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Distinct Roles of Opioid and Dopamine Systems in Lateral Hypothalamic Intracranial Self-Stimulation

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Abstract

Background: Opioid and dopamine systems play crucial roles in reward. Similarities and differences in the neural mechanisms of reward that are mediated by these 2 systems have remained largely unknown. Thus, in the present study, we investigated the differences in reward function in both μ-opioid receptor knockout mice and dopamine transporter knockout mice, important molecules in the opioid and dopamine systems.
Methods: Mice were implanted with electrodes into the right lateral hypothalamus (l hour). Mice were then trained to put their muzzle into the hole in the head-dipping chamber for intracranial electrical stimulation, and the influences of gene knockout were assessed.
Results: Significant differences are observed between opioid and dopamine systems in reward function. μ-Opioid receptor knockout mice exhibited enhanced intracranial electrical stimulation, which induced dopamine release. They also exhibited greater motility under conditions of "despair" in both the tail suspension test and water wheel test. In contrast, dopamine transporter knockout mice maintained intracranial electrical stimulation responding even when more active efforts were required to obtain the reward.
Conclusions: The absence of μ-opioid receptor or dopamine transporter did not lead to the absence of intracranial electrical stimulation responsiveness but rather differentially altered it. The present results in μ-opioid receptor knockout mice are consistent with the suppressive involvement of μ-opioid receptors in both positive incentive motivation associated with intracranial electrical stimulation and negative incentive motivation associated with depressive states. In contrast, the results in dopamine transporter knockout mice are consistent with the involvement of dopamine transporters in positive incentive motivation, especially its persistence. Differences in intracranial electrical stimulation in μ-opioid receptor and dopamine transporter knockout mice underscore the multidimensional nature of reward.

Keywords: ICSS, reward function, μ-opioid receptor, dopamine transporter, knockout mouse

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Significance Statement

We investigated the differences in reward function in both μ-opioid receptor knockout (MOP KO) mice and dopamine transporter knockout (DAT KO) mice, important molecules in the opioid and dopamine systems, by using lateral hypothalamic intracranial self-stimulation (lhICSS) procedures. MOP KO mice exhibited enhanced lhICSS, which induced dopamine release. They also exhibited greater motility under conditions of "despair" in both the tail suspension test and water wheel test. In contrast, DAT KO mice maintained lhICSS responding even when more active efforts were required to obtain the reward. The present results showed that MOP or DAT deficiency did not lead to the absence of lhICSS responsiveness but rather differentially altered it. Differences in lhICSS in MOP and DAT knockout mice underscore the multidimensional nature of reward.

Introduction

The reward system is a crucial base of emotion and behavior. The release of dopamine in the nucleus accumbens is thought to be one of the principal mechanisms for all rewards associated with food, water, and addictive drugs (Wise, 1996). Cocaine and other amphetamine-like psychostimulants are known to block dopamine transporters (DATs), which reuptake released dopamine and terminate dopamine signals. The DAT is undoubtedly one of the most important targets of psychostimulants (Giros et al., 1996; Sora et al., 2001b). Opioids, such as morphine, are another type of addictive drug. Previous studies that used μ-opioid receptor (MOP) knockout (KO) mice clearly showed that morphine induces reward via MOPs (Matthes et al., 1996; Sora et al., 1997). Opioids have also been reported to increase dopamine release in the nucleus accumbens (Di Chiara and Imperato, 1988; Piepponen et al., 1999) and are one of the principal mechanisms in the rewarding effects of opioids. Using dopamine-deficient mice, Hnasko et al. (2005) suggested that dopamine is actually not required for morphine-induced reward. Despite extensive investigations of the reward system, this discrepancy has not yet been resolved. Furthermore, although Belluzzi and Stein (1977) suggested that the opioid system mediates drive-reducing rewards, such as satisfaction and well-being, and the dopamine system mediates drive-inducing rewards, such as excitement and incentive, the precise roles of these neuronal systems in the different reward systems have remained unclear.

