokinin$_1$ receptor knock-out (NK$_1$−/−) and wildtype mice: exploratory behaviour and extracellular noradrenaline concentration in the cerebral cortex of anaesthetised subjects. *Neuropharmacology* **48**: 706–719.

Holmes A, Heilig M, Rupniak NMJ, Steckler T, Griebel G (2003). Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. *Trends Pharmacol Sci* **24**: 580–588.

Invernizzi RW, Pierucci M, Calcagno E, Di Giovanni G, Di Matteo V, Benigno A *et al* (2007). Selective activation of 5-HT$_{2C}$ receptors stimulates GABA-ergic function in the rat substantia nigra pars reticulata: a combined *in vivo* electrophysiological and neurochemical study. *Neuroscience* **144**: 1523–1535.

Kramer MS, Cutler NR, Keighner J, Shrivastava R, Carman J, Sramek JJ *et al* (1998). Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science* **281**: 1640–1645.

Kramer MS, Winokur A, Kelsey J, Preskorn SH, Rothschild AJ, Snavely D *et al* (2004). Demonstration of the efficacy and safety of a novel substance P (NK$_1$) receptor antagonist in major depression. *Neuropsychopharmacology* **29**: 385–392.

Lacoste B, Riad M, Descarries L (2006). Immunocytochemical evidence for the existence of substance P receptor (NK_1) in serotonin neurons of rat and mouse dorsal raphe nucleus. *Eur J Neurosci* **23**: 2947–2958.

Léger L, Gay N, Cespuglio R (2002). Neurokinin NK_1- and NK_3-immunoreactive neurons in serotonergic cell groups in the rat brain. *Neurosci Lett* **323**: 146–150.

Legutko R, Gannon RL (2001). Serotonin transporter localization in the hamster suprachiasmatic nucleus. *Brain Res* **893**: 77–83.

Lejeune F, Gobert A, Millan MJ (2002). The selective neurokinin $(NK)_1$ antagonist, GR205171, stereospecifically enhances mesocortical dopaminergic transmission in the rat: a combined dialysis and electrophysiological study. *Brain Res* **935**: 134–139.

Lejeune F, Millan MJ (2000). Pindolol excites dopaminergic and adrenergic neurons, and inhibits serotonergic neurons, by activation of 5-HT_{1A} receptors. *Eur J Neurosci* **12**: 3265–3275.

Lejeune F, Newman-Tancredi A, Audinot V, Millan MJ (1997). Interaction of (+)- and (−)-8- and 7-hydroxy-2-(di-n-propylamino)tetraline at human (h)D_3, hD_2 and h serotonin$_{1A}$ receptors and their modulation of the activity of serotonergic and dopaminergic neurones in rats. *J Pharmacol Exp Ther* **280**: 1241–1249.

Lessard A, Pickel VM (2005). Subcellular distribution and plasticity of neurokinin-1 receptors in the rat substantia nigra and ventral tegmental area. *Neuroscience* **135**: 1309–1323.

Lieb K, Fiebich BL, Herpfer I, Mantovani M, Löffler M, Feuerstein TJ (2005). No modulatory effect of neurokinin-$_1$ receptor antagonists on serotonin uptake in human and rat brain synaptosomes. *Eur Neuropsychopharmacol* **15**: 641–646.

Liu R, Ding Y, Aghajanian GK (2002). Neurokinins activate local glutamatergic inputs to serotonergic neurons of the dorsal raphe nucleus. *Neuropsychopharmacology* **27**: 329–340.

Lucki I, Singh A, Kreiss DS (1994). Antidepressant-like behavioral effects of serotonin receptor agonists. *Neurosci Biobehav Rev* **18**: 85–95.

Ma Q-P, Bleasdale C (2002). Modulation of brain stem monoamines and gamma aminobutyric acid by NK_1 receptors in rats. *Neuroreport* **13**: 1809–1813.

Maubach KA, Martin K, Chicchi G, Harrison T, Wheeldon A, Swain CJ *et al* (2002). Chronic substance P (NK_1) receptor antagonist and conventional antidepressant treatment increases burst firing of monoamine neurones in the locus coeruleus. *Neuroscience* **109**: 609–617.

