European Journal of Pharmacology 602 (2009) 66–72

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Behavioural Pharmacology

The MCH_1 receptor antagonist SNAP 94847 induces sensitivity to dopamine D_2/D_3 receptor agonists in rats and mice

Douglas A. Marsteller [a], Christophe P.G. Gerald [b], Ron Kong [c], Manuel Cajina [e], Douglas A. Craig [e,*], Chad J. Swanson [d]

[a] Clinical Pharmacology and Therapeutics, Children's Hospital of Philadelphia, 3615 Civic Center Boulevard, Abramson Building, Rm 918, Philadelphia, PA 19104, USA
[b] Transcription Diagnostics Inc. 1201 Rio Vista Drive, Mahwah NJ 07430, USA
[c] PTC Therapeutics, Inc, 100 Corporate Court, South Plainfield, NJ 07080, USA
[d] Spinal Muscular Atrophy Foundation, 888 Seventh Avenue, New York, NY 10019, USA
[e] Lundbeck Research USA, 215 College Road, Paramus, NJ 07652, USA

ARTICLE INFO

Article history:
Received 6 May 2008
Received in revised form 8 October 2008
Accepted 23 October 2008
Available online 6 November 2008

Keywords:
Locomotor activity
Depression
Melanin-concentrating hormone
Quinpirole
Fluoxetine
SNAP 94847

ABSTRACT

Antidepressant treatment of two or more weeks in rats has been shown to enhance the locomotor-stimulating effects of dopamine D_2/D_3 receptor agonists. This action has been attributed to an increased sensitivity of postsynaptic dopamine receptors in the nucleus accumbens, thought to represent an essential mechanism by which antidepressants act therapeutically to enhance reward and motivation. We tested whether the melanin-concentrating hormone $receptor_1$ (MCH_1) antagonist SNAP 94847, reported to have antidepressant-like activity in several preclinical behavioral models, mimics this key feature of established antidepressants. Locomotor responses to the dopamine D_2/D_3 agonist quinpirole following acute or chronic administration of fluoxetine (18 mg/kg/day) or SNAP 94847 (20 mg/kg/day) were assessed in habituated Sprague–Dawley rats, as well as BALB/c and CD-1 mice. Rats showed a significant increase in quinpirole-induced locomotor activity following chronic (2 weeks), but not acute (1 h) fluoxetine or SNAP 94847 administration. BALB/c mice treated for 21 days with fluoxetine or SNAP 94847 showed marked increases in quinpirole-induced locomotor activity, with the onset of hyper-locomotion appearing earlier in the time course after SNAP 94847 compared to fluoxetine. Administration of either compound for 7 days was also sufficient to augment the quinpirole response in BALB/c mice. Fluoxetine and SNAP 94847 (21 days) failed to modify quinpirole responses in CD-1 mice, and the compounds were ineffective after acute administration in both mouse strains. This report demonstrates in two rodent species that chronic treatment with an MCH_1 receptor antagonist, as with clinically proven antidepressants, produces sensitization to the locomotor effects of dopamine D_2/D_3 agonists.

1. Introduction

The widespread treatment of major depression with serotonin reuptake inhibitors, monoamine oxidase inhibitors, and tricyclic antidepressants underscores the therapeutic benefit of elevating monoamine neurotransmission. The mesolimbic dopaminergic system, originating in the ventral tegmental area and terminating in the nucleus accumbens, has received considerable attention for its role in reward behaviors and incentive motivation. Alterations in this system are considered to be of particular relevance to the pathogenesis of depression, and are identifiable in preclinical models used to assess antidepressant-like activity (Arnt et al., 1984; Maj et al., 1984; Serra et al., 1990). A common, if not universal, feature of clinically efficacious antidepressants is that they enhance the locomotor-stimulating effects of dopamine D_2/D_3 agonists in rats, following chronic but not acute treatment (Ainsworth et al., 1998; D'Aquila et al., 2000). A similar sensitization of the dopamine system can be produced in rats using electroconvulsive shock (D'Aquila et al., 1997) or sleep deprivation (Arriaga et al., 1988), two effective, non-pharmacological treatments for depression. In contrast, a decrease in sensitivity to the locomotor effects of dopamine agonists has been reported after chronic mild stress in rats. This protocol is considered to produce symptoms of anhedonia, as assessed by an animal's diminished preference for sucrose (Papp et al., 1993; Willner, 1997). The fact that these distinct clinically effective treatments, as well as a model of decreased hedonic drive in rodents, converge on this common mechanism indicates that sensitization of mesolimbic dopamine drive is a key feature of antidepressant-like activity.

Recent evidence indicates that melanin-concentrating hormone $receptor_1$ (MCH_1) antagonists exhibit antidepressant- and anxiolytic-like activities in multiple preclinical behavioral models in rats and

* Corresponding author. Tel.: +1 201 225 0892; fax: +1 201 261 0623.
E-mail address: dcraig1@optonline.net (D.A. Craig).

0014-2999/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2008.10.051

mice (Borowsky et al., 2002; Chaki et al., 2005; David et al., 2007; Georgescu et al., 2005; Smith et al., 2006), although there are conflicting reports (e.g., see Basso et al., 2006). MCH_1 receptor mRNA and protein are found throughout the rat brain (Hervieu et al., 2000). The potential for a significant interaction of MCH (melanin-concentrating hormone) and dopaminergic processes was suggested by the high density of MCH_1 receptor binding in the shell sub-region of the nucleus accumbens (Borowsky et al., 2002). Georgescu et al. (2005) showed that direct microinjection of MCH into the mouse nucleus accumbens increased immobility in the forced swim test, while a peptide MCH_1 receptor antagonist decreases immobility. They also found that MCH blocks D_1 agonist-induced phosphorylation of AMPA receptors in explants of the nucleus accumbens shell region. These observations suggest that an increased sensitivity to dopamine in the nucleus accumbens may also contribute to the antidepressant-like activity of MCH_1 receptor antagonists. In support of this idea, MCH_1 receptor knockout mice have been shown to display enhanced behavioral and neurochemical sensitivity to direct and indirect dopamine agonists (Smith et al., 2005).

