0022-3565/07/3211-237–248$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 321:237–248, 2007

Vol. 321, No. 1
109678/3192373
*Printed in U.S.A.*

# Efficacy of the MCHR1 Antagonist *N*-[3-(1-{[4-(3,4-Difluorophenoxy)phenyl]methyl}(4-piperidyl))-4-methylphenyl]-2-methylpropanamide (SNAP 94847) in Mouse Models of Anxiety and Depression following Acute and Chronic Administration Is Independent of Hippocampal Neurogenesis[S]

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Received June 21, 2006; accepted January 8, 2007

**ABSTRACT**

Melanin-concentrating hormone (MCH) is a hypothalamic neuropeptide that plays a role in the modulation of food intake and mood. In rodents, the actions of MCH are mediated via the MCHR1 receptor. The goal of this study was to investigate the effects of acute (1 h) and chronic (28 days) p.o. dosing of a novel MCHR1 antagonist, *N*-[3-(1-{[4-(3,4-difluorophenoxy)-phenyl]methyl}(4-piperidyl))-4-methylphenyl]-2-methylpropanamide (SNAP 94847), in three mouse models predictive of antidepressant/anxiolytic-like activity: novelty suppressed feeding (NSF) in 129S6/SvEvTac mice and light/dark paradigm (L/D) and forced swim test (FST) in BALB/cJ mice. A significant increase in the time spent in the light compartment of the L/D box was observed in response to acute and chronic treatment with SNAP 94847. An anxiolytic/antidepressant-like effect was found in the NSF test after acute and chronic treatment, whereas no effect was observed in the FST. Because neurogenesis in the dentate gyrus has been shown to be a requirement for the effects of antidepressants in the NSF test, we investigated whether neurogenesis was required for the effect of SNAP 94847. We showed that chronic treatment with SNAP 94847 stimulated proliferation of progenitors in the dentate gyrus. The efficacy of SNAP 94847 in the NSF test, however, was unaltered in mice in which neurogenesis was suppressed by X-irradiation. These results indicate that SNAP 94847 has a unique anxiolytic-like profile after both acute and chronic administration and that its mechanism of action is distinct from that of selective serotonin reuptake inhibitors and tricyclic antidepressants.

Depression and anxiety are major causes of disability worldwide. The major obstacles faced in treating these disorders with selective serotonin reuptake inhibitors (SSRI) are that the therapeutic response develops slowly (3–4 weeks), side effects often occur, and there is a significant percentage of nonresponders (≈30%) (Wong and Licinio, 2001). Neuropeptide receptors may offer alternative therapeutic targets for depression and anxiety disorders (Griebel, 1999), particularly those selectively localized in brain regions

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
doi:10.1124/jpet.106.109678.
[S] The online version of this article (available at http://jpet.aspetjournals.org) contains supplemental material.

**ABBREVIATIONS:** SSRI, selective serotonin reuptake inhibitor(s); MCH, melanin-concentrating hormone; GPCR, G protein-coupled receptor(s); EPM, elevated plus maze; SNAP 94847, *N*-[3-(1-{[4-(3,4-difluorophenoxy)phenyl]methyl}(4-piperidyl))-4-methylphenyl]-2-methylpropanamide; FST, forced swim test; ATC0065, $N^2$-[*cis*-4-({2-[4-bromo-2-(trifluoromethoxy)phenyl]ethyl}amino)cyclohexyl]-$N^4$, $N^4$-dimethylquinazoline-2,4-diamine dihydrochloride; ATC0175, *N*-(*cis*-4-{[4-(dimethylamino)quinazolin-2-yl]amino}cyclohexyl)-3,4-difluorobenzamide hydrochloride; GW3430, 6-(4′-chlorophenyl)-3-[3-methoxy-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-3*H*-thieno[3,2-*d*]pyrimidin-4-one; NSF, novelty suppressed feeding test; L/D, light-dark paradigm; TCA, tricyclic antidepressant(s); HEK, human embryonic kidney; BrdU, 5-bromo-2′-deoxyuridine; PBS, phosphate-buffered saline; DCX, doublecortin; TBS, Tris-buffered saline; ANOVA, analysis of variance; PLSD, protected least significant difference; SGZ, subgranular zone; SNAP 7941, (+)-methyl (4*S*)-3-{[(3-{4-[3-(acetylamino)phenyl]-1-piperidinyl}propyl)amino]carbonyl}-4-(3,4-difluorophenyl)-6-(methoxymethyl).

mediating emotional behavior and responses to stress. In this regard, tachykinins (substance P and neurokinin A), corticotropin-releasing factor, vasopressin, neuropeptide Y, and melanin-concentrating hormone (MCH) have received recent attention (Holmes et al., 2003).

MCH is a cyclic nonadecapeptide originally isolated from salmon pituitary and involved in food intake and body color change in fish (Kawauchi et al., 1983). In mammals, MCH is expressed predominantly in neurons of the lateral hypothalamus and the zona incerta, which project broadly throughout the brain (Bittencourt et al., 1992). Its effects are mediated by two receptors belonging to the superfamily of G protein-coupled receptors (GPCR): MCHR1 (originally SLC-1/GPR24) (Bachner et al., 1999; Chambers et al., 1999; Saito et al., 1999) and MCH2R (SLT/S643b) (An et al., 2001; Sailer et al., 2001); the latter is found in primates but not in rodents.

Although studied extensively in relation to food intake and body weight, MCH may also be involved in the modulation of anxiety. The literature regarding anxiety, however, is somewhat unclear because both anxiogenic and anxiolytic effects of MCH have been reported. MCH produced anxiolytic effects in the rat elevated plus maze (EPM), open field (Monzon and De Barioglio, 1999), and Vogel punished drinking tests (Kela et al., 2003). However, Gonzalez et al. (1996) reported an anxiogenic-like effect of MCH in the EPM. In addition, MCH is involved in the control of the hypothalamic-pituitary adrenal axis because intracerebroventricular administration of MCH increases circulating corticosterone, an effect blocked by pretreatment with an anticorticotropin–releasing factor antibody (Jezova et al., 1992). Supporting these data, Kennedy et al. (2003) showed that injection of MCH directly into the paraventricular nucleus increased both circulating adrenocorticotropin and corticosterone. Furthermore, a role for MCH in mood-related behaviors is supported by the expression of MCHR1 in the locus coeruleus and in limbic structures such as the hippocampus and basolateral amygdala (Hervieu et al., 2000).

The first reported selective, high affinity MCHR1 antagonist, SNAP 7941, had acute antidepressant- and anxiolytic-like effects in the rat forced swim test (FST) and social interaction tests and the guinea pig maternal separation-induced vocalization test (Borowsky et al., 2002). Two new MCHR1 antagonists, ATC0065 and ATC0175, were also shown to have anxiolytic- and antidepressant-like activity in rodents (Chaki et al., 2005). In addition, direct delivery of another MCHR1 antagonist to the nucleus accumbens shell produced antidepressant-like activity in the FST, whereas intra-accumbens shell injection of MCH produced the opposite effect (Georgescu et al., 2005). More recently, a pretreatment with the MCHR1 antagonist GW3430 reversed the anxiogenic effects of MCH in the EPM and stress-induced hyperthermia tests and restored plasma corticosterone to control levels (Smith et al., 2006).

Although these findings indicate that acute blockade of the MCHR1 receptor produced an antidepressant and anxiolytic profile (Borowsky et al., 2002; Chaki et al., 2005), the behavioral consequences of chronic MCHR1 antagonist administration have not been described. To investigate the effects of chronic MCHR1 antagonist treatment, as well as to distinguish between the anxiolytic- and antidepressant-like activities, we assessed the behavioral effects of a novel MCHR1 antagonist, SNAP 94847, after acute (1 h) or chronic (28 days) treatment in three mouse models of anxiety and depression: the novelty suppressed feeding test (NSF), the light-dark paradigm (L/D), and the FST. The NSF test has been shown to be sensitive to acute injection of anxiolytic drugs and to detect changes in mouse behavior after chronic but not acute treatment with SSRI and tricyclic antidepressants (TCA) (Santarelli et al., 2003). The L/D paradigm has proven useful for the investigation of both classic anxiolytics (benzodiazepines) and newer anxiolytic-like compounds (e.g., serotonergic drugs or drugs acting on neuropeptide receptors) (Bourin and Hascoët, 2003), whereas the FST is considered a primary screening test for antidepressants, with predictive validity across a range of compounds that are structurally and mechanistically diverse (Borsini and Meli, 1988).

Finally, because Santarelli et al. (2003) have used radiological methods to show that the behavioral effects of chronic SSRI and TCA in the NSF test may require hippocampal neurogenesis, we also examined whether a similar dependence exists for SNAP 94847 in this model.

