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α_2-Adrenergic receptor modulates 5-HT_{2A}-mediated behavioral effects of MDMA and psilocybin in mice

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Classic serotonergic psychedelics such as psilocybin act as agonists at cortical serotonin (5-HT) 2A receptors (5-HT_{2A}R), inducing psychedelic effects in humans and head-twitch responses (HTRs) in rodents. Another class of psychedelic drugs called entactogens, exemplified by MDMA, function primarily as monoamine releasers and typically evoke minimal HTR despite causing serotonin release. The polypharmacology of psychedelic drugs at receptors other than 5-HT_{2A}R may modulate their behavioral effects. Here, we report that MDMA, but not psilocybin, induces robust elevations of both 5-HT and norepinephrine (NE) in the medial prefrontal cortex. Blocking the release of extracellular NE unmasks MDMA-evoked HTR, suggesting that polypharmacology involving noradrenergic receptors may oppose the 5-HT_{2A}–mediated effects of MDMA. Artificially elevating NE also attenuates psilocybin-induced HTR, supporting this hypothesis. Selective agonism of the noradrenergic α_2 receptor (α_2R) is sufficient to suppress 5-HT_{2A}-mediated HTR, and also suppresses the HTR in locus coeruleus-ablated mice, suggesting that this effect is mediated by heteroreceptors. Moreover, psilocybin-induced effects in the forced swim test persisted in the presence of α_2R activation. Thus, these findings support a model in which some forms of 5-HT_{2A} signaling can be attenuated by α_2R activation without interfering with antidepressant-like effects. The ability to reduce potential side effects of 5-HT_{2A} activation while preserving antidepressant-like effects via α_2R and other analogous receptors may be relevant to therapeutic development.

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INTRODUCTION

Psychedelics have profound subjective effects and have shown substantial promise in clinical trials for psychiatric disorders. Mechanisms underlying such therapeutic effects with psychedelic drugs have remained unclear. Classic serotonergic psychedelics like psilocybin are compounds whose primary pharmacological action is agonism at the cortical serotonin 2A-type receptor (5-HT_{2A}R), eliciting characteristic hallucinogenic effects [1–3]. In rodents, 5-HT_{2A}R agonism robustly evokes the head-twitch response (HTR), characterized by a rapid side-to-side rotational head movement. The HTR is a well-established proxy for 5-HT_{2A}R activation in rodent models [4]. Recent studies demonstrate that psilocybin produces robust antidepressant- and anxiolytic-like effects in rodent models of stress, reducing stress-induced immobility and sucrose preference deficits in chronic unpredictable mild stress [5, 6], chronic despair [7], and chronic pain paradigms [8].

In contrast, another subgroup of psychedelic drugs called entactogens [9] (meaning "going within to bring out"), like MDMA, belong to a subclass of monoamine-releasing phenethylamines known to elicit introspection, emotional openness, and prosocial behavior [9–11]. Entactogens primarily function by elevating extracellular serotonin (5-HT), norepinephrine (NE), and dopamine (DA) levels via monoamine transporter (5-HT transporter (SERT); NE transporter (NET); DA transporter (DAT)) reuptake inhibition and reverse efflux [12–14], and through interactions with the vesicular monoamine transporter 2 (VMAT2) to redistribute vesicular stores to increase extracellular monoamines [15–17]. In contrast to serotonergic psychedelics, entactogens elicit minimal direct 5-HT_{2A}R activation [18, 19]. Though prior research has shown HTR counts increase after pharmacological induction of 5-HT release via indirect activation of the 5-HT_{2A}R [20], entactogens induce low HTR [21–23], and have fewer dissociative and hallucinogenic effects [9, 24]. In rodents, administration of MDMA prior to extinction training markedly facilitates long-term reduction of conditioned fear responses [25, 26]. These differences in pharmacological and behavioral effects reflect a fundamentally different balance of receptor engagement and neuromodulatory

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regulation of entactogens compared to serotonergic psychedelics. Therefore, the mechanisms by which entactogens differ from serotonergic psychedelics remains unclear.

