

# The melanin-concentrating hormone system modulates cocaine reward

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**Drug addiction is mediated by complex neuronal processes that converge on the shell of the nucleus accumbens (NAcSh). The NAcSh receives inputs from the lateral hypothalamus (LH), where self-stimulation can be induced. Melanin-concentrating hormone (MCH) is produced mainly in the LH, and its receptor (MCH1R) is highly expressed in the NAcSh. We found that, in the NAcSh, MCH1R is coexpressed with dopamine receptors (D1R and D2R), and that MCH increases spike firing when both D1R and D2R are activated. Also, injecting MCH potentiates cocaine-induced hyperactivity in mice. Mice lacking MCH1R exhibit decreased cocaine-induced conditioned place preference, as well as cocaine sensitization. Using a specific MCH1R antagonist, we further show that acute blockade of the MCH system not only reduces cocaine self-administration, but also attenuates cue- and cocaine-induced reinstatement. Thus, the MCH system has an important modulatory role in cocaine reward and reinforcement by potentiating the dopaminergic system in the NAcSh, which may provide a new rationale for treating cocaine addiction.**

G protein-coupled receptor | drug abuse | neuropeptide | MCH1R knockout mice | MCH1R antagonist

The shell of the nucleus accumbens (NAcSh) is known to be an important center for reward and motivation. It is a major terminal area of the mesolimbic dopaminergic system, which projects from the ventral tegmental area (VTA) (1). The NAcSh neurons express both dopamine D1 and D2 receptors, which have opposite effects on cAMP-dependent signaling; however, motivated behaviors require concomitant D1 and D2 receptor signaling in the NAcSh (2). The NAcSh receives inputs from different brain areas, in particular, from the lateral hypothalamus (LH) (3). The importance of the LH in reward and motivation has been shown by Olds (4), who discovered that electrical activation of the LH induced an extraordinarily intense self-stimulation response in rats. However, the transmitter(s) responsible for this action have not been established definitively. We now know of 2 neuropeptides that are uniquely synthesized in the LH: the orexins/hypocretins (5), and melanin-concentrating hormone (MCH) (6–8). The orexin/hypocretin neurons do not project prominently to the NAcSh, whereas the MCH neurons do (9). This data suggested to us that the MCH system may regulate the activities of the NAcSh neurons to influence reward.

MCH is a cyclic, 19-amino acid peptide originally isolated from salmon pituitary as a melanophore-concentrating factor (10). It is also present in rats and humans (11, 12), where it is viewed as important in regulating nutritional homeostasis (8, 13). In mammals, MCH is expressed predominantly centrally, and occurs only in 2 nuclei: the perikarya of the LH area (LHA), and the zona incerta (ZI). However, MCH fibers project in many areas throughout the CNS (6, 9), indicating that the MCH system may have a broad range of physiological roles. In rodents, MCH is known to interact with one G protein-coupled receptor, MCH1R (14–18). MCH1R is expressed in many brain regions (9), and the NAcSh has one of the highest MCH1R expression

levels. Indeed, the effects of the MCH system on feeding and mood behaviors already have been shown to depend on its activity in the NAcSh (19). Because motivated behaviors related to the NAcSh often require dopamine (2), the high MCH1R expression level in the NAcSh suggested to us that the MCH system might interact with the dopamine system in the NAcSh, and thereby regulate drug taking and/or seeking.

This report provides evidence for this hypothesis by showing that the dopamine and MCH systems can interact in the NAcSh at the level of their receptors, and that MCH administration can increase dopamine responses *in vitro* and *in vivo*. Also, chronic blockade of the MCH system attenuates motivation for cocaine, whereas acute blockade inhibits cocaine self-administration, as well as cue- and cocaine-induced reinstatement. Therefore, our results suggest that the MCH system is a powerful potentiator of the dopaminergic system in NAcSh neurons, exhibiting an important modulatory role in cocaine reward and reinforcement, which may provide a new rationale for treating cocaine addiction.

