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Pharmacology, Biochemistry and Behavior 71 (2002) 667–680

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Induction of hyperlocomotion in mice exposed to a novel environment by inhibition of serotonin reuptake A pharmacological characterization of diverse classes of antidepressant agents

Mauricette Brocco, Anne Dekeyne, Sylvie Veiga, Sylvie Girardon, Mark J. Millan*

Psychopharmacology Department, Institut de Recherches Servier, Centre de Recherches de Croissy, 125 chemin de Ronde, 78290 Croissy/Seine, Paris, France

Received 1 June 2001; received in revised form 20 August 2001; accepted 1 September 2001

Abstract

This study characterized the influence of acute administration of diverse classes of antidepressant agent upon the spontaneous locomotor activity (LA) of mice in a novel, open-field environment. The selective serotonin (5-HT) reuptake inhibitors (SSRIs), citalopram, fluoxetine, paroxetine, fluvoxamine, litoxetine and zimelidine, dose-dependently enhanced LA. Their actions were mimicked by the mixed 5-HT/noradrenaline (NA) reuptake inhibitors (SNRIs), venlafaxine, duloxetine and S33005. In contrast, clomipramine only slightly elevated LA and two further tricyclics, imipramine and amitriptyline, were inactive. Further, the selective NA vs. 5-HT reuptake inhibitors (NARIs), reboxetine, desipramine, maprotiline, nisoxetine and nortriptyline all failed to increase LA. The ''atypical antidepressants,'' mianserin and mirtazapine, neither of which modify 5-HT reuptake, as well as the mixed SSRI/5-HT_2 antagonists, nefazodone and trazodone, also failed to increase LA. Doses of SSRI and SNRI which increased LA did not modify motor performance in the rotarod test. Further, they did not enhance LA in rats, suggesting that this response is characteristic of mice. Finally, upon prehabituation of mice to the activity chamber, the SSRI, citalopram, and the SNRI, venlafaxine, *failed* to increase LA. In conclusion, in mice exposed to a *novel* environment, inhibition of 5-HT reuptake by SSRIs and SNRIs enhances spontaneous LA in the absence of a generalized influence upon motor function. This response provides a simple parameter for characterization of SSRIs and SNRIs, and differentiates them from other classes of antidepressant agent. Although an influence upon arousal and/or anxiety is likely related to the increase in LA, the functional significance of this response requires additional elucidation.

Keywords: Serotonin; 5-HT; SSRI; Depression; Antidepressant; Fluoxetine; Venlafaxine; Motor function; Locomotion; Arousal; Anxiety

1. Introduction

Serotonergic mechanisms play an important role in the modulation of motor behaviour. This role is expressed at numerous levels of the neuroaxis: notably, in the spinal cord, the basal ganglia, the nucleus accumbens and other limbic structures, and in the frontal cortex (FCX) (Geyer, 1996; Millan et al., 1997; Wallis, 1994). In addition to its influence upon motor function per se, 5-HT plays a crucial role in the modulation of nociception, mood and mnesic function, all of which reciprocally interact with motor behaviour (Barnes and Sharp, 1999; Geyer, 1996; Maes and Meltzer, 1995; Meneses 1999; Millan, 1995; Steckler and Sahgal, 1995). Correspondingly, a perturbation of serotonergic transmission is implicated in the motor as well as emotional and cognitive symptoms of many neurological and psychiatric disorders (see Bloom and Kupfer, 1995 for reviews).

In two principle respects, serotonergic mechanisms controlling motor behavior are of considerable pertinence to depressive states. *First*, in addition to despair and anhedonia, psychomotor retardation is considered a cardinal symptom of this disorder — while a subpopulation of patients may, at least transiently, display motor agitation (Caligiuri and Ellwanger, 2000; Sachdev and Aniss, 1994; Widlöcher and Ghozlan, 1989). *Second*, a perturbation of corticolimbic serotonergic pathways is strongly implicated

* Corresponding author. Tel.: +33-1-5572-2425; fax: +33-1-5572-2470.
E-mail address: mark.millan@fr.netgrs.com (M.J. Millan).

0091-3057/02/$ – see front matter
PII: S0091-3057(01)00701-8

in the etiology of depressive states, an amelioration of which may be clinically achieved by reinforcement of serotonergic transmission (Blier and de Montigny, 1999; Broekkamp et al., 1995; Maes and Meltzer, 1995; Millan et al., 2000b; Staley et al., 1998). In this regard, several classes of clinically active antidepressant inhibit 5-HT reuptake in the hippocampus, FCX and other structures via actions at the neuronal 5-HT transporter (SERT) (Barker and Blakely, 1995; Blakely and Baumann, 2000; Schloss and William, 1998). For example, selective 5-HT reuptake inhibitors (SSRI), such as fluoxetine, paroxetine, citalopram, litoxetine, zimelidine and fluvoxamine (Fuller et al., 1991; Frazer, 1997; Goodnick and Goldstein, 1998; Popik, 1999; Sánchez and Meier, 1997; Schloss and William, 1998), and the mixed 5-HT/NA reuptake inhibitors (SNRIs), venlafaxine, duloxetine and S33005 (Dawson et al., 1999; Harvey et al., 2000; Kasamo et al., 1996; Millan et al., 2001a,b; Muth et al., 1986; Schweizer et al., 1997) all elevate extracellular levels of 5-HT by actions at 5-HT transporters. Tricyclic agents, such as clomipramine (CMI), amitriptyline and imipramine, likewise inhibit 5-HT (and NA) reuptake, but they also interact with histamine$_1$ receptors and α_1-adrenoceptors (AR), blockade of which elicits sedation (Mir and Taylor, 1997; Owens et al., 1997; Tatsumi et al., 1997). Further, they display antagonist properties at 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, in common with trazodone and nefazodone. These phenylpiperazines, in addition to a novel antidepressant described by Yamanouchi, 3-{3-[4-(7-fluoro-indan-4-yloxy)-piperidin-1-yl]propylamino carbonyl}-pyridine fumarate (herein designated YM$_1$), all markedly inhibit 5-HT reuptake (Davis et al., 1997; Nutt, 1996; Pazzagli et al., 1999). Interestingly, it has been suggested that mianserin and mirtazapine, both of which display 5-HT$_{2C}$ and α_2-AR antagonist properties, may indirectly enhance serotonergic transmission via blockade of inhibitory α_2-ARs on serotonergic neurones, although this contention has been challenged (Bengtsson et al., 2000; De Boer et al., 1996; Haddjeri et al., 1995, 1996; see Millan et al., 2000a; Whale et al., 2000). Finally, several other classes of antidepressant, such as the selective NA reuptake inhibitors (NARIs), desipramine (DMI), reboxetine, nortriptyline, maprotiline and nisoxetine display low affinity for SERTs (Pawlowski and Nowak, 1987; Burrows et al., 1998; Cryan and Lucki, 1999; Owens et al., 1997; Wong et al., 2000).