Psychedelics directly bind to many other 5-HT, DA, and NE receptors [27]. This polypharmacology of psychedelics at receptors other than 5-HT_{2A}R may modulate their behavioral effects. Prior work demonstrates that entactogens act as potent inhibitors of NE reuptake and facilitators of NE release [9, 10, 19], whereas serotonergic psychedelics do not alter NE levels [28]. Activation of the α_2-adrenergic receptor (α_2R) also exerts potent presynaptic inhibitory control over NE release and increases postsynaptic responsiveness in pyramidal neurons in the prefrontal cortex (PFC) to enhance working memory [29–31]. Other monoamine transporter inhibitors (e.g., cocaine) demonstrate that α_2Rs activity attenuates 5-HT_{2A}R-mediated HTR [32]. This suggests a modulatory axis by which noradrenergic activity might regulate serotonergic output. Furthermore, blockade or activation of α_2Rs can differentially affect the behavioral and therapeutic effects of serotonergic compounds [33–35], suggesting that α_2R signaling may distinguish the mechanisms underlying entactogens and serotonergic psychedelics. This raises the question: Is co-released NE acting to suppress 5-HT_{2A}R–mediated behavioral outcomes? Our central hypothesis is that postsynaptic α_2R activation suppresses the 5-HT_{2A}R-mediated behavioral effects of serotonergic psychedelics and entactogens.

Here, we show that the entactogens MDMA, methylone, and MBDB produce minimal 5-HT_{2A}R-mediated HTR compared to classical psychedelics, indicating that entactogens elicit far fewer 5-HT_{2A}R-driven behaviors. We further demonstrate that MDMA, but not psilocybin, strongly elevates both 5-HT and NE in the medial PFC (mPFC), revealing divergent monoaminergic signatures between these drug classes. Importantly, we find that extracellular NE actively suppresses HTRs, pharmacological attenuation of NE release unmasks MDMA-induced HTRs, whereas increasing NE levels reduces psilocybin-elicited HTRs. Postsynaptic α_2R activation is sufficient to dampen 5-HT_{2A}R-mediated HTR and is independent of noradrenergic input from the locus coeruleus (LC). Lastly, psilocybin reduces the immobility duration in the forced swim test independent of α_2R activation. Together, these data reveal a noradrenergic gating mechanism via α_2R that constrains 5-HT_{2A}R signaling and helps explain the mechanistic and behavioral dissociation between entactogens and serotonergic psychedelics, ultimately contributing to our understanding of processes underlying therapeutic effects.

METHODS

Animals

Strains used include C57BL6/J and DBH-cre mice purchased from Jackson laboratories at 8 weeks of age. The DBH-cre strain was initially obtained from The Jackson Laboratory and subsequently bred in-house. Animals were group housed, kept on a 12 h/12 h light-dark cycle, and provided ad libitum chow and water. Both male and female mice were used for all experiments. All experiments were performed during the light cycle (7:00–19:00) to maintain standardized and reproducible experimental conditions.

Drug preparation and administration

Drugs were administered intraperitoneally (i.p.; 0.01 mL/kg) using insulin syringes. MDMA (34480), methylone (10986), MBDB (14218), psilocybin (14041), 2C-B (11734), reboxetine (15038), atipamezole (34289), and guanfacine (22907) (Cayman Chemical, Ann Arbor, MI) were dissolved in saline. For HTR dose–response experiments, drugs were given immediately before testing. For photometry, MDMA (10 mg/kg), methylone (10 mg/kg), MBDB (10 mg/kg), and psilocybin (1 mg/kg) were injected after a 5 min baseline, and recordings continued for 15 min post-injection based on published monoamine release and HTR time courses [4, 36, 37]. A crossover design was used for 5-HT photometry (N = 3 saline-MDMA-psilocybin; N = 3 saline-psilocybin-MDMA; N = 2 saline-MDMA) and NE photometry (N = 4 saline-MDMA-psilocybin; N = 4 saline-psilocybin-MDMA). Reboxetine was administered 1 min before MDMA or 30 min before psilocybin [38, 39]. Atipamezole (2 mg/kg) was given 20 min before MDMA [40, 41], and guanfacine (0.15 mg/kg) 30 min before reboxetine or psilocybin [42, 43]. In fear conditioning, MDMA (7.8 mg/kg) and guanfacine (0.15 mg/kg) were administered 30 min before testing [25, 26], whereas psilocybin was given at trial onset. Doses and timing were based on prior literature, pharmacokinetics, and experimental requirements.

