



NEUROSCIENCE

MDMA enhances empathy-like behaviors in mice via 5-HT release in the nucleus accumbens

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MDMA (3,4-methylenedioxymethamphetamine) is a psychoactive drug with powerful prosocial effects. While MDMA is sometimes termed an “empathogen,” empirical studies have struggled to clearly demonstrate these effects or pinpoint underlying mechanisms. Here, we paired the social transfer of pain and analgesia—behavioral tests modeling empathy in mice—with region-specific neuropharmacology, optogenetics, and transgenic manipulations to explore MDMA’s action as an empathogen. We report that MDMA, given intraperitoneally or infused directly into the nucleus accumbens (NAc), robustly enhances the social transfer of pain and analgesia. Optogenetic stimulation of 5-HT release in the NAc recapitulates the effects of MDMA, implicating 5-HT signaling as a core mechanism. Last, we demonstrate that systemic MDMA or optogenetic stimulation of NAc 5-HT inputs restores deficits in empathy-like behaviors in the *Shank3*-deficient mouse model of autism. These findings demonstrate enhancement of empathy-related behaviors by MDMA and implicate 5-HT signaling in the NAc as a core mechanism mediating MDMA’s empathogenic effects.

INTRODUCTION

3,4-Methylenedioxymethamphetamine (MDMA) is a psychoactive drug known for its powerful prosocial effects (1). Since the earliest investigations of MDMA, anecdotal clinical reports have noted the drug’s ability to influence empathy (2, 3), earning it a reputation as an “empathogen.” However, empirical studies examining MDMA’s empathogenic properties in humans have produced conflicting results (4–7), leaving it unclear how or under what conditions MDMA influences empathy. Moreover, the neurobiological mechanisms underlying these alleged effects remain unknown.

This topic is currently of particular relevance because MDMA is re-emerging as a potential therapeutic tool, showing great promise as an adjunct to psychotherapy for post-traumatic stress disorder (PTSD) (8, 9). MDMA’s psychotherapeutic value may lie in its ability to strengthen the patient-therapist relationship, thereby facilitating talk therapy sessions (9, 10). Given that empathy is a core component of this relationship (11), understanding MDMA’s empathogenic action may offer mechanistic insights into the drug’s utility in the context of psychiatry. However, human studies exploring MDMA’s behavioral effects on empathy have been limited by the use of static, image-based empathy assays and self-report measures.

We recently developed two “social transfer” paradigms to model empathy-like behaviors in mice. During the social transfer of pain, bystander (BY) mice acquire pain following a brief social interaction with a demonstrator (DEM) mouse experiencing inflammatory pain (12–14). In the social transfer of analgesia, BY mice acquire pain relief following a social interaction with a DEM experiencing inflammatory pain and concurrent morphine (MOR) analgesia (12). Here, we examine the impact of MDMA on these empathy-like behaviors and use brain region-specific pharmacology, optogenetics,

and transgenic manipulations to interrogate the underlying mechanisms. We find that MDMA robustly enhances both the social transfer of pain and analgesia and that the drug’s action in the nucleus accumbens (NAc) core alone is sufficient to produce these effects. Moreover, optogenetic stimulation of serotonin (5-HT) inputs in the NAc core similarly enhances empathy, suggesting a key role for MDMA-mediated 5-HT release in the NAc. Because empathy dysregulation has been reported in patients with autism spectrum disorder (ASD) (15) and MDMA is being explored as a therapeutic for ASD (16), we investigated social transfer in the *Shank3*-deficient mouse model of ASD and the impact of MDMA on this behavior. We find that *Shank3*-deficient mice do not display the social transfer of pain or analgesia, and remarkably, both MDMA and optogenetic activation of 5-HT inputs in the NAc restore this empathy-like behavior. These results provide mechanistic insights into MDMA’s action as an empathogen and the neurobiological mechanisms by which empathy may be pharmacologically restored in conditions associated with alterations in certain empathy behaviors.

RESULTS

MDMA enhances empathy-like behaviors in mice

We recently characterized the “social transfer of pain” in which BY mice display mechanical hypersensitivity after a 1-hour interaction with a DEM mouse experiencing inflammatory pain due to hind-paw injection of complete Freund’s adjuvant (CFA) (12, 13). Because empathy-like behavior may be mechanistically distinct from “social preference” as measured by the traditional three chamber social interaction assay (17), we chose to test the effects of MDMA on the social transfer of pain. Male BY mice were injected with MDMA (7.5 mg/kg, i.p.; MDMA-BY) or saline (SAL-BY) 15 min before an abbreviated 10-min social interaction with a CFA-injected male DEM mouse (CFA-DEM; Fig. 1A). Following this brief interaction, MDMA-BY mice displayed markedly reduced mechanical thresholds, whereas mechanical sensitivity in SAL-BY mice remained unchanged (Fig. 1, B and C). Hypersensitivity in MDMA-BY mice lasted beyond 24 hours with recovery to control levels by 48 hours (Fig. 1D), indicating that MDMA robustly facilitates the social

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transfer of pain. MDMA-injected control mice (MDMA-CON) displayed unchanged mechanical thresholds after interacting with a pain-naïve cage mate (Fig. 1, B to D), and MDMA alone did not affect mechanical thresholds at any dose tested (fig. S1A). The strength of the social transfer of pain was not correlated with the amount of time BY mice spent directly interacting with CFA-DEM (i.e., sniffing and chasing) (fig. S1B). Furthermore, CFA-DEM mice displayed similar mechanical thresholds after interacting with MDMA- or SAL-treated BY mice, suggesting no social buffering effect of pain in CFA-DEM mice (fig. S1C).

Unexpectedly, MDMA did not enhance the social transfer of pain in female mice (fig. S1D). We found that male and female mice showed an equivalent rise in 5-HT levels in the NAc following MDMA administration, suggesting that the pharmacological action of MDMA on 5-HT release is very unlikely to account for the observed sex differences (fig. S1, E to H). Although unexpected, this result is consistent with human reports suggesting that MDMA enhances emotional empathy only in male participants (5). As a result of this finding, all subsequent experiments were performed in male mice.

To determine whether MDMA's effects as a psychostimulant might account for its empathogenic effects in this assay, we tested whether a similar, yet distinct psychostimulant, methamphetamine (METH), also enhanced the social transfer of pain. We found that this was not the case, as mechanical thresholds did not differ between METH-treated bystanders (METH-BY) and SAL-BY mice following a 10-min social interaction with a CFA-DEM mouse (Fig. 1E), indicating that MDMA's behavioral effects are not simply a result of its psychostimulant properties.

We next tested whether MDMA also influences the social transfer of analgesia. All mice first received CFA injection (CFA-DEM and CFA-BY) to induce inflammatory pain, and DEM mice were also immediately given an analgesic dose of MOR (CFA-MOR-DEM; 10 mg/kg, s.c.). Before the social interaction, BY mice received either SAL (CFA-SAL-BY) or MDMA (CFA-MDMA-BY; Fig. 1F). CFA-MDMA-BY mice showed significantly higher mechanical thresholds 1 and 6 hours but not 24 hours after the brief social interaction, indicating that MDMA facilitates the social transfer of analgesia (Fig. 1, G to I). Notably, CFA-SAL-BY mice displayed similar mechanical