## Results

### Interactions Between the MCH and Dopamine Systems in the NAcSh.

Previously, we and others showed that MCH1R is expressed in many areas of the brain (9, 16). In particular, MCH1R is highly expressed in the NAcSh, an important region in regulating reward behavior due to dopamine transmission. We then determined whether the MCH1R is coexpressed with dopamine receptors in the NAcSh using double *in situ* hybridization. Two separate experiments were performed to analyze D1R/MCH1R and D2R/MCH1R expression (Fig. 1*A*). We found that the vast majority of the cells positive for D1R- or D2R- coexpress MCH1R (Fig. 1*A* and *B*), in agreement with the data of Georgescu et al. (19), which showed that MCH1R is colocalized with both dynorphin- and enkephalin-containing cells in the NAcSh. Our localization data suggest that the MCH system in the NAcSh may modulate dopamine-related responses and dopamine-mediated addictive behaviors.

To understand whether the MCH system interacts functionally with the dopamine system in the NAcSh, we used whole-cell patch-clamp electrophysiology to monitor spike firing in NAcSh neurons. Application of MCH (2.5  $\mu$ M) increased spike firing in the presence of threshold concentrations of a combination of D1 and D2 agonists (3  $\mu$ M of SKF 81297 and quinpirole, respectively) (20), whereas MCH alone or MCH in combination with only the D1 or the D2 agonist had no effect (D1 or D2 agonist alone previously have been shown to have no effect) (20). Also, the MCH/D1/D2-

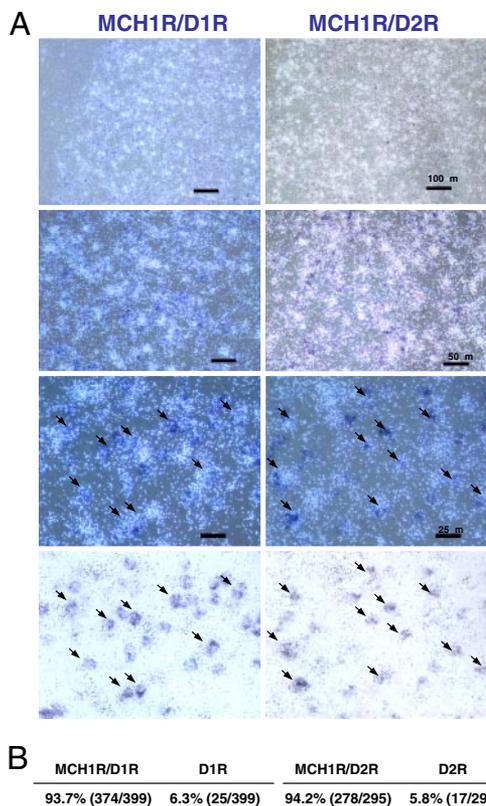
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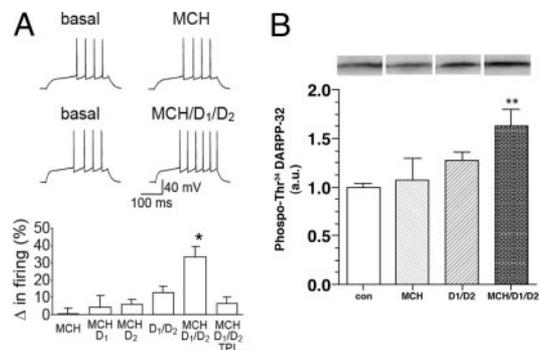


**Fig. 1.** Colocalization of MCH1R with D1R and D2R in the NAcSh. (A) Dark field images of MCH1R mRNA (white) with D1R mRNA or D2R mRNA (dark blue) in NAcSh. The panels at the bottom are bright field images of MCH1R (black grains) and D1R/D2R (blue). Arrows indicate examples of MCH1R-expressing cells that are colocalized either with D1R or D2R. (B) Percentages of cells expressing either MCH1R/D1R or D1R and MCH1R/D2R or D2R. D1R and D2R cells were counted first, and then matched for MCH1R expression (no. of colocalized cells/no. of D1R or D2R expressing cells counted).