In the present study, we examined whether behavioral sensitivity to dopamine D_2/D_3 receptor stimulation can be elicited in response to the MCH_1 receptor antagonist SNAP 94847 (David et al., 2007) in rats and compared the actions to those of fluoxetine. We also established the model in mice, where the effects of antidepressants were strain-dependent. In addition to allowing for confirmation of the effects in the rat, the mouse model affords a practical alternative for future investigations.

2. Materials and methods

2.1. Animals

All animal use was in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Lundbeck Research Animal Use and Care Committee. Experimentally naïve, age-matched, male Sprague–Dawley rats (Crl:SD; Charles River, Kingston, NY, USA), CD-1 mice (Crl:CD-1; Charles River) and BALB/c mice (BALB/cByJ; Jackson Labs, Bar Harbor, ME, USA) were used in the following experiments (rat start weight: 250–275 g; mouse start weight: 20–24 g). All animals were provided food and water *ad libitum* and were on a 12-h light:12-h dark cycle. Mice were housed 4/cage; rats were housed 2/cage.

2.2. Chemicals

All doses refer to the salt form. (−)-Quinpirole hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) was administered via i.p. injection in a saline vehicle (injection volume: rats, 1 ml/kg; mice, 10 ml/kg). For acute and chronic studies in rats, fluoxetine (Sigma-Aldrich, St. Louis, MO, USA) and SNAP 94847 (*N*-[3-(1-{[4-(3,4-difluorophenoxy)phenyl] methyl}(4-piperidyl))-4-methylphenyl]-2-methylpropanamide; Lundbeck Research) were administered via oral gavage in 20% beta-cyclodextrin vehicle (Sigma-Aldrich). For chronic studies in mice, fluoxetine and SNAP 94847 were administered in the drinking water containing 0.01% lactic acid.

2.3. Equipment

Locomotor activity was recorded using MotorMonitor software (Hamilton-Kinder, USA) with 15×7 photo beam SmartFrame hardware operating at 50 ms sampling rate. Data points were summed into 10 and 180 min bins and compiled into ambulations (centroid relocation), fine movements (beam breaks independent of centroid movement), and distance traveled. Therefore, direct comparison of animals of different size was not possible and movement relative to the control was used for evaluation between species and across strains.

2.4. Locomotor studies in treatment-naïve animals

Animals were habituated for 60 min to a novel 15″×7″ locomotor cage with a filter-top lid, after which they were injected with quinpirole and returned immediately to the locomotor cage. Locomotor activity was measured for 180 min. To establish the dose–response relationship for quinpirole, the following dose ranges were used: rats (0.1–10 mg/kg, i.p.), and mice (1–20 mg/kg, i.p.).

2.5. Locomotor studies in antidepressant-treated rats

Chronically treated Sprague–Dawley rats were administered fluoxetine (18 mg/kg/day) or SNAP 94847 (20 mg/kg/day) via oral gavage in 20% beta-cyclodextrin for 2 weeks (1 injection, 6 h into light cycle). For acute treatment, drugs were given orally in 20% beta-cyclodextrin 1 h before testing. On each test day, locomotor activity was recorded during a 60 min habituation to the novel locomotor cage, after which the rats were administered quinpirole (0.3 mg/kg, i.p.) and returned immediately to the cage, where locomotor activity was measured for an additional 180 min. In each case control animals were administered the corresponding vehicle, without drug.

2.6. Locomotor studies in antidepressant-treated mice

For chronic studies, CD-1 or BALB/c mice were given 7- or 21-days of fluoxetine (18 mg/kg/day) or SNAP 94847 (20 mg/kg/day). Compounds were delivered to mice in 0.01% lactic acid as drinking water solutions to avoid the stress of oral gavage. Compound concentrations were adjusted based on the average water intake per day for mice determined from previous experiments (3.5 l/day), to account for changes in average body weight and water intake. For acute drug studies, BALB/c or CD-1 mice were dosed at the beginning of the habituation period (60 min) with fluoxetine (18 mg/kg, i.p.) or SNAP 94847 (20 mg/kg, i.p.) in 20% cyclodextrin. On each test day, locomotor activity was recorded during a 60 min habituation to the novel locomotor cage, after which the mice were administered quinpirole (10 mg/kg, i.p.) and returned immediately to the cage, where locomotor activity was measured for an additional 180 min. Control animals were administered the corresponding vehicles, without drug.

2.7. Statistics

Locomotor data in 10 min bins were analyzed first, using two-way analysis of variance (ANOVA) in GraphPad Prism 4 (San Diego, CA) to determine the significance of drug treatments over time. When a significant compound effect was found, a one-way ANOVA was applied to data in 180 min bins, using Student–Newman–Keuls post-hoc for multiple comparisons to determine significance ($P<0.05$) for between-group means.

2.8. Bioanalysis of plasma and brain tissue samples

To assess steady state plasma and brain levels of fluoxetine and SNAP 94847, samples were collected after administration for 7 days in satellite groups. Additionally, in order to confirm that the effect of antidepressants to enhance the locomotor response to quinpirole is not related to a pharmacokinetic interaction that enhances quinpirole exposure, we determined the plasma and brain levels of quinpirole at 30 and 180 min after injection in vehicle-treated BALB/c mice and mice treated for 1 week with fluoxetine (18 mg/kg/day). Compound content in plasma samples was determined using LC/MS/MS, consisting of Spark-Holland Symbiosis HPLC (Spark Holland B.V., Netherlands) coupled with TSQ Quantum Ultra mass spectrometry (Thermo Electron Corp., San Jose, CA, USA). Prior to analysis, plasma and brain samples were thawed at room temperature. Brain homogenates were

prepared by homogenizing the tissue with an aqueous solvent at a ratio of 3/1 (volume/weight) for rat and at a ratio of 4/1 (volume/weight) for mouse using a Tomtec Autogizer (Tomtec, Hamden, CT, USA). Aliquots of 50 µl plasma and/or brain homogenate sample were mixed with 150 µl of DMSO solution containing an internal standard. The diluted plasma/brain samples were centrifuged (200 g; 15 min at 20 °C), and 10 µl supernatant was analyzed by LC/MS/MS, using a Gemini column (3 µ C18, 30×2 mm, Phenomenex, Torrance, CA, USA) for analytical separation. The mobile phase consisted of 0.1% formic acid in water and 0.01% formic acid in acetonitrile. Quantification of drug level in plasma and brain was performed using Xcaliber 2.0 (Thermo Electron Corp., San Jose, CA, USA).