## Materials and Methods

**Subjects.** Adult male 129S6/SvEvTac mice (Taconic Farms, Germantown, NY) were used for the NSF study because of their sensitivity to chronic antidepressants in this behavioral model (Santarelli et al., 2003). Male BALB/cJ mice (Jackson Laboratories, Bar Harbor, ME) were used for the FST and the L/D studies because of their high sensitivity to chronic antidepressant treatment in these models (Belzung and Griebel, 2001; Dulawa et al., 2004). All the mice were 7 to 8 weeks old and weighed 23 to 35 g at the beginning of the treatment and were maintained on a 12-h light/12-h dark schedule (lights on at 6:00 AM) and housed in groups of five of the same strain. Food and water were provided ad libitum. Behavioral testing occurred during the light phase between 7:00 AM and 7:00 PM. All the testing was conducted in compliance with the National Institutes of Health laboratory animal care guidelines and with protocols approved by the Institutional Animal Care and Use Committee.

**Drugs.** The behavioral effect of SNAP 94847 (Fig. 1A) (Lundbeck Research USA, Paramus, NJ) was compared in each behavioral test with escitalopram oxalate (H. Lundbeck A/S, Copenhagen, Denmark), imipramine hydrochloride (Sigma, St. Louis, MO), or diazepam (Sigma). For the acute study, SNAP 94847 (20 mg/kg) and vehicle (20% hydroxypropyl-$\beta$-cyclodextrin) were delivered p.o. (0.1 ml/10 g b.wt.), and diazepam (1 and/or 1.5 mg/kg), imipramine (20 mg/kg), or escitalopram (5 mg/kg) was delivered s.c. (0.1 ml/10 g b.wt.). For the chronic study, SNAP 94847 (2 and/or 20 mg/kg/day), imipramine (20 mg/kg/day), escitalopram (5 mg/kg/day), and vehicle (0.01% lactic acid) were delivered in opaque bottles to protect them from light, available ad libitum in the drinking water, and replaced weekly. The average water intake per day for 129S6/SvEvTac and BALB/cJ mice determined from previous experiments (3.5 ml/day) was used to adjust the concentration of each drug, and the brain levels of SNAP 94847 after acute or chronic exposure in 129S6/SvEvTac or BALB/cJ strains were measured (Table 1). For each experiment, the control group received the appropriate vehicle.

**Experiments.** The pharmacological activity of SNAP 94847 was characterized using in vitro binding and functional antagonism assays. It was then tested in mouse behavioral models predictive of antidepressant/anxiolytic-like activity: NSF test, the L/D paradigm, and the FST after acute (1 h before testing) or chronic (28 days) treatment. In each animal model, the effects of SNAP 94847 were compared with the benzodiazepine anxiolytic diazepam, the classic TCA imipramine, or the new SSRI escitalopram (12–15 animals per treatment). Naive mice were used only once for the acute and chronic

TABLE 1

Brain levels (ng/g tissue) of imipramine, escitalopram, or SNAP 94847 after acute (1 h) or chronic (28 days) exposure in 129S6/SvEvTac or BALB/cJ strains

Data represent mean ± S.E.M. of brain levels (ng/g tissue) of either imipramine, escitalopram, or SNAP 94847 after acute (1 h) or chronic (28 days) exposure in 129S6/SvEvTac or BALB/cJ strains. Brains were collected 15 min after beginning of the dark cycle.

| | 129S6/SvEvTac Strain | | BALB/cJ Strain | |
|---|---|---|---|---|
| | Acute | Chronic | Acute | Chronic |
| Imipramine (20 mg/kg/day) | 7761 ± 1173 | N.D. | N.D. | N.D. |
| Escitalopram (5 mg/kg/day) | N.D. | N.D. | 1352 ± 64 | 63 ± 12 |
| SNAP 94847 (20 mg/kg/day) | 533 ± 24 | 55 ± 1 | 1410 ± 238 | 190 ± 36 |

N.D., not determined.

studies, and experimenters were blind to treatment condition for all the tests.

**Receptor Binding and in Vitro Functional Antagonism.** Binding affinity for SNAP 94847 was measured in membranes from modified human embryonic kidney (HEK) 293 cells (PEAKRAPID cells, Edge Biosystems, Gaithersburg, MD) transfected transiently with either the mouse (ISS) or the rat (Sprague-Dawley) MCHR1 receptor. Membranes were labeled with the antagonist radioligand [$^3$H]SNAP 7941, and assays were performed as described previously (Borowsky et al., 2002). Furthermore, SNAP 94847 was tested for binding or functional antagonism in a broad cross-reactivity panel comprising 32 peptide GPCR, 51 nonpeptide GPCR, 21 ion channel binding sites, 14 enzymes, and 6 transporters (CEREP, Celle l'Evescault, France). Functional antagonism of MCH-evoked [$^3$H]-inositol phosphate formation was evaluated in HEK 293 cells stably transfected with the rat MCHR1 as described previously (Bonini et al., 2000). SNAP 94847 was incubated at varying concentrations with the cells for 20 min before addition of MCH; 30 min later, the assay was terminated and total inositol phosphate release was measured. Concerning the MCHR1 in different mouse strains, we present the alignments of our mouse MCHR1 sequence and other mouse strains (Supplemental Table 1).

**NSF Paradigm.** The NSF paradigm is a conflict test that elicits competing motivations: the drive to eat and the fear of venturing into the center of brightly lit arena. Latency to begin eating is used as an index of anxiety-like behavior because classic anxiolytic drugs decrease this measure. The NSF test was carried out during a 5-min period as described previously (Santarelli et al., 2001). In brief, the testing apparatus consisted of a plastic box (50 × 50 × 20 cm), the floor of which was covered with approximately 2 cm of wooden bedding. Twenty-four hours before behavioral testing, all the food was removed from the home cage. At the time of testing, a single pellet of food (regular chow) was placed on a white paper platform positioned in the center of the box. An animal was placed in a corner of the box, and a stopwatch was immediately started. The latency to eat (defined as the mouse sitting on its haunches and biting the pellet with the use of forepaws) was timed. Immediately after this test, the animal was transferred to its home cage, and the amount of food consumed by the mouse in 5 min was measured, serving as a control for change in appetite as a possible confounding factor.

**L/D Test.** The L/D test was conducted in an open field chamber measuring 43 × 43 cm (MED Associates, St. Albans, VT), having a white floor and clear walls with a dark plastic box insert opaque to visible light but transparent to infrared light covering half of the area of the chamber. Infrared tracking and data collection were controlled by a computer running Activity Monitor software (MED Associates). Based on the modifications proposed by Belzung et al. (1987), the open field box was divided into two equal areas with an opening located in the center of the dark wall at floor level, allowing passage between the light and dark chambers. The light compartment was brightly illuminated with an 8-W fluorescent tube (400 lux). The test was performed in a quiet, darkened room, and the mice were kept in this room at least 1 h before the test. Between each trial, the light/dark compartments were cleaned. At the beginning of the test, the mouse was placed in the dark compartment and allowed to freely explore both chambers for 5 min. During the test, the time spent in the dark and the light compartments was recorded.

**FST Procedure.** The FST procedure was modified relative to the traditional method, so as to enhance sensitivity for detecting the antidepressant activity of SSRI. The modifications consist of an increase in water depth (Dulawa et al., 2004). Mice were placed into plastic buckets (19 cm diameter, 23 cm deep, filled with 23–25°C water) and videotaped for 6 min. The last 4 min were scored for the duration of immobility. Mice were exposed for 6 min to the FST approximately 24 h before the actual test to increase sensitivity for detecting antidepressant behavioral effects (Borsini et al., 2002). We used a 6-min pre-exposure rather than the traditional 15-min exposure, because we have previously observed fluoxetine's effects with this shorter pretest exposure and because a shorter pretest swimming exposure minimizes stress experienced by the animals (Dulawa et al., 2004).

**Irradiation.** Mice were anesthetized with ketamine and xylazine (100 mg/ml ketamine, 20 mg/ml xylazine), placed in a stereotaxic frame, and exposed to cranial irradiation using a Siemens (Munich, Germany) Stabilopan X-ray system operated at 300 kVp and 20 mA. Animals were protected with a lead shield that covered the entire body but left unshielded a 3.22- × 11-mm treatment field above the hippocampus (interaural 3.00 to 0.00) (Fig. 7B) exposed to X-ray. Dosimetry was done using a Capintec (Ramsey, NJ) Model PR06G electrometer ionization chamber and Kodak Readypack Radiographic XV films (Rochester, NY). The corrected dose rate was approximately 1.8 Gy/min at a source to skin distance of 30 cm. The procedure lasted 2 min, 47 s, delivering a total of 5 Gy. Three 5-Gy doses were delivered on days 1, 4, and 8 (Fig. 7A).