induced increase in firing was reduced by the MCH1R antagonist, TPI 1361-17 (2  $\mu$ M) (Fig. 2A). These results indicate that the MCH and dopamine systems can interact to elevate spike firing in the NAcSh, and that blockade of the MCH system would inhibit that increase in spike firing. This hypothesis was confirmed by using a biochemical approach. Because dopamine receptor activation is known to increase DARPP-32 phosphorylation at Thr-34 in the dorsal striatum (21), we monitored DARPP-32 phosphorylation at Thr-34 by MCH alone (2.5  $\mu$ M) or after coincubation of MCH with D1 and D2 agonists (3  $\mu$ M of SKF 81297 and quinpirole, respectively) in NAcSh slices. Although MCH or D1 plus D2 agonists had no significant or few effects, MCH increased DARPP-32 phosphorylation when combined with both D1 and D2 agonists (\*\*,  $P < 0.01$  vs. control; see Fig. 2B). Together, these experiments led us to conclude that MCH and dopamine receptors can act in concert to enhance the activity of NAcSh neurons.

#### Effect of MCH Administration on Cocaine-Induced Locomotor Activity.

To analyze whether MCH system activation can potentiate the dopamine system in vivo, we investigated whether cocaine-induced hyperactivity is enhanced by exogenous MCH administration. Mice were injected either with vehicle or MCH (0.7 or 1 nmol i.c.v.) 5 min before cocaine injection (6 or 12 mg/kg i.p.), and their locomotor activity was measured for 60 min. Time course of horizontal activity after vehicle, MCH (1 nmol i.c.v.), cocaine (6 or 12 mg/kg i.p.), or MCH/cocaine (1 nmol i.c.v., and 6 or 12 mg/kg i.p., respectively) are shown in Fig. 3A and B. As expected, 6 and 12 mg/kg of cocaine increased locomotor



**Fig. 2.** Interaction of the MCH1R with D1R and D2R in the NAcSh. (A) Changes in NAcSh spike firing induced by MCH (2.5  $\mu$ M) alone, D1 agonist (SKF 81297 3  $\mu$ M) plus MCH, D2 agonist (Quinpirole 3  $\mu$ M) plus MCH, D1+D2 agonists plus MCH, and D1+D2 agonists plus MCH and TPI 1361-17 (2  $\mu$ M) (\*,  $P < 0.05$  for MCH/D1/D2 vs. each other condition,  $F_{(5, 25)} = 6.66$ ; \*,  $P < 0.001$ , for 1-way ANOVA, followed by Bonferroni multiple comparison test;  $n = 4-6$ ). Resting potential for MCH example was  $-82.4$  and  $-83.2$  mV before and after MCH, and resting potential for MCH/D1/D2 example was  $-80.2$  and  $-79.6$  mV before and after agonists. (B) Phosphorylation of DARPP-32 (Thr 34) in NAcSh slices after MCH (2.5  $\mu$ M) alone, D1+D2 agonists (SKF 81297 3  $\mu$ M and Quinpirole 3  $\mu$ M), and D1+D2 agonists plus MCH (\*\*,  $P < 0.01$  for MCH/D1/D2 vs. control, ANOVA followed by Bonferroni multiple comparison test;  $n = 6-10$ ). Immunoblots for detection of phospho-Thr-34 DARPP-32 are shown at the top. The levels of phospho-Thr-34 DARPP-32 were normalized to values obtained from control slices (a.u., arbitrary unit).