Studies of rewarding electrical brain stimulation in humans and animals have been invaluable for providing insights into the mechanisms of reward. The intracranial self-stimulation (ICSS) paradigm has distinct advantages, including (1) its independence from the rewarding effects of drugs and other natural rewards, such as food, water, and sex; (2) long-lasting responses; (3) rapid extinction; (4) very potent reinforcing effects; and (5) its direct involvement of limited neural circuits (Negus and Miller, 2014). Stimulation of the medial forebrain bundle of the lateral hypothalamus most reliably elicits ICSS responding. Stimulation of this brain area activates dopamine neurons in the ventral tegmental area and causes dopamine release in the nucleus accumbens. Therefore, analyses of lateral hypothalamic ICSS (lhICSS) appear to be promising for understanding reward mechanisms that involve the dopamine system and relationships between opioid and dopamine systems. Various addictive drugs, including both psychostimulants and opioids, have been investigated with regard to their rewarding effects using lhICSS (Negus and Miller, 2014). Although the deletion of endogenous molecules could be useful for investigating their role in reward systems, few studies have investigated changes in ICSS behavior in KO animals. Thus, in the present study, we analyzed lhICSS in MOP and DAT KO mice (i.e., one of the most important molecules in the opioid and dopamine systems). We found that the opioid and dopamine systems mediate distinct rewarding effects, likely through distinct neural mechanisms.

Materials and Methods

Animals

Heterozygote-heterozygote matings of MOP KO mice and DAT KO mice on a C57/129 background were used to produce wild-type, heterozygous, and homozygous MOP KO and DAT KO animals (Sora et al., 1997, 1998). The mice were housed in an environment at 23°C ± 1°C with 50% ± 5% relative humidity under a 12-h-light/-dark cycle (lights on 8:00 AM to 8:00 PM). Food and water were available ad libitum. The mice were >10 weeks of age at the time of the experiments. The experimental procedures and housing conditions were approved by each Institutional Animal Care and Use Committee, and all of the animals were cared for and treated humanely in accordance with our institutional guidelines on animal experimentation.

Surgery

A bipolar electrode was constructed of 2 tightly twisted strands of insulated stainless-steel wire and implanted in the right lateral hypothalamus under anesthesia at the following stereotaxic coordinates: 1.2 mm lateral, 0.9 mm posterior, and 4.9 mm ventral to bregma. The electrode position was verified after ICSS testing as described previously (Ikeda et al., 2001).

Lateral Hypothalamic ICSS Apparatus

Brain stimulation was delivered in a head-dipping chamber or zone-occupying chamber (O'Hara and Co., Ltd.) as described previously (Ikeda et al., 2001). A train of electrical stimulation was delivered to the subject when it put its muzzle into the hole in the head-dipping chamber. A train of electrical brain stimulation was delivered every 0.5 seconds as long as the animal remained in the stimulation zone in the zone-occupying chamber, unless otherwise specified.

LhICSS Test Procedure

One week after surgery, the mice were placed in the head-dipping chamber to determine the initial extent of the ICSS response as described previously (Ikeda et al., 2001). Some of the mice were also placed in the zone-occupying chamber to determine the initial extent of the ICSS response. In the head-dipping tests, at least 100 head-dips in a 600-second session were considered a stable response. In the zone-occupying tests, at least 60 seconds spent in the target zone in a 600-second session was considered

a stable response. After training, mice that presented stable ICSS responding 1 hour before each experiment were used.

In the experiments that were conducted to determine current-response relationships, the head-dipping rate was sequentially measured for 300 seconds at 0, 20, 40, 60, 80, 100, 120, 140, and 160 μA. In the experiments that were conducted to determine response decay that was caused by an increase in the response/reinforcement ratio, the head-dipping rate was sequentially measured for 600 seconds using progressive reinforcement ratios (Hodos, 1961) of 1, 2, 3, 4, 6, 8, 12, 18, and 28 (one head-dipping in the first session for one train of electrical stimulation, 2 head-dippings in the second session for one train of electrical stimulation, and so on) using same mice on another test day. The current intensity was fixed at 100 μA. At the end of the experiment, the head-dipping rate was measured for 600 seconds at a ratio of 1. The data were discarded when the mouse did not present stable ICSS responding during the second ratio-1 period. The largest ratio at which the response rate was still >10% of the rate for the first ratio-1 period was designated as the breakpoint for each mouse in this study.