**5-Bromo-2′-Deoxyuridine Labeling and Immunohistochemistry.** To assess the effect of SNAP 94847 or imipramine treatments on the number of 5-bromo-2′-deoxyuridine (BrdU)-positive cells, mice were administered BrdU (150 mg/kg, i.p. dissolved in saline) 2 h before sacrifice. After anesthesia with ketamine (100 mg/kg), mice were perfused transcardially (cold saline for 2 min, followed by 4% cold paraformaldehyde at 4°C). The brains were then removed and cryoprotected in 30% sucrose and stored at 4°C. Serial sections (35 $\mu$M) were cut through the entire hippocampus (plates 41–61) (Franklin and Paxinos, 1997) or the subgranular zone (SGZ) (plates 27–40) on a cryostat and stored in phosphate-buffered saline (PBS) with 0.1% $NaN_3$. For 3′3-diaminobenzidine HCl (DAB) staining, sections were mounted on slides and boiled in citric acid (pH 6.0) for 5 min, rinsed with PBS, and treated with 0.01% trypsin in Tris/$CaCl_2$ for 10 min. Brain sections were incubated for 30 min with 2 N HCl and blocked with 5% normal goat serum (NGS). Sections were then incubated overnight at room temperature with anti-mouse BrdU (1:100). After washing with PBS, sections were incubated for 1 h with secondary antibody (1:200 biotinylated goat anti-mouse) followed by amplification with an avidin-biotin complex. The staining was visualized with DAB. For the quantification of BrdU labeling, a stereological procedure was used as described previously (Malberg et al., 2000).

For doublecortin (DCX) staining, the procedure consisted of the following steps: 1-h incubation in 0.1 M Tris-buffered saline (TBS) with 0.5% Triton X-100 and 10% normal donkey serum, followed by

anti-rat DCX primary antibody (1:100) in TBS/Triton X-100 for 24 h at 4°C. The secondary antibody was biotinylated donkey anti-goat (1:500) in TBS/normal donkey serum for 1 h at room temperature, followed by a 1-h amplification step using an avidin-biotin complex (Vector Laboratories, Burlingame, CA).

**Drug Levels in Brain.** Whole-brain drug concentrations were measured in all the groups of mice. Either 1 h or 28 days after the drug treatment, mice were killed by cervical dislocation without anesthesia. The brain was removed after a rapid dissection of the cranium. Each brain sample was weighed and placed in homogenization solution (50% deionized water, 30% isopropanol, and 20% dimethyl sulfoxide) with a ratio of 4:1 (ml/g). After homogenization of the mixture, 50 $\mu$l of homogenized mixture was added with 3 volumes of precipitation solution (90% acetonitrile and 10% dimethyl sulfoxide) and centrifuged. The supernatant was injected into the mass spectrometer. The mass spectrometer signal was fitted to a standard curve generated by injecting standards of known concentration.

**Statistical Analysis.** Saturation and competition binding assays were analyzed using GraphPad Prism (GraphPad Software, San Diego, CA). Maximal binding ($B_{max}$) and equilibrium dissociation constant ($K_D$) were derived from a one-site ligand-binding model. Displacement curves were fit to a one-site equation of variable Hill slope to calculate $IC_{50}$ values; affinity constants ($K_i$) were derived from the $IC_{50}$ according to the relationship $K_i = IC_{50} / (1 + L/K_D)$, where L represents the concentration of radioligand and $K_D$ represents its equilibrium dissociation constant (Cheng and Prusoff, 1973). The antagonist affinity estimate $pA_2$ was derived by linear regression analysis of a plot of log CR-1 versus log B, according to the following equation: $pA_2 = \log (CR\text{-}1) - \log (B)$, where CR = the ratio of $EC_{50(test)} / EC_{50(control)}$, and B represents antagonist concentration (M). For behavioral assays, datasets were initially checked to ensure normality and homogeneity of variance using SPSS 13.0 (SPSS Inc., Chicago, IL). Then, data from behavioral experiments and the BrdU labeling were analyzed by one-way analysis of variance (ANOVA), followed by Fisher's protected least significant difference (PLSD) post hoc analysis. The effects of irradiation on SNAP 94847-induced decrease of latency to feed in the NSF were determined by a two-way ANOVA. Differences were considered significant when $p \leq 0.05$. All the analyses were conducted using Statview 5.0 (JMP Software, Cary, NC).

## Results

**In Vitro Pharmacological Characterization of SNAP 94847.** The antagonist radioligand [$^3$H]SNAP 7941 exhibited saturable, high affinity specific binding to membranes from PEAKRAPID 293 cells expressing the mouse MCHR1 ($B_{max}$ = 11.4 ± 4.6 pmol/mg protein; $K_D$ = 530 ± 45 pM; mean ± S.E.M. of three determinations) (Fig. 1B). SNAP 94847 displaced [$^3$H]SNAP 7941 with high affinity ($K_i$ = 1.69 ± 0.42 nM; Hill slope = 1.1; $n$ = 3) (Fig. 1C). This affinity agrees well with that determined using PEAKRAPID 293 cells expressing the rat MCHR1 ($K_i$ = 1.90 ± 0.08 nM, $n$ = 10; data not shown). A selectivity index [$K_{i\ (target)}/K_{i\ (mMCHR1)}$] of at least 100 was obtained when SNAP 94847 was profiled for binding or functional antagonism at 32 other peptide GPCR, 51 nonpeptide GPCR, 21 channel binding sites, 14 enzymes, and 6 transporters. Marginally higher cross-reactivity was seen at the 5-hydroxytryptamine$_{2B}$ receptor ($K_i$ = 137 nM). We also examined functional antagonism of MCH-evoked [$^3$H]inositol phosphate formation in HEK 293 cells stably expressing the rat MCHR1. SNAP 94847 (0.03–10 $\mu$M) produced concentration-dependent dextral shifts in the concentration curve to MCH, with a progressive reduction in the maximal response. A plot of the CR-1 versus log (SNAP 94847) afforded an estimated $pA_2$ value of 7.81 ± 0.21 ($n$ = 3) (Fig. 1D). These results are consistent with an orthosteric-insurmountable mode of antagonism (Kenakin et al., 2006), for which affinity estimates

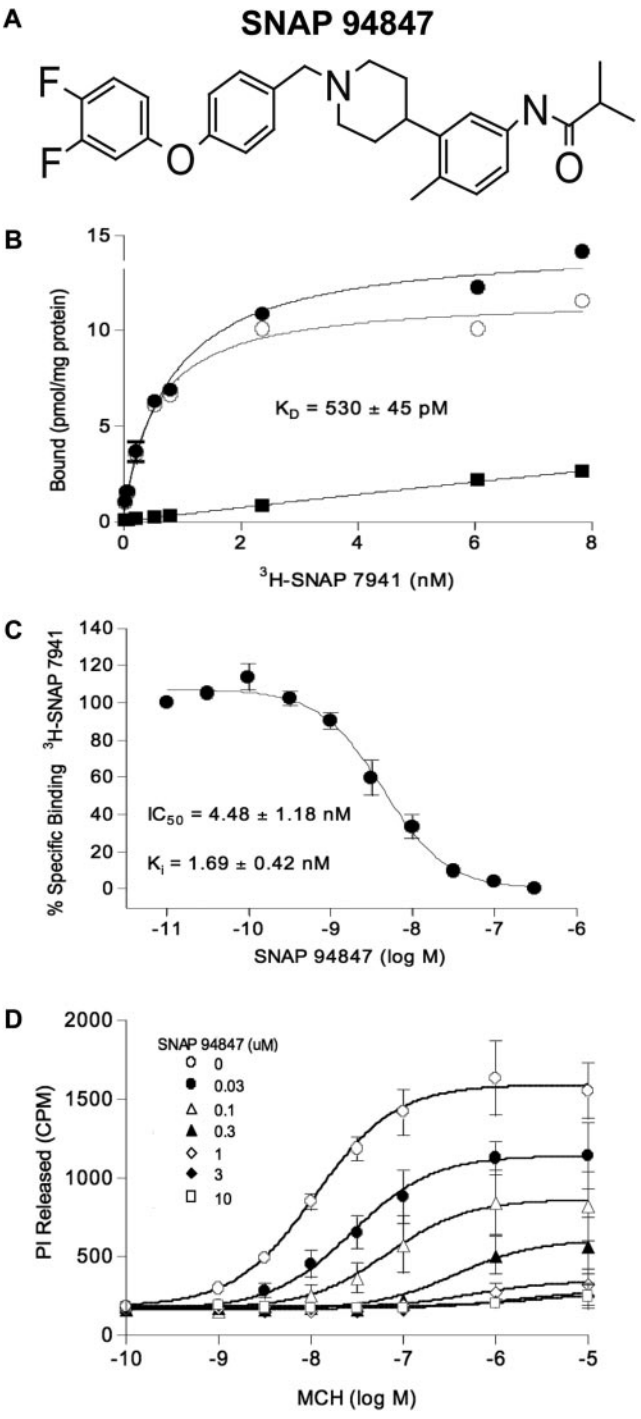


**Fig. 1.** A, chemical structure of SNAP 94847. B, binding of [$^3$H]SNAP 7941 to PEAKRAPID membranes expressing the mouse MCHR1: total binding (●), nonspecific binding in the presence of 1 $\mu$M unlabeled SNAP 7941 (■), and specific binding (○) ($n$ = 3 each). C, displacement of [$^3$H]SNAP 7941 binding by SNAP 94847 (points represent mean ± S.E.M. of six determinations). D, a representative dataset showing concentration-dependent antagonism of MCH-evoked [$^3$H]inositol phosphate production in HEK 293 cells stably expressing the rat MCHR1. The data shown represent a single experiment, each point representing the average of duplicate determinations; $pA_2$ represents the mean ± S.E.M. of three independent determinations.

using $pA_2$ determination may lead to small (maximal, ~2-fold) overestimates of affinity.