Michelgard A, Appel L, Pissiota A, Frans O, Langström B, Bergström M *et al* (2007). Symptom provocation in specific phobia affects the substance P neurokinin-$_1$ receptor system. *Biol Psychiatry* **61**: 1002–1006.

Millan MJ (2003). The neurobiology and control anxious states. *Prog Neurobiol* **70**: 83–244.

Millan MJ (2004). The role of monoamines in the action of established and 'novel' antidepressant agents: a critical review. *Eur J Pharmacol* **500**: 371–384.

Millan MJ (2006). Multi-target strategies for the improved treatment of depressive states: conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacol Ther* **110**: 135–370.

Millan MJ, Brocco M, Gobert A, Dorey G, Casara P, Dekeyne A (2001a). Anxiolytic properties of the selective, non-peptidergic CRF_1 antagonists, CP154 526 and DMP695: a comparison to other classes of anxiolytic agent. *Neuropsychopharmacology* **25**: 585–600.

Millan MJ, Gobert A (1999). (−)-Pindolol increases dialysate concentrations of dopamine and noradrenaline, but not serotonin, in the frontal cortex of freely-moving rats. *Neuropharmacology* **38**: 909–912.

Millan MJ, Hjorth S, Samanin R, Schreiber R, Jaffard R, De Ladonchamps B *et al* (1997a). S15535, a novel benzodioxopiperazine ligand of serotonin (5-HT)$_{1A}$ receptors: II. Modulation of hippocampal serotonin release in relation to potential anxiolytic properties. *J Pharmacol Exp Ther* **282**: 148–161.

Millan MJ, Lejeune F, De Nanteuil G, Gobert A (2001b). Selective blockade of neurokinin $(NK)_1$ receptors facilitates the activity of adrenergic pathways projecting to the frontal cortex and dorsal hippocampus in the rat. *J Neurochem* **76**: 1949–1954.

Millan MJ, Lejeune F, Gobert A (2000). Reciprocal autoreceptor and heteroceptor control of serotonergic, dopaminergic and adrenergic transmission in frontal cortex: a review, and relevance to the actions of antidepressant agents. *J Psychopharmacol* **14**: 114–138.

Millan MJ, Newman-Tancredi A, Rivet JM, Brocco M, Lacroix P, Audinot V *et al* (1997b). S15535, a novel benzodioxopiperazine ligand of serotonin (5-HT)1A receptors: I. Interaction with cloned human (h)5-HT_{1A}, dopamine hD2/hD3 and hα2A-adrenergic receptors in relation to modulation of cortical monoamine release and activity in models of potential antidepressant activity. *J Pharmacol Exp Ther* **282**: 132–147.

Molewijk HE, Hartog K, van der Poel AM, Mos J, Olivier B (1996). Reduction of guinea pig pup isolation calls by anxiolytic and antidepressant drugs. *Psychopharmacology* **128**: 31–38.

Morilak DA, Frazer A (2004). Antidepressants and brain monoaminergic systems: a dimensional approach to understanding their behavioural effects in depression and anxiety disorders. *Int J Neuropsychopharmacol* **7**: 193–218.

Newman-Tancredi A, Chaput C, Gavaudan S, Verrièle L, Millan MJ (1998). Agonist and antagonist action of (−)-pindolol at recombinant, human 5-HT_{1A} receptors. *Neuropsychopharmacology* **18**: 395–398.

Redrobe JP, Bourin M (1998). Dose-dependent influence of buspirone on the activities of selective serotonin reuptake inhibitors in the mouse forced swimming test. *Psychopharmacology* **138**: 198–206.

Renoldi G, Invernizzi RW (2006). Blockade of tachikinin NK_1 receptors attenuates stress-induced rise of extracellular noradrenaline and dopamine in the rat and gerbil medial prefrontal. *J Neurosci Res* **84**: 961–968.

Rupniak NM, Carlson EC, Harrison T, Oates B, Seward E, Owen S *et al* (2000). Pharmacological blockade or genetic deletion of substance P (NK_1) receptors attenuates neonatal vocalisation in guinea-pigs and mice. *Neuropharmacology* **39**: 1413–1421.

Rupniak NM, Carlson EJ, Webb JK, Harrison T, Porsolt RD, Roux S *et al* (2001). Comparison of the phenotype of $NK_1R-/-$ mice with pharmacological blockade of the substance P (NK_1) receptor in assays for antidepressant and anxiolytic drugs. *Behav Pharmacol* **12**: 497–508.