For the evaluation of response decay, the head-dipping experiment was conducted first using naive mice, followed by the zone-occupying experiment on another test day. In the experiments that evaluated response decay that was caused by a delay of electrical stimulation, the head-dipping rate was sequentially measured for 600 seconds with a delay between the response and the stimulation (0, 0.5, 1, 2, 4, 8, and 0 s) at 100 μA. In the experiments that evaluated response decay that was caused by a decrease in stimulation, the head-dipping rate was sequentially measured for 600 seconds at 100, 80, 60, 40, 20, 0, and 100 μA. In the experiments that evaluated response decay that was caused by the extension of time that was required for a train of stimulation in the zone-occupying paradigm, the time spent in the stimulation zone was sequentially measured for 600 seconds with the required time at 0.5, 1, 2, 4, 8, and 0.5 seconds at 100 μA.

Tests for Depressive-Like Behavior

The mice were suspended by their tails for 600 seconds, and motility was measured during the last 300 seconds using an acceleration detector (Neuroscience Inc., Tokyo). In the water-wheel test, the mice were placed into a pool of water (7 cm long × 30 cm wide × 15 cm deep; O'Hara and Co., Ltd.) that contained a water wheel (10 cm diameter × 7 cm wide). The water temperature was maintained at 25 ± 1°C. The mice were tested for 360 seconds, and the number of wheel rotations was measured during the last 300 seconds.

Statistical Analysis

All the data were normally distributed and are expressed as the mean ± SEM. The statistical analyses were performed using repeated-measures ANOVA. Posthoc comparisons, when appropriate, were conducted using the Tukey-Kramer test. The statistical analyses were performed using StatView software (SAS Instruments, Inc.).

Results

Current-Response Relationship of the lhICSS Response in MOP and DAT KO Mice

We examined the relationship between head-dipping rate and stimulation current in wild-type, heterozygous, and homozygous MOP KO mice (Figure 1a) and DAT KO mice (Figure 1b). The head-dipping rates at 20 and 40 μA in MOP KO mice were almost the same for all 3 genotypes. At currents between 60 and 160 μA, homozygous MOP KO mice exhibited an increase in their ICSS rates with currents up to 100 μA; above this current, ICSS rates slightly decreased. Wild-type mice exhibited no significant change in ICSS rate, and heterozygous MOP KO mice exhibited a gradual decrease in ICSS rates >60 μA. Although no significant interaction was found between the stimulation current and MOP genotype ($F_{16,216} = 1.16$, $P > .05$, repeated-measures ANOVA), a significant difference was found between wild-type and homozygous MOP KO mice at 80, 100, 120, and 160 μA ($P < .05$, Tukey-Kramer posthoc test).

The head-dipping rates in DAT KO mice increased at 40 μA for all 3 genotypes. Although no significant interaction was found between stimulation current and DAT genotype ($F_{16,232} = 1.30$, $P > .05$, 2-way mixed-design ANOVA) compared with wild-type DAT KO mice, homozygous DAT KO mice exhibited a significant increase in head-dipping rate at 0 and 60 μA ($P < .05$, Tukey-Kramer posthoc test). Although both MOP KO and DAT KO mice exhibited nearly the same increase in the maximum number of head dips, only DAT KO mice tended to show an increase in the number of head dips in response to the lower current intensity.