**Effects in the NSF Paradigm.** The effects of acute (20 mg/kg) and chronic (2 and 20 mg/kg/day) SNAP 94847 treatment were tested in 129S6/SvEvTac strain mice in the NSF test. One-way ANOVA followed by Fisher's PLSD post hoc test revealed a significant effect of the MCHR1 antagonist 1 h after injection [$F(3,51) = 13.13$, $p < 0.01$] and after 28 days of treatment [$F(3,50) = 5.32$, $p < 0.01$].

The acute p.o. administration of SNAP 94847 (20 mg/kg) reduced the latency to feed in the NSF test, and the magnitude of the effect was similar to that of the classic anxiolytic, diazepam (1.5 mg/kg s.c.) (Fig. 2A). In contrast, acute imipramine (20 mg/kg s.c.) exerted an effect opposite to SNAP 94847 or diazepam, increasing the latency to feed. The feeding drive of each mouse was assessed by returning it to the familiar environment of its home cage immediately after the NSF test and measuring the amount of food consumed over a period of 5 min. An acute administration of imipramine decreased the home food consumption [$F(3,51) = 2.82$, $p < 0.05$], whereas SNAP 94847 and diazepam produced no change on this value (Fig. 2B) ($p < 0.56$ and $p < 0.12$ for SNAP 94847 and diazepam, respectively). SNAP 94847 and diazepam acutely exhibited an anxiolytic-like effect, whereas imipramine did not. It is not clear from these data whether imipramine produced an acute anxiogenic-like effect or an acute decrease in appetite based on the change in home cage consumption.

The 28-day treatment period with either SNAP 94847 (20 mg/kg/day) or imipramine (20 mg/kg/day) led to a decrease in latency to feed in the NSF test, whereas the lowest dose of SNAP 94847 tested (2 mg/kg/day) did not modify the latency to feed [$F(3,50) = 5.32$, $p < 0.01$ and $p < 0.05$ for SNAP 94847 and imipramine, respectively] (Fig. 2C). Thus, similar anxiolytic-like effects were observed for both imipramine and SNAP 94847 after chronic administration in this model. Home cage food consumption was not modified by either drug [$F(3,50) = 0.61$] (Fig. 2D).

**Effects in the L/D Paradigm.** BALB/cJ mice were used for the L/D paradigm because this strain seems to be very responsive to anxiolytic treatment (Belzung and Griebel, 2001). SNAP 94847 was tested for both acute and chronic effects.

After acute treatment, one-way ANOVA followed by Fisher's PLSD post hoc test revealed a significant effect of SNAP 94847 (20 mg/kg) or diazepam (1 and 1.5 mg/kg) on the time

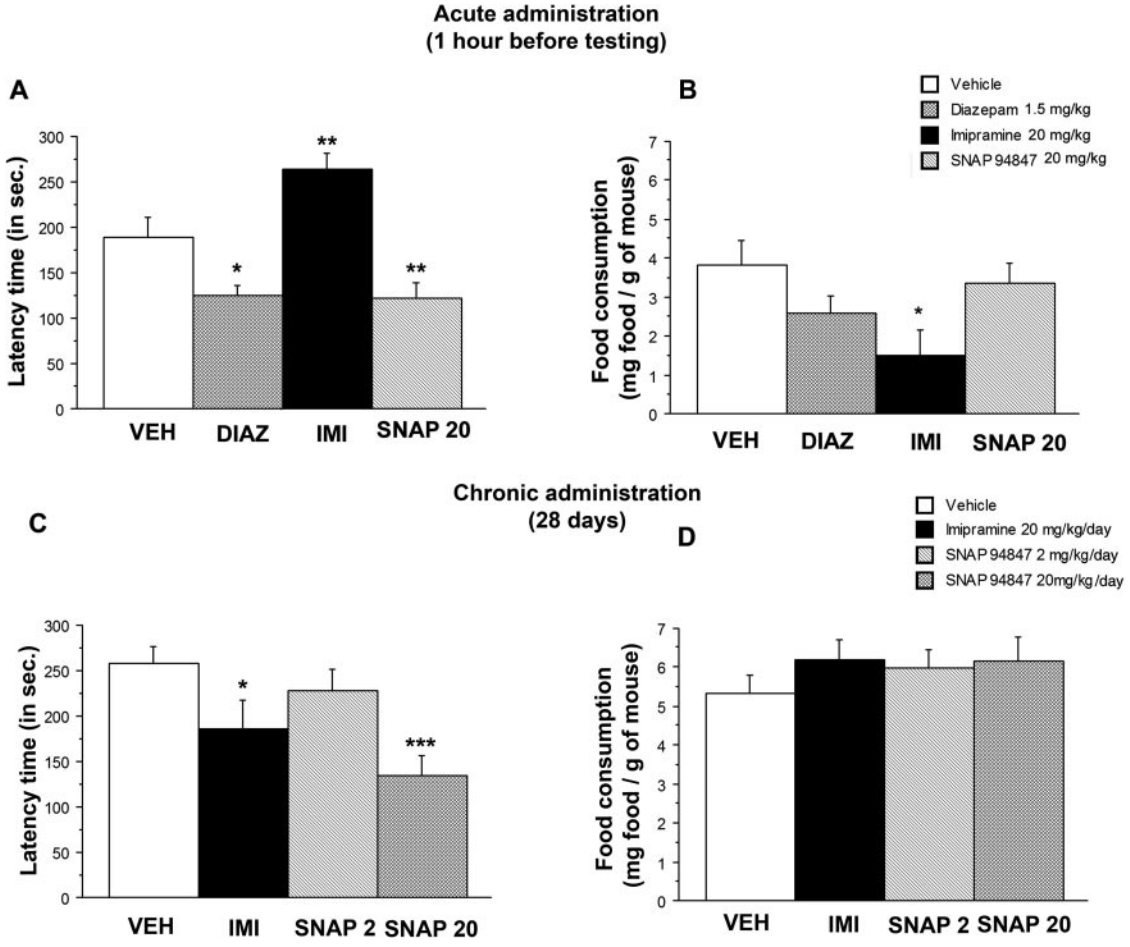


**Fig. 2.** The effects of vehicle (VEH), diazepam (DIAZ), imipramine (IMI), and SNAP 94847 on latency to feed in the NSF paradigm and food consumption in the home cage in 129S6/SvEvTac mice. A, mice were dosed with VEH, DIAZ (1.5 mg/kg s.c.), IMI (20 mg/kg s.c.), or SNAP 94847 (2, 20 mg/kg p.o.) and were tested 1 h later in the NSF paradigm. B, immediately after the NSF test, the same mice were placed in their home cage, and food consumption was monitored for 5 min. C, mice were tested in the NSF paradigm following 28 days of treatment with VEH, SNAP 94847 (20 mg/kg/day p.o.), or IMI (20 mg/kg/day p.o.). D, home cage food consumption for chronically dosed mice was monitored for 5 min immediately after the NSF test. All the data represent mean ± S.E.M.; $n = 12$ to 15 animals per group. *, $p < 0.05$; **, $p < 0.01$ from corresponding vehicle-treated group (ANOVA, Fisher's PLSD post hoc test).

spent in the light compartment [$F(4,49) = 2.66$, $p < 0.05$] (Fig. 3A) and on the number of transitions [$F(4,49) = 7.58$, $p < 0.01$] (Fig. 3B). The effect observed with SNAP 94847 is similar to the lowest dose of diazepam tested ($p < 0.05$). An acute dose of escitalopram did not increase the time spent in the light but increased transitions, even if the effect did not reach the significance ($p < 0.15$). Contrary to SNAP 94847 and diazepam (1 mg/kg), the highest dose of diazepam (1.5 mg/kg) increased the ambulatory distance ($p < 0.01$) [$F(4,49) = 3.25$, $p < 0.05$] (Fig. 3C).