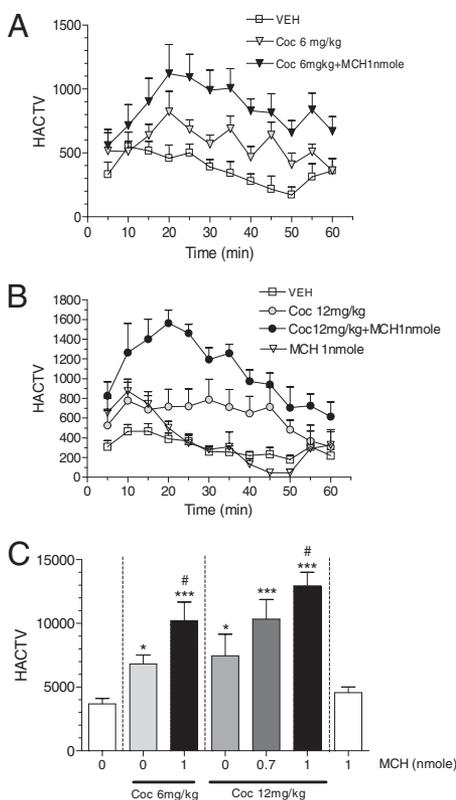
activity. However, i.c.v. injections of MCH potentiated the hyperactivity induced by cocaine injection in a dose-related manner (\*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$  vs. vehicle group; and #,  $P < 0.05$  vs. cocaine alone group; see Fig. 3C), showing that central injection of MCH can potentiate cocaine effects in vivo.

#### Decreased Sensitivity to the Rewarding Properties of Cocaine in MCH1R KO Mice.

To test whether the endogenous MCH system contributes to dopamine-mediated reward in vivo, MCH1R KO mice were tested for their cocaine-seeking behavior in the conditioned place preference (CPP) paradigm and for any changes in their locomotor activity during cocaine sensitization. Cocaine-induced CPP paradigm measures the ability of cocaine to produce a positive association with environmental cues, a response that is characteristic of rewarding/reinforcing stimuli, such as those created by drugs of abuse. We used a 2-chamber, nonbiased, CPP model. Mice were injected either with vehicle or cocaine on alternate days, and confined in one of the chambers during conditioning sessions. During the postconditioning session, increased time spent in the cocaine-paired chamber was taken to indicate increased cocaine-seeking behavior. We found that cocaine induced CPP in WT mice, as shown by the increase in the time spent in the cocaine-associated compartment during the postconditioning session (Fig. 4A, #,  $P < 0.05$  vs. saline). However, cocaine did not significantly induce CPP in MCH1R KO mice. Also, WT mice displayed a significant preference for the cocaine-paired compartment when compared with MCH1R KO mice (\*\*,  $P < 0.01$ , at 12 mg/kg vs. MCH1R KO mice).

MCH1R KO mice also were tested for sensitization of locomotor activity in response to repeated cocaine administration. WT mice showed significantly increased locomotor activity after repeated daily cocaine injections (Fig. 4B, ##,  $P = 0.0022$  vs. day 3), whereas MCH1R KO mice did not exhibit any significant changes in activity, in agreement with the previous report (22). Together, these results indicate that the lack of MCH1R results in a decreased sensitivity to the rewarding properties of cocaine in vivo. Therefore, MCH system activation is involved in the rewarding effect of cocaine.