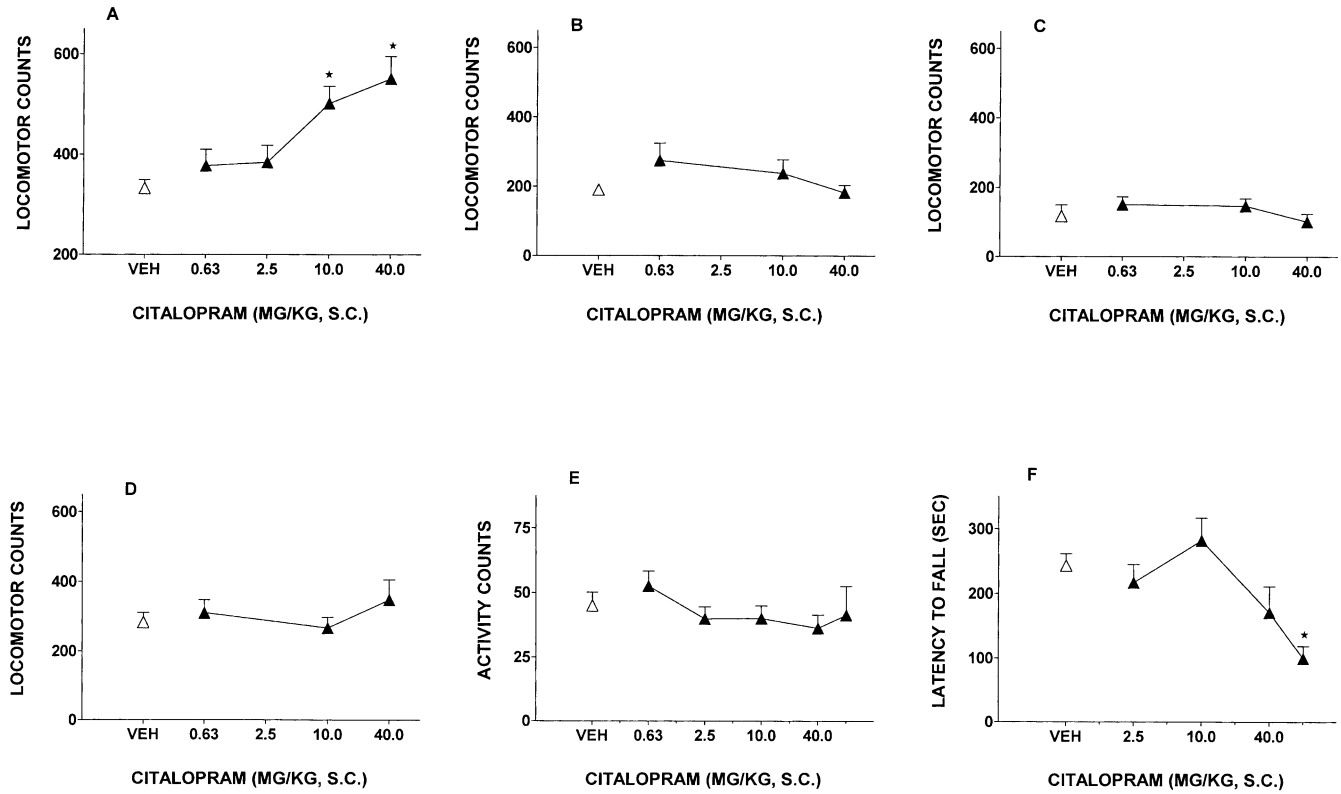

Fig. 1. Influence of the SSRI, citalopram, upon locomotor behaviour of mice exposed to a novel environment as compared to motor function under other experimental conditions (see Methods for details). Panel A: Locomotor activity of mice exposed to a novel environment. Panel B: Locomotor activity of mice following pre-exposure (30 min) to the environment. Panel C: Locomotor activity of mice following pre-exposure (60 min) to the environment. Panel D: Locomotor activity of mice following 4 days pre-exposure (10 min/day) to the environment. Panel E: Locomotor activity of rats exposed to a novel environment. Panel F: Induction of ataxia in the rotarod test in mice. Data are means ± S.E.M. $N > 6$ per value. ANOVA as follows. Panel A: $F(4,27) = 8.56$, $P < .001$; Panel B: $F(3,16) = 1.60$, $P > .05$; Panel C: $F(3,16) = 0.88$, $P > .05$; Panel D: $F(3,20) = 0.78$, $P > .05$; Panel E: $F(5,35) = 0.78$, $P > .05$; Panel F: $F(4,30) = 5.28$, $P < .01$. Asterisks indicate significance of drug to vehicle values in Dunnett's test following ANOVA. * $P < .05$.

In view of the significance of motor symptoms to depressive disorders, it is curious that the influence of serotonergic (and other) classes of antidepressant upon locomotor behaviour has been little examined. This is particularly true in view of the importance of locomotor activity (LA) as a potential variable in modifying the behaviour or rodents in diverse models of potential antidepressant activity (Willner, 1991). In fact, several studies have evaluated the influence of antidepressants upon LA in mice and rats in parallel with the performance of such studies (e.g., Griebel et al., 1994; Lightowler et al., 1994; Réméric and Lucki, 1998). Interestingly, an increase in LA in mice was observed with fluoxetine (5 mg/kg ip, De Angelis, 1996), although a high dose of 32 mg/kg was inactive in the study of Da-Rocha et al. (1997), as was fluvoxamine at doses of 2–16 (Da-Rocha et al., 1997) and 20 mg/kg (Njung'e and Handley, 1991). Citalopram (10 mg/kg ip, Griebel et al., 1994) was reported to increase LA in mice, although it was found to be inactive (20 mg/kg) by Njung'e and Handley (1991). Finally, venlafaxine (dose unspecified, Hascoët et al., 2000) also increased LA in mice. In contrast to these variable findings, there has been a highly consistent *failure* to find increases in LA with SSRIs and SNRIs in *rats* (e.g., Detke et al., 1995; Griebel et al., 1997; Joly and Sanger, 1986; Lightowler et al., 1994; Millan et al., in press-b; Réméric and Lucki, 1998; Silva and Brandão, 2000).

These data in mice, though intriguing, remain fragmentary in terms of the limited number of antidepressants for which data are available, the restricted dose ranges evaluated and, in particular, the lack of a significant body of comparative data simultaneously comparing actions of various classes of antidepressant agent. Nevertheless, inasmuch as increases in LA are elicited by drugs acting as 5-HT releasers, such as methylenedioxymethamphetamine and para-chloroamphetamine (Callaway et al., 1992; Fibiger and Campbell, 1971; Green et al., 1995; Rempel et al., 1993; White et al., 1996), they raise the possibility that an increase in extracellular levels of 5-HT via blockade of 5-HT reuptake may increase LA in mice.

The purpose of the present investigation was to systematically examine the influence of antidepressant agents upon spontaneous LA in mice. *First*, we employed a simple, automated and rapid system for evaluation of the influence of antidepressant agents upon the spontaneous LA of mice exposed to a *novel* environment. *Second*, in exploiting this parameter, we characterized the actions of a substantial number—and diverse classes—of antidepressant agents.