3. Results

3.1. Locomotor responses to quinpirole in treatment-naïve rats and mice

A dose–response relationship for quinpirole was determined in each species/strain, with the aim of identifying a dose producing a minimal effect on locomotor activity. In treatment-naïve rats, quinpirole (0.3–10 mg/kg) produced an initial reduction in locomotor activity, followed by a gradual dose-related increase in ambulation relative to saline-injected control animals (Fig. 1A). ANOVA revealed a significant effect of quinpirole [$F_{(3,44)}=3.10$, $P<0.05$]. When data were binned over 180 min, a significant increase in ambulation was seen only at a dose of 1 mg/kg ($P<0.05$) (Fig. 1B), though a trend for increased ambulation was apparent also at 0.3 and 10 mg/kg. A dose of 0.3 mg/kg was selected for subsequent studies.

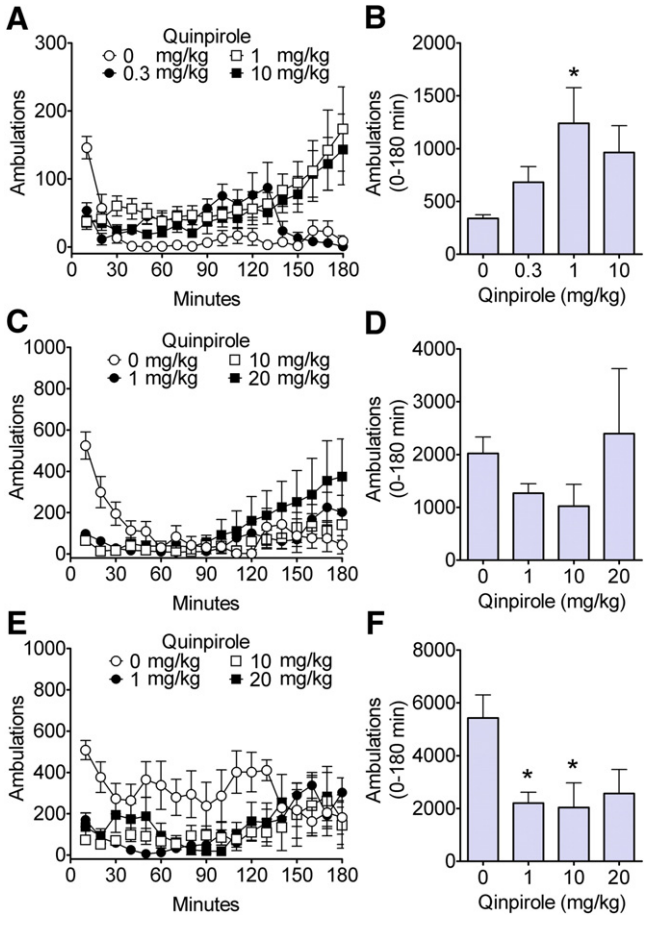

Fig. 1. Locomotor activity in response to quinpirole represented as time course and cumulative ambulatory count over 180 min in (A, B) Sprague–Dawley rats, (C, D) BALB/c mice, and (E, F) CD-1 mice. Data represent the mean±S.E.M. from 8 animals per group. *$P<0.05$ vs. saline only (0 mg/kg) group.

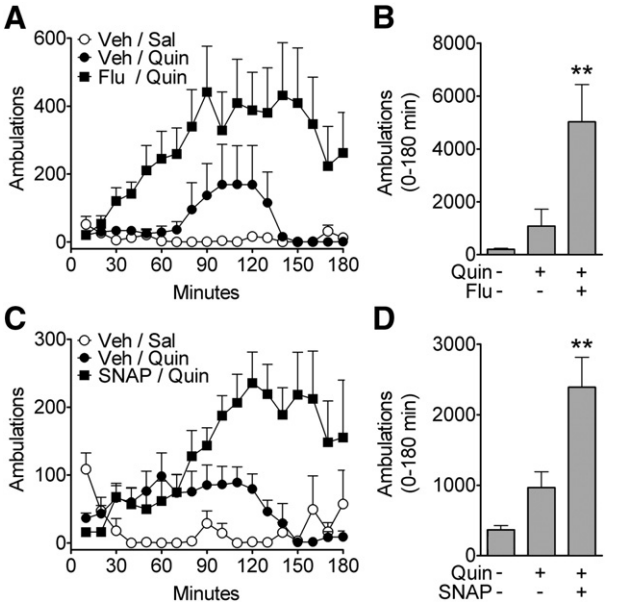

Fig. 2. Effects of chronic (14 days) treatment with fluoxetine (Flu, 18 mg/kg/day) and SNAP 94847 (SNAP, 20 mg/kg/day) on locomotor responses to acute challenge with saline or quinpirole (Quin, 0.3 mg/kg, i.p.) in rats. (A) Time course and (B) cumulative response in fluoxetine-treated rats. (C) Time course and (D) cumulative response in SNAP 94847-treated rats. Legends indicate chronic treatment/acute challenge. Data represent the mean±S.E.M. from 8 animals per group. **$P<0.01$ vs. Quin.