Chronic exposure to cocaine has been shown to increase



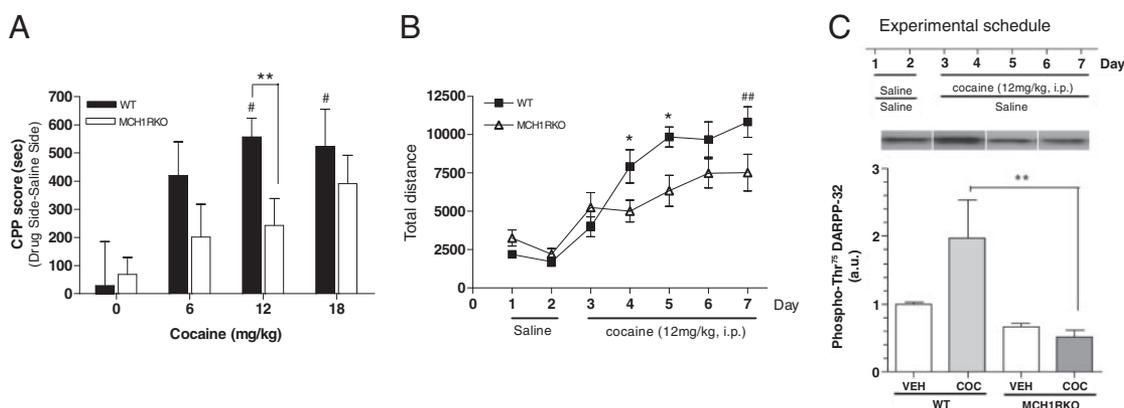
**Fig. 3.** Effect of MCH on cocaine induced locomotor activity. (A) Time course of horizontal activity after vehicle, cocaine (6 mg/kg i.p.) or MCH/cocaine (1 nmol i.c.v. and 6 mg/kg i.p., respectively). (B) Time course of horizontal activity after vehicle, MCH (1 nmol i.c.v.), cocaine (12 mg/kg i.p.), or MCH/cocaine (1 nmol and 12 mg/kg, respectively). (C) Cumulative horizontal activity. Effect of MCH on cocaine induced hyperactivity for 60 min (\*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$  vs. vehicle group; #,  $P < 0.05$  vs. cocaine alone group;  $n = 6-17$  per dose; ANOVA followed by Bonferroni multiple comparison test).

DARPP-32 phosphorylation at Thr 75 in the caudate putamen and nucleus accumbens (23). Here, we repeated this experiment, and show that WT mice exhibit an increased DARPP-32 phosphorylation level at Thr 75 in NAcSh with repeated cocaine administration (Fig. 4C). In contrast, we found that MCH1R KO mice do not exhibit a similar change in phosphorylation level

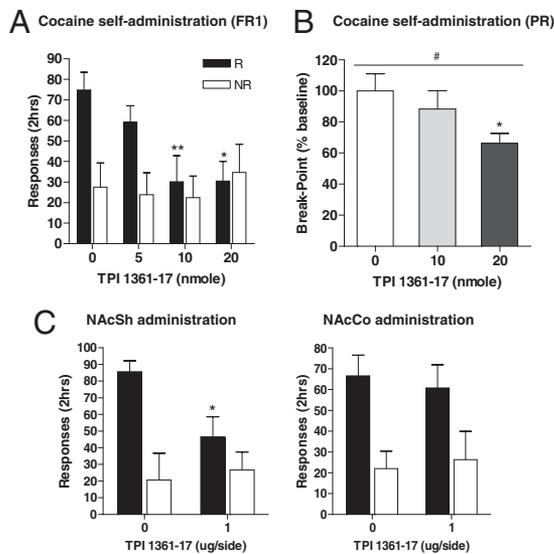
after chronic cocaine treatment. Indeed, the phosphorylation level of DARPP-32 at Thr 75 in NAcSh was significantly reduced in MCH1R KO mice when compared with the level in WT mice (\*\*,  $P < 0.01$  vs. WT/coc group), consistent with the decreased cocaine sensitivity of MCH1R KO mice found in the behavioral assays (Fig. 4B). Therefore, MCH system activation impacts the DARPP-32 pathway in response to chronic cocaine treatments.