Rupniak NMJ, Webb JK, Fisher A, Smith D, Boyce S (2003). The substance P (NK_1) receptor antagonist L-760735 inhibits fear conditioning in gerbils. *Neuropharmacology* **44**: 516–523.

Ryckmans T, Berton O, Grimée R, Kogej T, Lamberty Y, Pasau P *et al* (2002). Dual NK_1 antagonists-serotonin reuptake inhibitors as potential antidepressants. Part 2: SAR and activity of benzyloxyphenethyl piperazine derivatives. *Bioorg Med Chem Lett* **12**: 3195–3198.

Saffroy M, Torrens Y, Glowinski J, Beaujouan J-C (2003). Autoradiographic distribution of tachykinin NK_2 binding sites in the rat brain: comparison with NK_1 and NK_3 binding sites. *Neuroscience* **116**: 761–773.

Salchner P, Singewald N (2006). 5-HT receptor subtypes involved in the anxiogenic-like action and associated Fos response of acute fluoxetine treatment in rats. *Psychopharmacology* **185**: 282–288.

Sánchez C (2003). Stress-induced vocalization in adult animals. A valid model of anxiety? *Eur J Pharmacol* **463**: 133–143.

Sanchez C, Meier E (1997). Behavioral profiles of SSRIs in animal models of depression, anxiety and aggression. Are they all alike? *Psychopharmaocology* **129**: 197–205.

Santarelli L, Gobbi G, Debs PC, Sibille EL, Blier P, Hen R *et al* (2001). Genetic and pharmacological distribution of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function. *Proc Natl Acad Sci USA* **98**: 1912–1917.

Sartori SB, Burnet PWJ, Sharp T, Singewald N (2004). Evaluation of the effect of chronic antidepressant treatment on neurokinin-1 receptor expression in the rat brain. *Neuropharmacology* **46**: 1177–1183.

Sharp T, Boothman L, Raley J, Quérée P (2007). Important messages in the 'post': recent discoveries in 5-HT neurone feedback control. *TiNS* **28**: 629–636.

Sheehan TP, Chambers RA, Russell DS (2004). Regulation of affect by the lateral septum: implications for neuropsychiatry. *Brain Res Rev* **46**: 71–117.

Shirayama Y, Mitsushio H, Takashima M, Ichikawa H, Takahashi K (1996). Reduction of substance P after chronic antidepressants treatment in the striatum, substantia nigra and amygdala of the rat. *Brain Res* **739**: 70–78.

Sloviter RS, Ali-Akbarian L, Horvath KD, Menkens KA (2001). Substance P receptor expression by inhibitory interneurons of the rat hippocampus: enhanced detection using improved immunocytochemical methods for the preservation and colocalization of GABA and other neuronal markers. *J Comp Neurol* **430**: 283–305.

Spooren W, Riemer C, Meltzer H (2005). NK_3 receptor antagonists: the next generation of antipsychotics? *Nat Rev Drug Discov* **4**: 967–975.

Stacey AE, Woodhall GL, Jones RSG (2002). Activation of neurokinin$_1$ receptors GABA release at synapses in rat entorhinal cortex. *Neuroscience* **115**: 575–586.

Steinberg R, Alonso R, Griebel G, Bert L, Jung M, Oury-Donat F *et al* (2001). Selective blockade of neurokinin-$_2$ receptors produces antidepressant-like effects associated with reduced corticotrophin-releasing factor function. *J Pharmacol Exp Ther* **299**: 449–458.

Szeidemann Z, Jakab RL, Shanabrough M, Leranth C (1995). Extrinsic and intrinsic substance P innervation of the rat lateral septal area calbindin cells. *Neuroscience* **69**: 1205–1221.

Valentino RJ, Bey V, Pernar L, Commons KG (2003). Substance P acts through local circuits within the rat dorsal raphe nucleus to alter serotonergic neuronal activity. *J Neurosci* **23**: 7155–7159.

Varty GB, Cohen-Williams ME, Morgan CA, Pylak U, Duffy RA, Lachowicz JE *et al* (2002). The gerbil elevated plus-maze II: anxiolytic-like effects of selective neurokinin NK_1 receptor antagonists. *Neuropsychopharmacology* **27**: 371–379.