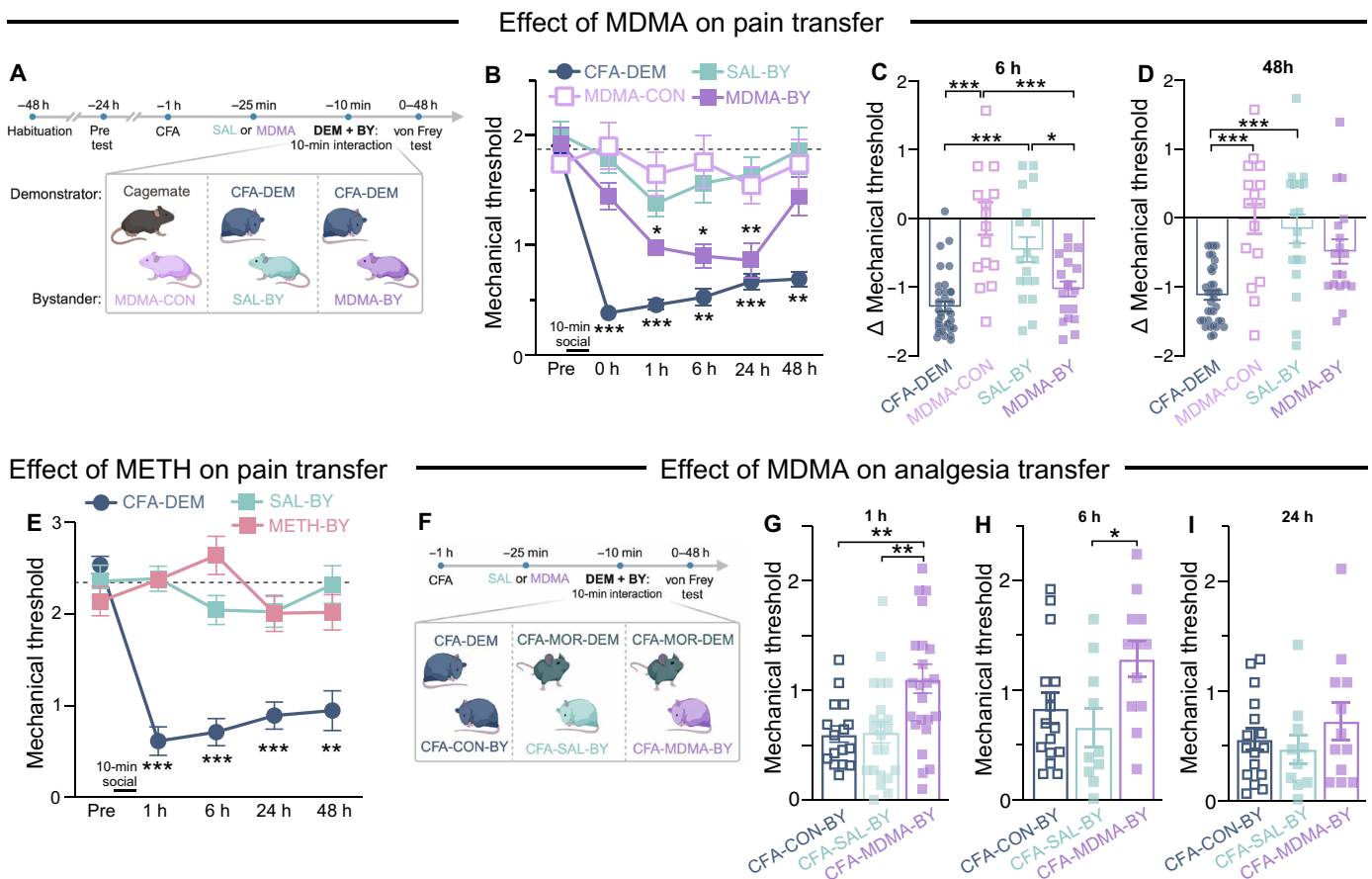


Fig. 1. MDMA enhances the social transfer of pain and analgesia in mice. (A) Schematic illustrating the timeline and all experimental groups for the MDMA-assisted social transfer of pain. (B) Time course of von Frey (vF) mechanical thresholds before the MDMA-assisted social transfer of pain and 0, 1, 6, 24, and 48 hours after the 10-min interaction. Asterisks indicate statistically significant differences from the SAL-BY control group. (C and D) Mechanical thresholds 6 hours (C) and 48 hours (D) after the social transfer of pain, presented as the change from the group baseline. (E) Time course of vF mechanical thresholds before the methamphetamine-assisted social transfer of pain and 1, 6, 24, and 48 hours after the 10-min interaction. Asterisks indicate statistically significant differences from the SAL-BY control group. (F) Schematic illustrating the timeline and all experimental groups for the MDMA-assisted social transfer of analgesia. (G to I) Mechanical thresholds 1 hour (G), 6 hours (H), and 24 hours (I) after the MDMA-assisted social transfer of analgesia. Statistical tests include one-way [(C), (D), (G), (H), and (I)] and two-way [(B) and (E)] analysis of variance (ANOVA) with Tukey's post hoc tests. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.0001$. All data presented as means \pm SEM.

thresholds as CFA-CON-BY mice at the 1- and 6-hour time points, suggesting that a 10-min interaction with a CFA-MOR-DEM mouse is not sufficient for a BY to acquire the social transfer of analgesia (Fig. 1, G and H). Furthermore, MDMA administration did not affect mechanical thresholds in CFA-injected mice (fig. S11). These results demonstrate that MDMA enhances both the social transfer of pain and analgesia, consistent with MDMA exhibiting empathogenic properties.

Local infusion of MDMA in the NAc enhances empathy

Previous work demonstrated that the social transfer of pain leads to increased neuronal activity in the NAc core of BY mice (12), and MDMA's prosocial effects in the three-chamber social preference test can be replicated by infusing MDMA directly into the NAc (18). To determine a role for the NAc in MDMA's empathogenic effects, we administered SAL or MDMA to Fos-Cre^{ERT2}/Ai14TdTomato [targeted recombination in active populations (TRAP)] mice and injected 4-hydroxytamoxifen (4-OHT) immediately before the social transfer of pain to fluorescently label active neurons and then counted the number of Fos-positive TRAPed cells (12, 19). Consistent with MDMA increasing NAc core neuronal activity, MDMA-BY mice displayed significantly more TRAPed cells in the NAc than SAL-BY mice after interacting with a CFA-DEM (Fig. 2A). In contrast, SAL-BY and MDMA-BY showed a comparable number of Fos-positive TRAPed cells in the anterior cingulate cortex (fig. S2A), another brain area involved in the social transfer of pain and analgesia (12).

To assess directly whether MDMA's action in the NAc alone is sufficient to replicate its systemic effects on the social transfer of pain and analgesia, we implanted microinjection cannulas into the NAc lateral core of BY mice (fig. S2B) and infused either SAL or MDMA immediately before a 10-min interaction with a CFA-DEM mouse (Fig. 2B). Because our behavioral characterization of the effects of systemic MDMA administration had revealed that the most robust effects on social transfer of pain and analgesia occurred 1 to 6 hours after the social interaction, we focused on these time points in our further neural manipulation experiments.

NAc-MDMA-BY mice displayed greater reductions in mechanical thresholds relative to NAc-SAL-BY mice 1 hour after the social interaction (Fig. 2, C and D), with recovery by 24 hours (Fig. 2E). These results indicate that MDMA infusion into the NAc is sufficient to replicate the ability of systemic MDMA to enhance the social transfer of pain. We also examined whether MDMA infusion into the NAc enhanced the social transfer of analgesia (Fig. 2F) and found that 1 hour after interacting with a CFA-MOR-DEM mouse, MDMA-infused bystanders (NAc-MDMA-BY) showed significantly higher mechanical thresholds than SAL-infused bystanders (NAc-SAL-BY; Fig. 2G); an effect that faded by 24 hours (Fig. 2H). Together, these results indicate that the NAc is a key mediator of MDMA's empathogenic effects as measured by the social transfer of pain and analgesia.

5-HT release in the NAc reproduces MDMA's empathogenic effects

MDMA promotes release of serotonin (5-HT), dopamine, and norepinephrine through modulation of their transporters, SERT, DAT, and NET (20). Previous studies show that MDMA's prosocial effects require 5-HT release in the NAc due to its interaction with SERT (18). To test whether 5-HT release in the NAc is similarly involved

in MDMA's enhancement of the social transfer of pain and analgesia, we injected Cre-dependent channel rhodopsin (AAV-DJ-EF1-DIO-hChR2 [H134R]-eYFP) or yellow fluorescent protein (YFP) (AAV-DJ-EF1-DIO-eYFP) into the dorsal raphe (DR) nucleus of SERT-Cre mice (fig. S3A) and implanted bilateral fiber optic cannulas over the NAc lateral core (fig. S3B) to selectively stimulate 5-HT inputs to the NAc (DR→NAc; Fig. 3A). Following DR→NAc stimulation during a 10-min interaction with a CFA-DEM mouse (Fig. 3B), ChR2-BY mice showed substantially enhanced social transfer of pain as represented by significantly reduced mechanical thresholds relative to YFP-BY control mice (Fig. 3, C and D). Mechanical thresholds returned to baseline in ChR2-BY mice by 24 hours (Fig. 3E). Optogenetic stimulation of DR→NAc 5-HT caused almost identical behavioral effects to those elicited by MDMA infusion into the NAc (fig. S3C). DR→NAc stimulation during mechanical testing did not affect the mechanical thresholds of ChR2 or YFP mice (fig. S3D), nor did it lead to a real-time place preference or aversion for the laser-paired side before or after the social transfer of pain (fig. S3E).

We next tested whether DR→NAc stimulation also enhanced the social transfer of analgesia (Fig. 3F) and found that CFA-ChR2-BY mice demonstrated stronger social transfer of analgesia relative to YFP controls as represented by significantly higher mechanical thresholds 1 hour after the interaction (Fig. 3G). Thresholds returned to baseline levels by 24 hours (Fig. 3H). These findings demonstrate that stimulation of 5-HT release alone in the NAc is sufficient to reproduce the behavioral effects of MDMA.

MDMA and NAc 5-HT ameliorate empathy deficits in a mouse model of ASD

Systemic MDMA (21) and 5-HT signaling in the NAc (22) have been shown to enhance sociability in mouse models of ASD. To test whether MDMA similarly enhances empathy-like behaviors in a mouse ASD model, we performed the social transfer of pain in *Shank3*-deficient mice, which carry heterozygous deletion of exons 4 to 22 in the *Shank3* gene (*Shank3*^{+/-}) (23). After first confirming that *Shank3*^{+/-} mice display social deficits in the three-chamber social preference test (fig. S4A), we paired wild-type (WT) (WT-BY) and *Shank3*^{+/-} bystanders (*Shank3*-BY) with a WT-CFA-DEM mouse for a 1-hour interaction in the absence of MDMA to assess whether they exhibit the social transfer of pain (Fig. 4A). *Shank3*-BY mice did not demonstrate any change in mechanical threshold following this social interaction, indicative of an inability to acquire the social transfer of pain (Fig. 4, B and C). Notably, baseline von Frey mechanical thresholds did not differ between *Shank3*^{+/-} and WT mice (fig. S4B).

Having established that the social transfer of pain is absent in *Shank3*^{+/-} mice, we next tested whether pretreatment with MDMA could rescue this deficit (Fig. 4D). Consistent with its effects in WT mice, MDMA-treated *Shank3*^{+/-} bystanders (*Shank3*-MDMA-BY) acquired the social transfer of pain as evidenced by significantly lower mechanical thresholds than SAL-treated *Shank3* bystanders (*Shank3*-SAL-BY) 4 hours, but not 48 hours, after the interaction (Fig. 4, E to G). *Shank3*-SAL-BY and *Shank3*-MDMA-BY mice did not differ in the amount of time spent interacting with the WT-CFA-DEM (fig. S4C). MDMA also rescued deficient social transfer of analgesia in *Shank3*^{+/-} mice (Fig. 4, H to J). Specifically, MDMA-treated *Shank3* bystanders (*Shank3*-CFA-MDMA-BY) showed significantly higher mechanical thresholds compared to SAL-treated *Shank3* bystanders (*Shank3*-CFA-SAL-BY) immediately after a 1-hour