An anxiolytic-like effect was also observed after p.o. administration of SNAP 94847 (20 mg/kg/day) for 28 days. Indeed, one-way ANOVA followed by Fisher's PLSD post hoc test revealed a significant effect of SNAP 94847 (20 mg/kg) on the time spent in the light compartment [$F(2,37) = 3.10$, $p < 0.05$] (Fig. 4A) and on the number of transitions [$F(2,37) = 2.94$, $p < 0.05$] (Fig. 4B). Although the effect after chronic treatment is weaker than the effect after acute treatment, the development of tolerance is not likely implicated, because after 5 days of treatment, the anxiolytic-like effect (increase of time spent in the light compartment) is similar to 28 days of treatment (Supplemental Fig. 1). Locomotor activity after chronic SNAP 94847 was not affected [$F(2,37) = 0.94$, $p > 0.05$].

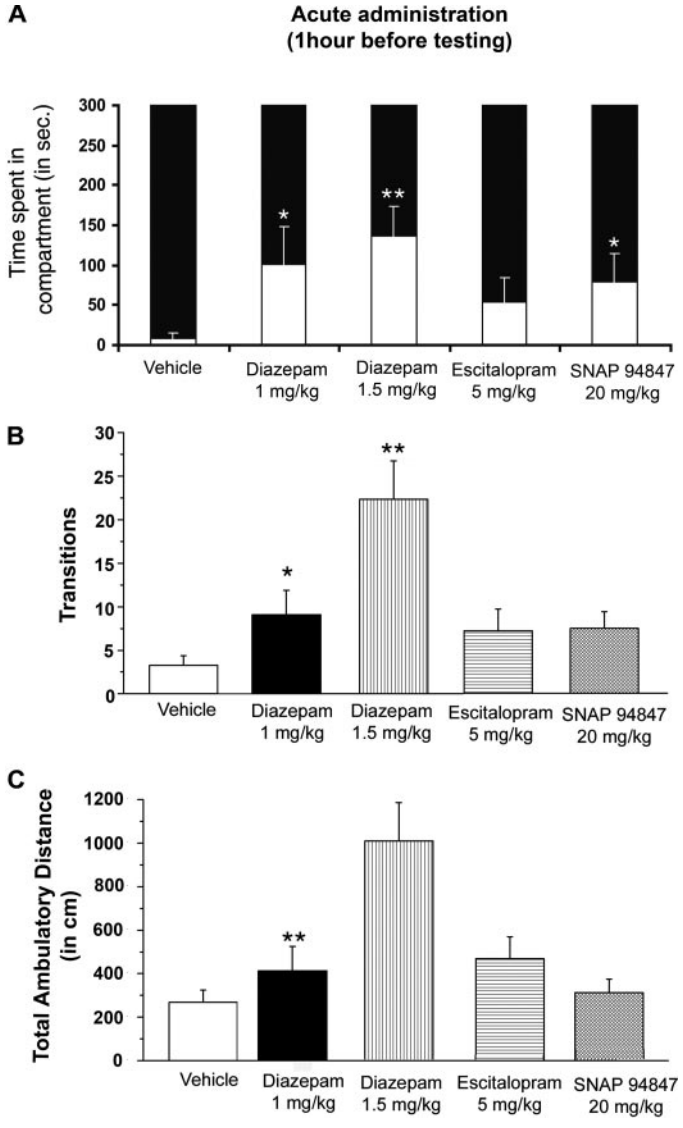


**Fig. 3.** Effects of acute (1 h) SNAP 94847 (20 mg/kg p.o.) treatment in the mouse L/D paradigm in BALB/cJ mice. The effects of 94847 were compared with vehicle treatment and the reference drugs escitalopram (5 mg/kg s.c.) and diazepam (1 and 1.5 mg/kg s.c.). A, total time spent in the light (□) and dark (■) compartments. B, number of transitions between light and dark compartments. C, total ambulatory distance. All the measurements were taken over a 5-min test period. Data represent the mean ± S.E.M. from $n = 12$ to 15 animals per group. *, $p < 0.05$, **, $p < 0.01$ from corresponding vehicle-treated group (ANOVA, Fisher's PLSD post hoc test).

**Effects in the Mouse FST.** We used the BALB/cJ mouse strain for the FST studies based on our previous observation that SSRI produce more robust effects in this mouse strain compared with others (Dulawa et al., 2004). The effects of acute and chronic SNAP 94847 treatment were compared with those of the SSRI escitalopram. One-way ANOVA followed by Fisher's PLSD post hoc test revealed a significant effect of escitalopram after acute (5 mg/kg) [$F(3,42) = 3.61$, $p < 0.01$] (Fig. 5A) and chronic (5 mg/kg/day) [$F(3,42) = 3.25$, $p < 0.05$] treatment (Fig. 5B) on the duration of immobility, indicating that escitalopram has acute and chronic antidepressant-like activity. However, SNAP 94847 had no significant effect on the duration of immobility after acute or chronic treatment compared with control treatments.

**Effects on Cell Proliferation.** Neurogenesis was shown to be required for the behavioral effects of SSRI and TCA in the NSF test (Santarelli et al., 2003). Because SNAP 94847 exhibited anxiolytic-like activity in the NSF test, we analyzed its effect on cell proliferation in the subgranular zone of the dentate gyrus (SGZ) after 28 days of treatment. Chronic p.o. treatment with SNAP 94847 (20 mg/kg/day) or imipramine (5 mg/kg/day) significantly increased the number of dividing cell progenitors in the SGZ of adult 129S6/SvEvTac mice [$F(3,23) = 4.07$, $p < 0.019$] (Fig. 6, A and B). No effect was seen with the lower dose of SNAP 94847 (2 mg/kg/day; $p < 0.18$).

**Effects of Irradiation on SNAP 94847-Induced Antidepressant/Anxiolytic-Like Activity in the NSF Test.** To test whether hippocampal neurogenesis was required for the effects of SNAP 94847, we sought to disrupt this process by using a focal X-irradiation (Santarelli et al., 2003). To directly evaluate the impact of irradiation on newly born neurons, we assessed immunoreactivity for DCX, a protein transiently expressed in young granule cells during the first postmitotic month (Brown et al., 2003). Irradiation drastically reduced the number of DCX-labeled cells in the SGZ (Fig. 7, C and D). However, a 28-day regimen of SNAP 94847 (20 mg/kg/day) reduced latency to feed in the NSF test, not only in sham ($p < 0.05$) but also in irradiated mice ($p < 0.05$) (Fig. 7E). Two-way ANOVA showed significant effects of MCHR1 treatment [$F(1,54) = 8.93$, $p < 0.01$], no effect of irradiation [$F(1,54) = 0.16$], and no interaction [$F(1,54) = 0.15$]. There was no significant effect on home cage food consumption of treatment [$F(1,54) = 1.44$, $p < 0.23$] or irradiation [$F(1,54) = 0.004$, $p < 0.94$], and no significant interaction [$F(1,54) = 0.003$, $p < 0.99$] (Fig. 7E). These results indicate that irradiation does not block the anxiolytic-like activity of SNAP 94847. Therefore, its anxiolytic-like behavioral effects in the NSF test seem to be independent of hippocampal neurogenesis.