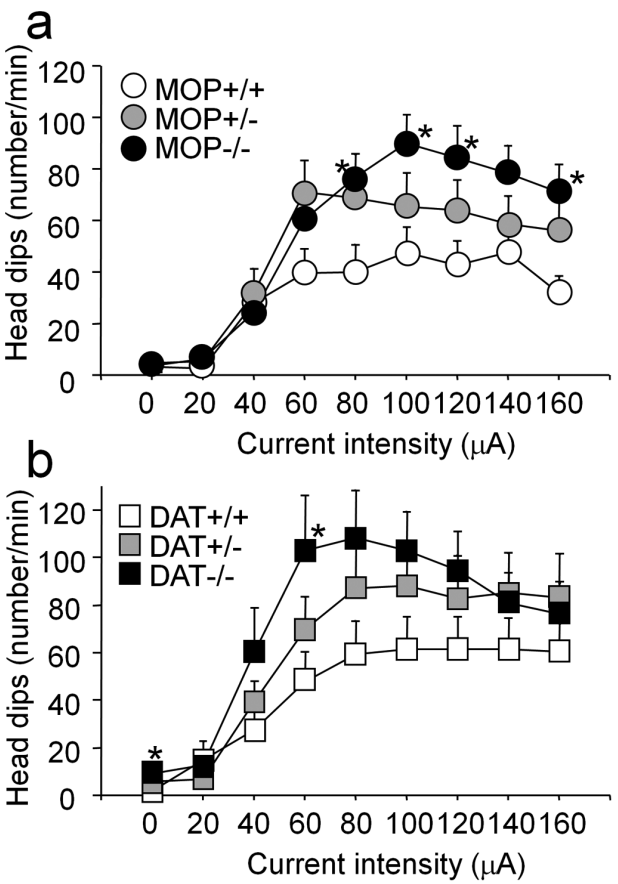

Figure 1. Current-response curves. (a) Head-dipping rates in μ-opioid receptor (MOP) knockout (KO) mice (+/+, $n = 11$; +/-, $n = 13$; -/-, $n = 13$). (b) Head-dipping rates in dopamine transporter (DAT) KO mice (+/+, $n = 11$; +/-, $n = 11$; -/-, $n = 10$). Each mark and vertical line represent the mean ± SEM. *$P < .05$, **$P < .01$, compared with wild-type mice.

Decay of lhICSS Responses Caused by a Decrease in Reinforcement Ratio

The analysis of lhICSS as the reinforcement ratio changes is useful for evaluating the relative reward strength of stimuli (Depoortere et al., 1999). We next compared the reduction of the head-dipping response as the reinforcement ratio decreased in wild-type, heterozygous, and homozygous MOP KO mice (Figure 2a) and DAT KO mice (Figure 2b). The ICSS responses decreased as the reward ratio decreased in all genotypes, with differences between genotypes. A weak interaction was found between the reinforcement ratio effect and MOP genotype ($F_{18,306} = 1.50$, $P = .0895$, 2-way mixed-design ANOVA). Homozygous MOP KO mice presented slower extinction of ICSS responses, especially at ratios of 2, 3, and 4 ($P < .01$ for ratio 2 and $P < .05$ for ratios 3 and 4, Tukey-Kramer posthoc test). The breakpoint increased in a MOP gene dose-dependent fashion (wild-type mice: 9.09 ± 2.43; heterozygous mice: 10.2 ± 2.56; homozygous mice: 12.1 ± 2.65). Recovery of the response rate in the last session with a reward ratio of 1 suggested that the decay was attributable not to fatigue but rather to extinction of ICSS responses that was caused by partial omission of brain stimulation reward.

In DAT KO mice, a significant difference was found between the reinforcement ratio effect and DAT genotype ($F_{18,243} = 2.55$, $P < .001$, repeated-measures ANOVA). The ICSS response decreased as the reward ratio decreased in wild-type and heterozygous DAT KO mice. Homozygous DAT KO mice maintained a high level of ICSS responding from a ratio of 1 to a ratio of 4 ($P < .01$ for ratio of 4, Tukey-Kramer posthoc test). The ICSS response in homozygous DAT KO mice began to decrease at a ratio of 6 and reached the same level as wild-type and heterozygous DAT KO mice at ratios of 8, 12, 18, and 28. The ICSS responses in the 3 genotypes recovered in the last session with a reward ratio of 1.

Increased Motility in MOP KO Mice in the Tail Suspension and Water Wheel Tests

The lhICSS response increased in MOP KO mice. We next investigated the converse behavior (i.e., escaping from conditions of "despair"). In the tail suspension and water wheel tests, homozygous MOP KO mice exhibited significant increases in the time of motility and the number of wheel rotations (Figure 3a-b). These similar observations in distinct tests, together with the results in the forced swim test and conditioned suppression of motility test (Filliol et al., 2000), may suggest the suppressive involvement of MOPs in negative incentive motivation associated with an attempt to avoid the depressive environment.