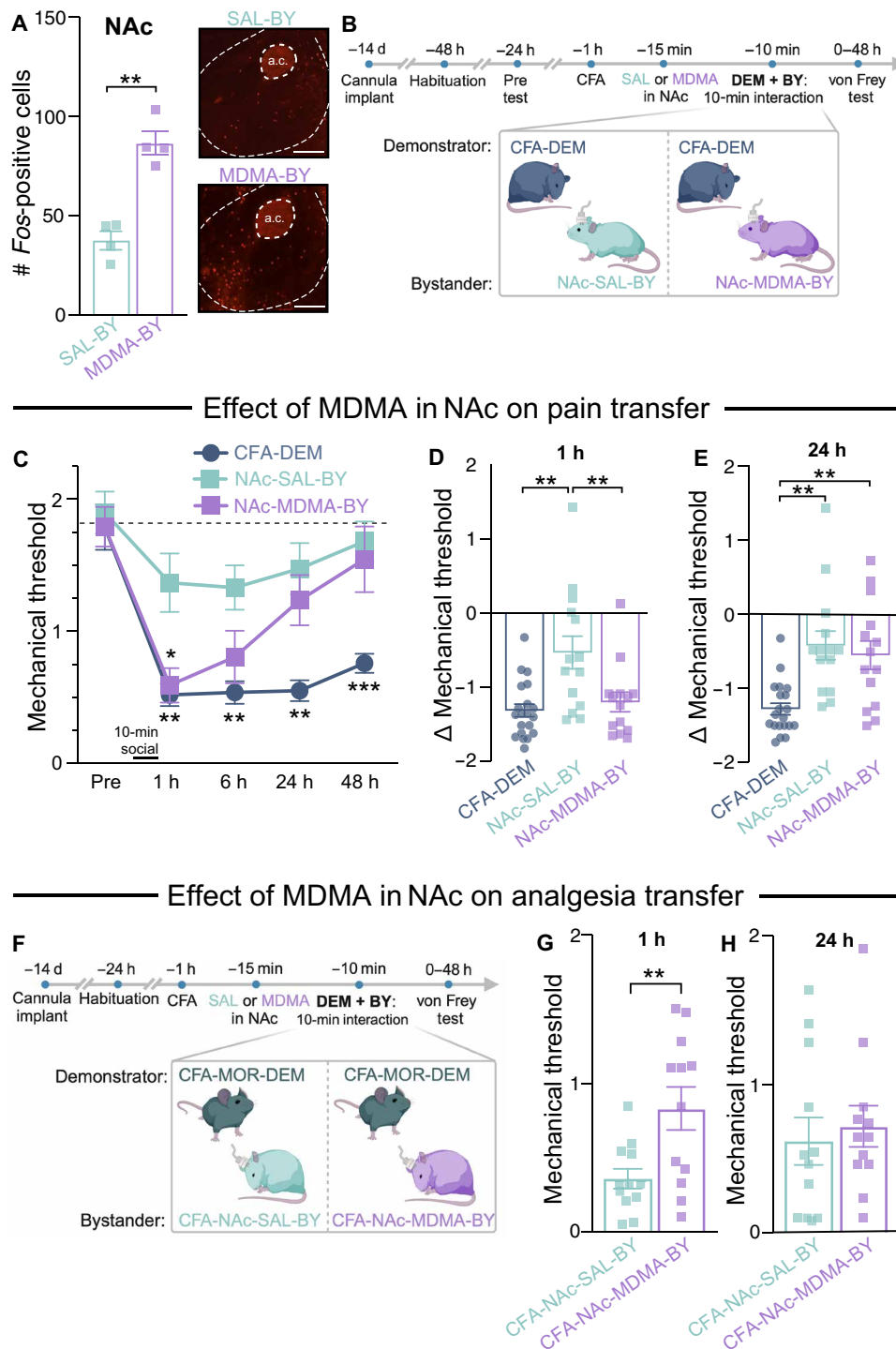


Fig. 2. MDMA infusion in NAc recapitulates the effects of systemic MDMA. (A) Quantification (left) and representative images (right) comparing the number of Fos-positive tdTomato-expressing neurons in the NAc in SAL- or MDMA-injected mice exposed to a CFA-DEM mouse. Scale bars, 250 μ m. (B) Schematic illustrating the timeline and all experimental groups for the social transfer of pain with local MDMA or SAL infusion in the NAc. (C) Time course of vF mechanical thresholds before the social transfer of pain with NAc infusion of MDMA or SAL and 1, 6, 24, and 48 hours after the 10-min interaction. Asterisks indicate statistically significant differences from the NAc-SAL-BY control group. (D and E) Mechanical thresholds 1 hour (D) and 24 hours (E) after the social transfer of pain, presented as the change from the group baseline. (F) Schematic illustrating the timeline and all experimental groups for the social transfer of analgesia with local MDMA or SAL infusion in the NAc. (G and H) Mechanical thresholds 1 hour (G) and 24 hours (H) after the social transfer of analgesia. Statistical tests include unpaired *t* test [(A), (G), and (H)], one-way [(D) and (E)], and two-way (C) ANOVA with Tukey's post hoc tests. **P* < 0.05, ***P* < 0.01, and ****P* < 0.0001. All data presented as means \pm SEM.

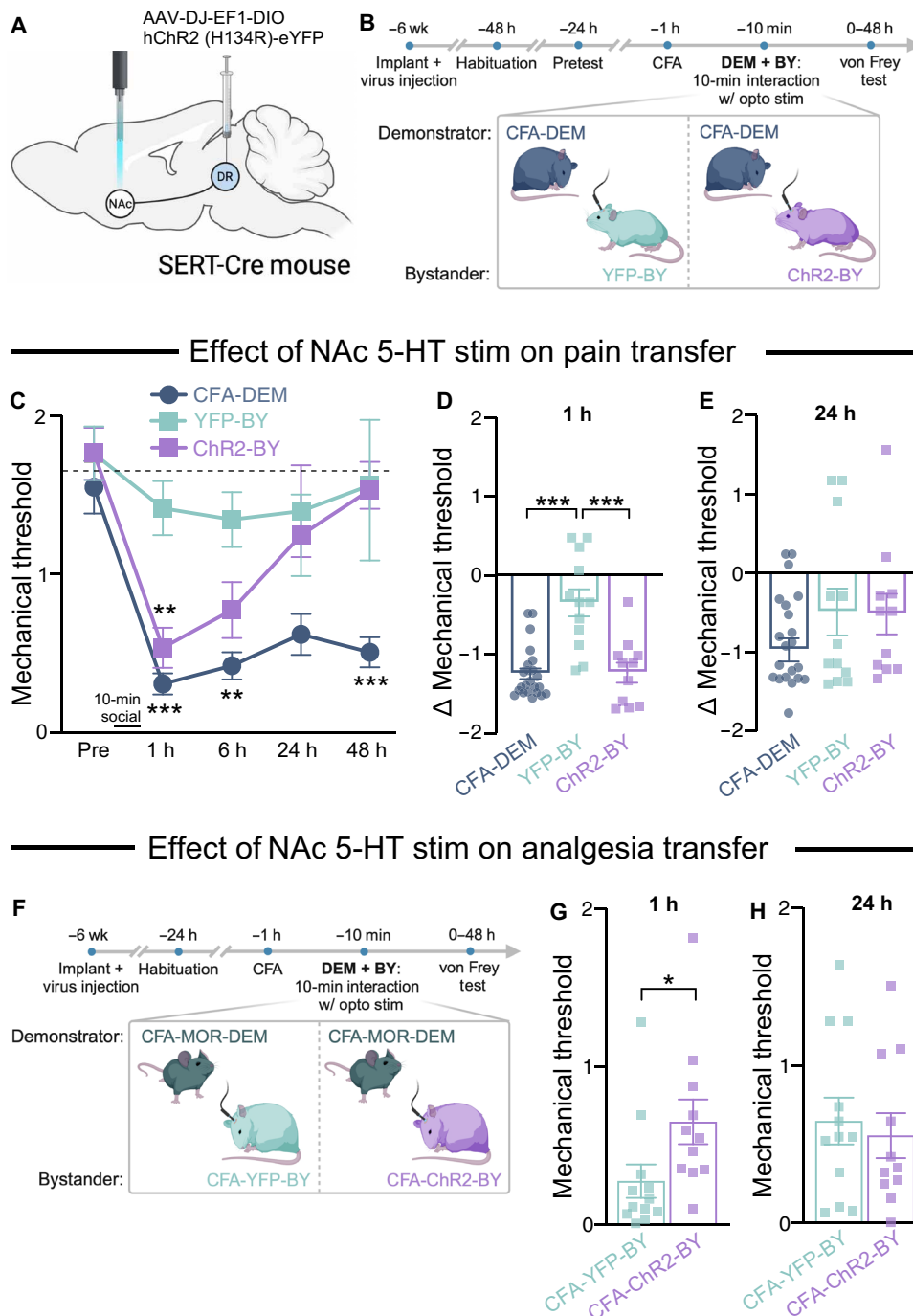


Fig. 3. Optogenetically-evoked 5-HT release in NAc reproduces the effects of intra-NAc MDMA on empathy behaviors. (A) Graphic showing viral strategy and placement of bilateral fiber optic cannula in SERT-Cre mice. (B) Schematic illustrating the timeline and all experimental groups for the social transfer of pain with optogenetic stimulation of DR 5-HT terminals in the NAc (DR→NAc). (C) Time course of vF mechanical thresholds before the social transfer of pain with DR→NAc stimulation and 1, 6, 24, and 48 hours after the 10-min interaction. Asterisks indicate statistically significant differences from the YFP-BY control group. (D and E) Mechanical thresholds 1 hour (D) and 24 hours (E) after the social transfer of pain with DR→NAc stimulation, presented as the change from group baseline. (F) Schematic illustrating the timeline and all experimental groups for the social transfer of analgesia with DR→NAc stimulation. (G and H) Mechanical thresholds 1 hour (G) and 24 hours (H) after the social transfer of analgesia. Statistical tests include unpaired t test [(G) and (H)], one-way [(D) and (E)], and two-way (C) ANOVA with Tukey's post hoc tests. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.0001$. All data presented as means \pm SEM.

