OXFORD

International Journal of Neuropsychopharmacology (2018) 21(12): 1128–1137

doi:10.1093/ijnp/pyy088
Advance Access Publication: October 18, 2018
Regular Research Article

REGULAR RESEARCH ARTICLE

Melanin-Concentrating Hormone (MCH) and MCH-R1 in the Locus Coeruleus May Be Involved in the Regulation of Depressive-Like Behavior

Hui Ye, Xiang-Yu Cui, Hui Ding, Su-Ying Cui, Xiao Hu, Yu-Tong Liu, Hui-Ling Zhao, Yong-He Zhang

Department of Pharmacology, Peking University, School of Basic Medical Science, Beijing, China.

H.Y. and X.Y.C. equally contributed to this paper.

Correspondence: Yong-He Zhang, PhD, Department of Pharmacology, Peking University, School of Basic Medical Science, Beijing, 100191, China (zhyh@hsc.pku.edu.cn).

Abstract

Background: Previous anatomical and behavioral studies have shown that melanin-concentrating hormone is involved in the modulation of emotional states. However, little is known about brain regions other than the dorsal raphe nucleus that relate the melanin-concentrating hormone-ergic system to depressive states. Numerous studies have shown that the locus coeruleus is involved in the regulation of depression and sleep. Although direct physiological evidence is lacking, previous studies suggest that melanin-concentrating hormone release in the locus coeruleus decreases neuronal discharge. However, remaining unclear is whether the melanin-concentrating hormone-ergic system in the locus coeruleus is related to depressive-like behavior.

Method: We treated rats with an intra-locus coeruleus injection of melanin-concentrating hormone, intracerebroventricular injection of melanin-concentrating hormone, or chronic subcutaneous injections of corticosterone to induce different depressive-like phenotypes. We then assessed the effects of the melanin-concentrating hormone receptor 1 antagonist SNAP-94847 on depressive-like behavior in the forced swim test and the sucrose preference test.

Results: The intra-locus coeruleus and intracerebroventricular injections of melanin-concentrating hormone and chronic injections of corticosterone increased immobility time in the forced swim test and decreased sucrose preference in the sucrose preference test. All these depressive-like behaviors were reversed by an intra-locus coeruleus microinjection of SNAP-94847.

Conclusions: These results suggest that the melanin-concentrating hormone-ergic system in the locus coeruleus might play an important role in the regulation of depressive-like behavior.

Keywords: depressive-like behavior, locus coeruleus, MCH, MCH-R1

Introduction

Melanin-concentrating hormone (MCH) is a 19-amino-acid cyclic neuropeptide that functions as a neuromodulator in rats. It was shown to be fully conserved in mammals, including humans (Forray, 2003; Saito and Nagasaki, 2008), and regulates feeding, energy homeostasis, mood, and the sleep-wake cycle (Verret et al., 2003; Monti et al., 2013). Neurons that

Received: May 9, 2018; **Revised:** October 11, 2018; **Accepted:** October 13, 2018

Significance Statement

Melanin-concentrating hormone (MCH) is claimed to be involved in the regulation of sleep and depressive-like behavior, and microinjecting MCH in the locus coeruleus (LC) could increase time spent in rapid-eye-movement sleep. In the present study, we found an intra-LC microinjection of MCH-Receptor 1 antagonist SNAP-94847 could block the depressive-like behavior of rats produced by repeated subcutaneous injection of corticosterone (CORT), i.c.v. injection of MCH, and intra-LC microinjection of MCH. Our results demonstrated that the MCH-ergic system in the LC is involved in the development of depressive-like behavior.

synthesize MCH are located mainly in the lateral hypothalamus and incerto-hypothalamic area and project throughout the central nervous system (Bittencourt et al., 1992; Saito et al., 2001; Torterolo et al., 2006).

MCH activates 2 types of receptors: MCH receptor 1 (MCH-R1) and MCH-R2. MCH-R1 is the only receptor subtype that is found in rodents (Tsunematsu et al., 2014). Because of the dense expression of MCH-R1 in areas of the brain that are involved in stress, reward, and emotional regulation (Saito et al., 2001), MCH signaling was suggested to regulate depressive-like behavior. Pharmacological support for this hypothesis was found when the MCH-R1 antagonist SNAP-7941 reduced immobility time in rats in the forced swim test (FST), and this effect was similar to fluoxetine (Borowsky et al., 2002). Additionally, the MCH-R1 antagonist *N*-[3-(1-[(4-[3,4-difluorophenoxy]phenyl)methyl]-4-piperidinyl)-4-methylphenyl]-2- methylpropanamide hydrochloride (SNAP-94847) was recently reported to effectively reverse the decrease in sucrose intake in an animal model of chronic mild stress-induced anhedonia (Smith et al., 2009). The MCH-R1 antagonist SNAP-94847 was shown to have a more rapid onset of action in the novelty-suppressed feeding test than a traditional antidepressant (David et al., 2007). The genetic deletion of MCH-R1 in female mice also resulted in antidepressant effects in the FST (Roy et al., 2007), highlighting a potential advantage of MCH-R1 as a target for the treatment of depression. However, the genetic inactivation of MCH-R1 in male mice did not result in an antidepressant-like effect (Roy et al., 2007). SNAP-7941 and 3 other MCH-R1 antagonists (T-226296, A-665798, and A-777903) did not have antidepressant efficacy in other paradigms (Basso et al., 2006). Thus, still debatable are the roles of MCH in depression-related behaviors. Moreover, the detailed mechanisms by which neuropeptide systems, including MCH, regulate depressive-like states are complex. Such complexity might be partially attributable to findings of studies that pharmacologically or genetically manipulated the MCH-ergic system and observed systemic effects, whereas MCH-ergic systems in specific brain regions may have distinct functions. Previous studies showed that microinjections of MCH in the dorsal raphe nucleus increased the duration of rapid-eye-movement (REM) sleep (Lagos et al., 2009) and increased immobility time in the FST (Lagos et al., 2011), thus demonstrating its pro-depressive effects.

Noradrenergic LC neurons are an important component of the sleep-wake cycle and have wake-promoting and REM-suppressive properties (Lu et al., 2006; Saper et al., 2010). The moderate innervation of MCH-containing neurons in tyrosine hydroxylase (TH)-positive cells has been identified in the LC (Del Cid-Pellitero and Jones, 2012). When injected directly in the LC, MCH increased the time spent in REM sleep (Monti et al., 2015). MCH has been suggested to silence LC neurons during REM sleep (Saper et al., 2010) while MCH-expressing neurons remain active (Hassani et al., 2009). Although direct physiological evidence is lacking, MCH release in the LC has been suggested to decrease neuronal discharge (Del Cid-Pellitero and Jones, 2012).

Numerous studies have indicated that the LC is involved in the regulation of depression. A minimal loss of LC neurons induced depressive-like behavior (Szot et al., 2016). However, unknown is whether the MCH-ergic system in the LC is functionally relevant to depressive-like behavior. The present study tested the hypothesis that the MCH-ergic system in the LC contributes to depressive-like behavior and that the pharmacological inhibition of MCH-R1 in the LC ameliorates depressive-like behavior. Rats received a direct intra-LC microinjection of MCH or intracerebroventricular (i.c.v.) injection of MCH, and depressive-like behavior was assessed in the sucrose preference test (SPT) and FST. We then examined whether an intra-LC injection of the MCH-R1 antagonist SNAP-94847 blocks depressive-like behavior that is induced by MCH or repeated corticosterone (CORT) administration.