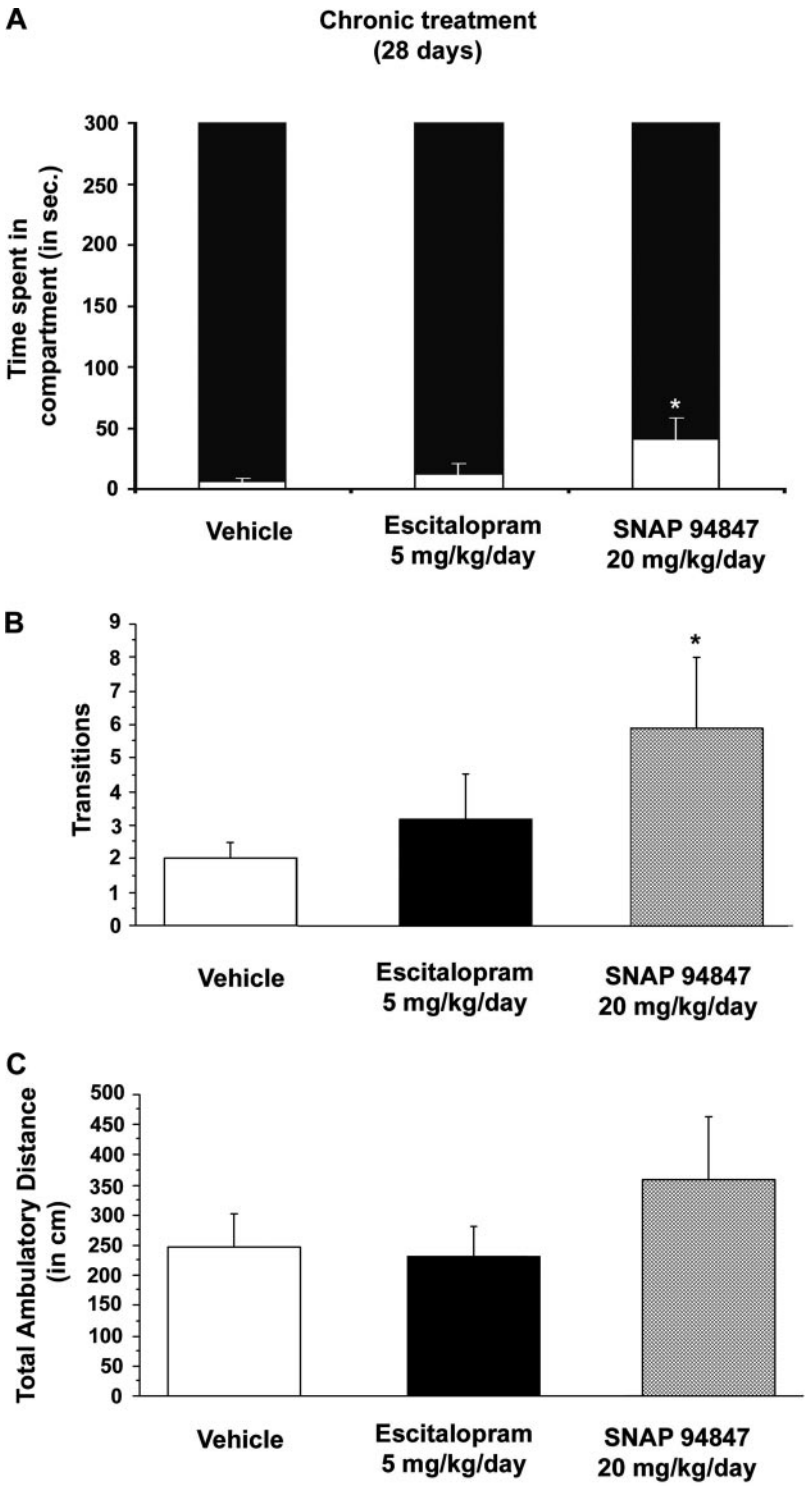


**Fig. 4.** Effects of chronic (28 days) SNAP 94847 (20 mg/kg/day p.o.) treatment in the mouse L/D paradigm in BALB/cJ mice. The effects 94847 were compared with vehicle treatment and the reference drug escitalopram (5 mg/kg/day p.o.). A, total time spent in the light (□) and dark (■) compartments. B, number of transitions between light and dark compartments. C, total ambulatory distance. All the measurements were taken over a 5-min test period. Data represent the mean ± S.E.M. from $n = 10$ to 15 animals per group. *, $p < 0.05$, from corresponding vehicle-treated group (ANOVA, Fisher's PLSD post hoc test).

## Discussion

Our results show that SNAP 94847 is a novel, high affinity selective antagonist at the MCHR1 with neurogenesis-independent actions in mouse behavioral models that differentiate it from classic anxiolytic and antidepressant drugs. SNAP 94847 binds with high (~2 nM) affinity to the mouse (ISS) and rat (Sprague-Dawley) MCHR1 with minimal cross-reactivity (selectivity ≥100-fold) to other GPCR, ion channels, enzymes, and transporters. In vitro functional studies show it to be a high affinity antagonist ($pA_2 = 7.81$) of MCH-evoked inositol phosphate formation, producing dextral shifts accompanied by a reduction of the maximal effect in the concentration-effect curve to MCH, consistent with an orthosteric-insurmountable antagonist interaction (Kenakin et al., 2006).

It was reported previously that MCH had divergent effects on stress and anxiety-related biological indices (Gonzalez et al., 1996; Monzon and De Barioglio, 1999; Kela et al., 2003). Recent data support the fact that MCH injection in rodent brain exerts anxiogenic-like behavioral effects and stimu-

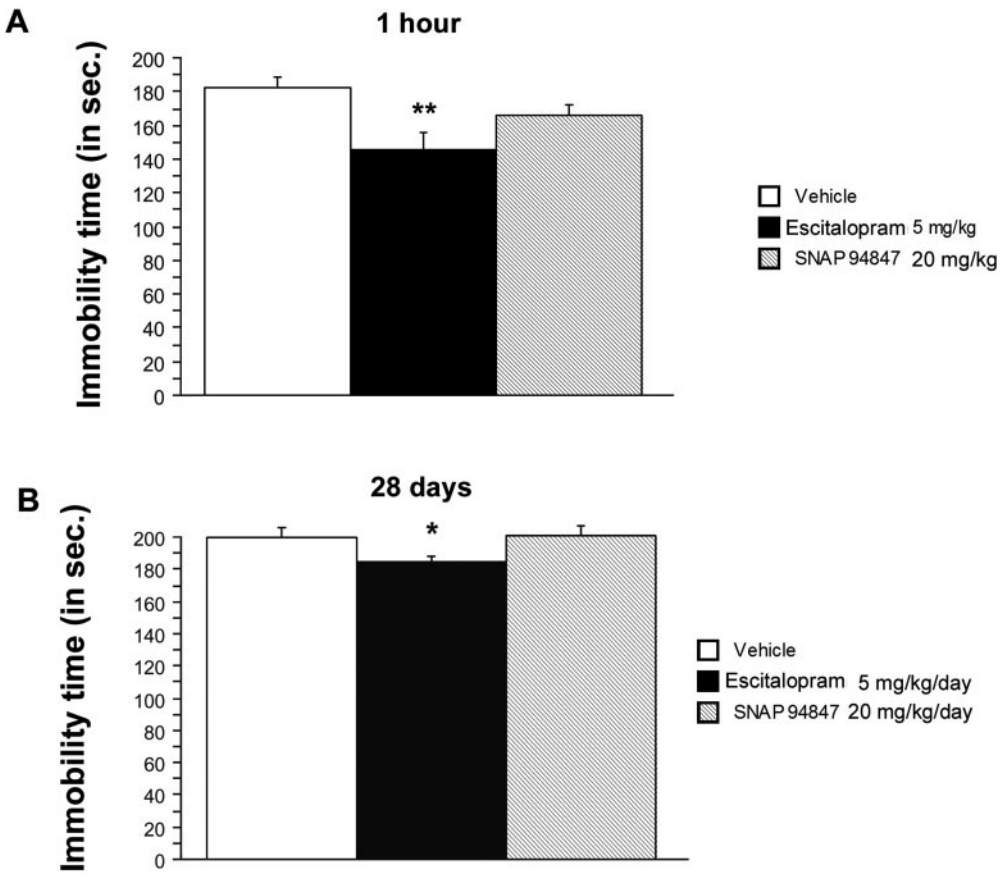


**Fig. 5.** Effects of vehicle, escitalopram, and SNAP 94847 in the FST following acute (1 h) or chronic (28 days) treatment in BALB/cJ mice. A, effects in FST after acute dosing of vehicle, escitalopram (5 mg/kg s.c.), and SNAP 94847 (20 mg/kg p.o.). B, effects in FST after chronic dosing of vehicle, escitalopram (5 mg/kg/day s.c.), and SNAP 94847 (20 mg/kg/day p.o.). Data are expressed as the mean ± S.E.M. of time spent immobile during the final 4 min of a 6-min swim session ($n = 14–15$ animals per group). *, $p < 0.05$; **, $p < 0.01$ from corresponding vehicle-treated group (ANOVA, Fisher's PLSD post hoc test).

lates the hypothalamic-pituitary adrenal axis, both of which are reversed by selective MCHR1 antagonists (Smith et al., 2006). Acute systemic administration of MCHR1 antagonists to rodents elicits either anxiolytic-like (Smith et al., 2006) or both anxiolytic- and antidepressant-like activity (Borowsky et al., 2002). In the present study, we characterized the behavioral effects of SNAP 94847 after acute (1 h) or chronic (28 days) administration in three rodent models used to detect anxiolytic- and antidepressant-like activity (FST, L/D paradigm, and NSF).

In the L/D paradigm, acute p.o. administration of SNAP 94847 (20 mg/kg) increased the time spent in the light compartment and the number of transitions compared with the vehicle-treated group. This acute anxiolytic-like effect is similar to that of diazepam, a classic anxiolytic. In addition, we showed that effect of SNAP 94847 persisted after chronic dosing (Fig. 4 and Supplemental Fig. 2). These results are consistent with recent data obtained in MCHR1 knockout mice (Chaki et al., 2005; Roy et al., 2006; Smith et al., 2006), which display anxiolytic-like behavior in various models such as the EPM, the open field, and a model of stress-induced hyperthermia (Roy et al., 2006). Recently, Smith et al. (2006) confirmed that anxiety-related responses were decreased by the MCHR1 antagonist GW3430 (30 and 100 mg/kg).