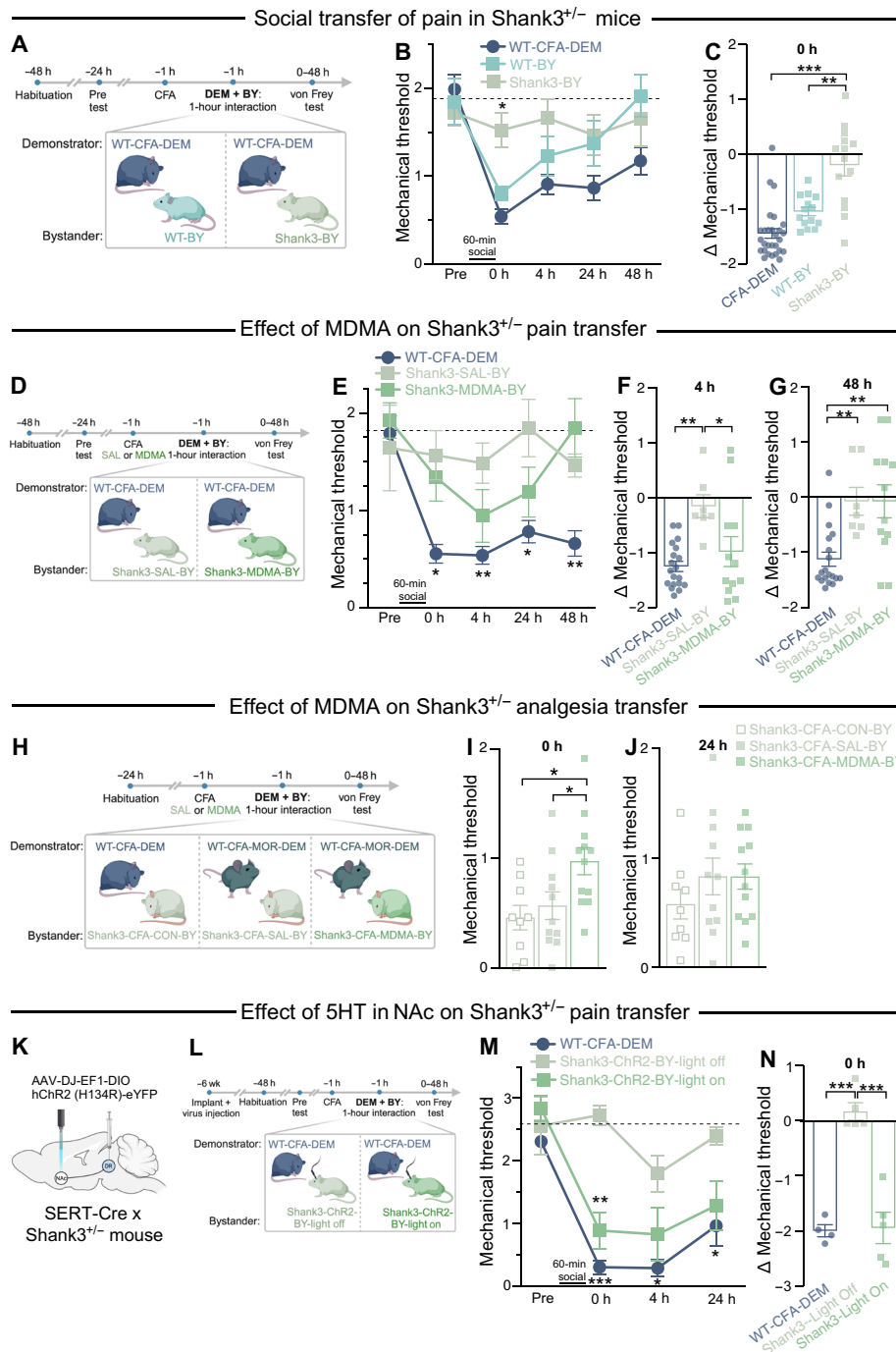


Fig. 4. Systemic MDMA and 5-HT in NAc reverse ASD-related empathy deficits in Shank3^{+/-} mice. (A) Timeline and experimental groups for the social transfer of pain in Shank3^{+/-} mice. (B) Time course of vF mechanical thresholds before the social transfer of pain and 0, 4, 24, and 48 hours after the 1-hour interaction. Asterisks indicate significant differences from WT-BY control group. (C) Mechanical thresholds immediately after the social transfer of pain presented as the change from group baseline. (D) Timeline and experimental groups for the MDMA-assisted social transfer of pain in Shank3^{+/-} mice. (E) Time course of vF mechanical thresholds before the MDMA-assisted social transfer of pain and 0, 4, 24, and 48 hours after the 1-hour interaction. Asterisks indicate significant differences from Shank3-SAL-BY control group. (F and G) Mechanical thresholds 4 hours (F) and 48 hours (G) after the social transfer of pain, presented as the change from group baseline. (H) Timeline and experimental groups for the MDMA-assisted social transfer of analgesia in Shank3^{+/-} mice. (I and J) Mechanical thresholds immediately (I) and 24 hours after (J) the social transfer of analgesia. (K) Viral strategy and placement of bilateral fiber optic cannula in SERT-Cre x Shank3^{+/-} mice. (L) Timeline and experimental groups for the social transfer of pain with DR→NAc stimulation in Shank3^{+/-} mice. (M) Time course of vF mechanical thresholds before the social transfer of pain with DR→NAc stimulation and 0, 4, and 24 hours after the 1-hour interaction. Asterisks indicate significant differences from light off control group. (N) Mechanical thresholds immediately after the social transfer of pain with DR→NAc stimulation, presented as the change from group baseline. Statistical tests include one-way [(C), (F), (G), (I), (J), and (N)] and two-way [(B), (E), and (M)] ANOVA with Tukey post hoc tests. *P < 0.05, **P < 0.01, and ***P < 0.0001. All data presented as means ± SEM.

interaction with a WT analgesia DEM (WT-CFA-MOR-DEM; Fig. 4I), but not 24 hours later (Fig. 4J). Thus, MDMA rescues the social transfer of both pain and analgesia in Shank3^{+/-} mice.

In a final experiment, we tested whether MDMA's effects on the social transfer of pain in Shank3-deficient mice could be reproduced by optogenetically stimulating 5-HT signaling in the NAc. By injecting AAV-DJ-EF1-DIO-hChR2 [H134R]-eYFP into the DR of SERT-Cre-expressing Shank3^{+/-} mice, which were obtained by crossing Shank3^{+/-} and SERT-Cre mice, and implanting bilateral fiber optic cannulas over the NAc core, we were able to selectively stimulate 5-HT inputs in the NAc core of Shank3^{+/-} mice (Fig. 4K). Optogenetic activation of DR 5-HT→NAc inputs in Shank3-ChR2-BY mice during their 1-hour interaction with a WT-CFA-DEM led to substantially reduced mechanical thresholds relative to Shank3-ChR2-BY mice that did not receive laser stimulation (Fig. 4, M and N). Thus, increasing 5-HT signaling in NAc core is sufficient to enhance an empathy-associated behavior in Shank3^{+/-} mice.

DISCUSSION

The question of whether MDMA promotes empathy in human subjects has been a topic of vigorous debate ever since its emergence as a possible tool to enhance the efficacy of psychotherapy (2, 3). The assertion that it does function as a robust “empathogen” is largely based on anecdotal clinical reports, which conflict with several human subject studies that have commonly failed to reveal robust empathogenic effects. For example, MDMA appears to only mildly potentiate empathy (5, 6) in the multifaceted empathy test, in which subjects are asked to self-report levels of arousal after exposure to emotionally charged images (24). This topic will become increasingly important with the anticipated Food and Drug Administration approval of MDMA as an adjunct to psychotherapy in the treatment of PTSD due to two successful phase 3 trials (8, 25). Once approved, it is almost certain that there will be increased off-label use of MDMA and further clinical trials testing its therapeutic efficacy in other neuropsychiatric disorders. Thus, determining the neural mechanisms underlying its potential empathy-enhancing properties has important implications for its future therapeutic uses.

Here, we addressed this topic in mice by assessing the effects of MDMA in two behavioral measures that we assert are behavioral correlates or antecedents of empathy: the social transfer of pain and the social transfer of analgesia. We find that MDMA enhances both of these measures and present evidence that this effect of MDMA is likely due to the release of 5-HT in the NAc, a conclusion that is consistent with the mechanism of MDMA underlying its prosocial effect in the three chamber social preference assay (18). The modest empathogenic effects of MDMA in human subject studies (5, 6) may be due to the static nature of the assays used to measure empathy and the use of self-reported measurements. Elucidating MDMA's influence on empathy may require more dynamic and specific assays, such as the social transfer of pain and analgesia assays. In addition, empathy is an umbrella term comprising many related yet functionally distinct behaviors, ranging from relatively simple behaviors such as emotional contagion or mimicry (i.e., “state matching”) to more sophisticated complex behaviors that are largely restricted to humans such as perspective taking and moral reasoning (i.e., “understanding”) (26).

Although previous studies have demonstrated MDMA's ability to elevate social preference and interest in rodents (18, 21, 27–29), this

is the first to explore MDMA's role in promoting empathy-like behaviors. MDMA induced an enhancement of the acquisition of another animal's state following a brief 10-min social interaction. This was observed in the context of both positively and negatively valenced states (pain and analgesia), with effects lasting for up to 24 hours after the social encounter. These results suggest that MDMA enhances an animal's sensitivity to the affective and physical status of a conspecific, facilitating a more robust and durable adoption of their state after only a brief interaction. Notably, the social transfer of pain lasted up to 24 hours after the interaction, while the social transfer of analgesia had returned to baseline by this time point. We speculate that with the social transfer of pain, it might be evolutionarily advantageous to be aware of and sensitive to a conspecific's discomfort for a longer period, as it could represent a danger signal and promote hypervigilance. This phenomenon has been demonstrated in our previous study, showing that hypersensitivity persists in BY mice even after pain has been terminated in DEMs via analgesia (14). From this perspective, the social transfer of analgesia would be disadvantageous and perhaps fade more rapidly because there would be severe consequences to neglecting one's own pain or injury.