Methods

Animals

Male Sprague-Dawley rats (250–300 g, Grade I, purchased from the Animal Center of Peking University, Beijing, China) were individually housed in plastic cages and maintained under an artificial 12-h-light/-dark cycle (lights on 9:00 AM to 9:00 PM) at 23°C ± 1°C and 50% ± 10% humidity. The rats had ad libitum access to food and water. All the experiments were conducted in accordance with the European Communities Council Directive (2010/63/EU) for the use of experimental animals and were approved by the Peking University Committee on Animal Care and Use.

Drugs

Corticosterone (Tokyo Chemical Industry Co., Ltd, Tokyo, Japan) was dissolved in 0.9% saline with 2% Tween 80 and administered s.c. MCH was purchased from Phoenix Pharmaceutical (Burlingame, CA) and dissolved in saline. The MCH-R1 antagonist SNAP-94847 was obtained from Tocris (Minneapolis, MN) and dissolved in 30% dimethylsulfoxide. We did not observe any adverse effects of dimethylsulfoxide in rats at this concentration, which is consistent with a previous report (Xu et al., 2009).

Experimental Design

In the first experiment, different doses of MCH (50 and 100 ng in 0.2 μL of saline/site) were bilaterally microinjected in the LC 30 minutes before the behavioral tests. SNAP-94847 (0.2 μL of 30 μg/μL) or vehicle was bilaterally microinjected in the LC 30 minutes before the microinjection of MCH (Figure 1A). The microinjections were performed with a Hamilton syringe that was connected to a 33-gauge injection cannula (Plastics One, Roanoke, VA). Drug or vehicle was delivered through the injection cannula that extended 1 mm beyond the guide cannula in a 0.2-μL volume for 2 minutes. The 0.2-μL volume is not excessive

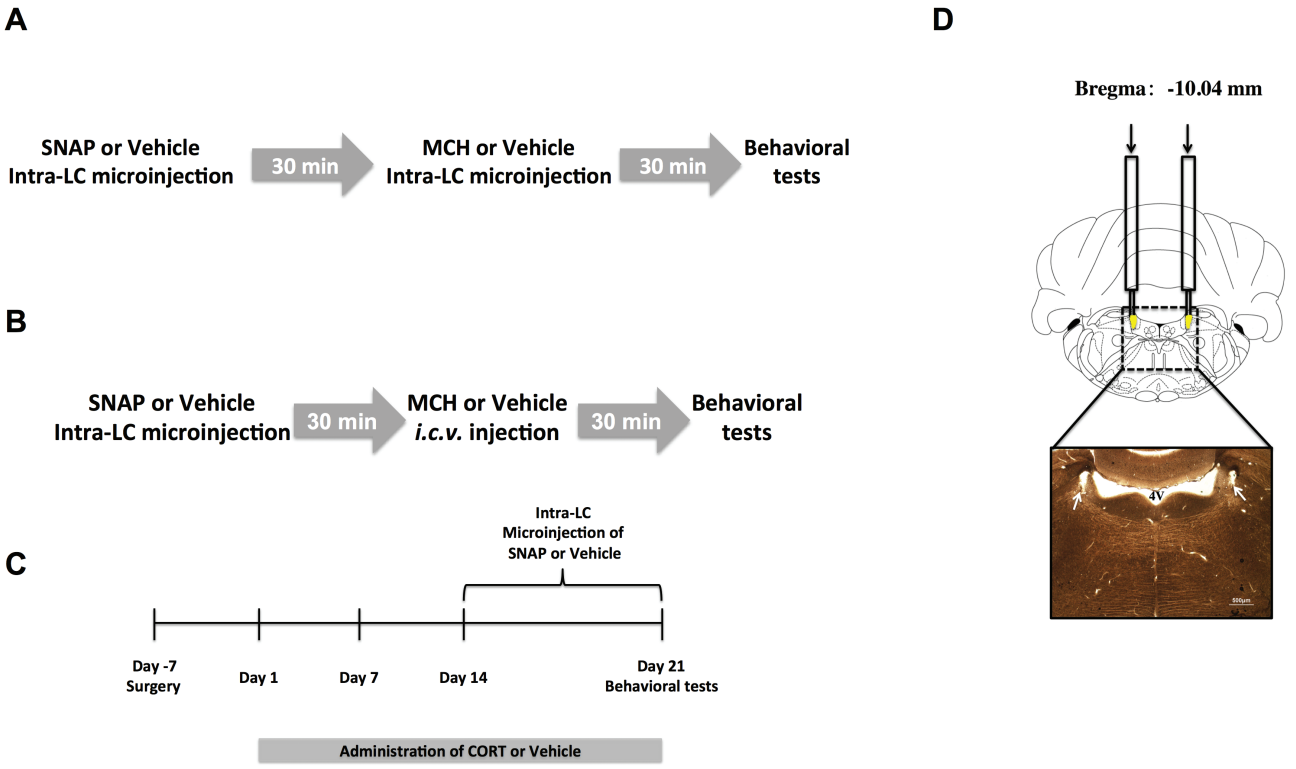

Figure 1. Time course of the experiment and histological identification of the microinjection sites. (A–C) The time courses of the first, second, and third experiments. (D) Photomicrograph scan of a coronal section illustrating the localization of locus coeruleus (LC). Arrows indicate the site of bilateral injection. 4V, fourth ventricle.

for the LC, as demonstrated by previous reports (Felippotti et al., 2011; Monti et al., 2015; Wang et al., 2015). The injection cannula was kept in place for another 2 minutes to allow the drug to completely diffuse from the tip. After the behavioral tests, 18 rats were perfused and prepared for further histological verification of the cannula placements. Data from 2 rats were excluded because of cannula misplacements, and 2 rats were excluded because of death during surgery. The dose of MCH was based on a previous study (Monti et al., 2015) with minor modification. At least 7 days elapsed between experiments.

In the second experiment (Figure 1B), different doses of MCH (0.4, 0.8, and 1.6 μg) and vehicle were dissolved in 5 μL saline and microinjected in the lateral ventricle (i.c.v. injection) 30 minutes before the behavioral test. SNAP-94847 or vehicle was bilaterally microinjected in the LC 30 minutes before the i.c.v. injection of MCH. The i.c.v. injections were performed with a polyethylene tubing (RWD Life Science, Shenzhen, China) that was connected to a 28-gauge injection cannula (RWD Life Science, Shenzhen, China). Drug or vehicle was administered through the injection cannula in a 5-μL volume for 3 minutes. The injection cannula was kept in place for another 2 minutes to allow the drug to completely diffuse from the tip. After the behavioral tests, 18 rats were perfused and prepared for further histological verification of cannula placements. Data from 2 rats were excluded because of cannula misplacements. The doses of MCH, SNAP-94847, and vehicle were based on previous reports (Verret et al., 2003; Xu et al., 2011; Sun et al., 2013). At least 7 days elapsed between experiments.

In the third experiment, 58 rats received vehicle or 40 mg/kg CORT (2 mL/kg) s.c. at 9:00 AM daily for 21 days (Figure 1A). SNAP-94847 (0.2 μL of 30 μg/μL) or vehicle was bilaterally microinjected in the LC 30 minutes before the CORT injection from day 15 to day 21 for 7 consecutive days. Our previous results indicated that rats receiving repeated CORT injections exhibit depressive-like behavior beginning on day 15 (Cui et al., 2018). All the rats underwent the behavioral tests 30 minutes after CORT administration on day 21. After the behavioral tests, the rats were perfused and prepared for further histological verification of cannula placements. Data from 2 rats were excluded because of cannula misplacements. The dose of CORT was based on a previous study (Wang et al., 2015) with minor modification.