To generalize our findings about SNAP 94847 to other anxiety-related paradigms, we subjected mice to the NSF test, a test in which mice deprived food for 24 h find conflict between their aversion to the center of a novel, brightly lit field and their attraction to a food pellet in the center of the field (Santarelli et al., 2001). The latency to begin eating in this test is reduced by acute anxiolytic or chronic antidepressant drugs but not by acute SSRI and TCA. In this paradigm, the response to acute SNAP 94847 treatment (20 mg/kg) was similar to that produced by diazepam, significantly decreasing the latency to feed, consistent with an anxiolytic-like activity. The acute effect of SNAP 94847 in the NSF model contrasted with that of imipramine, which exerted an acute anxiogenic-like response, in agreement with other reports of acute anxiogenic-like actions in mice (Cole and Rodgers, 1995) and humans (Nutt and Glue, 1989). The anxiolytic effect of SNAP 94847 persisted after chronic treatment (28 days, 20 mg/kg/day). The reduction in latency to feeding in this test seems to be unrelated to any possible effect of SNAP 94847 on appetite because home cage feeding, assessed immediately after the NSF test, was not affected significantly by drug treatment. A decrease in body weight in 129S6/SvEvTac mice, however, was found after chronic administration of SNAP 94847 at 20 mg/kg (Supplemental Fig. 3). These results are in accord with previously published data, showing that chronic MCHR1 blockade produces a modest reduction of food consumption compared with fenfluramine but a reduction of body weight throughout the treatment period (Borowsky et al., 2002). This suggests that energy expenditure could also play a role in the effects on body weight. Taken together, the results from the L/D and NSF tests confirm acute and chronic anxiolytic-like activity of this MCHR1 antagonist.

We investigated the effects of SNAP 94847 in the FST using BALB/cJ mice that have been found to respond robustly to SSRI after chronic treatment (Dulawa et al., 2004). SNAP 94847 (20 mg/kg) did not reduce immobility time after either acute or chronic treatment, whereas escitalopram had

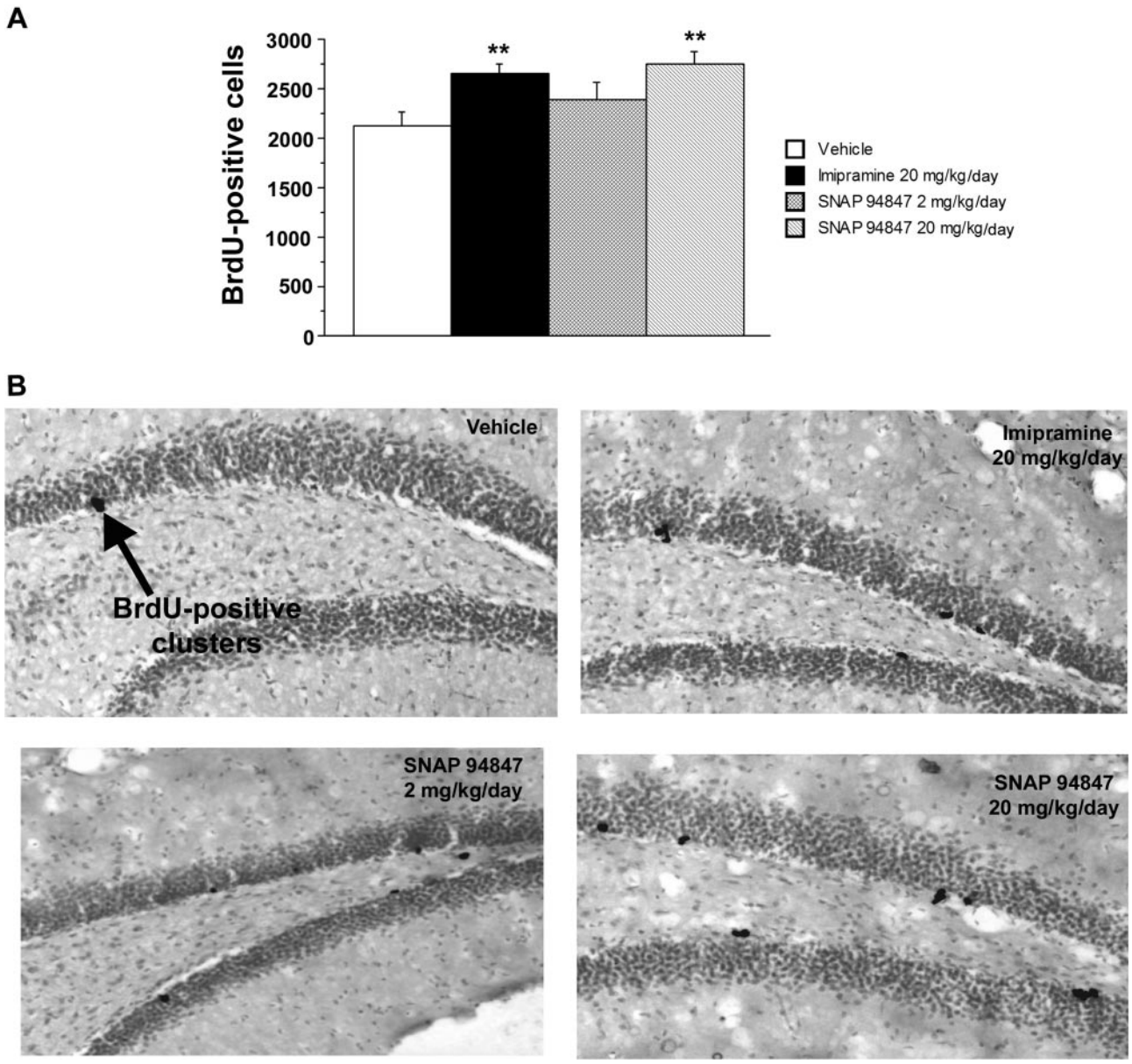


**Fig. 6.** Effects of chronic SNAP 94847 treatment on cell proliferation in the dentate gyrus 129S6/SvEvTac mice. Mice were treated for 28 days with vehicle, SNAP 94847 (20 mg/kg/day p.o.), or imipramine (20 mg/kg/day p.o.). Two hours after a single BrdU injection (150 mg/kg), labeled cells were found as clusters in the SGZ. A, effects of vehicle or drug treatments on total BrdU-positive cells per dentate gyrus. Data represent the mean ± S.E.M. of the cell counts from six or seven animals per treatment group (*, $p < 0.05$, from corresponding vehicle-treated group; ANOVA, Fisher's PLSD post hoc test). B, representative illustrations of BrdU immunoreactivity (20× magnification) in the dentate gyrus after 28 days of treatment with vehicle, SNAP 94847, or imipramine. BrdU-positive cells counts were made within the SGZ and adjacent zone defined as a two-cell body wide zone along the hilar border (40× magnification).

antidepressant-like activity after acute (5 mg/kg) and chronic administration (5 mg/kg/day). SNAP 94847 was ineffective when tested again in the mouse FST, similar in comparison with fluoxetine (Supplemental Fig. 4). Interestingly, acute administration of an MCHR1 antagonist produced an antidepressant-like effect in the FST in rats (Borowsky et al., 2002). Therefore, our results with SNAP 94847 in the mouse are different from those obtained in rats in the FST model. Recently, Georgescu et al. (2005) confirmed the MCH pathway as a promising target for antidepressant development as they have observed a decrease of immobility in the FST in rats after injection of an MCHR1 antagonist into the shell of the nucleus accumbens. Therefore, there may be species and/or strain differences in the effects of MCH antagonists in the FST. However, our data clearly show a difference in the responsiveness to SNAP 94847 in the FST and NSF tests. SNAP 94847 has no effect in the FST, whereas it has an effect in the NSF test both acutely and chronically (Table 2). Together with the fact that the NSF test is sensitive to acute anxiolytics whereas the FST is not, our data may indicate that the NSF is more responsive to anxiolytic drugs (both acutely and chronically) than to antidepressants. In that context, the chronic effect of SSRI and TCA seen in both the NSF and the FST may have different meanings. The NSF test may detect the anxiolytic effect of chronic SSRI and TCA, whereas the FST may be sensitive to the antidepressant effect of SSRI and TCA.