An unexpected observation was that systemic MDMA did not increase the amount of time that BY mice spent socially interacting with the CFA DEM during the social transfer of pain. This was true for both WT (fig. S1B) and Shank3 mice (fig. S4C). There are several possible explanations for these results. First, unlike previous studies that found that MDMA increased interaction time in the three-chamber assay, our studies used freely interacting partners, and most notably, one of the mice was experiencing inflammatory pain. This quality may occlude MDMA-induced social interest, especially as our findings seem to indicate that MDMA-treated bystanders are more sensitive and show heightened empathic responses to the distress of the social partner. Second, the social partner in our studies is a familiar cagemate, whereas previous studies showing MDMA's prosocial effects have used an unfamiliar juvenile mouse as the social stimulus (18). Last, previous studies showed that MDMA enhances social preference indexes in the three-chamber social preference test (18), whereas the interaction here was in a free-moving context. These important differences in the experimental design likely affect the observed social interaction times.

Another unexpected result was that the MDMA-dependent enhancement of social transfer of pain and analgesia occurred in male, but not female, mice. We have previously demonstrated that in the absence of MDMA, male and female mice both exhibit similar magnitudes of response in the social transfer of pain (12). This suggests that the lack of effect of MDMA on the social transfer of pain in females is not due to a baseline difference in this empathic capacity. Our fiber photometry experiments revealed that systemic MDMA evoked comparable levels of 5-HT signaling in the NAc of male and female mice, suggesting that the observed sex differences are not likely due to differential pharmacological action. This is consistent with human data showing that males and females display equivalent rises in plasma MDMA and oxytocin following MDMA administration (5). While we cannot provide any mechanistic explanation for this sex difference, other rodent studies also report sex differences in behavioral and physiological responses to MDMA (28, 29). Furthermore, human subjects studies report that MDMA increases implicit and explicit emotional empathy only in male participants (5), while female subjects report greater perceptual changes and thought disturbances and more adverse effects (30, 31).

MDMA infusion into the NAc core and optogenetic stimulation of DR 5-HT inputs in the NAc were sufficient to recapitulate the drug's effects on the social transfer of pain and analgesia, which strongly implicates a critical role for 5-HT release in the NAc in mediating MDMA's empathogenic effects. This conclusion is consistent with previous work demonstrating a critical role for NAc 5-HT release in mediating both prosocial behaviors and the enhancement of social preference by MDMA (20–22, 32, 33). In addition to role of the NAc in the social transfer of pain and analgesia in the absence of MDMA (12), NAc core neurons are activated when mice observe another mouse receiving foot shocks (31), and several human studies have implicated the NAc in trait empathic happiness (34) and motivating prosocial/helping behaviors (35, 36).

BY mice that received local manipulations restricted to the NAc core showed a more rapid onset and shorter overall duration than those that received systemic MDMA. We suspect that this difference is due to pharmacokinetics and the pharmacodynamic time courses of systemic versus intra-accumbens MDMA administration or optogenetic release of 5-HT in NAc. Systemically administered MDMA is subject to first-pass metabolism and will take longer to reach the brain and ultimately increase 5-HT release in NAc, resulting in delayed onset of the behavioral effect. In addition, MDMA is likely to remain psychoactive for longer when systemically administered. In contrast, both NAc infusion of MDMA and optogenetic stimulation of DR→NAc 5-HT inputs should produce nearly immediate and short-lived localized 5HT release, resulting in a more rapid onset and faster return to baseline. The fact that the time course of effects for the intra-NAc MDMA infusion and optogenetic stimulation of 5-HT inputs were nearly identical supports this supposition. However, it is possible that other factors may contribute including systemic MDMA's effects on other regions upstream or downstream of NAc 5HT inputs.

Our laboratory previously reported that individual NAc MSNs are contacted by multiple glutamatergic synaptic inputs from medial prefrontal cortex, ventral hippocampus, periventricular thalamus, and basolateral amygdala and that bath application of 5-HT diminished excitatory postsynaptic current amplitudes for all inputs except those from medial prefrontal cortex (37). We therefore suspect that 5-HT in NAc selectively alters synaptic transmission on certain inputs, but the exact mechanism through which 5-HT selectively modulates NAc inputs to enhance empathy is unclear. Resolving this will require a comprehensive set of experiments, which will be an important focus of future studies.

The effects of MDMA are markedly different than those of selective serotonin reuptake inhibitors (SSRIs), which, in contrast, have been reported to reduce affective empathy with long-term use (38). The most likely explanation is that while MDMA and SSRIs both act upon 5-HT signaling, they do so at very different magnitudes and time courses, resulting in distinct subjective effects. MDMA causes very large and rapid increases in 5-HT levels in the NAc (39) through molecular interactions with SERT that are independent of activity in 5-HT inputs. In contrast, SSRIs inhibit reuptake of 5-HT following action-potential-dependent release, resulting in a more gradual and less robust build-up of 5-HT, which is dependent upon endogenous signaling.

We also explored whether MDMA could restore deficits in empathy in heterozygous *Shank3*^{+/-} mice, a genetic manipulation that has construct validity for mimicking one form of ASD. In contrast to WT mice (15), *Shank3*^{+/-} animals failed to show the social transfer

of pain or analgesia even after a 1-hour interaction; deficiencies that were rescued by MDMA. Consistent with a critical role of 5-HT action in the NAc, optogenetic stimulation of DR 5-HT inputs in the NAc core rescued the social transfer of pain in *Shank3*^{+/-} mice. Although we are not aware of any studies measuring NAc 5-HT release in *Shank3*^{+/-} mice, the *Shank3* protein has been reported to interact with SERT (32), creating the possibility that *Shank3*-deficient mice could have altered SERT function, which in turn may influence the magnitude of 5-HT release. Further studies will be necessary to address whether *Shank3*^{+/-} mice show basal differences in NAc 5-HT signaling, which could underlie the observed empathy deficits.

Together, the results of the current study suggest that MDMA enhances empathy-like behaviors by stimulating 5-HT release in the NAc, a mechanism also capable of reversing empathy deficits in a mouse model of ASD. These findings are consistent with other recent studies to implicate 5-HT action in the NAc as a broad signaling mechanism for motivating and encoding prosocial behaviors. Future studies should explore the downstream mechanisms by which 5-HT release in the NAc drives prosocial and empathy-like behaviors with the goal of stimulating similar experiments in human subject studies. The results also provide motivation for further study of the specific NAc 5-HT receptors mediating the effects of MDMA with the assumption that these will be potential druggable targets for the treatment of empathy deficits in a range of neuropsychiatric disorders.

MATERIALS AND METHODS

Statistics

All statistical analyses were performed with GraphPad Prism software. Experiments with more than two groups were subject to one-way analysis of variance (ANOVA) or two-way ANOVA with Tukey's correction for multiple post hoc comparisons. For all experiments using repeated measures ANOVAs, groups were compared to their respective control at each time point. Experiments with two groups were analyzed using two-tailed unpaired *t* tests, unless the dataset failed Shapiro-Wilk tests for normality, in which case the data were subjected to Mann-Whitney *U* tests. All data are presented as means ± SEM. Data points identified as statistically significant outliers (determined by Grubb's test, *P* < 0.05) were removed from the analyses.

Animals

All experimental procedures were approved by the Stanford University Administrative Panel on Laboratory Animal Care in accordance with American Veterinary Medical Association Guidelines and the International Association for the Study of Pain. Male C57Bl6/J mice (strain 000664, the Jackson Laboratory) aged 8 to 16 weeks were used for all experiments, unless otherwise specified. Mice arrived at the laboratory at 7 weeks old and were allowed at least 1 week to habituate in their home cages before experiments. Mice were housed in groups of two or four per cage on a 12-hour light/12-hour dark cycle with food and water ad libitum, on a ventilated rack (Innovive Innorack 3.5). All behavioral experiments were performed during the same circadian period (7:00 a.m. to 6:00 p.m.).

For TRAP experiments, male TRAP mice (*Fos*^{2A-iCreER}) were generously donated by the Luo Laboratory at Stanford University and crossed with Ai14-TdTomato Cre-reporter mice (strain 007914m, the Jackson Laboratory) to visualize active neurons. All mice were 8 to 12 weeks old at the start of experiments.

To enable optogenetic manipulation of serotonergic neurons, we used male SERT-Cre mice (TG[Slc6a4-cre]ET33Gsat; GENSAT Project at Rockefeller University; MGI: 3836639) (33). All SERT-Cre mice were bred and genotyped in house.

Male *Shank3*-deficient mice (*Shank3*^{+/-}) carrying heterozygous deletion of exons 4 to 22 in the *Shank3* gene (B6.CG-*Shank3*^{tm2.1Bux}/J; strain 032169, the Jackson Laboratory) were obtained from the Jackson Laboratory and subsequently maintained in-house. To enable optogenetic manipulations of serotonergic neurons in *Shank3*^{+/-} mice, *Shank3*^{+/-} animals were crossed with SERT-Cre mice. All *Shank3*^{+/-} animals used were heterozygous for *Shank3* deletion and aged 8 to 16 weeks at the time of experiments. WT littermates were used as DEMs and BY controls.

Drug treatments/preparation

All drugs were dissolved or diluted in 0.9% SAL and administered at a volume of 0.01 ml/g. MDMA (7.5 mg/kg; Organix Inc., O-8571) and METH (2 mg/kg; Sigma-Aldrich M8750) were administered intraperitoneally. The dose of METH was selected on the basis of previous studies (18), which found that this dose (2 mg/kg) was sufficient to induce locomotor sensitization and conditioned place preference, but not an effect in the three-chamber social preference assay. MOR (10 mg/kg; Sigma-Aldrich, M8777, MOR sulfate salt pentahydrate) and meloxicam (10 mg/kg; VetOne, Ostilox, 5 mg/ml solution) were administered subcutaneously. For TRAP experiments, 4-OHT (Sigma-Aldrich, H6278) was prepared in a mixture of castor and sunflower oil (20:80 ratio) and injected intraperitoneally at a dose of 50 mg/kg.