Intracerebroventricular Injection Surgery

Anesthetized rats were positioned in a stereotaxic apparatus (Aguilar et al., 2014; Lipski et al., 2017). A single guide cannula (23 gauge, RWD Life Science) was implanted in a hole that was drilled in the skull above the appropriate targeted structures according to the following coordinates: anterior/posterior, -0.9 mm; lateral, 1.5 mm; dorsal/ventral, -0.33 mm (Paxinos and Watson, 2005). The cannula was secured to the skull with 4 stainless-steel screws and dental acrylic. After surgery, the rats were injected with penicillin for 3 days and allowed to recover for 7 days before the behavioral experiments.

Intra-LC Microinjection Surgery

Similar to the i.c.v surgery, for the intra-LC microinjections, anesthetized rats were positioned in a stereotaxic apparatus, and a double-guide cannula (26 gauge, C/C dist. 2.4 mm, Plastics One, Roanoke, VA) was implanted with the tip 1 mm above the LC at the following coordinates (distance from lambda): anterior/posterior, -3.4 mm; lateral, ±1.2 mm; dorsal/ventral, -6.0 mm below the brain surface (15° inclination of vertical stereotaxic bar). After the experiment, the cannula placements were histologically verified by light microscopy in 50-μm sections. The

injection placements were verified according to Paxinos and Watson (2005). All the data that are presented in this study were derived from animals whose injection sites were within the LC (Figure 1D). A total number of 6 rats were excluded because of cannula misplacements.

Behavioral Tests

All the animals were randomly divided into 2 separate cohorts for the behavioral tests. One cohort underwent only the FST, and the other cohort underwent the open field test (OFT) and SPT.

Forced Swim Test

The FST was performed according to a modified version of the paradigm (Wang et al., 2014; Fenton et al., 2015). On the pretest day, each rat was individually placed for 15 minutes into a 25-cm-diameter × 60-cm-high Plexiglas cylinder that was filled with 23°C to 25°C water to a depth of 40 cm. On the test day, the rat was placed into the same cylinder again and recorded for 5 minutes, 30 minutes after CORT administration on day 21 in the first experiment, 30 minutes after the i.c.v. MCH injection in the second experiment, and 30 minutes after the intra-LC MCH microinjection in the third experiment. Behavior was recorded by 2 video cameras (1 on top and 1 on the side). After the experiment, the rat was removed from the water, dried with a towel, and returned to its home cage. The videotapes were analyzed by a researcher who was blind to each rat's treatment condition. Behavior in the last 4 minutes of the test was classified into 3 types. Immobility was defined as the minimum movement that was necessary to keep the rat's head above the water. Climbing was defined as vigorous vertical forepaw movements. Swimming was defined as large and horizontal forepaw movements that displaced water to move the rat's body around the cylinder.

Open Field Test

Locomotor activity was measured in the OFT, which was performed according to a modified version of the paradigm (Wang et al., 2014). The OFT was conducted 30 minutes after CORT administration on day 21 in the first experiment, 30 minutes after the i.c.v. MCH injection in the second experiment, and 30 minutes after the intra-LC MCH microinjection in the third experiment. The rats were placed in a Plexiglas chamber (40 cm × 40 cm × 65 cm), and behavior was recorded by an automated video tracking system (DigBehv-LM4, Shanghai Jiliang Software Technology, Shanghai, China). The video files were later analyzed using DigBehv analysis software. Locomotor activity is expressed as the total distance traveled in 10 minutes (supplementary Figure 2; Harrell et al., 2013). After the OFT, the rats underwent the SPT.

Sucrose Preference Test

The SPT was used to determine anhedonia-like behavior, which is considered a core symptom of clinical depression. In the present study, the rats were habituated to drink from 2 bottles for 48 hours. One bottle was filled with water, and the other bottle was filled with 1% sucrose solution. The position of the bottles was changed every 4 hours to avoid side preference. After training, the rats were water deprived for 12 hours before the SPT. On the test day, after the OFT, the water and 1% sucrose bottles were placed in the rat's home cages, and rats were allowed to drink freely from both bottles for 1 hour. Water and sucrose consumption were measured by comparing the weight difference of the bottles before and after the test. Anhedonia was assessed as sucrose preference, which was calculated according to the following formula: sucrose preference = sucrose intake (g) / (sucrose intake [g] + water intake [g]) × 100%. To assess the nonspecific suppression of drinking, total fluid consumption was calculated as the sum of sucrose intake and water intake.

Immunofluorescence Staining

Anesthetized rats were perfused with 250 mL of phosphate buffered saline (PBS) and 250 mL of 4% paraformaldehyde. Whole brains were immediately removed, post-fixed in the same fixative at 4°C for 24 hours, and then immersed in 30% sucrose at 4°C for cryoprotection. The brains were rapidly frozen in liquid n-hexane that was cooled with a mixture of solid carbon dioxide and ethanol. Coronal sections (20 μm) that encompassed the LC (Bregma: -9.7 to -10.2 mm) were cut using a freezing microtome (Leica CM1850, Leica Microsystems UK, Milton Keynes, UK).

Each slide-mounted tissue section was double stained with TH and MCH according to standard procedures (Cao et al., 2016; Wang et al., 2017). The sections were first washed in PBS (3 × 5 minutes) and then incubated in cold acetone for 30 minutes, followed by washing in PBS (3 × 5 minutes). The antigen retrieval procedure was conducted in citrate buffer in a microwave (0.01 mol/L, pH = 6.0, 100°C). After cooling to room temperature, the sections were blocked with 5% donkey serum at room temperature for 1 hour. The sections were then incubated with a mixture of 2 primary antibodies overnight at 4°C. The primary antibodies were mouse anti-TH (SantaCruze, 1:1000) and rabbit anti-pro-MCH antibody (Phoenix Pharmaceuticals, 1:500). After washing in PBS (3 × 5 minutes), the sections were incubated with respective fluorophore-conjugated secondary antibodies (donkey anti-rabbit IgG Alexa Fluor 488, Abcam, 1:1000; Cy3 donkey anti-mouse IgG, Jackson Laboratories, 1:1000) for 120 minutes at room temperature, followed by washing in PBS (3 × 5 minutes). For negative control, another set of brain sections was processed without any anti-MCH antibody, and its secondary antibody was retained. Images of negative control were captured with te same settings as the sections with primary antibody for MCH. Finally, the sections were mounted with fluorescent mounting medium with 4', 6-diamidino-2-phenylindole.

The sections were examined under a TCS SP8 confocal microscope (Leica Microsystems, Wetzlar, Germany). The brightness and contrast of the captured images were adjusted using ImageJ software. Tyrosine hydroxylase was labeled red and MCH was labeled green.

Statistical Analysis

The data are expressed as mean ± SEM and were analyzed using SPSS 21.0 software (SPSS Inc., Chicago, IL). The data were analyzed using 1-way ANOVA followed by the Bonferroni posthoc test or 2-way ANOVA. In all the tests, $P < .05$ was considered statistically significant.