In BALB/c mice quinpirole (1–20 mg/kg) produced a significant ($P<0.05$) decrease in locomotor activity over the first 20 min [ANOVA 0–90 min: $F_{(3,28)}=17.02$, $P<0.001$]. The 20 mg/kg dose showed a trend to increase ambulation only over the final 30 min of the test (Fig. 1C). When data were summed over 180 min, there were no significant differences in ambulation between saline- and quinpirole-treated animals (Fig. 1D). Saline-treated CD-1 mice were slow to acclimate to the novel environment. As a result quinpirole-treated CD-1 mice exhibited significantly reduced activity relative to controls throughout most of the test [treatment: $F_{(3,28)}=3.9$, $P<0.05$) (Fig. 1E). Doses of 1 and 10 mg/kg produced a significant decrease in ambulation relative to control animals over the testing period ($P<0.05$) (Fig. 1F). A dose of 10 mg/kg was selected for subsequent antidepressant drug testing in CD-1 and BALB/c mice.

3.2. Locomotor responses to quinpirole in antidepressant-treated rats and mice

The locomotor activity measured during the 60 min habituation period for Sprague–Dawley rats treated for 14 days with fluoxetine (18 mg/kg/day) or SNAP 94847 (20 mg/kg/day) was not different from that of vehicle-treated animals (data not shown). Rats treated for 14 days with fluoxetine exhibited a greater, time-dependent locomotor response to quinpirole (0.3 mg/kg), relative to that in rats receiving vehicle [treatment: $F_{(2,21)}=8.156$, $P<0.01$; treatment×time: $F_{(34,357)}=2.274$, $P<0.001$] (Fig. 2A). When data were binned over the total 180 min, fluoxetine increased the response to quinpirole significantly ($P<0.01$) (Fig. 2B). Similarly, rats treated for 14 days with SNAP 94847 showed an exaggerated locomotor response to acute quinpirole [treatment: $F_{(2,19)}=11.31$, $P<0.001$; treatment×time: $F_{(34,323)}=4.061$, $P<0.0001$] (Fig. 2C). The effect of SNAP 94847 on quinpirole-evoked ambulations over the entire observation period was significant compared to the untreated animals ($P<0.01$) (Fig. 2D). Although it may appear that fluoxetine produced a larger response to quinpirole, with an earlier onset of action than seen with SNAP 94847, we cannot

conclude the differences are real because the compounds were tested in separate experiments. Neither fluoxetine nor SNAP 94847 treatment modified the tendency for quinpirole to inhibit locomotor activity over the initial 20 min of observations. Additionally, when tested after acute (1 h) exposure to fluoxetine or SNAP 94847, the locomotor response to quinpirole was not different from that of vehicle-treated animals (data not shown).

As with rats, the locomotor activity measured during the 60 min habituation period for BALB/c mice treated for 21 days with fluoxetine (18 mg/kg/day) or SNAP 94847 (20 mg/kg/day) in the drinking water was not different from that of vehicle-treated animals (data not shown). In BALB/c mice treated for 21 days with fluoxetine or SNAP 94847, quinpirole (10 mg/kg) produced a significant increase in ambulation relative to untreated animals [treatment: $F_{(3,28)}=8.971$, $P<0.001$; treatment×time: $F_{(51,476)}=11.50$, $P<0.0001$] (Fig. 3A). Interestingly, a marked increase in locomotion was apparent after only 40 min in the SNAP 94847-treated group, whereas a comparable effect in fluoxetine-treated animals was not evident until 100 min post-quinpirole. The effects of both compounds were significant over 180 min: fluoxetine ($P<0.05$), and SNAP 94847 ($P<0.001$) (Fig. 3B). To determine if there were differences in the duration of treatment required to augment the response to quinpirole, we tested the effects of fluoxetine and SNAP 94847 after 7 days or acute (1 h) exposure. Both compounds enhanced the response to quinpirole after 7 days. The rapid development of hyperactivity that was seen after 21 days of SNAP 94847 treatment was not observed after 7 days, and significant hyperactivity to quinpirole in both groups developed only after 110 min [treatment $F_{(3,26)}=6.523$, $P<0.01$; treatment×time: $F_{(51,442)}=7.865$, $P<0.0001$] (Fig. 3C). A significant effect for both compounds was attained over the 180 min test period: fluoxetine ($P<0.05$), and SNAP 94847 ($P<0.05$) (Fig. 3D). An increased locomotor response to quinpirole was not seen in animals treated for 1 h with either fluoxetine or SNAP 94847 (data not shown). The immediate reduction in locomotor activity in response to quinpirole was not modified by treatment with either fluoxetine or SNAP 94847 at 7 or 21 days.

The treatment of CD-1 mice for 21 days with fluoxetine (18 mg/kg/day) or SNAP 94847 (20 mg/kg/day) in drinking water had no effect on basal locomotor activity measured during the 60 min habituation period (data not shown) and also failed to significantly affect the locomotor response to quinpirole (10 mg/kg) (Fig. 3E, F). Similarly, no effects of fluoxetine or SNAP 94847 were observed following either 7 days or 1 h treatments (data not shown).