Social transfer of pain

On the 2 days before the social interaction, all DEM and BY mice were habituated to the testing room (30 to 60 min their home cage) and von Frey rack (20 to 30 min). The day before the social interaction, all mice were pretested for baseline mechanical thresholds. All social interactions were performed in the morning between 7:00 a.m. and 11:00 a.m. The day of the experiment, mice were again habituated to the room for 30 min. One hour before the social interaction, all DEM mice were lightly restrained and injected with 10 μ l of CFA (Sigma-Aldrich, F5881), an inflammatory medium, into the left hind paw to induce long-lasting, local inflammatory pain (40, 41). DEM mice were then placed into a separate housing cage to avoid interaction with cage mates who would soon become pain BY mice. Fifteen minutes before the social interaction, all BY mice were injected intraperitoneally with SAL or MDMA (7.5 mg/kg) and placed individually into clean housing cages without food or water. Fifteen minutes later, one CFA DEM mouse—a familiar cage mate—was placed into the cage for a 10-min social interaction. Notably, SAL-BY and MDMA-BY groups were tested at different times or in different rooms to avoid any intergroup influence that could occur due to simultaneous, side-by-side social interactions. After the 10-min interaction, all mice were returned to their home cages, but BY and DEM mice were divided and subsequently housed only with treatment-matched cage mates (i.e., SAL-BY together and MDMA-BY together) to avoid the continuous social transfer of pain. SAL- and MDMA-BY mice were housed separately to avoid any intergroup influence. Thus, each cage of four mice at the beginning of the experiment was split into two cages of two mice (one cage contained two CFA-DEM mice, while the other contained two BY treated with either MDMA or SAL) (13). All mice

were subjected to mechanical threshold testing at various time points following the social transfer. CFA-DEM mice were tested separately from BY mice to avoid social transfer of pain during brief exposures on the rack.

For social transfer of pain experiments using METH, mice were injected with METH (2 mg/kg, i.p.) or SAL 15 min before a 10-min interaction with a CFA DEM.

For social transfer of pain experiments in *Shank3*^{+/-} mice, WT littermates were used as CFA-DEM mice, and heterozygous *Shank3*^{+/-} mice were used as BY mice. DEM mice were lightly restrained and injected with CFA in their left hindpaw before being immediately placed into a clean housing cage without food or water. *Shank3*^{+/-} BY mice were then injected intraperitoneally with either SAL or MDMA (7.5 mg/kg) and placed immediately into the cage for a 1-hour interaction with the CFA DEM. After 1 hour, all mice were immediately subjected to mechanical threshold testing.

Social transfer of analgesia

On the 2 days before the social interaction, mice were habituated to both the testing room (30 to 60 min in their home cage) and the von Frey rack (20 to 30 min). Mice were again habituated to the room for 30 min on the day of the interaction. One hour before the social interaction, all mice were lightly restrained and injected with 10 μ l of CFA into the left hindpaw. Fifteen minutes before the interaction, all analgesia DEM mice received MOR (10 mg/kg, s.c.) and were placed into a separate housing cage. BY mice received either SAL or MDMA (7.5 mg/kg, i.p.) injection and were placed individually into clean housing cages without food or water. Fifteen minutes later, one MOR-DEM mouse was placed into this cage for a 10-min social interaction with the BY mouse. Control CFA bystanders (CFA-CON-BY) received SAL injection and interacted with a SAL-injected (rather than MOR-injected) CFA-DEM social partner. After the 10-min interaction, all mice were returned to their respective home cages, but MOR-injected DEM mice were housed separately to avoid the continuous social transfer of analgesia. This resulted in each cage of four being separated into two cages of two (one containing both BY mice and the other containing both DEM mice). BY mice were subjected to mechanical threshold testing at various time points following the social interaction.

For all social transfer of analgesia experiments in *Shank3*^{+/-} mice, WT littermates were used as analgesia DEMs and heterozygous *Shank3*^{+/-} mice were used as bystanders. WT-CFA-MOR-DEM mice received CFA in their left hindpaw and subcutaneous MOR before being immediately placed into a clean housing cage without food or water. *Shank3*^{+/-} BY mice received CFA in their left hindpaw and either SAL or MDMA (7.5 mg/kg, i.p.) before being immediately placed into the cage for a 1-hour interaction with the DEM mouse. Control mice (*Shank3*-CFA-CON-BY) were paired with a SAL-injected (rather than MOR-injected) WT-CFA-DEM mouse to control for the demonstration of analgesia. After the interaction, all BY mice were immediately subjected to mechanical threshold testing.

Social interaction time

Social interaction times between DEM and BY mice during the social transfer of pain were manually scored by experimentally blind researchers. Interaction time was defined as any period during which the BY mouse was actively sniffing any region of the DEM (including the snout, body, anogenital area, and tail) or grooming.

For quantification of interaction times during the social transfer of pain in Shank3-deficient mice, only the 10-min period used for the social transfer of pain in wild-type mice was scored. Thus, interaction times were quantified from 15 to 25 min of the social interaction.

von Frey mechanical sensitivity testing

Mice were habituated to homemade plexiglass enclosures on top of a homemade wire testing rack for 20 min 2 days before the start of the experiment. On the day of testing, mice were habituated to the apparatus for 10 to 20 min before mechanical sensitivity testing. The plantar surface of the left hind paw was mechanically stimulated using von Frey hairs (0.01 to 2 g of plastic fibers, North Coast). Paw withdrawal, shaking, and licking were all considered responses. The up-down technique was used to calculate mechanical threshold values (42). This method uses stimulus oscillation around the response threshold to determine the median 50% threshold of response. The testing rack was placed in the same behavioral room, in which the social transfer occurred and was illuminated with a dim lamp. For social transfer of pain experiments involving a 10-min social interaction, mechanical sensitivity testing occurred at 1, 6, 24, and 48 hours after social interaction. For experiments using a 1-hour social interaction, testing occurred at 0, 4, 24, and 48 hours after the social interaction.

Targeted recombination in active populations

For TRAP experiments, Fos^{2A-iCreER};TRAP2 mice were crossed with Ai14 reporter mice to enable tdTomato expression in active neuronal populations. On the 2 days before the social experience, TRAP2/Ai14 mice received intraperitoneal SAL injections and were placed in the behavioral room for at least 1 hour to habituate to the environment and injection. On the day of the experiment, TRAP2/Ai14 mice were habituated to the testing room for 20 min in their home cage before being injected with 4-OHT and either SAL or MDMA (7.5 mg/kg, i.p.) and then placed into a clean cage with a CFA-injected cage mate for a total of 4 hours. All TRAP2/Ai14 mice used for experiments were heterozygous. The 4-OHT was prepared fresh on the morning of the experiment. One week after the social interaction, all TRAP2;Ai14 BY mice were perfused and brains were sliced and imaged to quantify regional counts of tdTomato-expressing neurons.

Three-chamber social preference testing

The three-chamber apparatus (length, 72 cm; width, 23 cm; height, 25 cm) was built in-house using 0.635-cm-thick sheets of clear extruded acrylic for walls, white Komatex for floors, and black barrier walls (TAP Plastics). The apparatus consisted of two outer chambers (28 cm by 23 cm) connected by a center chamber (16 cm by 23 cm). Testing was performed as previously described with modification (43). The test mouse was first placed into the three-chamber apparatus for a 10-min habituation period, during which two empty inverted pencil cups were placed in the center of both outer chambers. On the following day, the mouse was reintroduced to the apparatus for a 10-min trial, in which one cup contained a novel object (nonsocial stimulus), while the other contained an age- and sex-matched WT mouse (social stimulus). Between experimental sessions, all cups, objects, and the three-chamber apparatus were wiped down with 70% ethanol. The cups used to hold mice were regularly rotated to minimize the effect of residual scents. Video was acquired by a computer-controlled ceiling-mounted digital camera and analyzed offline using Biobserve tracking software (Biobserve

GmbH). The amount of time spent in the “social” and “nonsocial” outer chambers was automatically scored by Biobserv. The preference index was calculated as (social chamber time – nonsocial chamber time)/(social chamber time + nonsocial chamber time).

Intracerebral drug infusions

Double-lumen, 26-gauge threaded cannula guides were custom-ordered (P1 Technologies) to enable bilaterally infusions into the NAc lateral core. Cannula guide dimensions were as follows: 2.4-mm separation, 8-mm pedestal, cut 4 mm below pedestal. The cannula guide was lowered into the craniotomy sites to a position 1 mm above the target structure and secured to the skull using a skull screw (Antrin Miniature Specialties; 00-90 x 1/16) followed by application of C&B Metabond (Parkell S371, S398, and S396) and light-cured dental adhesive cement (Geristore A&B paste, DenMat). For infusions into the NAc lateral core, the following coordinate was used: 0.98 anterior-posterior (AP), 1.2 medial-lateral (ML), and –3.0 dorsal-ventral (DV) (note that the drug infusion cannula extended 1 mm below the guide, targeting a –4.0 DV coordinate). After securing the cannula guide, a bilateral dummy cannula was inserted into the infusion ports and the device was sealed with an aluminum cap. Drug infusion experiments were performed 2 to 4 weeks after surgery.

For drug infusions, the dummy cannula was replaced with a bilateral infusion cannula measuring 1 mm longer than the corresponding cannula guide. Infusions were delivered by inserting drug-primed bilateral infusers into the implanted cannula guide. Infusers were connected via PE-50 tubing to a 23-gauge needle of a syringe (Hamilton, model 7641 RN and 7786-01) mounted in a dual syringe pump (Harvard Apparatus, model 55-2222). MDMA was dissolved in 0.9% SAL to achieve a concentration of 0.5 µg in 500 nl of infusate, delivered bilaterally at a rate of 350 nl/min. After a 90-s infusion, the infuser was held in place for an additional 30 s, then the dummy cannula was reinserted into the cannula guide and the implant was resealed with an aluminum cap.

Stereotactic surgeries

All surgeries were performed under aseptic conditions using a small animal digital stereotaxic instrument (David Kopf Instruments). Mice were anesthetized with isoflurane (5% induction and 1% maintenance). A small incision was made in the scalp, and burr holes were drilled in the skull at the appropriate stereotaxic coordinates using a 0.5-mm drill bit (Fine Science Tools, 19007-05). Mice recovered from anesthesia individually on a heating pad before being returned to group housing. Meloxicam (5 mg/kg, s.c.) was given for postoperative analgesia.