Results

Microinjection of SNAP-94847 in the LC Blocked Depressive-Like Behavior That Was Induced by an Intra-LC Microinjection of MCH

In the first experiment, we examined the effects of a direct microinjection of MCH in the LC. Different doses of MCH were microinjected in the LC. Melanin-concentration hormone

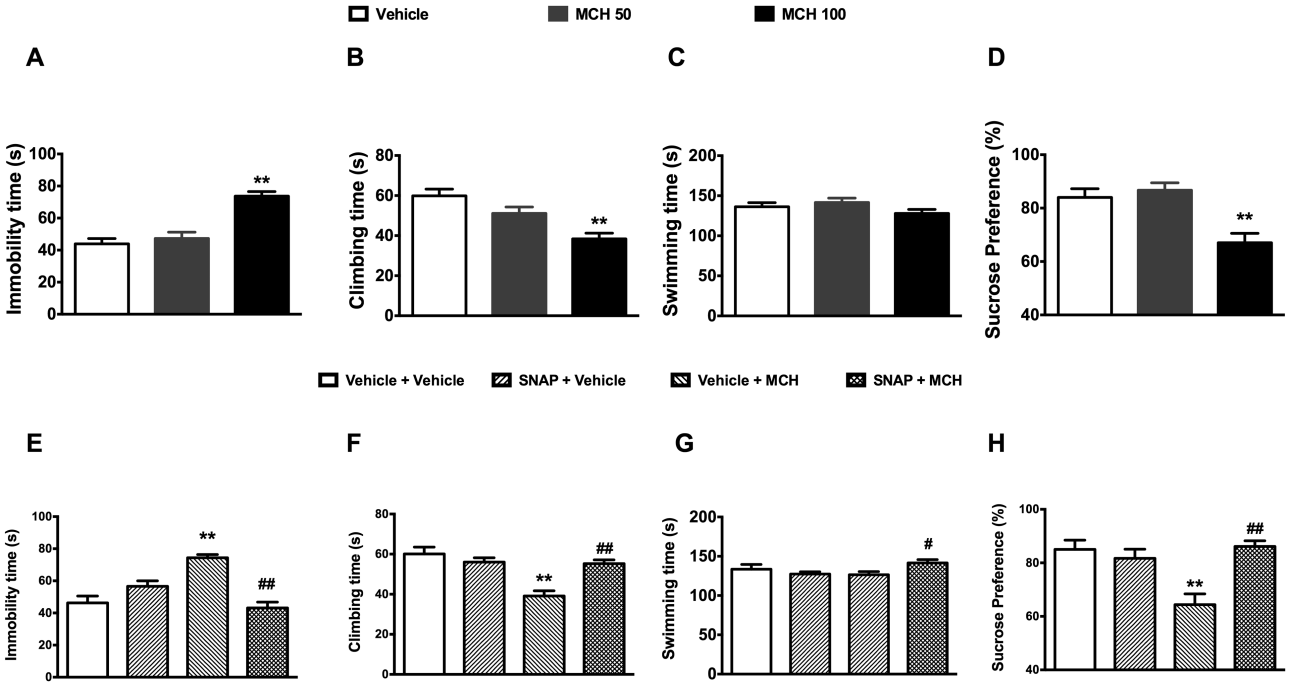

Figure 2. Effects of intra-locus coeruleus (LC) injection of SNAP-94847 on the behavioral alterations induced by microinjection of melanin-concentrating hormone (MCH) in the LC. Microinjection of 100 ng MCH into the LC increased the immobility time (A) and decreased the climbing time (B) in the forced swim test (FST) and decreased sucrose preference in the sucrose preference test (SPT) (D). No significant alterations were found in the swimming time in the FST (C). Pretreatment of microinjecting SNAP-94847 in the LC could block the MCH-induced depressive-behavior in the FST and SPT (E–H). All data are presented as mean ± SEM, n = 6–8/group. $^{**}P < .01$ as compared with the Vehicle group and ##$P < .01$ as compared with the Vehicle + MCH group.

at a dose of 100 ng significantly increased immobility time ($F_{2,17} = 24.685$, $P < .001$; Figure 2A) and decreased climbing time ($F_{2,17} = 12.401$, $P < .001$; Figure 2B). The microinjection of 100 ng MCH in the LC also significantly decreased sucrose preference in the SPT ($F_{2,17} = 13.397$, $P < .001$; Figure 2D) compared with the vehicle group. No significant effects of intra-LC microinjection of different doses of MCH on swimming time were observed in the FST ($P > .05$, Figure 2C).

The MCH-R1 antagonist SNAP-94847 was then microinjected in the LC before the intra-LC injection of MCH to determine whether MCH-R1 in the LC is involved in the regulation of depressive-like behavior. The ANOVA of immobility time in the FST (Figure 2E) revealed significant effects of MCH ($F_{1,24} = 4.684$, $P < .05$) and SNAP-94847 ($F_{1,24} = 9.545$, $P < .01$) and a significant MCH × SNAP-94847 interaction ($F_{1,24} = 37.633$, $P < .001$). The posthoc analysis showed that the intra-LC injection of MCH (100 ng) significantly increased immobility time compared with the vehicle group ($P < .01$), which was blocked by the intra-LC microinjection of SNAP-94847 ($P < .01$). The ANOVA of climbing time (Figure 2F) revealed significant effects of MCH ($F_{1,24} = 17.278$, $P < .01$) and SNAP-94847 ($F_{1,24} = 5.316$, $P < .05$) and a significant MCH × SNAP-94847 interaction ($F_{1,24} = 14.900$, $P < .01$). The posthoc analysis showed that MCH decreased climbing time ($P < .01$), which was blocked by SNAP-94847 ($P < .01$). The ANOVA of swimming time (Figure 2G) revealed no significant effects of the intra-LC injection of MCH ($P > .05$) or SNAP-94847 ($P > .05$) but showed a significant MCH × SNAP-94847 interaction ($F_{1,24} = 5.603$, $P < .05$). In the SPT, the ANOVA of sucrose preference (Figure 2H) revealed significant effects of MCH ($F_{1,24} = 5.580$, $P < .05$) and SNAP-94847 ($F_{1,24} = 7.223$, $P < .05$) and a significant MCH × SNAP-94847 interaction ($F_{1,27} = 13.404$, $P < .01$). The posthoc analysis showed that MCH decreased sucrose preference ($P < .01$), which was blocked by SNAP-94847 ($P < .01$).

Microinjection of SNAP-94847 in the LC Blocked Depressive-Like Behavior That Was Induced by an Intracerebroventricular Injection of MCH

In the second experiment, the i.c.v. injection of MCH induced depressive-like behavior in the FST and SPT. In the FST, the 0.8- and 1.6-μg doses of MCH significantly increased immobility time ($F_{3,24} = 30.786$, $P < .001$; Figure 3A), decreased climbing time ($F_{3,24} = 5.083$, $P = .007$; Figure 3B), and decreased swimming time ($F_{3,24} = 8.594$, $P < .001$; Figure 3C) compared with the vehicle group. The 0.4 μg dose of MCH exerted no significant effects on these parameters. The 0.8- and 1.6-μg doses of MCH decreased sucrose preference in the SPT ($F_{3,24} = 8.594$, $P < .001$; Figure 3D).

To determine the involvement of MCH-R1 in the LC in the regulation of depressive-like behavior, we pretreated the rats with SNAP-94847 in the LC before i.c.v. MCH administration (0.8 μg). The ANOVA of immobility time in the FST (Figure 3E) revealed significant effects of MCH ($F_{1,27} = 11.379$, $P < .01$) and SNAP-94847 ($F_{1,27} = 37.990$, $P < .001$) and a significant MCH × SNAP-94847 interaction ($F_{1,27} = 43.592$, $P < .001$). The posthoc analysis showed that the i.c.v. injection of MCH increased immobility time compared with the vehicle group ($P < .01$), which was blocked by the intra-LC microinjection of SNAP-94847 ($P < .01$). The ANOVA of climbing time (Figure 3F) revealed significant effects of MCH ($F_{1,27} = 8.589$, $P < .01$) and SNAP-94847 ($F_{1,27} = 4.193$, $P < .05$) and a significant MCH × SNAP-94847 interaction ($F_{1,27} = 6.009$, $P < .05$). The posthoc analysis showed that MCH decreased climbing time ($P < .01$), which was blocked by SNAP-94847 ($P < .01$). The ANOVA of swimming time (Figure 3G) revealed significant effects of the i.c.v. injection of MCH ($F_{1,27} = 0.000$, $P = .985$) and intra-LC microinjection of SNAP-94847 ($F_{1,27} = 10.253$, $P < .05$) and a significant MCH × SNAP-94847 interaction ($F_{1,27} = 10.098$, $P < .05$). The posthoc analysis showed that MCH decreased swimming time ($P < .05$),