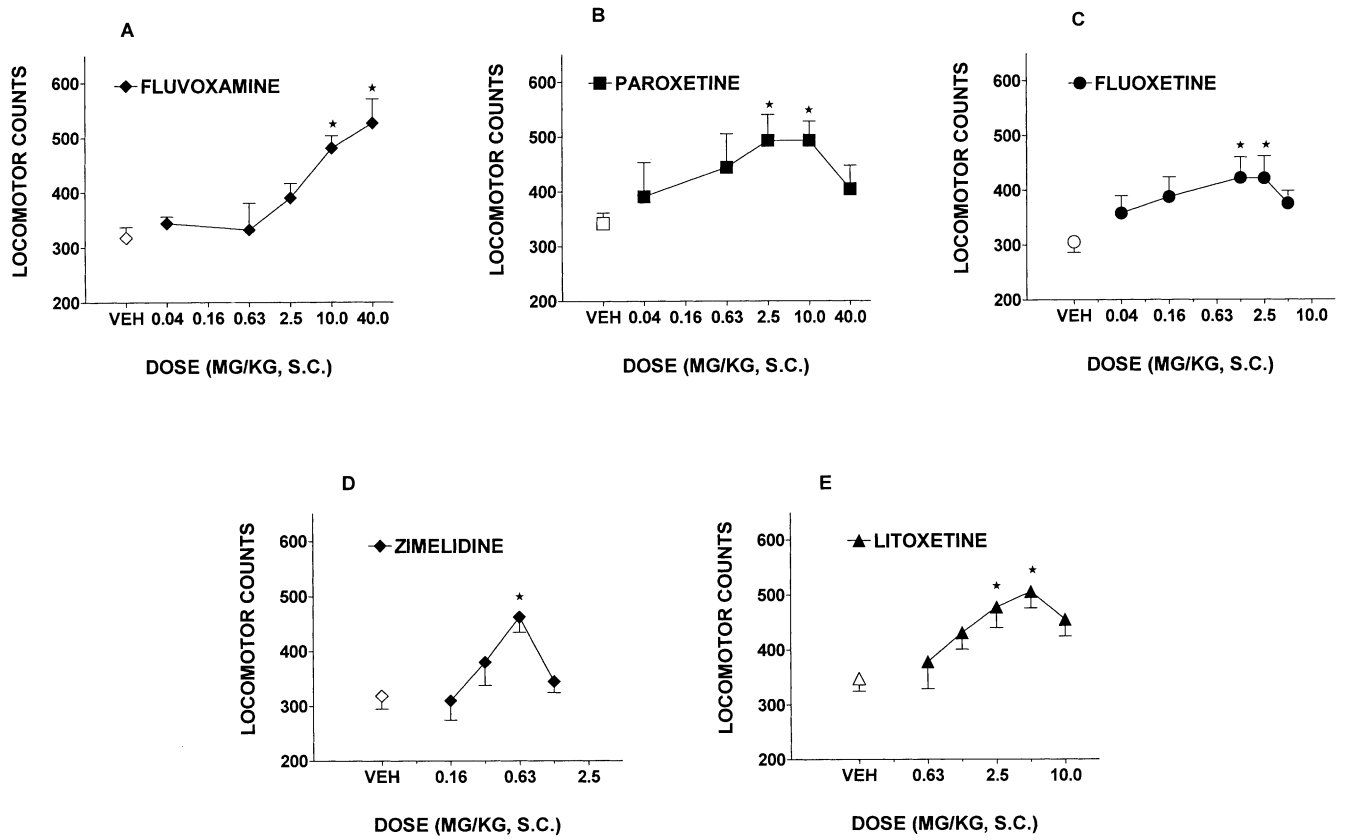

Fig. 2. Influence of the SSRIs, fluoxetine, fluvoxamine, litoxetine, paroxetine and zimelidine, upon locomotor activity of mice exposed to a novel environment. Data are means ± S.E.M. $N > 6$ per value. ANOVA as follows. Fluoxetine: $F(5,53) = 3.03$, $P < .05$; fluvoxamine: $F(5,41) = 8.50$, $P < .001$; litoxetine: $F(5,54) = 3.79$, $P < .01$; paroxetine: $F(5,36) = 2.49$, $P < .05$; zimelidine: $F(4,34) = 4.59$, $P < .01$. Asterisks indicate significance of drug to vehicle values in Dunnet's test following ANOVA. * $P < .05$.

Table 1
Summary of the influence of antidepressant agents upon locomotor behaviour

SPLOC mouse						ROTAROD		SPLOC rat	
Class	Drug	MED ↑	(% MOE)	MED ↓	(% MOE)	MED ↓	(% MOE)	MED ↓	(% MOE)
SSRI	Fluoxetine	1.25	38	>5	0	80	51	40	71
	Paroxetine	2.5	44	>40	18	40	59	40	44
	Citalopram	10	66	>40	0	80	59	>80	20
	Litoxetine	2.5	45	>10	31	40	80	10	71
	Zimelidine	0.63	45	>1.25	0	>10	12	NT	NT
	Fluvoxamine	10	66	>40	0	80	44	>40	23
SNRI	Venlafaxine	2.5	84	>40	0	80	49	40	60
	S33005	0.16	93	>10	0	40	35	80	22
	Duloxetine	2.5	101	>40	0	NT	NT	NT	NT
NARI	Reboxetine	>40	20	2.5	33	40	77	0.63	55
	Desipramine	>40	4	40	49	40	78	10	64
	Nisoxetine	>40	0	40	36	40	81	NT	NT
	Nortriptyline	>40	0	10	51	10	60	10	81
	Maprotiline	>40	15	40	71	>10	23	>40	35
TRICYCLIC	Amitriptyline	>40	0	2.5	69	2.5	87	>40	32
	Clomipramine	10	33	>40	32	40	70	>80	13
	Imipramine	>40	16	>40	11	>10	41	2.5	40
SSRI/5-HT_{2C}	Nefazodone	>40	0	40	53	40	85	NT	NT
	Trazodone	>40	0	2.5	80	0.63	91	0.63	91
	YM_1	>40	0	10	84	10	54	10	71
5-HT_{2C}/α_2-AR	Mianserin	>10	6	2.5	78	10	54	40	76
	Mirtazapine	>40	0	10	73	20	58	10	49

MED = minimal effective dose (mg/kg sc); % MOE = % maximal observed effect; SPLOC = spontaneous locomotion of mice or rats unfamiliar with the activity chamber; ROTAROD = ataxia of mice in the rotarod test; NT = not tested.

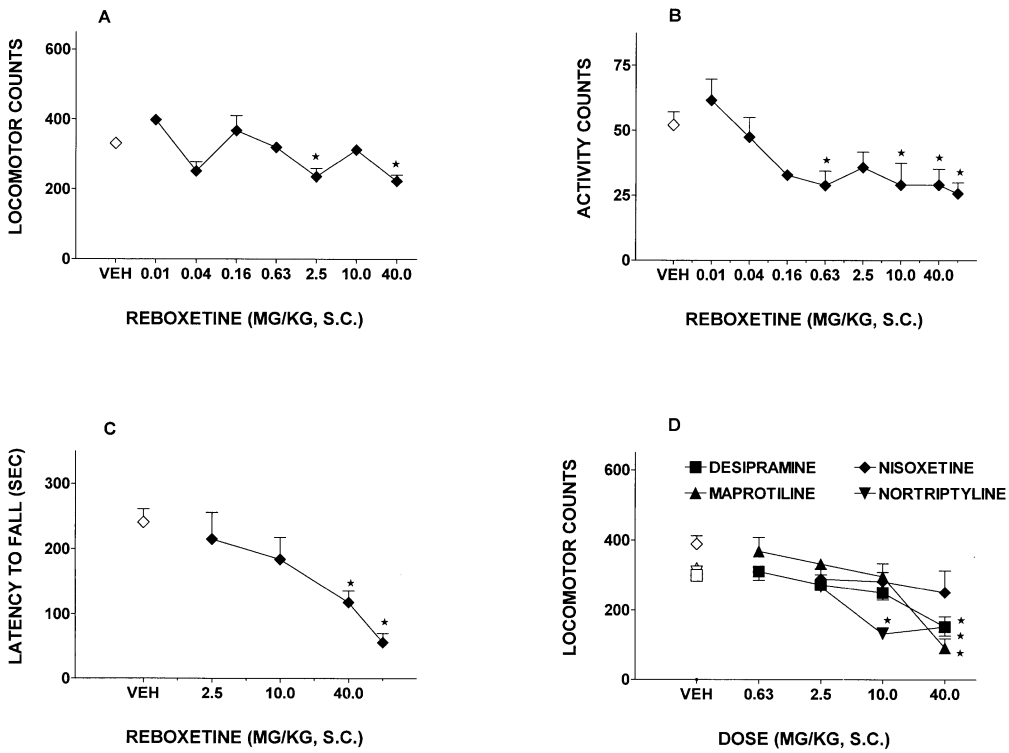

Fig. 3. Influence of the NARIs, reboxetine, desipramine, maprotiline, nisoxetine and nortriptyline, upon locomotor behaviour of mice exposed to a novel environment as compared (reboxetine only) to motor function under other experimental conditions. Panels A and D: Locomotor activity of mice exposed to a novel environment. Panel B: Locomotor activity of rats exposed to a novel environment. Panel C: Induction of ataxia in the rotarod test in mice. Data are means ± S.E.M. $N > 6$ per value. ANOVA as follows. Panel A: reboxetine, $F(7,48) = 4.85$, $P < .001$; Panel B: reboxetine, $F(7,43) = 3.79$, $P < 0.01$; Panel C: reboxetine, $F(4,31) = 8.65$, $P < .001$; Panel D: desipramine, $F(4,35) = 8.16$, $P < .001$; maprotiline, $F(4,33) = 14.83$, $P < .001$; nisoxetine, $F(3,15) = 2.63$, $P > .05$; nortriptyline, $F(3,22) = 11.86$, $P < .001$. Asterisks indicate significance of drug to vehicle values in Dunnett's test following ANOVA. * $P < .05$.