Distinct Decay of lhICSS Responding in DAT KO Mice

To further investigate the remarkable resistance to lhICSS response decay in DAT KO mice, we analyzed lhICSS response decay in 3 different experiments. In the experiment that evaluated the response decay that was caused by a delay of electrical stimulation, the head-dipping responses in DAT KO mice gradually decreased as the stimulation delay increased (Figure 4a). Although no significant difference was found between the delay and DAT genotype ($F_{12,162} = 0.77$, $P > .05$, 2-way mixed-design ANOVA), the responses in DAT KO mice at delays of 0.5, 1, 4, and 8 seconds were significantly higher than those in wild-type mice. In the experiment that evaluated the response decay that was caused by a decrease in stimulation, the responses in DAT KO mice decreased when the current intensity decreased (Figure 4b). No significant interaction was found between current intensity and genotype ($F_{10,140} = 0.42$,

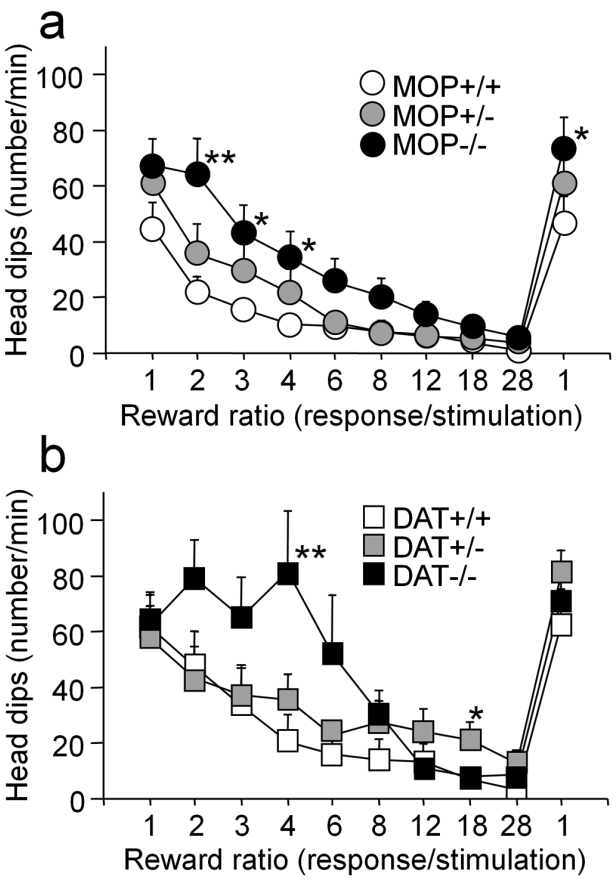

Figure 2. Response decay in a progressive-ratio schedule. (a) Slight resistance to extinction of head-dipping at 100 μA in μ-opioid receptor (MOP) knockout (KO) mice (+/+, $n = 11$; +/-, $n = 13$; -/-, $n = 13$). (b) Significant resistance to extinction of head-dipping at 100 μA in dopamine transporter (DAT) KO mice (+/+, $n = 11$; +/-, $n = 11$; -/-, $n = 10$). Each mark and vertical line represent the mean ± SEM. *$P < .05$, **$P < .01$, compared with wild-type mice.