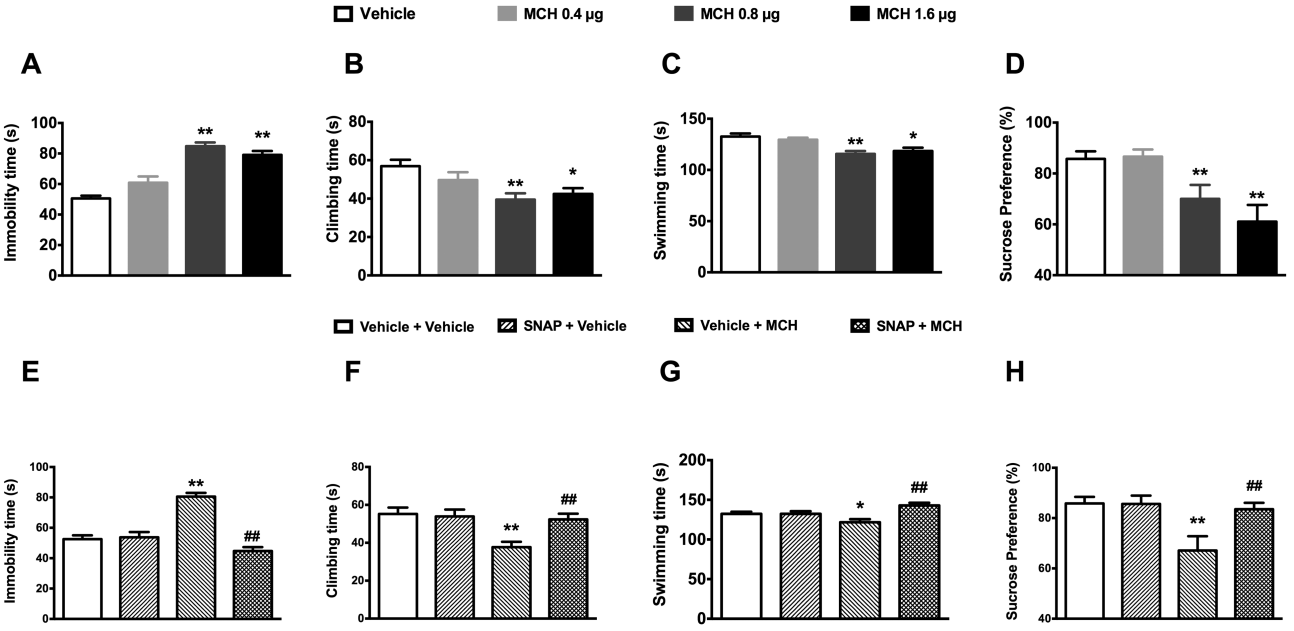

Figure 3. Effects of intra-locus coeruleus (LC) microinjection of SNAP-94847 on the behavioral alterations induced by i.c.v. injection of melanin-concentrating hormone (MCH). Intracerebroventricular injection of MCH (0.8 and 1.6 μg) increased the immobility time and decreased the climbing time and swimming time in the forced swim test (FST) (A–C) and decreased the sucrose preference index in the sucrose preference test (SPT) (D). Pretreatment of microinjecting SNAP-94847 in the LC could block the depressive-behavior in the FST and SPT (E–H). All data are presented as mean ± SEM, n = 7–8/group. * $P < .05$, ** $P < .01$ as compared with the Vehicle group, ## $P < .01$ as compared with the Vehicle + MCH group.

which was blocked by SNAP-94847 ($P < .01$). In the SPT, the ANOVA of sucrose preference (Figure 3H) revealed significant effects of MCH ($F_{1,27} = 7.421$, $P < .05$) and SNAP-94847 ($F_{1,27} = 4.499$, $P < .05$) and a significant MCH × SNAP-94847 interaction ($F_{1,27} = 4.742$, $P < .05$). The posthoc analysis showed that MCH decreased sucrose preference ($P < .01$), which was blocked by SNAP-94847 ($P < .01$).

Microinjection of SNAP-94847 in the LC Blocked Depressive-Like Behavior That Was Induced by Repeated CORT Administration

In the third experiment, the ANOVA of immobility time in the FST (Figure 4A) revealed significant effects of CORT ($F_{1,24} = 59.336$, $P < .001$) and SNAP-94847 ($F_{1,24} = 43.989$, $P < .001$) and a significant CORT × SNAP-94847 interaction ($F_{1,24} = 51.701$, $P < .001$). The posthoc analysis showed that repeated CORT administration for 21 days increased immobility time compared with the vehicle group ($P < .01$), which was blocked by the intra-LC microinjection of SNAP-94847 ($P < .01$). The ANOVA of climbing time (Figure 4B) revealed significant effects of CORT ($F_{1,24} = 8.881$, $P < .01$) and SNAP-94847 ($F_{1,24} = 5.108$, $P < .05$) and a significant CORT × SNAP-94847 interaction ($F_{1,24} = 21.122$, $P < .001$). The posthoc analysis showed that CORT decreased climbing time ($P < .01$), which was blocked by SNAP-94847 ($P < .01$). The ANOVA of swimming time (Figure 4C) revealed significant effects of CORT ($F_{1,24} = 26.278$, $P < .001$) and SNAP-94847 ($F_{1,24} = 22.167$, $P < .001$) and a significant CORT × SNAP-94847 interaction ($F_{1,24} = 9.432$, $P < .01$). The posthoc analysis showed that CORT decreased swimming time compared with the vehicle group ($P < .01$), which was blocked by SNAP-94847 ($P < .01$). In the SPT, the ANOVA of sucrose preference (Figure 4D) revealed significant effects of CORT ($F_{1,24} = 21.285$, $P < .001$) and SNAP-94847 ($F_{1,24} = 6.369$, $P < .05$) and a significant CORT × SNAP-94847 interaction ($F_{1,24} = 5.479$, $P < .05$). The posthoc analysis showed that CORT decreased sucrose preference ($P < .01$), which was blocked by SNAP-94847 ($P < .01$).

Immunofluorescent Evidence of the Expression of MCH-Ergic Neurofibers in the LC

Sections of the LC were double-immunostained for MCH and TH to demonstrate the expression of MCH-ergic neurofibers in the LC. A schematic diagram of double staining is shown in Figure 5A–E. Confocal laser scanning microscopy of the LC revealed a small number of MCH-immunopositive neurofibers throughout the LC nucleus (Figure 5A). Consistent with previous studies (Del Cid-Pellitero and Jones, 2012; Yoon and Lee, 2013), we also found that some MCH-immunopositive neurofibers appeared to contact TH-immunopositive soma or proximal dendrites (Figure 5E). The existence of MCH fibers in the double-immunostained sections was reinforced by result of negative control (Figure 5F–J), which showed no MCH signal was detected throughout the LC area.

Discussion

The innervation of MCH-ergic fibers and expression of MCH-R1 in the LC were previously reported (Saito et al., 2001; Yoon and Lee, 2013). In the present study, we also observed the expression of MCH-ergic neurofibers in the LC (Figure 5). The present results showed that the microinjection of MCH in the LC increased immobility time in the FST and decreased sucrose preference in the SPT. This depressive-like behavior was blocked by pretreatment with the MCH-R1 antagonist SNAP-94847 in the LC. The microinjection of SNAP-94847 in the LC also ameliorated depressive-like behavior that was induced by chronic CORT administration, an i.c.v. injection of MCH, and an intra-LC injection of MCH. These results indicate that the MCH-R1/MCH-ergic system in the LC might play an important role in the regulation of depressive behavior.

Multiple theories have been proposed with regard to the ways by which the LC is involved in the pathology of depression.