Their influence upon LA in rats was also determined. *Third*, we determined the specificity of any changes observed to these conditions in evaluating the actions of the highly selective SSRI, citalopram, and the SNRI, venlafaxine, upon LA of mice already familiarized with the locomotor chamber. *Fourth*, for all drugs, doses modulating LA were compared to those eliciting a generalized perturbation of motor function in the rotarod test in mice.

2. Methods

2.1. Animals

Locomotion experiments used male Wistar rats weighing 250–300 g (Iffa-Credo, L'Arbresle, France) and male NMRI mice weighing 22–28 g (Iffa-Credo, L'Arbresle, France). Rotarod studies were carried out on male NMRI mice weighing 25–30 g at the time of the experiment. They were maintained in sawdust-lined cages with unrestricted access to food and water. Laboratory temperature was 21 ± 1 °C and humidity, 60 ± 5%. There was a 12-h light/dark cycle, with lights "on" at 7:30 a.m. All animal use procedures conformed with international European ethics standards (86/609-CEE) and the French National Committee (décret 87/848) for the care and use of laboratory animals.

2.2. Evaluation of LA in mice

Twenty hours before testing, the mice were isolated into transparent polycarbonate cages (23 × 13 × 13 cm) with sawdust floor covering and free access to chow and water. Testing was performed in the morning, starting at 09:00 h. The test cage was made of white plexiglass (27 × 27 × 30 cm) and was illuminated with a 6-W light. Photocells (four on each of two walls facing each other) were located 6 cm apart, 2 cm above the floor and connected via an interface (Osys-Orga System, Changé, France) to a computer. Software was written by Hesperid, Loiron, France. The interruption of two adjacent beams corresponded to a locomotion count.

2.3. Testing of mice unfamiliar with the activity chamber

In the majority of studies, mice unfamiliar with the activity chamber were treated either with drug or vehicle in their home cage. Thirty minutes later, they were placed into the illuminated test cage for a 10-min period of observation.

2.4. Testing of mice habituated to the activity chamber

The influence of "novelty" upon the locomotor effects of citalopram and venlafaxine was evaluated in mice which had been pre-exposed to the test chamber using one of the

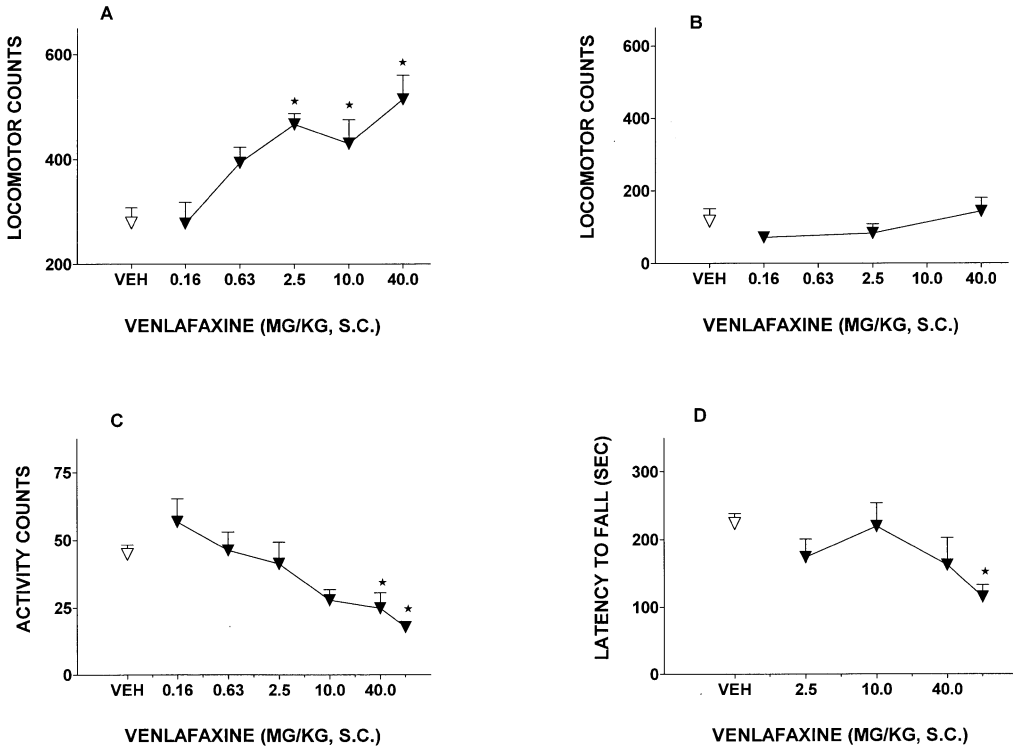

Fig. 4. Influence of the SNRI, venlafaxine, upon locomotor behaviour of mice exposed to a novel environment as compared to motor function under other experimental conditions. Panel A: Locomotor activity of mice exposed to a novel environment. Panel B: Locomotor activity of mice following pre-exposure (60 min) to the environment. Panel C: Locomotor activity of rats exposed to a novel environment. Panel D: Induction of ataxia in the rotarod test in mice. Data are means ± S.E.M. $N > 6$ per value. ANOVA as follows. Panel A: $F(5,32) = 7.61$, $P < .001$; Panel B: $F(3,16) = 1.28$, $P > .05$; Panel C: $F(5,41) = 3.64$, $P < .01$; Panel D: $F(3,41) = 1.49$, $P > .05$. Asterisks indicate significance of drug to vehicle values in Dunnett's test following ANOVA. * $P < .05$.

following habituation protocols: *Protocol 1*, on the test day, mice were injected with drug or vehicle and immediately placed into the activity cage. Thirty minutes later, locomotor behaviour was recorded during a 10-min period; *Protocol 2*, on the test day, mice were placed into the activity cage for 30 min. Then, they were administered either drug or vehicle. Thirty minutes later, locomotor behaviour was recorded during a 10-min period; *Protocol 3*, over the 4 days preceding the test day, mice (housed per six in a cage) were placed individually into the activity chamber for a daily 10-min period of habituation. After the fourth pre-exposure to the test cage, i.e., 20 h before test day, they were transferred into individual transparent polycarbonate cages, and testing was performed as described above for unfamiliar conditions.

2.5. LA in rat

As described previously (Dekeyne et al., 2000a; Millan et al., 2001a,b), rats were administered drug or vehicle and maintained (three or four per cage) in their home cages for 30 min. Thereafter, they were placed individually into sawdust-lined transparent polycarbonate cages ($30 \times 18 \times 19$ cm) between two infrared beams (18 cm apart, 4 cm above the floor) in the activity chambers, for a 12-min period of ambulatory recording. The photocells were connected through an interface (Coulbourn Instruments, PA, USA) to a computer with appropriate software. The consecutive interruption of two beams was recorded as a locomotion count.

2.6. Induction of ataxia in mice

As described previously (Millan et al., 1997), 30 min after drug or vehicle injection, mice were placed on the bar of a Rotarod apparatus (Ugo Basile, Varese, Italy), which rotated with a gradual acceleration from 4 to 40 rpm over a period of 300 s. The latency of mice to fall was determined with a cut-off of 360 s.

2.7. Statistics

Dose–effects were analyzed employing a one-way ANOVA followed by Dunnett's test. Inhibitory doses (ID_{50}) plus 95% confidence limits (CL) and minimal effective doses (MEDs) were calculated when appropriate. The percentage maximal observed effect (% MOE) corresponded to the largest increase (or decrease) relative to vehicle values.