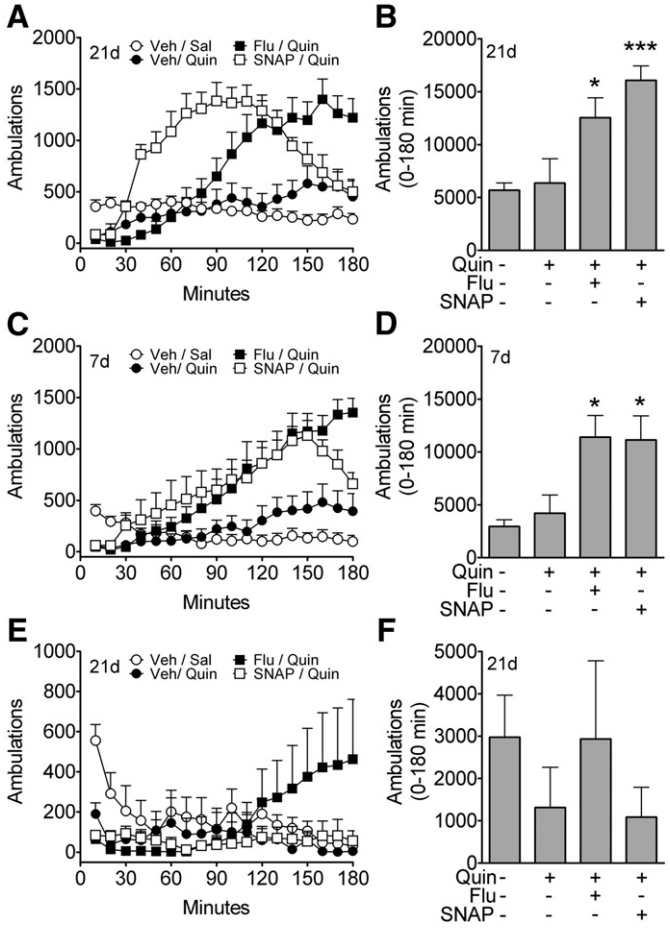

Fig. 3. Effects of chronic (21 days) treatment with fluoxetine (Flu, 18 mg/kg/day) and SNAP 94847 (SNAP, 20 mg/kg/day) on locomotor responses to acute challenge with saline or quinpirole (Quin, 10 mg/kg, i.p.) in mice. (A) Time course and (B) cumulative response following 21 days of drug treatment in BALB/c mice. (C) Time course and (D) cumulative response after 7 days of drug treatment in BALB/c mice. (E) Time course and (F) cumulative response in following 21 days of drug treatment in CD-1 mice. Legends indicate chronic treatment/acute challenge. Data represent the mean±S.E.M. from 8 animals per group. *$P<0.05$, ***$P<0.001$ vs. Quin.

Table 1
Plasma (ng/ml) and brain (ng/g tissue) exposure of fluoxetine and SNAP 94847 after chronic administration (1 week) to rats and mice

	Plasma		Brain	
SD rat				
Fluoxetine	397±135	(3)	12,712±3975	(3)
SNAP 94847	93±51	(4)	160±90	(4)
BALB/c mice				
Fluoxetine	688±24	(4)	5943±575	(4)[a]
SNAP 94847	28±11	(4)	31±11	(4)[a]
CD-1 mice				
Fluoxetine	1054±70	(8)	9323±374	(4)[a]
SNAP 94847	54±2	(8)	26±9	(4)[a]

Data are expressed as mean±S.E.M. (*N*).
[a] *N*=2 each from 2 separate experiments.

3.3. Pharmacokinetic measurements

The exposure levels of fluoxetine and SNAP 94847 in plasma (ng/ml) and brain (ng/g tissue) are shown in Table 1, and are within previously reported efficacious ranges.

The effect of fluoxetine treatment (18 mg/kg/day, 7 days) on the pharmacokinetics of quinpirole in BALB/c mice is shown in Table 2. In drug-naïve BALB/c mice, plasma and brain levels of quinpirole reached

Table 2
Effect of fluoxetine treatment on exposure levels of quinpirole in plasma (ng/ml) and brain (ng/g)

	Plasma	Brain
Untreated mice		
Quinpirole (30 min)	879	3771
Quinpirole (180 min)	36	287
Fluoxetine-treated mice (7 days)		
Quinpirole (30 min)	818	4293
Quinpirole (180 min)	58	298
Fluoxetine (30 min)	706	19,322
Fluoxetine (180 min)	764	17,248

Untreated mice and mice treated for 7 days with fluoxetine in the drinking water (18 mg/kg/day) received a single injection of quinpirole (10 mg/kg, i.p.). Plasma and brain exposure of quinpirole and fluoxetine were measured 30 min and 180 min after a single injection of 10 mg/kg in BALB/c mice. Data represent the means of measurements from 2 mice each.

high levels within 30 min of a single i.p. injection (10 mg/kg). Plasma and brain levels of quinpirole decreased to ~10% of the initial value after 180 min. Mice treated for 7 days with fluoxetine (18 mg/kg/day) exhibited the expected high plasma and brain levels of the antidepressant, which, as expected, were similar at the 30 and 180 min time points. Plasma and brain levels of quinpirole in fluoxetine-treated mice did not differ substantially from the levels measured in untreated mice.

4. Discussion

The ability to augment behavioral sensitivity to dopamine D_2/D_3 agonists in rats is a mechanistic feature shared by all classes of clinically effective antidepressants (D'Aquila et al., 2000), as well as the non-medicinal antidepressant interventions electroconvulsive shock (D'Aquila et al., 1997) and sleep deprivation (Arriaga et al., 1988). The phenomenon is believed to arise from an increase in sensitivity of dopamine D_2/D_3 receptors within the nucleus accumbens, and is postulated to represent the neurophysiological process underlying the pro-hedonic effects of antidepressants in humans (Serra et al., 1992; Willner et al., 2005). At present there are no data to indicate that antidepressants of different classes differ in the degree to which they induce sensitivity to dopamine D_2/D_3 agonists, but it would be of interest to determine whether differences exist and, if so, how they relate to clinical efficacy within distinct patient populations. The primary objective of this investigation was to determine whether the MCH_1 receptor antagonist SNAP 94847 promotes a similar sensitization in rats challenged with the dopamine D_2/D_3 agonist quinpirole. In addition to studying rats, we established the model in mice, as this not only provides confirmation of the effect in a second species, but also offers the practical advantages of reduced cost, housing space, and drug requirements.

The overall acute effects of the dopamine D_2/D_3 agonist were consistent with literature reports from studies with habituated animals. These demonstrate that acute dopamine D_2/D_3 agonist-evoked behavior in rats is characterized by a dose-dependent and biphasic locomotor response (low dose: inhibition; higher dose: stimulation), whereas reduced locomotor activity is the predominant response across a wide range of dopamine D_2/D_3 agonist doses in mice (Halberda et al., 1997; Ralph and Caine, 2005). The reason for this species-dependent difference is not clear, and may relate to species differences in basal spontaneous locomotor activity, drug metabolism or receptor distribution.