Viral reagents and injections

All viral reagents were purchased from the Stanford Neuroscience Gene Vector and Virus Core. For optogenetic experiments in SERT-Cre mice, AAV-DJ-EF1-DIO-eYFP and AAV-DJ-EF1-DIO-hChR2(H134R)-eYFP were used. Fiber photometry experiments in WT mice used AAV9-hSyn-GRAB-5HT3.6(5HT3) (WZ Biosciences, Columbia, MD).

For experiments involving optogenetic manipulations, viruses (1 µl) were infused into the DR nucleus (–4.35 AP, 0.0 ML, and –2.95 DV). For fiber photometry experiments, virus was injected unilaterally into the NAc (+1.0 AP, +0.8 ML, and –3.7 DV from brain surface). All viruses were infused at a rate of 0.150 µl/min using a glass micropipette connected to a Hamilton syringe with tubing backfilled

with mineral oil. The injector tip was lowered an additional 100 μm below the planned injection site and then raised to the final coordinate before infusion to facilitate virus diffusion at the site of injection. After infusion, the pipette was raised 100 μm for 5 min to allow for diffusion before being removed slowly. For all optogenetics experiments, mice were allowed 6 weeks between the virus injection and behavioral testing to allow for viral expression at synaptic terminals.

Optogenetic manipulations

Optogenetic fibers targeting the NAc lateral core were custom-ordered (Doric Lenses, two Ferrule cannula, 2.5-mm pitch, 4.5-mm fiber optic length, 200- μm fiber core diameter, FLT fiber tip). Cannulas were lowered until just above the NAc lateral core (0.98 AP, 1.2 ML, and -3.75 DV) and secured to the skull using a skull screw (Antrin Miniature Specialties; 00-90 x 1/16) with sequential application of C&B Metabond (Parkell S371, S398, and S396) and light-cured dental adhesive cement (Geristore A&B paste, DenMat).

For optogenetic stimulation of ChR2, ferrules were connected to a 473-nm laser diode (OEM Laser Systems) through a FC/PC adaptor and a fiber optic rotary joint (Doric Lenses). Laser output was controlled using a Pulse Pal (Sanworks) to deliver 5-ms light pulses at 20 Hz. Light output was adjusted to 12 to 15 mW to stimulate synaptic terminals using a digital power meter console (Thorlabs). Mice were acclimated to optogenetic tethers during the acclimation-habituation periods before the test day. For social transfer of pain and analgesia experiments involving optogenetic stimulation, light stimulation was delivered for the full duration of the 10-min interaction period. Light was not delivered during von Frey mechanical sensitivity testing.

For optogenetic experiments involving Shank3^{+/-} \times SERT-Cre mice, all procedures were performed as described above, but with an extended social interaction (1 hour rather than 10 min). Laser photostimulation (20 Hz, 5-ms pulses) was applied throughout the full 1-hour interaction.

Fiber photometry

Optical fibers (Doric Lenses, 5.0 mm in length, 400- μm core, 0.66 numerical aperture, FLT) were unilaterally implanted over the NAc (+1.0 AP, +0.8 ML, and -3.7 DV from brain surface). Optical fibers were secured to the skull with stainless steel screws (thread size 00-90 x 1/16, Antrin Miniature Specialties), C&B Metabond, and light-cured dental adhesive cement (Geristore A&B paste, DenMat). Mice were group housed to recover for at least 3 weeks before recordings began.

Fiber photometry was performed as previously described (18, 22, 39). AAV9-hSyn-GRAB 5-HT was injected into the NAc with a fiber directed above. After at least 3 weeks, mice were habituated to the photometry setup. On the test day, mice were connected to patch cables and allowed to habituate alone in the homecage for a 10-min baseline recording. Mice were then injected with MDMA (7.5 mg/kg, i.p.), and recordings continued for another 40 min.

Data were acquired using Synapse software controlling an RZ5P lock-in amplifier (Tucker-Davis Technologies). GRAB 5-HT was excited by frequency-modulated 465- and 405-nm light-emitting diodes (Doric Lenses). Optical signals were bandpass-filtered with a fluorescence mini cube (Doric Lenses), and signals were digitized at 6 kHz. Signal processing was performed with custom scripts in MATLAB (MathWorks). Briefly, signals were debleached by fitting with a mono-exponential decay function, and the resulting fluorescence traces were smoothed and z-scored. Peri-event time histograms were constructed by taking the average of the 50-min recording

consisting of the 10 min before and 40 min after MDMA administration, which was defined as time = 0. Area under the curve was defined as the integral between 0 and 40 min. Photometry data were processed and analyzed in MATLAB with custom scripts. Area under the curve was calculated, and photometry data were graphed with GraphPad Prism 9.

Real-time place preference

The real-time place preference test was performed in a rectangular Plexiglas apparatus (length, 72 cm; width, 23 cm; height, 25 cm) with three chambers. The outer chambers had different wall patterns and flooring (walls: black and white stripes versus black and white squares; flooring: smooth versus rough plastic), while the center chamber was plain (no wall pattern, smooth floor). The subject mouse was placed in the center compartment for 2 min before it was allowed to explore the entire apparatus for 15 min. During this time, the mouse received photostimulation (20 Hz, 5-ms pulses) whenever it was in a designated chamber. The chamber used was alternated between each testing session. Immediately after the initial test, a reversal test was conducted and the side paired with stimulation was switched. There was no interruption between the two phases of the experiment. The average time spent in each chamber during the initial and reversal sessions was used for analysis. Video tracking software (Biobserve) was used to record animal movement and automatically quantify chamber time. An eight-channel TTL output box (Biobserve GmbH, TTL-8-091120-1) was used to automate light delivery in the photostimulation-associated zone.

General histological procedures and imaging

Mice were anesthetized with isoflurane and transcardially perfused with 1 \times phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS. Brains were removed and postfixed for 24 hours in 4% paraformaldehyde in PBS. Brains were sectioned at 75 μm on a vibratome and collected in PBS. For localization of viral injections, sections were washed three times for 10 min in PBS and then incubated in PBS with 0.5% Triton X-100, 10% normal goat serum, and 0.2% bovine serum albumin (BSA) for 1 hour. After a 5-min wash in PBS, the tissue was then incubated overnight in chicken anti-green fluorescent protein (GFP) (1:1000; Aves Labs, GFP-1010) in carrier solution containing 0.5% Triton X-100, 1% normal goat serum, and 0.2% BSA in PBS with agitation. After four 10-min washes in PBS, sections were incubated in secondary antibody (1:750; goat anti-chicken Alexa Fluor 488, Invitrogen, A-11039) for 2 hours at room temperature. Sections were then washed four times in PBS, mounted onto SuperFrost Plus glass slides, and coverslipped with VECTASHIELD HardSet Mounting Media with 4',6-diamidino-2-phenylindole. Imaging was performed on a Keyence BZ-X800 fluorescence microscope.

Supplementary Materials

This PDF file includes:

Figs. S1 to S4

REFERENCES AND NOTES

1. R. de la Torre, M. Farré, P. N. Roset, N. Pizarro, S. Abanades, M. Segura, J. Segura, J. Camí, Human pharmacology of MDMA: Pharmacokinetics, metabolism, and disposition. *Ther. Drug Monit.* **26**, 137–144 (2004).
2. S. J. Peroutka, H. Newman, H. Harris, Subjective effects of 3,4-methylenedioxyamphetamine in recreational users. *Neuropsychopharmacology* **1**, 273–277 (1988).