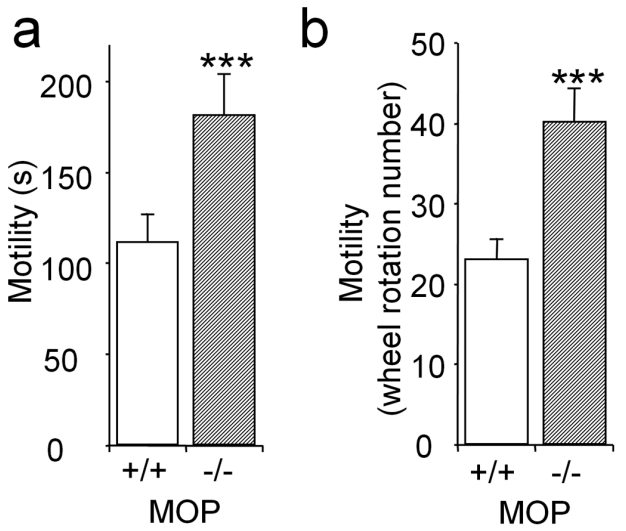

Figure 3. Increased motility in μ-opioid receptor (MOP) knockout (KO) mice in tests of depressive-like behavior. (a) Tail-suspension test. Motility in mice (+/+, $n = 31$; -/-, $n = 27$) was measured during the last 5 minutes of the 10-minute session. (b) Water wheel test. Motility in mice (+/+, $n = 33$; -/-, $n = 27$) was measured during the last 5 minutes of the 6-minute session. Each bar and vertical line represent the mean ± SEM. ***$P < .001$ (Student's t test).

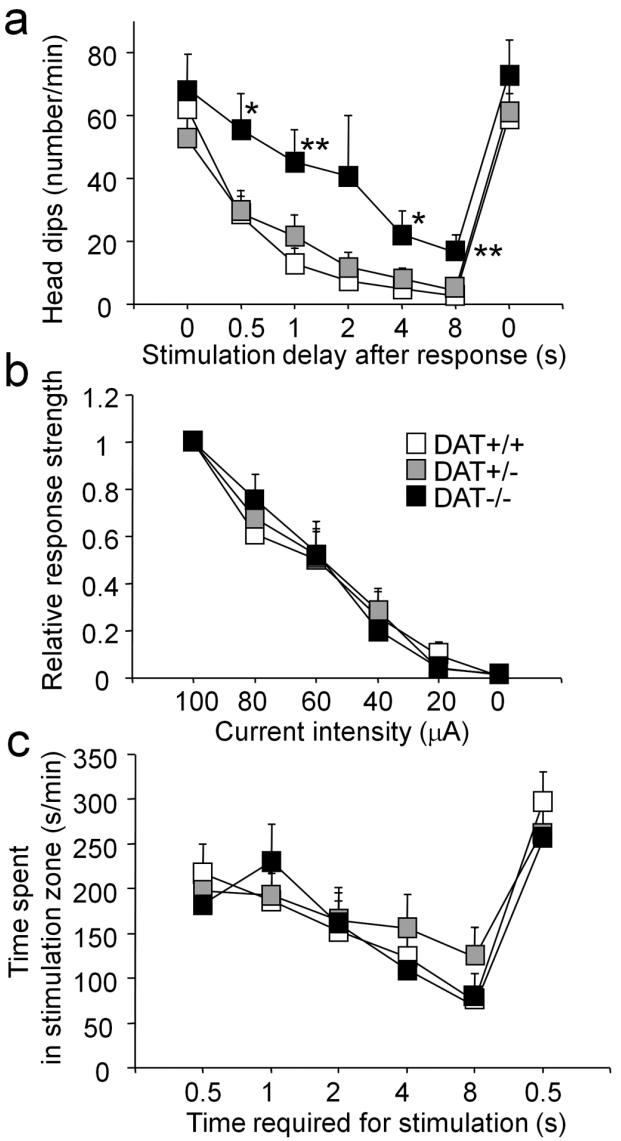

Figure 4. Distinct response decay of intracranial self-stimulation (ICSS) in 3 schedules in dopamine transporter (DAT) knockout (KO) mice. (a) Significant resistance to extinction of head-dipping at 100 μA in a delay schedule in which the delay between the response and the stimulation increased. +/+, $n = 10$; +/-, $n = 10$; -/-, $n = 10$. (b) Extinction of head-dipping according to decrease in current intensity. +/+, $n = 10$; +/-, $n = 11$; -/-, $n = 10$. (c) Extinction of zone-occupying at 100 μA caused by an increase in the time necessary for stimulation. +/+, $n = 12$; +/-, $n = 12$; -/-, $n = 10$. Each mark and vertical line represent the mean ± SEM. *$P < .05$, **$P < .01$, compared with wild-type mice.

$P > .05$, 2-way mixed-design ANOVA). In the experiment that evaluated the response decay that was caused by extension of the time that was required for a train of stimulation in the zone-occupying paradigm, DAT KO mice exhibited a similar pattern of the time spent in the stimulation zone compared with their littermates as the required time changed (Figure 4c). No significant interaction was found between the time required and genotype ($F_{10,155} = 1.40$, $P > .05$, 2-way mixed-design ANOVA).