A dose of 0.3 mg/kg quinpirole in rats produced an initial inhibition of ambulation, followed by a marginal increase over basal ambulatory activity. This dose was selected to test the effects of SNAP 94847 and fluoxetine. The dose of 20 mg/kg SNAP 94847 was chosen based on previous results with chronic administration in behavioral models (David et al., 2007). In Sprague–Dawley rats, SNAP 94847 produced a marked increase in the locomotor response to quinpirole after 2 weeks of treatment, but had no effect after acute (1 h) treatment. The effect was comparable to that of the positive control, fluoxetine, and is consistent with previous results for diverse antidepressants (see D'Aquila et al., 2000; Willner, 1997). Neither fluoxetine nor SNAP 94847 affected basal ambulatory activity, measured during the 60 min habituation period.

Untreated BALB/c and CD-1 mice responded to quinpirole primarily with a reduction in locomotor activity. We selected the dose of 10 mg/kg quinpirole for antidepressant drug testing, because it was an intermediate dose showing behavioral evidence of dopamine D_2/D_3 receptor activation (inhibition of ambulation) without overt stimulatory effects. Despite the inhibitory effect of quinpirole alone, chronic (21, 7 days) treatment of BALB/c mice with either SNAP 94847 or fluoxetine resulted in a marked increase in ambulation in response to quinpirole. While both the magnitude and onset of ambulatory activity was comparable in mice treated for 7 days with either drug, after 21 days of treatment the ambulatory effects of quinpirole appeared nearly 60 min earlier in mice receiving SNAP 94847. The significance of this difference is unclear, but since this was a within-experiment comparison, the observation may have mechanistic significance that deserves further exploration. As in rats, sensitization to quinpirole was not observed in BALB/c mice treated for only 1 h with either compound.

The antidepressant effect in mice was strain-specific. Locomotor responses to quinpirole were not modified significantly in CD-1 mice receiving acute or chronic treatment with SNAP 94847 or fluoxetine. The difference appears unrelated to drug exposure, as the level of each compound was equivalent in the two strains. There were specific instances of sensitivity to antidepressant effects, as a marked effect was seen after 21 days in 1 of 8 mice in the SNAP 94847 group, and in 2 of 8 mice in the fluoxetine group, giving rise to the large variability in Fig. 3E. This may reflect the increased individual variability associated with outbred strains in general. BALB/c mice possess a polymorphism in the tryptophan hydroxylase-2 (*tph-2*) gene, resulting in decreased serotonin synthesis and tissue levels (Zhang et al., 2004), and are generally characterized as more anxious relative to CD-1 mice in behavioral models (Ennaceur et al., 2008). Whether these factors play a role in the strain-dependent sensitivity to antidepressants in the present study remains to be determined.

The suppression of locomotor activity seen over the first 10–30 min after quinpirole dosing in this study, and in general to low doses of quinpirole in previous studies, has been attributed to the reduction in dopamine release resulting from activation of pre-synaptic dopamine D_2/D_3 autoreceptors (Millan et al., 2004). One potential mechanism for producing an overall increase in the locomotor response to dopamine D_2/D_3 activation would be to reduce the sensitivity of the inhibitory presynaptic receptors. The fact that neither SNAP 94847 nor fluoxetine modified the quinpirole-induced suppression of locomotor activity suggests that presynaptic dopamine signaling is not affected, and the main effect of both compounds is on postsynaptic dopamine D_2/D_3 signaling.

Data from our experiments in rats and mice, together with previous evidence from experiments in rats (Ainsworth et al., 1998; D'Aquila et al., 2000), indicate that the enhanced ambulation in response to quinpirole cannot be attributed to the additive locomotor effects of the antidepressant plus quinpirole. Neither fluoxetine nor SNAP 94847 resulted in any increase in basal locomotor activity compared to vehicle-treated animals, measured during the 60 min habituation period for each experiment. Similarly, several lines of evidence indicate that the enhanced response to quinpirole is not the result of a pharmacokinetic interaction between the antidepressant and quinpirole. First, as shown in Fig. 1, an increase in the dose of quinpirole from that used in the combination studies (i.e., 0.3 mg/kg in rat and 10 mg/kg in mice) does not result in an appreciable increase in locomotor activity. Additionally, if the effect resulted from a pharmacokinetic interaction, it might also be expected to occur after acute antidepressant administration; however, acute antidepressant administration did not enhance quinpirole-induced locomotor activity. Finally, the results of our experiments with the combination of fluoxetine and quinpirole in BALB/c mice (Table 2) demonstrate that chronic exposure to the antidepressant does not affect brain exposure to quinpirole. Thus, the most parsimonious conclusion remains that antidepressants act at the level of D_2D_3 receptors or their signaling pathways to enhance the dopamine agonist-evoked locomotor response.

The precise mechanism by which MCH_1 receptor antagonists produce sensitization to quinpirole is likely to be distinct from that of monoamine-related antidepressants. Elevated levels of monoamines in response to MCH_1 receptor antagonists have not been reported, and in our laboratory acute MCH_1 receptor antagonist administration did not affect levels of dopamine, norepinephrine, or 5-HT in the shell of the nucleus accumbens, nor 5-HT levels in the prefrontal cortex (unpublished observations). In mice lacking the MCH_1 receptor,

Smith et al. (2005) detected no differences in basal or amphetamine-evoked levels of 5-HT, norepinephrine or dopamine in the nucleus accumbens, although reduced basal and stress-evoked 5-HT levels were found by Roy et al. (2006) in the prefrontal cortex of MCH_1 receptor knockout mice.