Surgeries

For stereotaxic surgeries, mice were anesthetized with isoflurane (3–1% in oxygen) and placed in a stereotaxic frame (Stoelting). For LC ablation, 0.8 µL of AAV1-EF1a-Flex-taCasp3-TEVP (UNC) was injected bilaterally at AP −0.9 mm and ML ± 0.9 mm from lambda, with 0.4 µL delivered at each of two depths (DV 3.1 and 3.3 mm below dura). For fiber photometry, 0.5 µL of AAV9-hSyn-NE2h or AAV9-hSyn-5HT3.5(h-S06) (1 × 10^13 gc/mL; WZ Biosciences) was injected into the mPFC (AP + 1.6 to +2.1 mm, ML ± 0.3 mm, DV 1.3 mm). Fiberoptic implants (0.2-mm core; Neurophotometrics) were placed at the injection site and secured with Metabond (Parkell). Mice received carprofen (5 mg/kg) for 2 days after surgery.

Fiber photometry

Mice underwent fiber photometry surgery 4–8 weeks before testing and were studied in a within-subject crossover design with 2-day intervals between drug conditions. Recordings were collected using a Neurophotometrics FP3002 system with interleaved 470 nm and 415 nm excitation (40 Hz total, 20 Hz/channel). Because 415 nm is not a true isosbestic point for GRABNE or GRAB5HT, only the 470 nm signal was analyzed. Baseline fluorescence was defined as the average signal from 120–240 s after recording onset, and ΔF/F was calculated as [(signal − baseline) / baseline] × 100. Drug-evoked GRABNE and GRAB5HT responses were expressed as percent change from baseline.

Histology

After fiber photometry and LC ablation experiments, mice were deeply anesthetized with isoflurane and transcardially perfused with phosphate-buffered saline followed by 4% paraformaldehyde. Brains were post-fixed, cryoprotected in 30% sucrose, and sectioned at 100 µm on a cryostat (Leica CM3050S). For photometry experiments, frontal cortex sections were imaged to verify viral expression and fiber placement (Olympus FV3000). For LC ablation, free-floating sections were immunostained for tyrosine hydroxylase using chicken anti-TH (1:1000; Aves Labs) and goat anti-chicken Alexa Fluor 488 (1:500; ThermoFisher), then mounted with Vectashield. Confocal z-stacks (20 µm) were acquired on an Olympus FV3000 using 4× and 60× objectives, and TH+ cells were quantified from 12 planes across three non-consecutive sections per animal using Imaris 9.6.1.

Head twitch response

Small magnets (SuperMagnetMan - N45 magnet 3 mm diameter, 0.5 mm thickness) were glued to ear tags (Stoelting - La Pias Aluminum Ear Tags) for automated HTR behavior assay. Mice were then ear tagged 3 days before HTR testing. The experimental setup was adapted from Gonzales-Maeso [44] and Kwan [45] including experimental apparatus and MATLAB code for analysis. Doses were randomly assigned using a shuffled list, and age-matched controls were injected with saline and randomly interspersed with drug conditions. Between each recording, the plastic boxes were cleaned with 70% ethanol.

Fear conditioning

On Day 1, mice underwent fear conditioning (Med Associates chamber) in context A (opaque white wall, eucalyptus scent). After 120 s, a 30 s tone (79.5–80.5 dB, 5.5 kHz) was presented and co-terminated with a 1 s, 1 mA foot shock, the session ended 120 s later. On Day 2, mice received atipamezole (2 mg/kg) or saline 20 min before MDMA, followed by MDMA (7.8 mg/kg) or saline 30 min before testing, and underwent extinction training in context B (no wall, orange scent). After 120 s, eight 30 s tones were presented with 45–75 s intertrial intervals, and the session ended 120 s after the last tone. Days 3–5 extinction recall sessions were identical to Day 2. For methylone (10 mg/kg), MBDB (10 mg/kg), and psilocybin (1 mg/kg), the same procedure was used except extinction training consisted of six tones on Day 2 followed by recall on Day 3. Chambers were

cleaned with 70% ethanol after conditioning and Formula 409 after extinction sessions. Freezing was quantified with VideoFreeze and validated against blinded manual scoring (inter-rater $r > 0.95$; automated vs. manual $r > 0.93$); freezing was defined as immobility >1 s, and locomotion as total distance traveled.