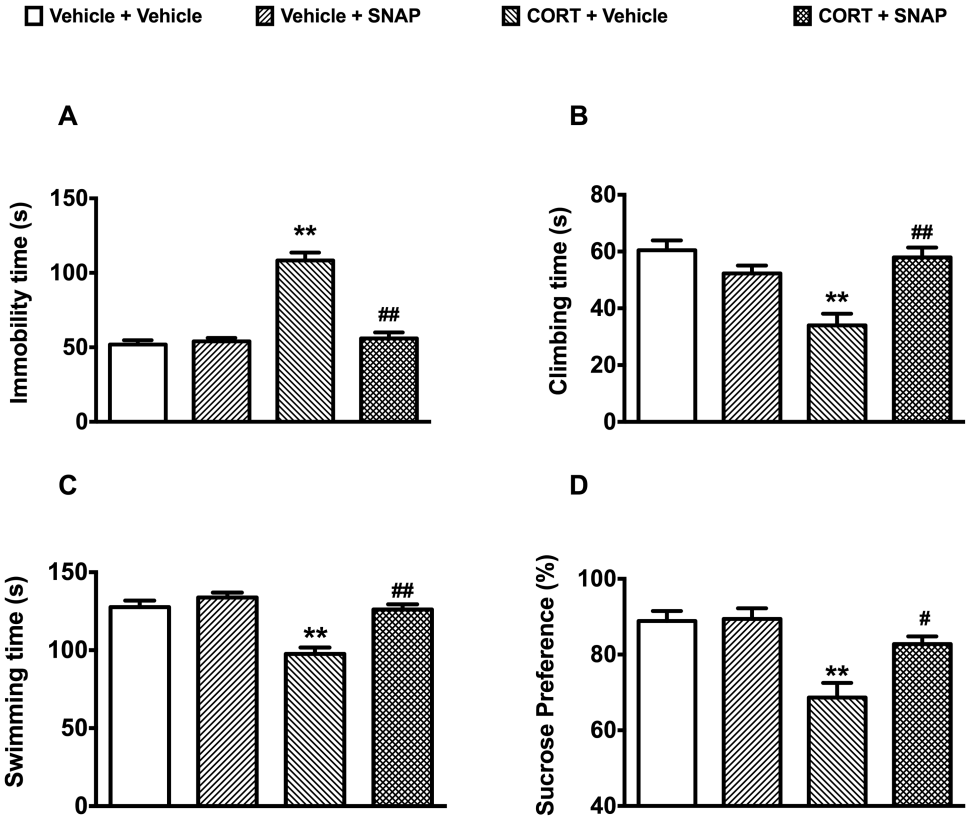

Figure 4. Effects of repeated corticosterone (CORT) administration on the behavior in forced swim test (FST) and sucrose preference test (SPT). Repeated CORT administration induced an increase of immobility time (A) and a decrease of climbing time and swimming time (B,C) in the FST and a decrease of sucrose preference in the SPT (D), which were blocked by microinjecting SNAP-94847 in the LC. All data are presented as mean ± SEM, n = 7–8/group. **$P < .01$ as compared with the Vehicle group and # $P < .05$ as compared with the Vehicle + CORT group.

Abundant noradrenergic neurons are located in the LC, and the first-line treatment for patients with major depressive disorder includes selective norepinephrine reuptake inhibitors (Cipriani et al., 2009; Croom et al., 2009). Recent evidence suggests that numerous inputs to the LC, including glutamatergic and corticotropin-releasing factor projections, are related to depression (Bissette et al., 2003; Bernard et al., 2011; Chandley and Ordway, 2012). The present results indicated that the MCH-ergic system that acts through MCH-R1 in the LC might also be an important component of the link between the LC and the regulation of depressive-like behavior.

The exact mechanism by which MCH-R1 in the LC is involved in regulating depressive-like behavior remains unclear. One possibility is that MCH plays an inhibitory role in the LC. Neurons that express MCH also contain glutamic acid decarboxylase for the synthesis of γ-aminobutyric acid (GABA; Sapin et al., 2010). Although direct physiological evidence is lacking, MCH release in the LC may decrease neuronal discharge, in part through the synaptic release and postsynaptic effects of GABA in the LC (Del Cid-Pellitero and Jones, 2012). Monti et al. (2015) proposed that MCH and GABA that is released by MCH-ergic neurons inhibit noradrenergic neurons in the LC, thus increasing REM sleep time (Monti et al., 2015). Depressive-like behavior that is induced by MCH in the LC may result from the inhibition of noradrenergic activity. Likewise, the antidepressant effect of MCH-R1 antagonism in the LC in the present study may be related to an enhancement of noradrenergic activity. Further studies are needed to demonstrate whether such effects of MCH-R1 antagonism occur only in this model of depressive-like behavior.

Chronic exposure to CORT or stress induces depressive-like behavior and affects the activity of LC noradrenergic neurons. Previous studies reported that more than 3 weeks of CORT or stress exposure is needed to decrease the levels of TH (Duncko et al., 2001; Yunan Zhao et al., 2008), which is the rate-limiting enzyme in the biosynthesis of norepinephrine. In the present study, the 21-day administration of CORT may have suppressed the activity of LC noradrenergic neurons, and treatment of MCH-R1 antagonist SNAP-94847 blocked this effect, thus demonstrating antidepressant efficacy.

Climbing behavior in the FST has been considered to be related mostly to an increase in noradrenergic system activation (Detke et al., 1995). In the present study, climbing behavior was significantly suppressed in rats that received a microinjection of MCH in the LC, repeated CORT administration, or an i.c.v. injection of MCH. The MCH-R1 antagonist SNAP-94847 reversed this increase in climbing time in all 3 experiments. These results support the hypothesis that the LC noradrenergic pathway in conjunction with MCH and MCH-R1 may be an important element in the regulation of depressive-like behavior.

In conclusion, the present study investigated the involvement of the LC MCH-ergic system in depressive-like behavior in rats. Depressive-like behavior was induced in rats by chronic CORT administration, an i.c.v. injection of MCH, and an intra-LC microinjection of MCH. The expression of depressive-like behavior was reversed by an intra-LC microinjection of the MCH-R1 antagonist SNAP-94847. Thus, the MCH-ergic system in the LC might be involved in the regulation of depressive-like behavior. Notably, however, the biological function of MCH in humans is