MCH_1 receptors are expressed prominently in the nucleus accumbens (Borowsky et al., 2002), and distinct actions of MCH within this nucleus are being discovered. MCH has been shown to increase, and an MCH_1 receptor antagonist to decrease, immobility in the forced swim test (Georgescu et al., 2005). In addition, MCH was shown in isolated brain slices to inhibit D_1 agonist-mediated phosphorylation of AMPA channels. Mice lacking the MCH_1 receptor are known to exhibit hyperactivity in novel and familiar settings (Marsh et al., 2002; Smith et al., 2005), and this has been attributed to an increased expression of both dopamine D_1- and D_2-like receptors, as defined by the binding of antagonist radioligands selective for each subtype (Smith et al., 2005). It is conceivable that a similar up-regulation of dopamine receptors underlies the sensitized locomotor response to SNAP 94847 seen in this study.

MCH_1 receptor antagonists have been reported to exhibit anxiolytic- and antidepressant-like activities in multiple pre-clinical models in rats and mice (Borowsky et al., 2002; Chaki et al., 2005; Georgescu et al., 2005; Smith et al., 2006). In particular, SNAP 94847 was shown to normalize sucrose consumption in the chronic mild stress paradigm and to increase social interaction time in rats, while reducing stress-induced hyperthermia and forced swim test immobility time in mice (Wolinsky et al., 2004). More recently David et al. (2007) demonstrated that chronic administration of SNAP 94847 to mice promoted neuronal progenitor cell proliferation in the dentate gyrus, reduced the latency to feed in the novelty suppressed feeding test, and increased time spent in the light compartment in the light/dark paradigm. It was not efficacious, however, in the forced swim test. Furthermore, in contrast to the neurogenesis-dependent activity of imipramine and fluoxetine in the novelty suppressed feeding test (Santarelli et al., 2003), the efficacy of SNAP 94847 was not affected by the elimination of neurogenesis by hippocampal irradiation (David et al., 2007). Based on the behavioral profile and neurogenesis-independent actions of SNAP 94847, David et al. (2007) proposed that SNAP 94847 might be more appropriately characterized as having anxiolytic-like rather than antidepressant-like properties. The present data demonstrate that increased behavioral sensitivity to a dopamine D_2/D_3 agonist is induced following chronic administration of SNAP 94847 to rats and mice. Considering the link between mesolimbic dopaminergic mechanisms and hedonic drive (Willner et al., 2005), the present study provides additional evidence for the potential antidepressant actions of MCH antagonists.

We have demonstrated that increased behavioral sensitivity to a dopamine D_2/D_3 agonist is induced following chronic administration of SNAP 94847 to rats and mice. The model in BALB/c mice may provide an attractive and practical alternative for further studies examining the mechanism of action of MCH antagonists, their modulation of dopaminergic circuitry, and their potential involvement in the neurobiology of motivation and reward. Finally, in view of reports that sensitization of dopamine D_2/D_3 receptors in the nucleus accumbens underlies antidepressant efficacy in the chronic mild stress model (Papp et al., 1993), as well as the role played by the chronic mild stress model in estimating the relative onset of action of novel antidepressant candidates (Sanchez et al., 2003), future studies should evaluate the utility of the present model as a surrogate for the labor intensive chronic mild stress model.

References

Ainsworth, K., Smith, S.E., Sharp, T., 1998. Repeated administration of fluoxetine, desipramine and tranylcypromine increases dopamine D2-like but not D1-like receptor function in the rat. J. Psychopharmacol. 12, 252–257.

Arnt, J., Hyttel, J., Overo, K.F., 1984. Prolonged treatment with the specific 5-HT-uptake inhibitor citalopram: effect on dopaminergic and serotonergic functions. Pol. J. Pharmacol. Pharm. 36, 221–230.

Arriaga, F., Dugovic, C., Wauquier, A., 1988. Effects of lithium on dopamine behavioural supersensitivity induced by rapid eye movement sleep deprivation. Neuropsychobiology 20, 23–27.

Basso, A.M., Bratcher, N.A., Gallagher, K.B., Cowart, M.D., Zhao, C., Sun, M., Esbenshade, T.A., Brune, M.E., Fox, G.B., Schmidt, M., Collins, C.A., Souers, A.J., Iyengar, R., Vasudevan, A., Kym, P.R., Hancock, A.A., Rueter, L.E., 2006. Lack of efficacy of melanin-concentrating hormone-1 receptor antagonists in models of depression and anxiety. Eur. J. Pharmacol. 540, 115–120.

Borowsky, B., Durkin, M.M., Ogozalek, K., Marzabadi, M.R., DeLeon, J., Lagu, B., Heurich, R., Lichtblau, H., Shaposhnik, Z., Daniewska, I., Blackburn, T.P., Branchek, T.A., Gerald, C., Vaysse, P.J., Forray, C., 2002. Antidepressant, anxiolytic and anorectic effects of a melanin-concentrating hormone-1 receptor antagonist. Nat. Med. 8, 825–830.

Chaki, S., Funakoshi, T., Hirota-Okuno, S., Nishiguchi, M., Shimazaki, T., Iijima, M., Grottick, A.J., Kanuma, K., Omodera, K., Sekiguchi, Y., Okuyama, S., Tran, T.A., Semple, G., Thomsen, W., 2005. Anxiolytic- and antidepressant-like profile of ATC0065 and ATC0175: nonpeptidic and orally active melanin-concentrating hormone receptor 1 antagonists. J. Pharmacol. Exp. Ther. 313, 831–839.

D'Aquila, P.S., Collu, M., Gessa, G.L., Serra, G., 1997. Dizocilpine prevents the enhanced locomotor response to quinpirole induced by repeated electroconvulsive shock. Eur. J. Pharmacol. 330, 11–14.

D'Aquila, P.S., Collu, M., Gessa, G.L., Serra, G., 2000. The role of dopamine in the mechanism of action of antidepressant drugs. Eur. J. Pharmacol. 405, 365–373.