**Effect of Acute MCH Blockade on Cocaine Self-Administration.** Cocaine self-administration is the most common paradigm for studying drugs of abuse. We studied how pharmacological blockade of the MCH system affects cocaine self-administration. These experiments used a high-affinity MCH1R antagonist, TPI 1361-17. This antagonist inhibits MCH1R with a nanomolar affinity, it is highly specific, showing no affinity for a panel of receptors and channels, is active in vivo, and does not exert unwanted side effects (24). The cocaine self-administration paradigm measures the primary reinforcing effect of cocaine. Animals were trained to self-administer cocaine (0.5 mg/kg per injection) on a fixed ratio 1 (FR1) schedule of reinforcement for 2 h/d for 4 days. Vehicle or TPI 1361-17 (5, 10, or 20 nmol, i.c.v.) was injected 5 min before the session, and the nose-poke responses were measured during the session. TPI 1361-17 inhibited cocaine self-administration dose-dependently (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$  vs. control; see Fig. 5A). TPI 1361-17 also was tested in rats working on a progressive ratio (PR) schedule. Under this schedule, rats are required to increase the number of nose-pokes required for each successive cocaine injection. The breakpoint was defined as the number of completed nose pokes before a 30-min period when no infusions were obtained by the rat. An increase in the breakpoint after TPI 1361-17 injection indicates that blockade of the MCH system increases the reinforcing properties of cocaine, whereas a decrease indicates that MCH blockade reduces the motivation for cocaine. The steps of the exponential progression were the same as those used previously (25). As shown in Fig. 5B, pharmacological blockade of the MCH system decreased breakpoint (percentage baseline) dose-dependently (#,  $P < 0.05$ , repeated measures 1-way ANOVA; \*,  $P < 0.05$  vs. control, repeated measures 1-way ANOVA followed by Dunnett's post hoc comparison; see Fig. 5B). The decrease in PR breakpoint suggests that blockade of the MCH system leads to a reduction in the rewarding efficacy of cocaine and in motivational properties for cocaine-taking behavior.

The strong inhibitory effect of TPI 1361-17 on important aspects of cocaine addiction, as well as high levels of MCH1Rs in the NAcSh suggest that the MCH system may modulate dopamine-related responses in the NAcSh. To analyze this



**Fig. 4.** Behavioral responses to cocaine in MCH1R KO mice. (A) Cocaine-induced place preference in WT and MCH1R KO mice. CPP score: Time spent on the drug side minus time spent on the saline side during post conditioning (#,  $P < 0.05$  vs. saline, ANOVA followed by Dunnett's test for multiple comparison; \*\*,  $P < 0.01$  vs. MCH1R KO mice, unpaired  $t$  test;  $n = 5-9$ ). (B) Cocaine sensitization in WT and MCH1R KO mice (##,  $P = 0.0022$  vs. day 3, paired  $t$  test; \*,  $P < 0.05$  vs. MCH1R KO mice, unpaired  $t$  test;  $n = 5-6$ ). (C) Changes of phosphorylation of DARPP-32 (Thr 75) in WT and MCH1R KO mice after 5 days of saline or cocaine treatment. The data were normalized to saline treated WT group (a.u., arbitrary unit) (\*\*,  $P < 0.01$  vs. WT/coc mice, ANOVA followed by Bonferroni multiple comparison test;  $n = 4-6$ ).



**Fig. 5.** Effect of MCH system blockade on cocaine self-administration. (A) TPI 1361-17 (5–20 nmol i.c.v.) inhibition of cocaine self-administration under a FR1 measured by the number of cocaine infusions obtained (■) and the number of inactive responses (□) (\*,  $P < 0.05$ ; \*\*,  $P < 0.01$  vs. control, ANOVA followed by Dunnett's test for multiple comparison;  $n = 6-11$ ). (B) Effect of TPI 1361-17 on cocaine self-administration under a PR schedule in rats ( $n = 6$ ). Data are presented as the breakpoint (percentage baseline) (mean  $\pm$  SEM). There was a significant TPI 1361-17 effect on breakpoint (#,  $P < 0.05$ , repeated measures ANOVA). Significant differences were determined by repeated measures 1-way ANOVA with Dunnett's post hoc comparison (\*,  $P < 0.05$  vs. control). (C) Cocaine self-administration on TPI 1361-17 administration into the NAcSh (1  $\mu$ g/side) or into NAcCo (\*,  $P < 0.05$ , unpaired  $t$  test;  $n = 6-7$ ). R and NR indicate reinforced (■) and nonreinforced responses (□), respectively. Injection placements for NAcSh (○) and NAcCo (●) shown in Fig. S1.