2.8. Drugs

In all procedures, full dose–response relationships were evaluated, with a maximal dose of 40 mg/kg, solubility permitting, for studies of LA in mice, and of 80 mg/kg for the rotarod procedure in mice and LA in rats. All drug doses are in terms of the base. Drugs were dissolved in distilled water. For duloxetine, fluoxetine, fluvoxamine, maprotiline, mianserin, mirtazapine, nortriptyline and paroxetine, a few drops of lactic acid were added and the pH adjusted to as close to neutrality as possible (>5.0). Drugs were administered subcutaneously in a volume of 1 ml/kg (rat) or 10 ml/kg (mice). Drug names, sources and salts were as follows. Amitriptyline, clomipramine, desipramine, imipramine, maprotiline and nortriptyline hydrochlorides (Sigma Chimie, St. Quentin-Fallavier, France); trazodone hydrochloride and zimelidine dihydrochloride (Research Biochemicals International, MA, USA); fluvoxamine maleate (Tocris, Bristol, UK); fluoxetine hydrochloride (Cilag, Schaffhausen, Switzerland); duloxetine HCl and nisoxetine HCl (Eli Lilly and Co., USA) and nefazodone HCl (Bristol-Meyers, USA). Mirtazapine base, citalopram HBr, YM_1 (3-{3-[4-(7-Fluoro-indan-4-yloxy)-piperidin-1-yl]}propylamino carbonyl}-pyridine fumarate, mianserin HCl, paroxetine HCl and venlafaxine HCl were synthetised by G. Lavielle (Institut de Recherches Servier). Reboxetine methanesulfonate, and the isomers, (−)S33005 HCl and (+)S33004 HCl (1-(*N*-*N*-dimethylaminomethyl)-1-(1-hydroxycyclohex-1-yl)-5-methoxy benzo cyclobutene) were synthetised by J.-L. Péglion (Institut de Recherches Servier).

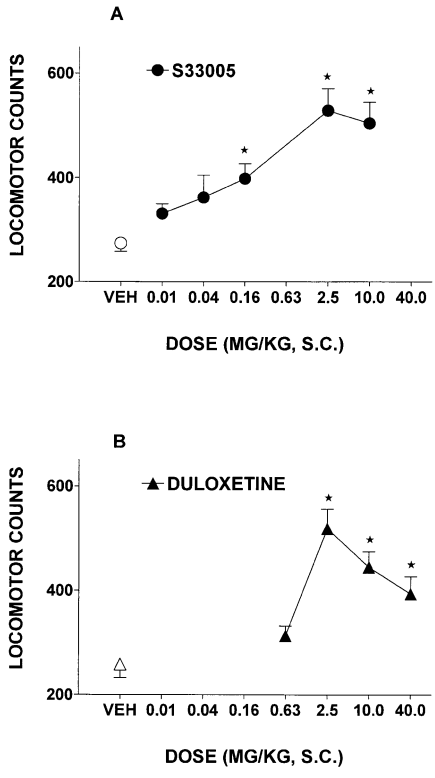

Fig. 5. Influence of the SNRIs, duloxetine and S33005, upon locomotor activity of mice exposed to a novel environment. Data are means ± S.E.M. $N > 6$ per value. ANOVA as follows. Panel A: S33005, $F(6,40) = 7.58$, $P < .001$ and Panel B: duloxetine, $F(4,27) = 13.01$, $P < .001$. Asterisks indicate significance of drug to vehicle values in Dunnett's test following ANOVA. * $P < .05$.

3. Results

3.1. Influence of SSRIs upon LA in mice exposed to a novel activity chamber, as compared to motor function under other conditions

The SSRI, citalopram (Fig. 1), elicited a dose-dependent and pronounced increase in LA in mice exposed to the novel activity chamber. In mice treated with a similar dose-range but pre-exposed to the activity chamber under three different conditions, no significant increase in LA was observed. In rats tested under equivalent conditions and naive to the test chamber, no increase in LA was seen. At doses, which elevated LA in mice unfamiliar with the activity chamber, citalopram did not elicit ataxia in the rotarod test in mice. A similar pattern of data was obtained for several other SSRIs, fluoxetine, fluvoxamine, paroxetine, litoxetine and zimelidine (Fig. 2), all of which significantly increased LA in mice exposed to a novel environment, although dose–responses were biphasic with an inflection at the highest dose tested. Likewise, in analogy to citalopram, they did not elevate LA in rats introduced into a novel chamber, and they did not elicit ataxia in the rotarod test at doses enhancing LA in naive mice (Table 1).

3.2. Influence of NARIs upon LA in mice exposed to a novel activity chamber, as compared to motor function under other conditions

In contrast to SSRIs, the NARI, reboxetine, did not significantly modify LA of mice exposed to a novel environment. At several doses, LA was significantly reduced. Similarly, reboxetine reduced LA in naive rats placed in an activity chamber. At the highest doses tested, it elicited a modest ataxia in the rotarod test. Similarly, several other NARIs, desipramine, maprotiline, nisoxetine and nortriptyline failed to elevate LA in mice exposed to novel environment and the latter actually reduced LA. Nortriptyline also elicited marked ataxia in the rotarod procedure (Fig. 3 and Table 1).

3.3. Influence of SNRIs upon LA in mice exposed to a novel activity chamber as compared to motor function under other conditions

The SNRI, venlafaxine (Fig. 4), elicited a dose-dependent, significant and marked enhancement in LA of naive mice placed in the activity chamber, whereas pre-exposed mice showed no such increase. At high doses, venlafaxine slightly decreased LA in rats exposed to a novel envi-

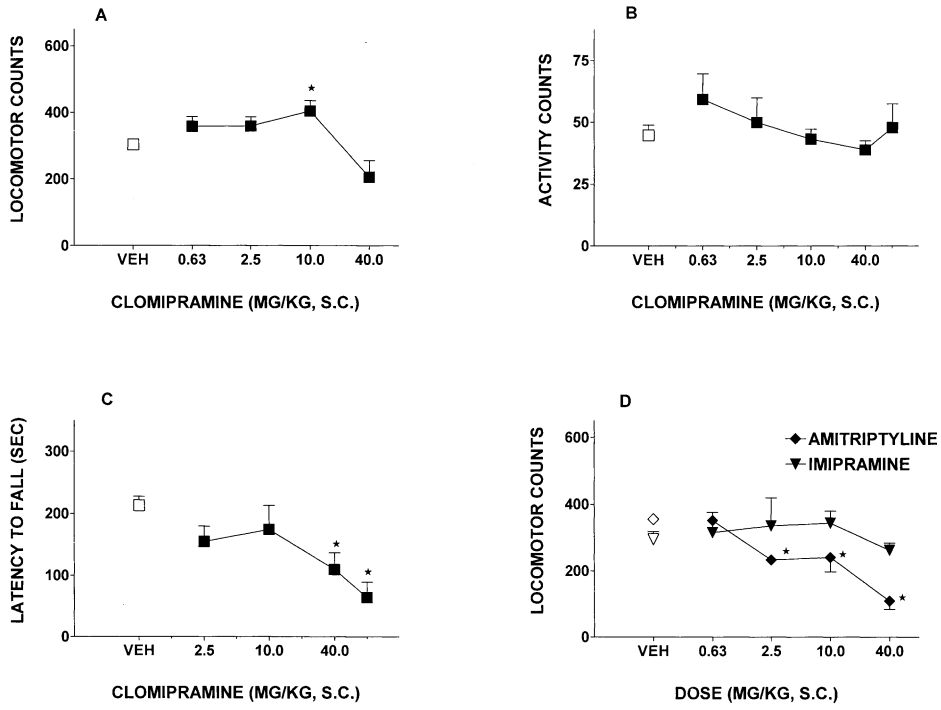

Fig. 6. Influence of the tricyclic agents, amitriptyline, clomipramine and imipramine upon locomotor behaviour of mice exposed to a novel environment as compared (clomipramine only) to motor function under other experimental conditions. Panels A and D: Locomotor activity of mice exposed to a novel environment. Panel B: Locomotor activity of rats exposed to a novel environment. Panel C: Induction of ataxia in the rotarod test in mice. Data are means ± S.E.M. $N > 6$ per value. ANOVA as follows. Panel A: clomipramine, $F(4,47) = 5.89$, $P < .001$; Panel B: clomipramine, $F(4,32) = 1.22$, $P > .05$; Panel C: clomipramine, $F(3,38) = 3.04$, $P < .05$; Panel D: amitriptyline, $F(4,31) = 18.5$, $P < .001$; imipramine, $F(4,28) = 0.49$, $P > .05$. Asterisks indicate significance of drug to vehicle values in Dunnett's test following ANOVA. * $P < .05$.