David, D.J., Klemenhagen, K.C., Holick, K.A., Saxe, M.D., Mendez, I., Santarelli, L., Craig, D.A., Zhong, H., Swanson, C.J., Hegde, L.G., Ping, X.I., Dong, D., Marzabadi, M.R., Gerald, C.P., Hen, R., 2007. Efficacy of the MCHR1 antagonist N-[3-(1-{[4-(3,4-difluorophenoxy)phenyl]methyl}(4-piperidyl))-4-methylphen yl]-2-methylpropanamide (SNAP 94847) in mouse models of anxiety and depression following acute and chronic administration is independent of hippocampal neurogenesis. J. Pharmacol. Exp. Ther. 321, 237–248.

Ennaceur, A., Michalikova, S., van, R.R., Chazot, P.L., 2008. Are benzodiazepines really anxiolytic? Evidence from a 3D maze spatial navigation task. Behav. Brain Res. 188, 136–153.

Georgescu, D., Sears, R.M., Hommel, J.D., Barrot, M., Bolanos, C.A., Marsh, D.J., Bednarek, M.A., Bibb, J.A., Maratos-Flier, E., Nestler, E.J., DiLeone, R.J., 2005. The hypothalamic neuropeptide melanin-concentrating hormone acts in the nucleus accumbens to modulate feeding behavior and forced-swim performance. J. Neurosci. 25, 2933–2940.

Halberda, J.P., Middaugh, L.D., Gard, B.E., Jackson, B.P., 1997. Synapse 26, 81–92.

Hervieu, G.J., Cluderay, J.E., Harrison, D., Meakin, J., Maycox, P., Nasir, S., Leslie, R.A., 2000. The distribution of the mRNA and protein products of the melanin-concentrating hormone (MCH) receptor gene, slc-1, in the central nervous system of the rat. Eur. J. Neurosci. 12, 1194–1216.

Maj, J., Rogoz, Z., Skuza, G., Sowinska, H., 1984. Repeated treatment with antidepressant drugs increases the behavioural response to apomorphine. J. Neural Transm. 60, 273–282.

Marsh, D.J., Weingarth, D.T., Novi, D.E., Chen, H.Y., Trumbauer, M.E., Chen, A.S., Guan, X.M., Jiang, M.M., Feng, Y., Camacho, R.E., Shen, Z., Frazier, E.G., Yu, H., Metzger, J.M., Kuca, S.J., Shearman, L.P., Gopal-Truter, S., Macneil, D.J., Strack, A.M., Macintyre, D.E., Van der Ploeg, L.H., Qian, S., 2002. Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic and have altered metabolism. Proc. Natl. Acad. Sci. U.S.A 99, 3240–3245.

Millan, M.J., Seguin, L., Gobert, A., Cussac, D., Brocco, M., 2004. The role of dopamine D3 compared with D2 receptors in the control of locomotor activity: a combined behavioural and neurochemical analysis with novel, selective antagonists in rats. Psychopharmacology (Berl.) 174, 341–357.

Papp, M., Willner, P., Muscat, R., 1993. Behavioural sensitization to a dopamine agonist is associated with reversal of stress-induced anhedonia. Psychopharmacology (Berl.) 110, 159–164.

Ralph, R.J., Caine, S.B., 2005. Dopamine D1 and D2 agonist effects on prepulse inhibition and locomotion: comparison of Sprague–Dawley rats to Swiss–Webster, 129X1/SvJ, C57BL/6J, and DBA/2J mice. J. Pharmacol. Exp. Ther. 312, 733–741.

Roy, M., David, N.K., Danao, J.V., Baribault, H., Tian, H., Giorgetti, M., 2006. Genetic inactivation of melanin-concentrating hormone receptor subtype 1 (MCHR1) in mice exerts anxiolytic-like behavioral effects. Neuropsychopharmacology 31, 112–120.

Sanchez, C., Gruca, P., Papp, M., 2003. R-citalopram counteracts the antidepressant-like effect of escitalopram in a rat chronic mild stress model. Behav. Pharmacol. 14, 465–470.

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C., Hen, R., 2003. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301, 805–809.

Serra, G., Collu, M., D'Aquila, P.S., De Montis, G.M., Gessa, G.L., 1990. Possible role of dopamine D1 receptor in the behavioural supersensitivity to dopamine agonists induced by chronic treatment with antidepressants. Brain Res. 527, 234–243.

Serra, G., Collu, M., D'Aquila, P.S., Gessa, G.L., 1992. Role of the mesolimbic dopamine system in the mechanism of action of antidepressants. Pharmacol. Toxicol. 71 (Suppl. 1), 72–85.

Smith, D.G., Tzavara, E.T., Shaw, J., Luecke, S., Wade, M., Davis, R., Salhoff, C., Nomikos, G.G., Gehlert, D.R., 2005. Mesolimbic dopamine super-sensitivity in melanin-concentrating hormone-1 receptor-deficient mice. J. Neurosci. 25, 914–922.

Smith, D.G., Davis, R.J., Rorick-Kehn, L., Morin, M., Witkin, J.M., McKinzie, D.L., Nomikos, G.G., Gehlert, D.R., 2006. Melanin-concentrating hormone-1 receptor modulates

neuroendocrine, behavioral, and corticolimbic neurochemical stress responses in mice. Neuropsychopharmacology 31, 1135–1145.
Willner, P., 1997. The mesolimbic dopamine system as a target for rapid antidepressant action. Int. Clin. Psychopharmacol. 12 (Suppl. 3), S7–14.
Willner, P., Hale, A.S., Argyropoulos, S., 2005. Dopaminergic mechanism of antidepressant action in depressed patients. J. Affect. Disord. 86, 37–45.
Wolinsky, T.D., Craig, D.A., Patel, J., Lichtblau, H., Papp, M., Gerald, C.P., 2004. Anxiolytic and antidepressant-like activity of SNAP94847, a novel MCH-1 antagonist. 34th Society for Neuroscience Meeting: #356.14.
Zhang, X., Beaulieu, J.M., Sotnikova, T.D., Gainetdinov, R.R., Caron, M.G., 2004. Tryptophan hydroxylase-2 controls brain serotonin synthesis. Science 305, 217.