possibility, we first tested whether TPI 1361-17 elicits its inhibitory action by acting in the NAcSh. TPI 1361-17 was injected into the NAcSh or into the closely located nucleus accumbens core (NAcCo). We found that TPI 1361-17 reduced cocaine self-administration only when injected into the NAcSh (\*,  $P < 0.05$ ; see Fig. 5C), showing that the NAcSh was a primary target of the modulatory role of the MCH system.

**Effects of MCH Blockade on Cue-, Cocaine-, and Stress-Induced Reinstatement.** Relapse is an important aspect of drug addiction (26). It is generally studied after cocaine self-administration and then extinction of response to cocaine; after this time, cocaine response can be reinstated by exposing the animals to cocaine-associated cues, priming injection of cocaine or stress. In our experiments, the

cue was a house light that predicted drug availability and a 5.6-s light that was associated with cocaine infusion; 10 to 12 days of cocaine self-administration under these conditions were followed by an extinction phase, in which nose poking had no consequences and the house light was turned off. Rats were allowed to reach an extinction baseline for 3 consecutive days (25% of their responses during cocaine self-administration) before being tested on cue-induced reinstatement. In these tests, the house light was turned on at the start of the session, and every nose poke resulted in activation of the discrete cue light, but not in cocaine delivery. We found that exposure to the cocaine-associated cues reinstated extinguished response on the previously cocaine-paired hole (\*\*\*,  $P < 0.001$ ), and that TPI 1361-17 was able to significantly reduce this response (\*\*\*,  $P < 0.001$ ; see Fig. 6A).

Next, we determined the effect of TPI 1361-17 on reinstatement of cocaine-seeking primed by injections of cocaine. Cocaine self-administration training and extinction were carried out as described above. However, on the test days, rats were primed by an injection of cocaine (10 mg/kg i.p.) before being put in the test chamber. The cocaine injection reinstated response on the previously cocaine-paired hole (\*\*\*,  $P < 0.001$ ), an effect that was inhibited by TPI 1361-17 (\*\*\*,  $P < 0.001$ ; see Fig. 6B). These 2 experiments indicate that the endogenous MCH system is critical for relapse induced by cocaine-associated cues, as well as a priming injection of cocaine.

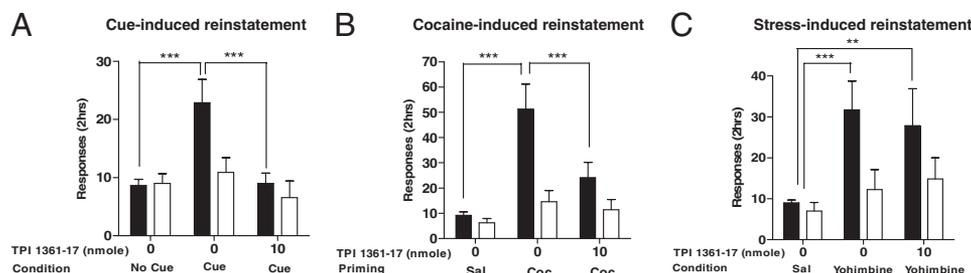
Another stimulus that triggers relapse-like behavior in rats is brief exposure to stress. Therefore, we tested the effect of TPI 1361-17 on stress-induced reinstatement of cocaine seeking. To trigger stress, we used yohimbine, an  $\alpha 2$  adrenoreceptor antagonist, which induces stress-like symptoms in humans and laboratory animals (27). Priming injections of yohimbine (2.5 mg/kg i.p.) reinstated responses to the previously cocaine-paired hole (\*\*\*,  $P < 0.001$ ). However, in contrast to the data above, this effect was not affected by the injections of TPI 1361-17 ( $P > 0.05$ ; see Fig. 6C). Thus, MCH1R activation does not appear to contribute to relapse induced by a stressor.