ronment. It was inactive in the rotarod test. The SNRIs, duloxetine and S33005, also increased LA in mice placed in a novel environment (Fig. 5). Duloxetine exerted this action at doses well below those eliciting ataxia in the rotarod procedure (Table 1). Further, S33005 did not elicit ataxia in the rotarod test, S33005 did not modify LA of naive rats (not shown). Like venlafaxine, further, LA of mice pre-exposed to the activity chamber was not significantly modified by S33005 (not shown). The action of S33005 was expressed stereospecifically inasmuch as its inactive isomer, S33004, tested at a dose maximally effective for S33005, did not significantly increase LA: vehicle, $n=7$, 298.6 ± 27.2 locomotor counts vs. S33004 (2.5 mg/kg sc), $n=7$, 347.4 ± 21.8, $P>.05$.

3.4. Influence of tricyclic agents upon LA in mice exposed to a novel activity chamber as compared to motor function under other conditions

The tricyclic, CMI, elevated LA of mice placed in the unfamiliar activity chamber only at a single dose (10.0 mg/kg), and this effect was modest. Further, the LA of naive rats was not enhanced by CMI, which also did not elicit ataxia. Amitriptyline and imipramine, two further tricyclic agents, failed to increase LA of naive mice at doses lower than those perturbing performances in the rotarod test. They did not modify LA of rats in a novel environment (Fig. 6 and Table 1).

3.5. Influence of SSRIs/5-HT$_2$ antagonists upon LA in mice exposed to a novel activity chamber as compared to motor function under other conditions

Trazodone, a mixed inhibitor of 5-HT reuptake and antagonist of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, significantly decreased LA of naive mice placed in the activity chamber. It also reduced LA in naive rats and elicited ataxia. Nefazodone, which possesses a similar pharmacological profile as trazodone, also failed to provoke LA in naive mice and elicited ataxia in the rotarod test. YM$_1$, which also acts as an inhibitor of 5-HT reuptake and 5-HT$_{2A/2C}$ receptor antagonist reduced LA of naive mice. It also elicited mild ataxia, but did not modify LA in naive rats (Fig. 7 and Table 1).

3.6. Influence of 5-HT$_2$/α_2-AR antagonists upon LA in mice exposed to a novel activity chamber as compared to motor function under other conditions

Mirtazapine, which is devoid of affinity for SERTs and which behaves as an antagonist at 5-HT$_{2A/2C}$ receptors and α_2-AR receptors, diminished LA of naive mice introduced into the activity chamber. It also elicited ataxia and signifi-

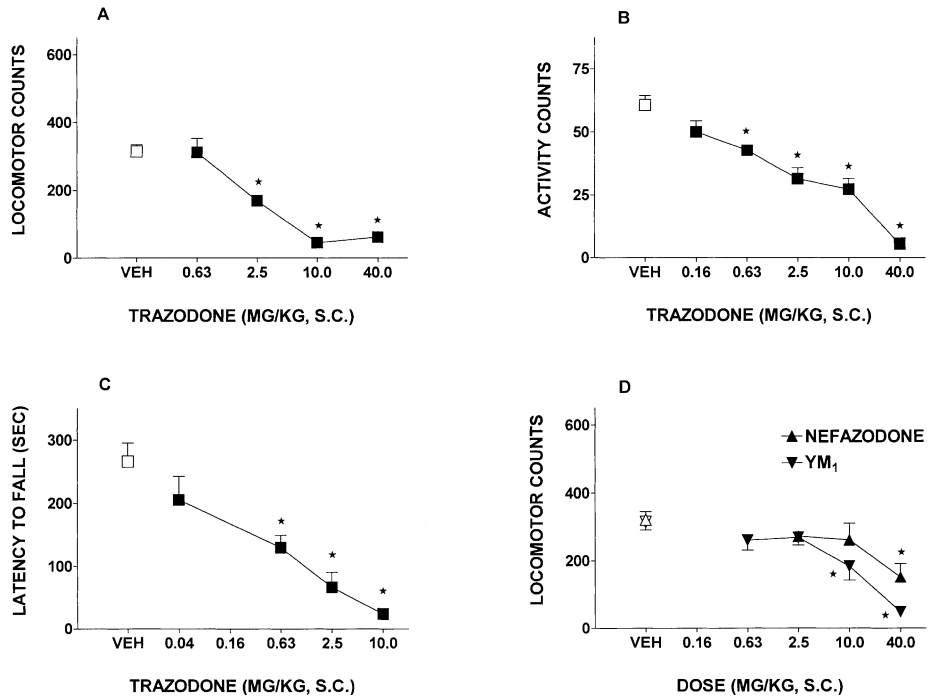

Fig. 7. Influence of the SSRI/5-HT$_2$ antagonists, nefazodone, trazodone and YM$_1$, upon locomotor behaviour of mice exposed to a novel environment as compared (trazodone only) to motor function under other experimental conditions. Panels A and D: Locomotor activity of mice exposed to a novel environment. Panel B: Locomotor activity of rats exposed to a novel environment. Panel C: Induction of ataxia in the rotarod test in mice. Data are means ± S.E.M. $N>6$ per value. ANOVA as follows. Panel A: trazodone, $F(4,26)=29.2$, $P<.001$; Panel B: trazodone, $F(5,35)=31.83$, $P<.001$; Panel C: trazodone, $F(4,29)=14.9$, $P<.001$; Panel D: nefazodone, $F(3,26)=5.03$, $P<.01$; YM$_1$, $F(3,24)=18.2$, $P<.001$. Asterisks indicate significance of drug to vehicle values in Dunnett's test following ANOVA. $*P<.05$.