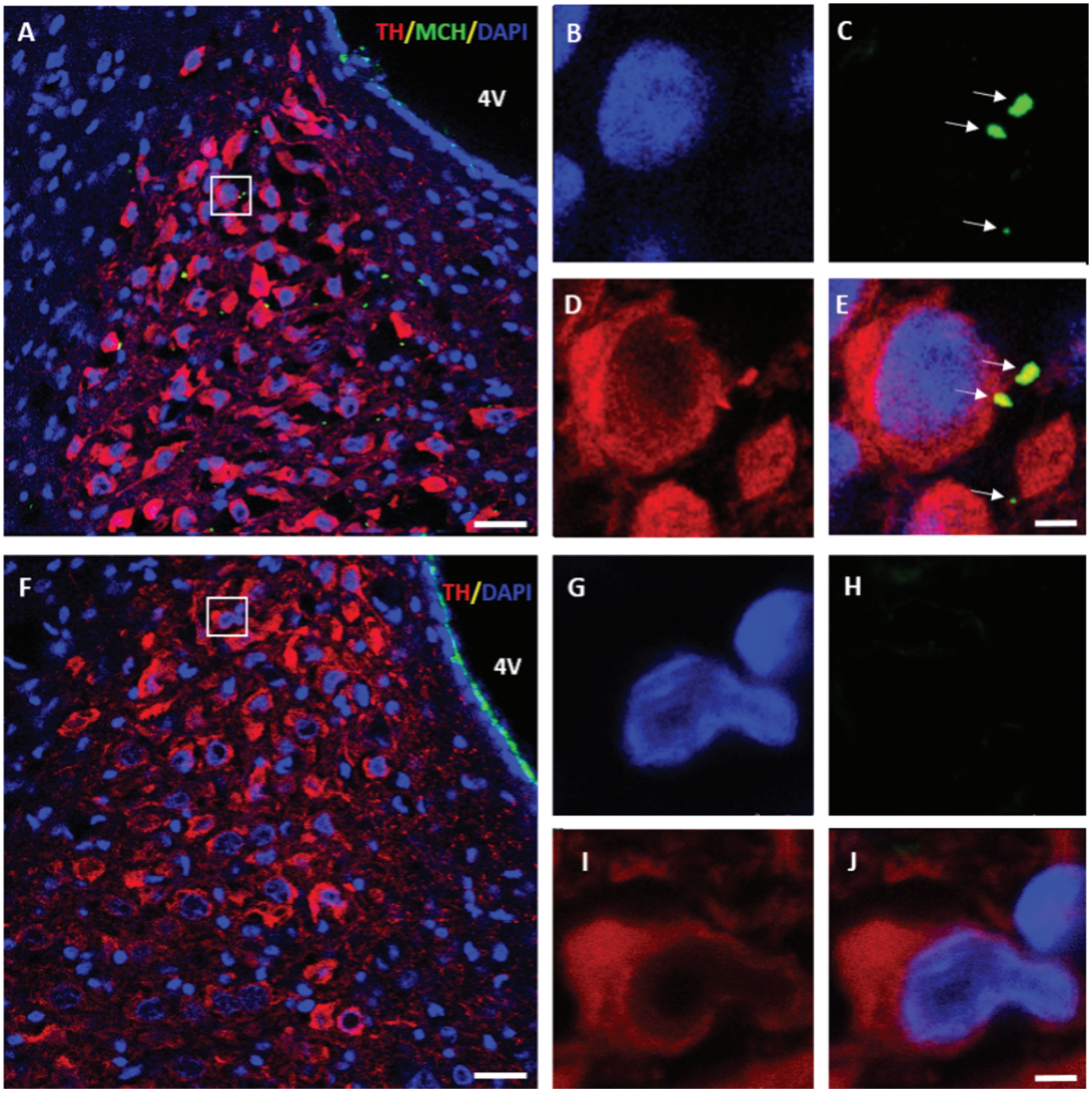

Figure 5. Fluorescent images of immunostaining for melanin-concentrating hormone (MCH) and tyrosine hydroxylase (TH) in the locus coeruleus (LC). MCH was labeled with Alexa Fluor 488 (green), TH was labeled with Cy3 (red), and the cell nucleus was counterstained with 4', 6-diamidino-2-phenylindole (blue) (A). (B–E) A single neuron from (A) at high magnification. The arrows in C and E indicate the MCH-ergic neurofibers. A few MCH-immunopositive neurofibers are located in the LC, some of which contact with the TH-immunopositive soma and dendrites. For negative control, TH was labeled with Cy3 (red) and the cell nucleus was counterstained with 4', 6-diamidino-2-phenylindole (blue) (F). (G–J) A single neuron from (F) at high magnification, and no MCH-immunopositive labeling was detected. Scale bars = 50 μm (for large panels), 5 μm (for small panels). 4V, fourth ventricle.

mediated by 2 G-protein-coupled receptors, MCH-R1 and MCH-R2. Therefore, more studies are needed to elucidate the functional relationship between the MCH-ergic system and other neural systems in the LC in patients with depression.

Acknowledgments

This study was funded by grants from the National Natural Science Foundation of China (nos. 81573407, 81872851, 81871038, and 81302746).

Statement of Interest

None.

References

Aguilar DD, Chen L, Lodge DJ (2014) Increasing endocannabinoid levels in the ventral pallidum restore aberrant dopamine neuron activity in the subchronic PCP rodent model of schizophrenia. Int J Neuropsychopharmacol 18:1–9.

Basso AM, Bratcher NA, Gallagher KB, Cowart MD, Zhao C, Sun M, Esbenshade TA, Brune ME, Fox GB, Schmidt M, Collins CA, Souers AJ, Iyengar R, Vasudevan A, Kym PR, Hancock AA, Rueter LE (2006) Lack of efficacy of melanin-concentrating hormone-1 receptor antagonists in models of depression and anxiety. Eur J Pharmacol 540:115–120.

Bernard R, Kerman IA, Thompson RC, Jones EG, Bunney WE, Barchas JD, Schatzberg AF, Myers RM, Akil H, Watson SJ (2011) Altered expression of glutamate signaling, growth factor, and glia genes in the locus coeruleus of patients with major depression. Mol Psychiatry 16:634–646.

Bissette G, Klimek V, Pan J, Stockmeier C, Ordway G (2003) Elevated concentrations of CRF in the locus coeruleus of depressed subjects. Neuropsychopharmacology 28:1328–1335.

Bittencourt JC, Presse F, Arias C, Peto C, Vaughan J, Nahon JL, Vale W, Sawchenko PE (1992) The melanin-concentrating hormone system of the rat brain: an immuno- and hybridization histochemical characterization. J Comp Neurol 319:218–245.

Borowsky B, Durkin MM, Ogozalek K, Marzabadi MR, DeLeon J, Lagu B, Heurich R, Lichtblau H, Shaposhnik Z, Daniewska I, Blackburn TP, Branchek TA, Gerald C, Vaysse PJ, Forray C (2002) Antidepressant, anxiolytic and anorectic effects of a melanin-concentrating hormone-1 receptor antagonist. Nat Med 8:825–830.

Cao Q, Jiang Y, Cui SY, Tu PF, Chen YM, Ma XL, Cui XY, Huang YL, Ding H, Song JZ, Yu B, Sheng ZF, Wang ZJ, Xu YP, Yang G, Ye H, Hu X, Zhang YH (2016) Tenuifolin, a saponin derived from Radix Polygalae, exhibits sleep-enhancing effects in mice. Phytomedicine 23:1797–1805.

Chandley MJ, Ordway GA (2012) Noradrenergic dysfunction in depression and suicide. In: The neurobiological basis of suicide (Dwivedi Y, ed), pp 29–63. Boca Raton-London-New York: CRC Press-Taylor & Francis.

Cipriani A, Furukawa TA, Salanti G, Geddes JR, Higgins JP, Churchill R, Watanabe N, Nakagawa A, Omori IM, McGuire H, Tansella M, Barbui C (2009) Comparative efficacy and acceptability of 12 new-generation antidepressants: a multiple-treatments meta-analysis. Lancet 373:746–758.

Croom KF, Perry CM, Plosker GL (2009) Mirtazapine: a review of its use in major depression and other psychiatric disorders. CNS Drugs 23:427–452.

Cui XY, Yang G, Cui SY, Huang YL, Ding H, Ye H, Zhang XQ, Wang ZJ, Zhang YH (2018) Sleep patterns deteriorate over time in chronic corticosterone-treated rats. Neurosci Lett 682:74–78.

David DJ, Klemenhagen KC, Holick KA, Saxe MD, Mendez I, Santarelli L, Craig DA, Zhong H, Swanson CJ, Hegde LG, Ping XI, Dong D, Marzabadi MR, Gerald CP, Hen R (2007) 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. J Pharmacol Exp Ther 321:237–248.

Del Cid-Pellitero E, Jones BE (2012) Immunohistochemical evidence for synaptic release of GABA from melanin-concentrating hormone containing varicosities in the locus coeruleus. Neuroscience 223:269–276.

Detke MJ, Rickels M, Lucki I (1995) Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology (Berl) 121:66–72.