Discussion

We examined the influence of MOPs and DATs on the control of reward and motivation using a KO mouse model. Compared with wild-type mice, homozygous and heterozygous MOP KO mice exhibited increases in lhICSS response rates in the head-dipping experiments. These effects depended on the KO-gene copy number. Although the threshold of current intensity was unaltered, homozygous MOP KO mice tended not to decrease their responding compared with wild-type mice. This observation suggests that MOP activity affects the lhICSS response rate and response extinction as the reinforcement ratio increases but does not alter the threshold of current intensity. The increases in lhICSS responding are likely not attributable to an increase in the general activity of MOP KO mice, because locomotor activity has been shown to be unaltered (Sora et al., 2001a) or to be specifically reduced (Filliol et al., 2000) in the open field test.

Morphine and other opioids are known to affect ICSS responding in a complicated way. Olds and Travis (1960) were the first to analyze the effects of morphine on ICSS responding. They found that acute morphine administration facilitated ICSS responses to stimulation of the tegmentum but inhibited ICSS responses to stimulation of the hypothalamus and septal area. Lorens and Mitchell (1973) reported that morphine initially produced depression, followed by the facilitation of lhICSS. Subsequent studies revealed that the effects of opioids on ICSS depend on several factors, such as the site of stimulation, the method of measuring ICSS, and the routes and timing of drug administration (Esposito and Kornetsky, 1978). Additionally, difficulties in interpretation have arisen in many of these studies. For example, acute and systemic administration of moderate doses of MOP agonists appears to enhance (Marcus and Kornetsky, 1974; Wise, 1996) or suppress (Wauquier and Niemegeers, 1976; Schaefer and Holtzman, 1977) lhICSS in rats. More recent studies that have used rate-frequency procedures have also confirmed that MOP agonists produce complex effects on ICSS, and these effects are influenced by dose, pretreatment time, and agonistic efficacy (O'Neill and Todtenkopf, 2010; Altarifi and Negus, 2011; Altarifi et al., 2012, 2013). The involvement of MOPs in lhICSS remains unclear. The clear lhICSS response in MOP KO mice that was observed in the present study indicates that MOPs are not an indispensable molecule in lhICSS. Moreover, the increase in lhICSS response rates in MOP KO mice in the present study suggests novel relationships between MOP systems and lhICSS. MOP systems would be involved in brain reward processes, including those that are not directly triggered by opiates.

Dopamine release in the nucleus accumbens is essential for the induction of lhICSS (Carlson, 1994). Several researchers have proposed that dopamine release in the nucleus accumbens is also a critical step for the rewarding effects of addictive drugs, including opioids (Pontieri et al., 1995; Wise, 1996). However, the present results indicate that the lack of MOPs enhances lhICSS behavior, suggesting complicated relationships between lhICSS and opioid-induced reward. Multiple reward systems have also been suggested to exist in the brain (Belluzzi and Stein, 1977; Van Ree and Ramsey, 1987; Koob and Le Moal, 1997). Belluzzi and Stein (1977) proposed an interesting hypothesis that there are 2 rewards: "drive-inducing" reward (i.e., a state of incentive and a process of motivation in pursuing a goal that might be mediated by the dopamine system) and "drive-reducing" reward (i.e., a state of satisfaction or well being and a process of attainment and consumption of a goal that might be mediated by the opioid system). According to their hypothesis, the present results could be interpreted as the following. The lack of MOPs might cause a loss of satisfaction, resulting in an increase in lhICSS responding in MOP KO mice. Although dopamine release in the nucleus accumbens is the central mechanism in lhICSS, it does not seem to be the sole mechanism in opioid-induced reward. Other brain regions could also play an important role in

opioid-induced reward. The hypothesis could also be supported by the study by Hnasko et al. (2005), which reported that dopamine-deficient mice exhibited robust morphine-induced conditioned place preference. Further investigations of active and passive ICSS responses to the stimulation of other brain regions using MOP KO mice could reveal additional mechanisms that underlie opioid-induced reward.