Together, our results strongly suggest that endogenous MCH has a critical role during reinstatement of cocaine-seeking by a cocaine-associated cue or by a cocaine priming injection, but not by stress.

## Discussion

The MCH system has been implicated in modulating several behavioral responses (13); most notably, MCH has been shown to induce increases in food intake when administered centrally (8). This effect has been linked to a high MCH1R expression level in the NAcSh (19). In this article, we report another role for the MCH system related to its receptor expression in the NAcSh: namely, a modulatory role on dopamine-related responses and, in particular, on cocaine addiction.

First, we report that practically all dopamine receptor-positive neurons of the NAcSh also express MCH1R. This data is in



**Fig. 6.** Effect of MCH system blockade on cue-, cocaine-, and stress-induced reinstatements. (A) Cue-induced reinstatement: TPI 1361-17 inhibition of reinstatement induced by cocaine-associated cues, measured by the number of responses in the previously cocaine-paired hole (■) and in the inactive hole (□) (\*\*\*,  $P < 0.001$ ;  $n = 7-10$ ). (B) Cocaine-induced reinstatement: TPI 1361-17 inhibition of reinstatement induced by priming injection of cocaine (10 mg/kg i.p.) (\*\*\*,  $P < 0.001$ ;  $n = 8$ ). (C) Stress-induced reinstatement: TPI 1361-17 inhibition of reinstatement induced by priming injection of yohimbine (2.5 mg/kg i.p.) (\*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ;  $n = 7-8$ ). Data expressed as mean  $\pm$  SEM.



scribed therapeutic tool for treating cocaine addiction or other disorders that result from dopamine imbalances in the NAcSh.

## Materials and Methods

Detailed methods are described in the *SI Materials and Methods*.

**Animals.** MCH1R KO mice (backcrossed to a C57BL/6 background; age-matched males; obtained from Merck) (52), or male C57BL/6 mice (National Cancer Institute) were used. Male Sprague–Dawley (Charles River) rats were used for all of the other experiments.

**Biochemical and Physiological Analyses.** Double in situ hybridization was carried out on brain coronal sections that were hybridized with <sup>35</sup>S-labeled MCH1R riboprobe and DIG-labeled riboprobes of D1R or D2R (provided by Dr. Stanley Watson) as described previously (53) with modifications (see *SI Materials and Methods*). Electrophysiology experiments were performed by using whole-cell recording as described previously (20). Phospho DARPP-32 assays were carried out as described previously (21) with slight modifications (see *SI Materials and Methods*) by using phospho-Thr 34 DARPP-32 antibody or phospho-Thr 75 DARPP-32 antibody.

**Behavioral Analyses.** Mice were habituated to the activity monitor (VERSAMEX system) for 1 h, then injected i.c.v. either with vehicle or MCH (total volume 2

μL) as described before (53). After 5 min, they were injected either with saline or cocaine (6, 12 mg/kg i.p.), and their locomotion was monitored for 60 min. CPP was conducted in 2-chambered boxes (containing visual, olfactory, and tactile cues) with the unbiased method for drug conditioning (see *SI Materials and Methods*). The time spent in each compartment during pre and post-conditioning tests (20 min) was measured.

In cocaine self-administration experiments, rats were trained to administer cocaine (500 μg/kg per injection) under an FR1 schedule for 2-h daily sessions. Response in the active hole resulted in illumination of the signal light (5.6 s) above the reinforced nose poke and the infusion of cocaine, after which the house light shut off for a 20-s time-out (a maximum of 100 cocaine infusions per session). Response in the inactive hole was counted, but had no consequences (see *SI Materials and Methods*).

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