Duncko R, Kiss A, Skultétyová I, Rusnák M, Jezová D (2001) Corticotropin-releasing hormone mRNA levels in response to chronic mild stress rise in male but not in female rats while tyrosine hydroxylase mRNA levels decrease in both sexes. Psychoneuroendocrinology 26:77–89.

Felippotti TT, dos Reis Ferreira CM, de Freitas RL, de Oliveira RC, de Oliveira R, Paschoalin-Maurin T, Coimbra NC (2011) Paradoxical effect of noradrenaline-mediated neurotransmission in the antinociceptive phenomenon that accompanies tonic-clonic seizures: role of locus coeruleus neurons and α(2)- and β-noradrenergic receptors. Epilepsy Behav 22:165–177.

Fenton EY, Fournier NM, Lussier AL, Romay-Tallon R, Caruncho HJ, Kalynchuk LE (2015) Imipramine protects against the deleterious effects of chronic corticosterone on depression-like behavior, hippocampal reelin expression, and neuronal maturation. Prog Neuropsychopharmacol Biol Psychiatry 60:52–59.

Forray C (2003) The MCH receptor family: feeding brain disorders? Curr Opin Pharmacol 3:85–89.

Harrell CS, Hardy E, Boss-Williams K, Weiss JM, Neigh GN (2013) Sex and lineage interact to predict behavioral effects of chronic adolescent stress in rats. Behav Brain Res 248:57–61.

Hassani OK, Lee MG, Jones BE (2009) Melanin-concentrating hormone neurons discharge in a reciprocal manner to orexin neurons across the sleep-wake cycle. Proc Natl Acad Sci U S A 106:2418–2422.

Lagos P, Torterolo P, Jantos H, Chase MH, Monti JM (2009) Effects on sleep of melanin-concentrating hormone (MCH) microinjections into the dorsal raphe nucleus. Brain Res 1265:103–110.

Lagos P, Urbanavicius J, Scorza MC, Miraballes R, Torterolo P (2011) Depressive-like profile induced by MCH microinjections into the dorsal raphe nucleus evaluated in the forced swim test. Behav Brain Res 218:259–266.

Lipski WJ, Dibble SM, Rinaman L, Grace AA (2017) Psychogenic stress activates C-fos in nucleus accumbens-projecting neurons of the hippocampal ventral subiculum. Int J Neuropsychopharmacol 20:855–860.

Lu J, Sherman D, Devor M, Saper CB (2006) A putative flip-flop switch for control of REM sleep. Nature 441:589–594.

Monti JM, Torterolo P, Lagos P (2013) Melanin-concentrating hormone control of sleep-wake behavior. Sleep Med Rev 17:293–298.

Monti JM, Lagos P, Jantos H, Torterolo P (2015) Increased REM sleep after intra-locus coeruleus nucleus microinjection of melanin-concentrating hormone (MCH) in the rat. Prog Neuropsychopharmacol Biol Psychiatry 56:185–188.

Paxinos G, Watson C (2005) The rat brain in stereotaxic coordinates 5th ed. San Diego: Elsevier Academic Press.

Roy M, David N, Cueva M, Giorgetti M (2007) A study of the involvement of melanin-concentrating hormone receptor 1 (MCHR1) in murine models of depression. Biol Psychiatry 61:174–180.

Saito Y, Cheng M, Leslie FM, Civelli O (2001) Expression of the melanin-concentrating hormone (MCH) receptor mRNA in the rat brain. J Comp Neurol 435:26–40.

Saito Y, Nagasaki H (2008) The melanin-concentrating hormone system and its physiological functions. Results Probl Cell Differ 46:159–179.

Saper CB, Fuller PM, Pedersen NP, Lu J, Scammell TE (2010) Sleep state switching. Neuron 68:1023–1042.

Sapin E, Bérod A, Léger L, Herman PA, Luppi PH, Peyron C (2010) A very large number of gabaergic neurons are activated in the tuberal hypothalamus during paradoxical (REM) sleep hypersomnia. Plos One 5:e11766.

Smith DG, Hegde LG, Wolinsky TD, Miller S, Papp M, Ping X, Edwards T, Gerald CP, Craig DA (2009) The effects of stressful stimuli and hypothalamic-pituitary-adrenal axis activation are reversed

by the melanin-concentrating hormone 1 receptor antagonist SNAP 94847 in rodents. Behav Brain Res 197:284–291.

Sun LL, Zhang Y, Liu JF, Wang J, Zhu WL, Zhao LY, Xue YX, Lu L, Shi J (2013) Role of melanin-concentrating hormone in the nucleus accumbens shell in rats behaviourally sensitized to methamphetamine. Int J Neuropsychopharmacol 16:1767–1780.

Szot P, Franklin A, Miguelez C, Wang Y, Vidaurrazaga I, Ugedo L, Sikkema C, Wilkinson CW, Raskind MA (2016) Depressive-like behavior observed with a minimal loss of locus coeruleus (LC) neurons following administration of 6-hydroxydopamine is associated with electrophysiological changes and reversed with precursors of norepinephrine. Neuropharmacology 101:76–86.

Torterolo P, Sampogna S, Morales FR, Chase MH (2006) MCH-containing neurons in the hypothalamus of the cat: searching for a role in the control of sleep and wakefulness. Brain Res 1119:101–114.

Tsunematsu T, Ueno T, Tabuchi S, Inutsuka A, Tanaka KF, Hasuwa H, Kilduff TS, Terao A, Yamanaka A (2014) Optogenetic manipulation of activity and temporally controlled cell-specific ablation reveal a role for MCH neurons in sleep/wake regulation. J Neurosci 34:6896–6909.

Verret L, Goutagny R, Fort P, Cagnon L, Salvert D, Léger L, Boissard R, Salin P, Peyron C, Luppi PH (2003) A role of melanin-concentrating hormone producing neurons in the central regulation of paradoxical sleep. BMC Neurosci 4:19.

Wang D, Song Y, Zhang J, Pang W, Wang X, Zhu Y, Li X (2017) AMPK-KLF2 signaling pathway mediates the proangiogenic effect of erythropoietin in endothelial colony-forming cells. Am J Physiol Cell Physiol 313:C674–C685.

Wang ZJ, Yu B, Zhang XQ, Sheng ZF, Li SJ, Huang YL, Cao Q, Cui XY, Cui SY, Zhang YH (2014) Correlations between depression behaviors and sleep parameters after repeated corticosterone injections in rats. Acta Pharmacol Sin 35:879–888.

Wang ZJ, Zhang XQ, Cui XY, Cui SY, Yu B, Sheng ZF, Li SJ, Cao Q, Huang YL, Xu YP, Zhang YH (2015) Glucocorticoid receptors in the locus coeruleus mediate sleep disorders caused by repeated corticosterone treatment. Sci Rep 5:9442.

Xu CM, Wang J, Wu P, Zhu WL, Li QQ, Xue YX, Zhai HF, Shi J, Lu L (2009) Glycogen synthase kinase 3beta in the nucleus accumbens core mediates cocaine-induced behavioral sensitization. J Neurochem 111:1357–1368.

Xu CM, Wang J, Wu P, Xue YX, Zhu WL, Li QQ, Zhai HF, Shi J, Lu L (2011) Glycogen synthase kinase 3β in the nucleus accumbens core is critical for methamphetamine-induced behavioral sensitization. J Neurochem 118:126–139.

Yoon YS, Lee HS (2013) Projections from melanin-concentrating hormone (MCH) neurons to the dorsal raphe or the nuclear core of the locus coeruleus in the rat. Brain Res 1490:72–82.

Zhao Y, Ma R, Shen J, Su H, Xing D, Du L (2008) A mouse model of depression induced by repeated corticosterone injections. Eur J Pharmacol 581:113–120.