Numerous clinical observations support the involvement of the opioid system in the morbidity of depression (Verebey et al., 1978; Gold et al., 1982). Animal studies have shown that acute morphine administration increases immobility in the forced swim test (Amir, 1982; Zurita and Molina, 1999) and water wheel test (Kastin et al., 1984). In the present study, the increase in ICSS responding and increase in motility under conditions of "despair" in MOP KO mice, together with previous findings of a reduction of anxiety-like and depressive-like behavior in MOP KO mice (Filliol et al., 2000), suggest that MOPs are involved in controlling behaviors that are related to negative incentive motivation. Furthermore, we previously found that the antidepressant effect of the serotonin and norepinephrine reuptake inhibitor venlafaxine was abolished in MOP KO mice in the forced swim test (Ide et al., 2010). Although the role of MOPs in the molecular and neural mechanisms that underlie depression has remained largely unknown, MOPs might suppress negative incentive motivation in an attempt to avoid the aversive environment.

DAT KO mice also exhibited an increase in lhICSS response rates in the head-dipping experiments compared with their littermates. lhICSS responding was resistant to the decrease in reinforcement ratio in DAT KO mice compared with their littermates. In DAT KO mice, extracellular dopamine levels are remarkably high in the caudate putamen and nucleus accumbens (Shen et al., 2004), with correspondingly high spontaneous locomotor activity (Sora et al., 1998). The increase in lhICSS responding and delayed lhICSS response decay may have resulted from an increase in extracellular dopamine. This is also consistent with previous studies that showed that dopamine D_1 receptors are an important determinant in brain stimulation reward (Tran et al., 2005). The increase in dopamine may augment ICSS responding in DAT KO mice by activating D_1 receptors.

Differences in lhICSS response rates were observed of DAT KO mice in the present head-dipping experiments (100 μA, reinforcement ratio = 1) (Figures 1 and 2). DAT KO mice are well known to exhibit hyperlocomotion (Giros et al., 1996). In the present study, DAT KO mice also exhibited hyperlocomotion in the ICSS chamber and tended to delay the start of head-dipping behavior, thus possibly resulting in lower head-dipping rates in DAT KO mice early in the ICSS sessions. Other ICSS methodologies may need to be employed to confirm the effects of drugs or gene adaptations that affect locomotion.

Using DAT KO mice, we determined that dopamine release could be involved in reward-related behavior, especially its persistence. Dopamine is a principal neurotransmitter that mediates locomotor and motivated behavior. Thus, the DAT KO mouse exhibits an increase in extracellular dopamine levels and is a useful animal model for studying the contributions of dopamine to the mechanisms of reward-related behavior. Mice with lifelong deletion of the DAT exhibited an increase in ICSS responding and retarded response decay, suggesting an important role for dopamine in controlling reward-related behavior. However, the retarded response decay only occurred in the case of increasing the stimulation delay after the responses. Interestingly, decreasing the current intensity or increasing the stimulation time did not alter the response decay in homozygous DAT KO mice. Thus, DATs may be postulated to modulate positive incentive motivation, especially its persistence or extinction. This may have resulted from developmental adaptations. Thus, it is necessary to create conditional DAT KO mice for further investigations.

In conclusion, MOP and DAT deficiency did not lead to the absence of lhICSS responsiveness but rather differentially altered it. The activation of MOPs led to a reduction of lhICSS, suggesting differences in reward processing between the opioid and dopamine systems. The present results in MOP KO mice (i.e., an increase in lhICSS responding and increase in mobility in tests of depression) are consistent with the suppressive involvement of MOPs in both positive incentive motivation associated with lhICSS and negative incentive motivation associated with aversive states. The results in DAT KO mice (i.e., enhanced persistence of lhICSS) are consistent with the involvement of DAT in positive incentive motivation, especially its persistence. The results suggest that MOP and DAT KO mouse strains are useful animal models for investigating the molecular mechanisms of these two pathways in the brain reward system. To further elucidate the precise molecular mechanisms associated with the brain reward system, future studies should employ double MOP/DAT mice to gain a better understanding of the cross talk between these two important components of the brain reward system.

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Statement of Interest

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