3. L. Hermle, M. Spitzer, D. Borchardt, K. A. Kovar, E. Gouzoulis, Psychological effects of MDE in normal subjects. Are entactogens a new class of psychoactive agents? *Neuropsychopharmacology* **8**, 171–176 (1993).
4. A. Borissova, B. Ferguson, M. B. Wall, C. J. Morgan, R. L. Carhart-Harris, M. Bolstridge, M. A. Bloomfield, T. M. Williams, A. Feilding, K. Murphy, R. J. Tyacke, D. Erritzoe, L. Stewart, K. Wolff, D. Nutt, H. V. Curran, W. Lawn, Acute effects of MDMA on trust, cooperative behaviour and empathy: A double-blind, placebo-controlled experiment. *J. Psychopharmacol.* **35**, 547–555 (2021).
5. C. M. Hysek, Y. Schmid, L. D. Simmler, G. Domes, M. Heinrichs, C. Eisenegger, K. H. Preller, B. B. Quednow, M. E. Liechti, MDMA enhances emotional empathy and prosocial behaviour. *Soc. Cogn. Affect. Neurosci.* **9**, 1645–1652 (2014).
6. Y. Schmid, C. M. Hysek, L. D. Simmler, M. J. Crockett, B. B. Quednow, M. E. Liechti, Differential effects of MDMA and methylphenidate on social cognition. *J. Psychopharmacol.* **28**, 847–856 (2014).
7. K. P. C. Kuypers, R. de la Torre, M. Farre, S. Yubero-Lahoz, I. Dziobek, W. Van den Bos, J. G. Ramaekers, No evidence that MDMA-induced enhancement of emotional empathy is related to peripheral oxytocin levels or 5-HT1a receptor activation. *PLOS ONE* **9**, e100719 (2014).
8. J. M. Mitchell, M. Bogenschutz, A. Lilenstein, C. Harrison, S. Kleiman, K. Parker-Guilbert, M. Ot'Alora G., W. Garas, C. Paleo, I. Gorman, C. Nicholas, M. Mithoefer, S. Carlin, B. Poulter, A. Mithoefer, S. Quevedo, G. Wells, S. K. Klaire, B. Van Der Kolk, K. Zarfaty, R. Amiaz, R. Worthy, S. Shannon, J. D. Woolley, C. Marta, Y. Gelfand, E. Hapke, S. Amar, Y. Wallach, R. Brown, S. Hamilton, J. B. Wang, A. Coker, R. Matthews, A. De Boer, B. Yazar-Klosinski, A. Emerson, R. Doblin, MDMA-assisted therapy for severe PTSD: A randomized, double-blind, placebo-controlled phase 3 study. *Nat. Med.* **27**, 1025–1033 (2021).
9. K. W. Smith, D. J. Sicignano, A. V. Hernandez, C. M. White, MDMA-assisted psychotherapy for treatment of posttraumatic stress disorder: A systematic review with meta-analysis. *J. Clin. Pharmacol.* **62**, 463–471 (2022).
10. J. B. Luoma, B. Shahar, M. Kati Lear, B. Pilecki, A. Wagner, Potential processes of change in MDMA-Assisted therapy for social anxiety disorder: Enhanced memory reconsolidation, self-transcendence, and therapeutic relationships. *Hum. Psychopharmacol.* **37**, e2824 (2022).
11. J. C. Watson, "The role of empathy in psychotherapy: Theory, research, and practice." in *Humanistic Psychotherapies: Handbook of Research and Practice (2nd Ed.)*, D. J. Cain, K. Keenan, S. Rubin, Eds. (American Psychological Association, 2016), pp. 115–145; <http://content.apa.org/books/14775-005>.
12. M. L. Smith, N. Asada, R. C. Malenka, Anterior cingulate inputs to nucleus accumbens control the social transfer of pain and analgesia. *Science* **371**, 153–159 (2021).
13. B. Rein, E. Jones, S. Tuy, C. Boustani, J. A. Johnson, R. C. Malenka, M. L. Smith, Protocols for the social transfer of pain and analgesia in mice. *STAR Protoc.* **3**, 101756 (2022).
14. M. L. Smith, C. M. Hostetler, M. M. Heinricher, A. E. Ryabinin, Social transfer of pain in mice. *Sci. Adv.* **2**, 1600855 (2016).
15. I. E. Harmsen, Empathy in autism spectrum disorder. *J. Autism Dev. Disord.* **49**, 3939–3955 (2019).
16. H. Kaur, S. Karabulut, J. W. Gauld, S. A. Fagot, K. N. Holloway, H. E. Shaw, W. E. Fantegrossi, Balancing therapeutic efficacy and safety of MDMA and novel MDXX analogues as novel treatments for autism spectrum disorder. *Psychodetic Med.* **1**, 166–185 (2023).
17. M. Yang, J. L. Silverman, J. N. Crawley, Automated three-chambered social approach task for mice. *Curr. Protoc. Neurosci.* **Chapter 8**, Unit 8.26 (2011).
18. B. D. Heifets, J. S. Salgado, M. D. Taylor, P. Hoerbel, D. F. Cardozo Pinto, E. E. Steinberg, J. J. Walsh, J. Y. Sze, R. C. Malenka, Distinct neural mechanisms for the prosocial and rewarding properties of MDMA. *Sci. Transl. Med.* **11**, eaaw6435 (2019).
19. W. E. Allen, L. A. DeNardo, M. Z. Chen, C. D. Liu, K. M. Loh, L. E. Feno, C. Ramakrishnan, K. Deisseroth, L. Luo, Thirst-associated preoptic neurons encode an aversive motivational drive. *Science* **357**, 1149–1155 (2017).
20. A. R. Green, A. O. Mechan, J. M. Elliott, E. O'Shea, M. I. Colado, The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharmacol. Rev.* **55**, 463–508 (2003).
21. J. J. Walsh, P. Llorach, D. F. Cardozo Pinto, W. Wenderski, D. J. Christoffel, J. S. Salgado, B. D. Heifets, G. R. Crabtree, R. C. Malenka, Systemic enhancement of serotonin signaling reverses social deficits in multiple mouse models for ASD. *Neuropsychopharmacology* **46**, 2000–2010 (2021).
22. J. J. Walsh, D. J. Christoffel, B. D. Heifets, G. A. Ben-Dor, A. Selimbeyoglu, L. W. Hung, K. Deisseroth, R. C. Malenka, 5-HT release in nucleus accumbens rescues social deficits in mouse autism model. *Nature* **560**, 589–594 (2018).
23. X. Wang, A. L. Bey, B. M. Katz, A. Badea, N. Kim, L. K. David, L. J. Duffney, S. Kumar, S. D. Mague, S. W. Hulbert, N. Dutta, V. Hayrapetyan, C. Yu, E. Gaidis, S. Zhao, J.-D. Ding, Q. Xu, L. Chung, R. M. Rodriguez, F. Wang, R. J. Weinberg, W. C. Wetsel, K. Dzirasa, H. Yin, Y. Jiang, Altered mGluR5-Homer scaffolds and corticostriatal connectivity in a Shank3 complete knockout model of autism. *Nat. Commun.* **7**, 11459 (2016).
24. I. Dziobek, K. Rogers, S. Fleck, M. Bahnemann, H. R. Heekeren, O. T. Wolf, A. Convit, Multifaceted empathy test. *APA PsychNet* **10** 10377/t54435-000, (2008).
25. J. M. Mitchell, M. Ot'Alora G., B. van der Kolk, S. Shannon, M. Bogenschutz, Y. Gelfand, C. Paleo, C. R. Nicholas, S. Quevedo, B. Balliett, S. Hamilton, M. Mithoefer, S. Kleiman, K. Parker-Guilbert, K. Zarfaty, C. Harrison, A. de Boer, R. Doblin, B. Yazar-Klosinski; MAPP2 Study Collaborator Group, MDMA-assisted therapy for moderate to severe PTSD: A randomized, placebo-controlled phase 3 trial. *Nat. Med.* **29**, 2473–2480 (2023).
26. F. B. M. De Waal, S. D. Preston, Mammalian empathy: Behavioural manifestations and neural basis. *Nat. Rev. Neurosci.* **18**, 498–509 (2017).
27. H. Esaki, Y. Sasaki, N. Nishitani, H. Kamada, S. Mukai, Y. Ohshima, S. Nakada, X. Ni, S. Deyama, K. Kaneda, Role of 5-HT1A receptors in the basolateral amygdala on 3,4-methylenedioxymethamphetamine-induced prosocial effects in mice. *Eur. J. Pharmacol.* **946**, 175653 (2023).
28. M. R. Thompson, P. D. Callaghan, G. E. Hunt, J. L. Cornish, I. S. McGregor, A role for oxytocin and 5-HT1A receptors in the prosocial effects of 3,4-methylenedioxymethamphetamine ("ecstasy"). *Neuroscience* **146**, 509–514 (2007).
29. S. Mukai, S. Nakada, H. Kamada, R. Yaguchi, S. Deyama, K. Kaneda, Differential sensitivity to detect prosocial effects of 3,4-methylenedioxymethamphetamine (MDMA) in different social approach paradigms in mice. *Neuropsychopharmacol. Rep.* **40**, 297–301 (2020).
30. M. E. Liechti, A. Gamma, F. X. Vollenweider, Gender differences in the subjective effects of MDMA. *Psychopharmacology* **154**, 161–168 (2001).
31. O. Dincok, J. E. Zachry, M. G. Kutlu, Nucleus accumbens core single cell ensembles bidirectionally respond to experienced versus observed aversive events. *bioRxiv* 2023.07.17.549364 [Preprint]. 19 July 2023. <https://doi.org/10.1101/2023.07.17.549364>.
32. M. A. Quinlan, M. J. Robson, R. Ye, K. L. Rose, K. L. Schey, R. D. Blakely, Ex vivo quantitative proteomic analysis of serotonin transporter interactome: Network impact of the SERT Ala56 coding variant. *Front. Mol. Neurosci.* **13**, 89 (2020).
33. S. Gong, M. Doughty, C. R. Harbaugh, A. Cummins, M. E. Hatten, N. Heintz, C. R. Gerfen, Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J. Neurosci.* **27**, 9817–9823 (2007).
34. G. Mirabito, Z. Taiwo, M. Bezdek, S. N. Light, Fronto-striatal activity predicts anhedonia and positive empathy subtypes. *Brain Imaging Behav.* **13**, 1554–1565 (2019).
35. S. M. Shdo, K. G. Ranasinghe, K. A. Gola, C. J. Mielke, P. V. Sukhanov, B. L. Miller, K. P. Rankin, Deconstructing empathy: Neuroanatomical dissociations between affect sharing and prosocial motivation using a patient lesion model. *Neuropsychologia* **116**, 126–135 (2018).
36. L. T. Rameson, S. A. Morelli, M. D. Lieberman, The neural correlates of empathy: Experience, automaticity, and prosocial behavior. *J. Cogn. Neurosci.* **24**, 235–245 (2012).
37. D. J. Christoffel, J. J. Walsh, P. Hoerbel, B. D. Heifets, P. Llorach, R. C. Lopez, C. Ramakrishnan, K. Deisseroth, R. C. Malenka, Selective filtering of excitatory inputs to nucleus accumbens by dopamine and serotonin. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2106648118 (2021).
38. M. Rütgen, C. Pletti, M. Tik, C. Kraus, D. M. Pfabigan, R. Sladky, M. Klöbl, M. Woletz, T. Vanicek, C. Windischberger, R. Lanzenberger, C. Lamm, Antidepressant treatment, not depression, leads to reductions in behavioral and neural responses to pain empathy. *Transl. Psychiatry* **9**, 164 (2019).
39. M. B. Pomrenze, D. F. Cardozo Pinto, P. A. Neumann, P. Llorach, J. M. Tucciarone, W. Morishita, N. Eshel, B. D. Heifets, R. C. Malenka, Modulation of 5-HT release by dynorphin mediates social deficits during opioid withdrawal. *Neuron* **110**, 4125–4143.e6 (2022).
40. A. A. Larson, D. R. Brown, S. El-Atrash, M. M. Walser, Pain threshold changes in adjuvant-induced inflammation: A possible model of chronic pain in the mouse. *Pharmacol. Biochem. Behav.* **24**, 49–53 (1986).
41. K. Ren, R. Dubner, Inflammatory Models of Pain and Hyperalgesia. *ILAR J.* **40**, 111–118 (1999).
42. S. R. Chaplan, F. W. Bach, J. W. Pogrel, J. M. Chung, T. L. Yaksh, Quantitative assessment of tactile allodynia in the rat paw. *J. Neurosci. Methods* **53**, 55–63 (1994).
43. B. Rein, K. Ma, Z. Yan, A standardized social preference protocol for measuring social deficits in mouse models of autism. *Nat. Protoc.* **15**, 3464–3477 (2020).

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