Stimulation of hippocampal neurogenesis has been sug-

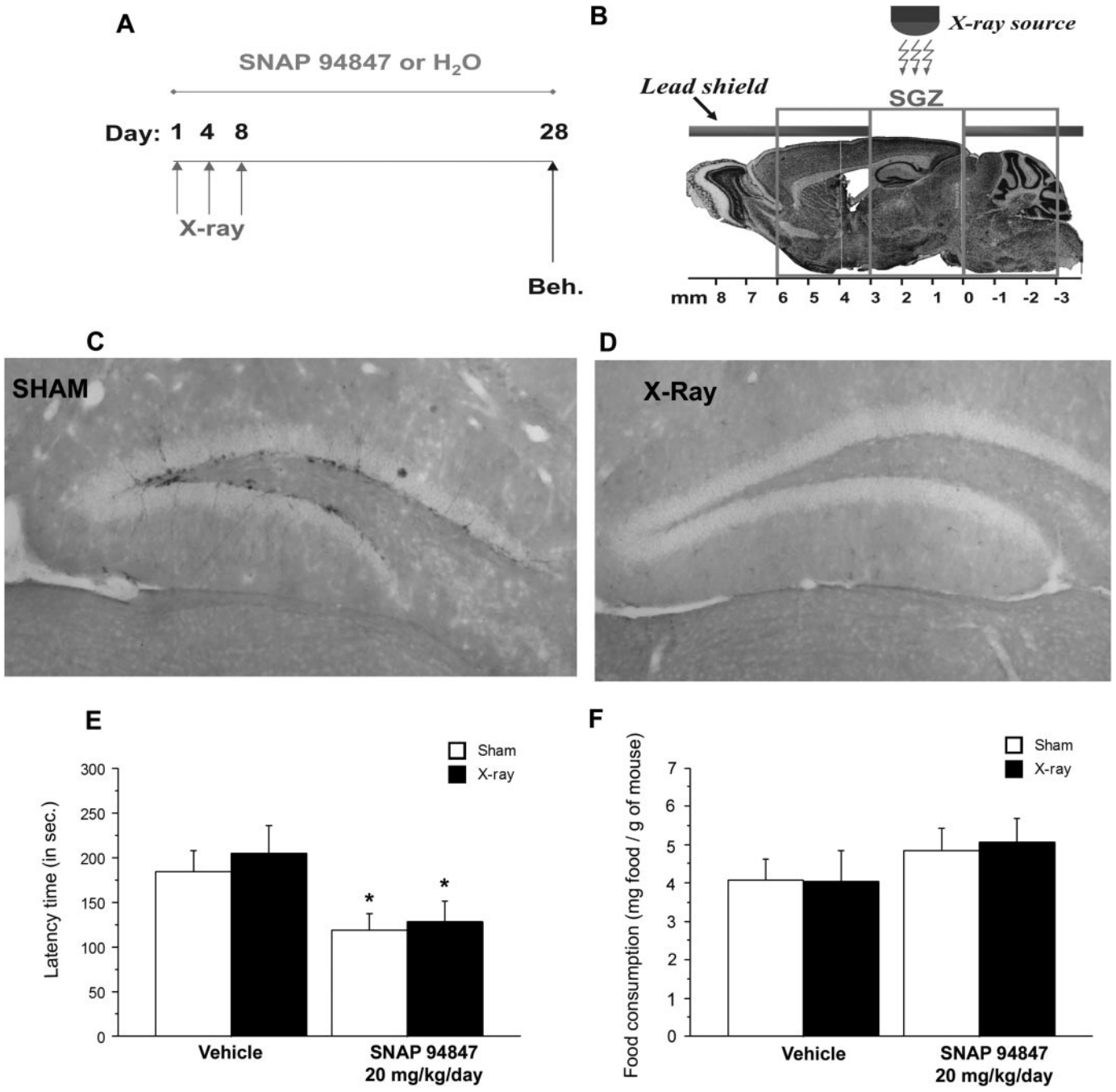


**Fig. 7.** Experimental design and effects of hippocampal irradiation on cell proliferation and responses to SNAP 94847 in the NSF test in 129S6/SvEvTac mice. A, mice were treated for 28 days with SNAP 94847 (20 mg/kg/day p.o.) and X-radiation (5 Gy) was delivered on days 1, 4, and 8. The NSF test was conducted on day 28. B, a shield protected the mouse's body while exposing the SGZ to X-rays (figure adapted from Franklin and Paxinos, 1997). C and D, representative images of DCX immunoreactivity in the dentate gyrus of sham mice (C) or 4 weeks after X-irradiation (D) (20× magnification). E, effects of vehicle and SNAP 94847 on latency to feed in the NSF test in sham and irradiated mice. F, effects of vehicle and SNAP 94847 on food consumption in the home cage in mice. Sham and irradiated mice were transferred to their home cage immediately following the NSF test. Data represent mean ± S.E.M. of 12 to 15 animals per group. Data were analyzed with a two-way ANOVA [pretreatment (irradiation or sham) and treatment factors]. There was a main effect of treatment but not effect of irradiation and no interaction. Planned comparisons test revealed a significant difference between each treated group and the corresponding vehicle-treated group (*, $p < 0.05$).

gested to underlie the delayed onset of therapeutic efficacy of SSRI and TCA (Duman et al., 1999; Malberg et al., 2000; Santarelli et al., 2003). Therefore, we investigated whether treatment for 28 days with SNAP 94847 stimulates neurogenesis in the mouse dentate gyrus. SNAP 94847 (20 mg/kg/day) and imipramine (20 mg/kg/day) stimulated progenitor cell proliferation in the SGZ, as evidenced, respectively, by a 30 and 25% increase in the number of BrdU-positive cells in the dentate gyrus. To assess whether hippocampal neurogenesis participates in the behavioral action of SNAP 94847 in the NSF test, we used an X-ray irradiation paradigm shown previously to suppress behavioral responses to SSRI and TCA in the NSF paradigm (Santarelli et al., 2003). The number of DCX-labeled cells was reduced significantly in

TABLE 2
Summary of the effects of SNAP 94847 in various behavioral models compared with classical anxiolytic and antidepressant drugs

| | 129S6/SvEvTac Strain | | BALB/cJ Strain | | | |
|---|---|---|---|---|---|---|
| | NSF (Latency to Feed) | | FST (Immobility Time) | | L/D Paradigm (Time in Light Compartment) | |
| | Acute | Chronic | Acute | Chronic | Acute | Chronic |
| Imipramine (20 mg/kg) | ↑ | ↓ | N.D. | N.D. | N.D. | N.D. |
| Escitalopram (5 mg/kg) | N.D. | N.D. | ↓ | ↓ | — | — |
| Diazepam (1 mg/kg) | N.D. | N.D. | N.D. | N.D. | ↑ | N.D. |
| Diazepam (1.5 mg/kg) | ↓ | N.D. | N.D. | N.D. | ↑ | N.D. |
| SNAP 94847 (2 mg/kg) | N.D. | — | N.D. | N.D. | N.D. | N.D. |
| SNAP 94847 (20 mg/kg) | ↓ | ↓ | — | — | ↑ | ↑ |

↑, increased parameter; ↓, decreased parameter; —, no significant effect on parameter; N.D., not determined; acute, 1-h treatment; chronic, 28-day treatment.

irradiated mice treated with SNAP 94847 (20 mg/kg/day, 28 days). Surprisingly, X-ray irradiation of the hippocampus did not suppress the effects of SNAP 94847 on behavior. Indeed, a 28-day regimen of this drug equally reduced latency to feed in the NSF test in both sham and hippocampal-irradiated mice. These results suggest that the mechanisms underlying the anxiolytic-like effects of SNAP 94847 are distinct from these underlying the effects of SSRI and TCA. Consistent with a distinct mechanism of action for SNAP 94847, we have shown that the onset of effect for SNAP 94847 in the NSF test is rapid, whereas SSRI and TCA have a slower onset. Furthermore, SNAP 94847 is effective in tests such as the L/D paradigm, in which SSRI and TCA are ineffective; conversely, SSRI and TCA are effective in the FST, whereas SNAP 94847 is not. Finally, the hippocampus seems to function as a primary locus of action for SSRI and tricyclics, whereas alternate brain systems acting within higher-order brain regions and pathways are likely to be important for the actions of SNAP 94847. Recent data suggest, for example, that some effects of SNAP 94847 may be mediated by receptors localized in the nucleus accumbens (Georgescu et al., 2005).

One of the questions we are raising in this study is whether behavioral paradigms can discriminate between the anxiolytic and antidepressant effects of compounds such SSRI and TCA. Our data, together with previous reports, suggest that although the NSF test may capture the anxiolytic effects of chronic SSRI, the FST may better model the antidepressant effects of chronic SSRI. In addition, the L/D test responds to acute anxiolytics but seems to be rather unresponsive to SSRI even when given chronically. It is interesting in this respect that SNAP 94847 works both in the NSF test and the L/D test but not in the FST. This may indicate that this compound has an anxiolytic profile after both acute and chronic administration but possibly does not have antidepressant activity. As a result, the new specific MCHR1 antagonist, SNAP 94847, may be more effective for the treatment of anxiety disorders than for depression. One potential advantage of MCHR1 antagonists over SSRI for the treatment of anxiety may be an acute onset of action compared with the delayed onset of therapeutic efficacy of SSRI.

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