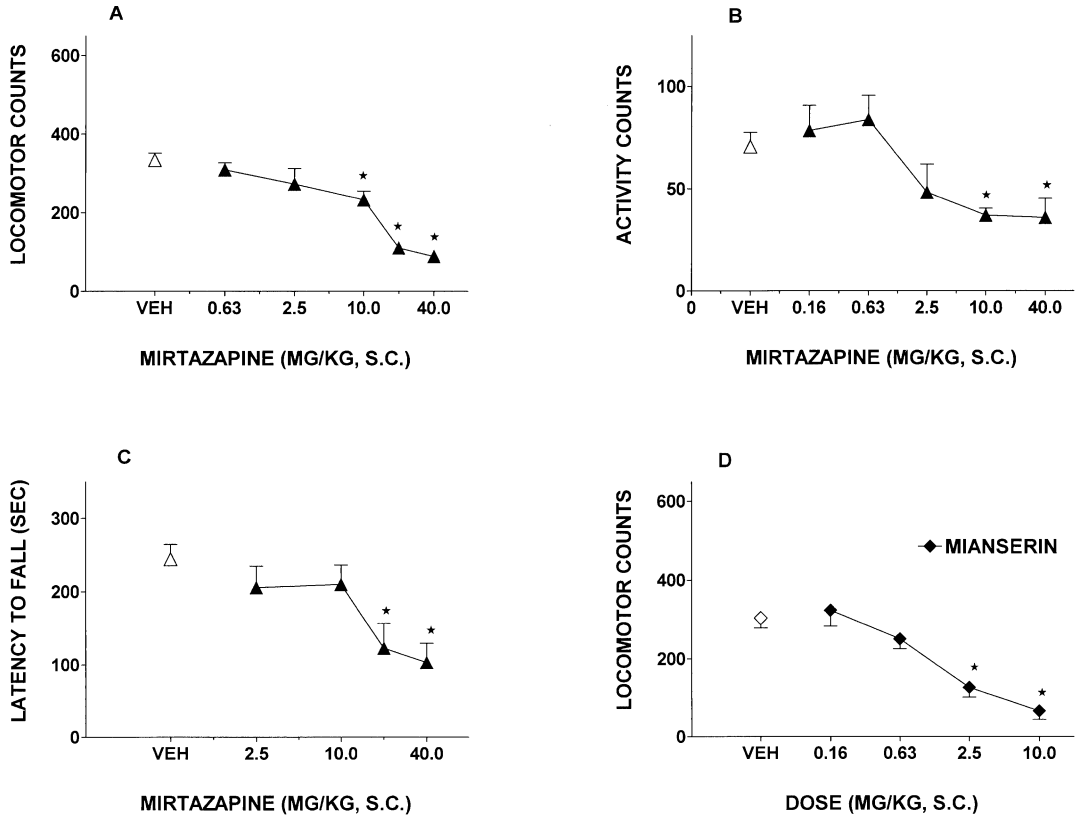

Fig. 8. Influence of the 5-HT_{2A}/α_2-AR antagonists, mianserin and mirtazapine, upon locomotor behaviour of mice exposed to a novel environment as compared (mirtazapine only) to motor function under other experimental conditions. Panels A and D: Locomotor activity of mice exposed to a novel environment. Panel B: Locomotor activity of rats exposed to a novel environment. Panel C: Induction of ataxia in the rotarod test in mice. Data are means ± S.E.M. $N>6$ per value. ANOVA as follows. Panel A: mirtazapine, $F(5,45)=21.04$, $P<.001$; Panel B: mirtazapine, $F(5,50)=4.35$, $P<.01$; Panel C: mirtazapine, $F(4,38)=5.48$, $P=.001$; Panel D: mianserin, $F(4,33)=13.77$, $P<.001$. Asterisks indicate significance of drug to vehicle values in Dunnett's test following ANOVA. * $P<.05$.

cantly diminished LA in naive rats. The chemically related mianserin, which likewise acts as an 5-$HT_{2A/2C}$ and α_2-AR antagonist and lacks affinity for SERTs, decreased LA in naive mice and rats, and elicited ataxia in the rotarod procedure (Fig. 8 and Table 1).

4. Discussion

4.1. Induction of LA in mice exposed to a novel environment by blockade of 5-HT reuptake: pharmacological specificity

The present study constitutes the first systematic and comparative evaluation of the influence of diverse classes of antidepressant agent upon LA in rodents, and specifically focusses on their modulation of spontaneous LA of mice exposed to a novel environment (activity chamber). The data demonstrates a consistent and robust elevation in LA with chemically diverse SSRIs and SNRIs. Several observations provide compelling evidence for a role of SERTs in this effect.

First, an interaction with SERTs is a common property of all drugs which enhanced LA. Possible direct actions at 5-HT receptors are unlikely. Indeed, citalopram is the most selective SSRI described to date (Popik, 1999; Millan et al., 2000a, 2001a). Although citalopram and other SSRIs display weak antagonist activity at 5-HT_{2A} and 5-HT_{2C} receptors (Jenck et al., 1998; Pälvimäki et al., 1996), this property cannot underlie an increase in LA under the present conditions inasmuch as (1) selective 5-HT_{2C} antagonists, such as SB-242084 and SB-206553, do not elevate LA (Dekeyne et al., 2000b; Kennett et al., 1997; Vickers et al., 2000); (2) the SNRIs, venlafaxine and S33005, are highly selective agents devoid of affinity for these receptors and all other sites examined (Millan et al., 2001a); (3) despite their 5-$HT_{2A/2C}$ antagonist properties, the tricyclic agents, mirtazapine and mianserin (Frazer, 1997; Millan et al., 2000a; Owens et al., 1997), failed to enhance LA and 4), Nefazodone, trazodone and YM_1, SSRIs with 5-$HT_{2A/2C}$ antagonist properties (Owens et al., 1997; Tatsumi et al., 1997), also did not elevate LA. Indeed, it remains unclear why the latter agents did not similarly enhance LA in naive mice. One possibility is that blockade of 5-HT_2 receptors interferes with an induction of LA via SERTs. This is unlikely, however, since 5-HT_{2C} receptor activation *decreases* LA (Kennett et al., 1997; Martin et al., 1998).

Further, blockade of 5-HT_{2A} and 5-HT_{2C} receptors do not prevent induction of LA by citalopram (Brocco, unpublished observations). Alternatively, antagonist properties at α_1-ARs and/or histamine$_1$ receptors (Mir and Taylor, 1997; Owens et al., 1997; Tatsumi et al., 1997), may mask SERT-mediated increases in LA not only for trazodone, nefazodone and YM_1, but also for the tricyclics, CMI, amitriptyline and imipramine which, despite their interaction with SERTs did not consistently increase LA. Indeed, mianserin and mirtazapine, which are similarly potent antagonists at H_1 receptor and α_1-ARs, both decreased LA herein (Millan et al., 2000a; Owens et al., 1997; Tatsumi et al., 1997).

Second, the induction of LA was observed with 6 chemically diverse SSRIs, all of which show markedly lower affinity for NA transporters- and negligible affinity for dopamine (DA) transporters (Owens et al., 1997; Tatsumi et al., 1997). SSRIs selectively enhance extracellular levels of 5-HT versus NA (and DA) throughout the limbic system, excluding a direct interaction with subcortical adrenergic and dopaminergic pathways in the mediation of LA. Although high doses of SSRIs elicit significant elevations in extracellular levels of NA and DA in FCX, there is no evidence that such effects facilitate LA (Jordan et al., 1994; see Millan et al., 2000b; Pozzi et al., 1999). Further, as shown herein, NARIs, which elicit more potent and more pronounced increases in extracellular levels of NA (and DA) in FCX than SSRIs, *failed* to elicit LA. Moreover, via blockade of inhibitory 5-HT_{2C} and α_2-ARs on frontocortical adrenergic pathways, mirtazapine and mianserin similarly facilitate NA and DA release in FCX (Millan et al., 2000a), yet they also did *not* increase LA.

Third, the novel cyclohexanol derivative, S33005, stereospecifically elicited LA, as compared to its less active isomer, S33004, which possesses substantially ($\sim$100-fold) lower affinity for native, rat and cloned, human SERTs (Millan et al., 2001a, b).

Fourth, doses of SSRIs and SNRIs effective in eliciting LA correspond well to those active upon acute administration in other models of potential antidepressant activity in rodents (Detke et al., 1995; Millan et al., 2001a, b; Popik, 1999; Schweizer et al., 1997).

Fifth, doses of SSRI and SNRIs increasing LA were all below those which elicit generalized alterations in motor function as revealed by the rotarod procedure. Interestingly, the increase in LA was a species-specific phenomenon inasmuch as LA was not increased in rats introduced into a novel environment, in line with previous observations (see Section 1). This provides an interesting distinction to the 5-HT releasers, PCA and MDMA, which elevate LA in both species (Callaway et al., 1991; Brocco, unpublished observations). Moreover, likewise in contrast to MDMA and PCA, an elevation in LA upon blockade of 5-HT reuptake was specifically expressed by naive mice, with pre-exposure to the activity chamber eliminating this action (see below).

4.2. Physiological significance and psychological bases of increases in LA in a novel environment

"Psychological" factors may underlie the elevation in LA. In this regard, previous studies have emphasized the role of increased "arousal" and "exploratory drive" in the facilitation of LA via enhancement of serotonergic transmission (Callaway et al., 1992; Fibiger and Campbell, 1971; Paulus and Geyer, 1992). However, these terms remain somewhat nebulous, and several, more precisely defined variables may be evoked.

First, it is unlikely that the increase in LA purely reflects an enhancement of *motor* function. Thus, for drugs which facilitate subcortical dopaminergic activity, such as selective DA reuptake inhibitors, their enhancement of LA in rodents (Millan et al., 2000b; Rahman et al., 2001) is equally robust in rats and mice, is associated with a massive elevation in extracellular levels of DA in subcortical regions and is expressed in animals both unfamiliar *and familiar* with the environment (Millan et al., 2000c; Rahman et al., 2001). On the other hand, the present elevation in LA was restricted to mice exposed to a novel chamber, and, as alluded to above, SSRIs and SNRIs do *not* potentiate mesolimbic dopaminergic transmission (Millan et al., 2000b, 2001b; Prisco and Esposito, 1995; Sakaue et al., 2000). Indeed, there is no experimental evidence for "psychostimulant"-like effects of SSRIs and SNRIs, an assertion underpinned by extensive clinical experience (Burke and Preskorn, 1995; Frazer, 1997).

Second, mice may display improved cognitive-attentional function, being more responsive to and more actively exploring a novel environment. Indeed, there is an extensive literature implicating serotonergic mechanisms in mnesic processes (Meneses, 1999; Steckler and Sahgal, 1995). However, there is little direct evidence to support a facilitatory influence of increases in extracellular levels of 5-HT upon attentional processes per se, and experimental and clinical studies of SSRIs have yielded complex, situation-dependent patterns of data as regards their variable, positive and/or negative influence upon cognitive processes (Amado-Boccara et al., 1995; Meneses, 1999; Ruotsalainen et al., 2000; Steckler and Sahgal, 1995). Further, in the present paradigm, several classes of "pro-cognitive" agent, including muscarinic agonists and 5-HT_{1A} antagonists, do *not* enhance LA (Brocco, unpublished observations). Thus, the increase in LA cannot be attributed purely to cognitive-attentional factors.

Third, as concerns the possible role of "anxiety" (Griebel, 1995), it should be emphasized that the present studies employed *acute* administration. Initial treatment with SSRIs and SNRIs tends to exacerbate anxious symptoms in patients yet, upon long-term administration, they alleviate anxious states (Brunello et al., 2000; Feighner, 1999; Mason et al., 1997; Millan et al., 2001b; Nutt, 2000). Studies of methylenedioxymethamphetamine have indicated that 5-HT may exert anxiogenic or anxiolytic actions dependent upon the

test conditions (Morley and McGregor, 2000) and it appears that SSRIs similarly act differentially as a function of the experimental paradigm. Thus, while acute administration of SSRIs and SNRIs elicits robust anxiogenic actions in certain models, notably social interaction and plus-maze paradigms in rats (Bristow et al., 2000; Dekeyne et al., 2000a,b; Duxon et al., 1998; Millan et al., unpublished observations), certain studies have reported anxiolytic actions of SSRIs in conflict paradigms, exploratory models and the ultrasonic vocalization test (Griebel et al., 1994; Hascoët et al., 2000; Hashimoto et al., 1996; Sánchez and Meier, 1997; Schreiber et al., 1998; Silva and Brandão, 2000). It is conceivable, thus, that a reduction in anxiety is involved in the increase in LA seen in naive mice. This possibility is further supported by the absence of an increase of LA in pre-adapted mice and by the enhancement of LA elicited in unfamiliar mice under the present conditions by the anxiolytic agents, clorazepate and alprazolam (Brocco, unpublished observations). However, not all anxiolytic agents increase LA in the present model: for example, 5-HT_{1A} agonists, CRF_1 antagonists and 5-HT_{2C} antagonists are inactive (Dekeyne, 2000a; Millan et al., 2000a; Dekeyne, unpublished observations). Further, although mirtazapine, mianserin, trazodone and nefazodone all possess anxiolytic actions (Brocco, unpublished observations; Hascoët et al., 2000; Mason et al., 1997; Nutt, 1996), they also did *not* increase LA under the present conditions.

4.3. Differentiation of SSRIs/SNRIs versus NARIs

It is important to emphasize the contrasting actions of SSRIs as compared to NARIs in the present paradigm. Inasmuch as behavioural paradigms exploited for the detection of antidepressant agents are generally designed to respond to *all* clinically effective antidepressant drugs (Willner, 1991), there is little information concerning behavioural models which clearly differentiate various classes (Millan et al., 1999, 2000b, 2001a,b). This is unfortunate, since tryptophan versus tyrosine depletion studies indicate that certain patients present deficits of either serotonergic *or* adrenergic transmission (Berman et al., 1999; Delgado and Moreno, 1999; Smith et al., 1999). Further, several recent studies have permitted differentiation of the clinical actions of NARIs as compared to SSRIs in depressed patients (Dubini et al., 1997; Eriksson, 2000; Healy and McMonagle, 1997; Massana et al., 1999; Schatzberg, 2000). Clearly, the simple paradigm described herein unambiguously distinguishes SSRIs (and SNRIs) from NARIs.

4.4. Limitations of the present study

Finally, several limitations of the present study should be recognized. *First*, while the automated procedure employed focussing on a single, invariant behavioural parameter, has the advantages of rapidity, simplicity and efficiency, it obviously provides only restricted information concerning the behavioural repertoire of subjects. Extensive characterization of open-field, plus-maze and ''defensive behaviour'' paradigms have exemplified the importance of a fuller characterization of behaviour (Blanchard et al., 1997; Cruz et al., 1994; Rodgers, 1997) and such information would be of interest to generate with the present paradigm. *Second*, as for many other empirical models pragmatically utilized for evaluation of potential antidepressant agents, the present protocol employed single, acute administration of drugs. However, several weeks of treatment is required for full expression of their therapeutic activity, so studies of the actions of SSRIs and SNRIs in the present model upon long-term administration would be of interest. *Third*, the present study adopted systemic drug administration in view of its clinical relevance. Thus, additional mechanistic studies are required to identify precise neuronal substrates, and 5-HT receptor subtypes, underlying the induction of LA. *Fourth*, it has been suggested that the inhibition by SSRIs, SNRIs and NARIs of α_2-AR-mediated sedation may provide insights into their influence upon psychomotor retardation, though *not* providing a model of this deficit per se (Gower and Marriott, 1980; Millan et al., 2001b; Von Voigtlander et al., 1978). It is obviously tempting, thus, to relate the elevation in LA with SSRI and SNRI to the ability of antidepressant agents to improve symptoms of psychomotor retardation. However, such extrapolations should be made only cautiously, in particular in light of the lack of influence of other classes of antidepressant upon LA. *Finally*, no increase in LA was elicited by drugs inhibiting 5-HT reuptake in rats and this study was undertaken with a *single* mice strain (NMRI). To our knowledge, the influence of SSRIs upon spontaneous LA has not previously been described for this strain, while a variable pattern of increases and decreases in spontaneous LA have been obtained with SSRIs in CD (Charles River) (De Angelis, 1996; Da-Rocha et al., 1997; Griebel et al., 1994) and MF1 (Aston-bred) (Njung'e and Handley, 1991) strains (see Section 1). Notably, differences in (basal) LA have been reported amongst various mice strains in other studies (Misslin et al., 1989; Crawley and Davis, 1982). Correspondingly, caution should be exercised in extrapolating the present observations to man.

4.5. Conclusion

In conclusion, the present study concretizes several, disparate, anecdotic and generally neglected observations (see Introduction) in rigorously demonstrating that SSRIs and SNRIs enhance the LA of mice exposed to a novel environment. Further, they demonstrate that this response clearly differentiates drugs inhibiting 5-HT as compared to NA reuptake, that it is unique to mice as compared to rats and that it is *specific* to mice introduced into a novel as compared to a familiar enviroment. The latter observation strongly suggests that purely motor factors are unlikely to account for this increase in LA, which likely reflects an enhancement of arousal incorporating a reduction of anxiety and, possibly a facilitation of attentional processes. This remains to be

further clarified. This procedure provides a rapid, simple and instructive paradigm for characterization and differentiation of various classes of antidepressant agent and may offer interesting insights both into their influence upon psychomotor function and their mechanisms of activity.

Acknowledgments

We thank Jimmy Mulot and Huguette Gressier for technical assistance and Marianne Soubeyran for secretarial assistance.

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