Chronic multimodal stress (CMMS)

On Day 1, mice were singly housed and habituated overnight to a two-bottle choice setup with 1.5% sucrose and water. Baseline sucrose preference was assessed on Days 2–3 using 1% sucrose during 14 h dark-phase sessions, with bottle positions alternated and intake measured by weight. From Day 4, mice underwent 15 days of unpredictable stress consisting of 5 h restraint under 2 Hz strobe light and 75 dB white noise. On Days 18–19, sucrose preference was reassessed to evaluate stress-induced changes. The protocol was adapted from Hesselgrave [46].

Sucrose preference test (SPT). Sucrose preference was calculated as [sucrose intake / (sucrose + water intake)] × 100. A 1.5% sucrose solution was used during the initial habituation phase, and a 1% sucrose solution was used for all baseline, post-stress, and post-treatment testing. Bottles were weighed before and after each session, and positions were alternated to control for side preference.

Post-treatment SPT. Four hours after the final injection, mice remained in their home cages and were exposed to a 14 h overnight sucrose preference test using a 1% sucrose solution. Bottle positions were alternated, and intake was measured by weight before and after the session.

Tail suspension test (TST). The day after the final stress session, mice underwent the TST to assess immobility duration. Mice were suspended by the tail (~1 cm from the tip) from a 10 cm horizontal bar for 6 min, and behavior was video recorded. Immobility was scored manually during the first 5 min of the test. Immobility was defined as the absence of any active movement. Animals were tested in a quiet room under consistent lighting conditions to minimize external stimuli.

Forced swim test (FST). On Day 21 of the CMMS protocol (24 h after treatment), mice underwent the forced swim test to assess immobility duration. Each mouse was placed in a 20 cm diameter glass cylinder filled with 21 ± 1 °C water (15 cm depth) for 6 min and video recorded. Immobility, defined as floating with minimal movement to keep the head above water, was scored manually during minutes 2–6. Tests were conducted under consistent conditions, and beakers were cleaned and refilled between trials.

Statistics

All experiments and analyses were performed blinded and conducted once in our laboratory. Statistical analyses were performed in Prism 9 (GraphPad). N values refer to independently treated animals, and sample sizes were based on prior literature. Normality was assessed with the Shapiro–Wilk test. Two-group comparisons used unpaired two-tailed Student's t tests, and comparisons across three or more groups used one-way ANOVA with Dunnett's post hoc test; nonparametric alternatives were used when assumptions were not met. Two-way ANOVA with Tukey's post hoc test was used for HTR and fear extinction data. Data are shown as individual values and mean ± SEM.

RESULTS

Entactogens do not significantly induce 5-HT$_{2A}$R-mediated head-twitches

To measure the HTR, we employed an automated magnetometer system in which a mouse is ear-tagged with a magnet and rapid head movements lead to induction of electric current in a wire surrounding an experimental box (Fig. 1A, B). Using this approach, we assessed HTR following administration of three entactogens: MDMA, methylone, and MBDB, and two serotonergic psychedelics: psilocybin and 2C-B. MDMA induced a suppression of HTRs at 5 mg/kg relative to saline, which was sustained at higher doses (Fig. 1C). Similarly, methylone induced a suppression of HTRs at 5 mg/kg relative to saline, which was intensified at higher doses (Fig. 1D). In contrast, MBDB displayed a significant increase in HTR at 5 mg/kg relative to saline, which returns to saline levels at higher doses (Fig. 1E). On the other hand, psilocybin produced a pronounced increase in HTR beginning at 0.3 mg/kg and peaking at 3 mg/kg (Fig. 1F), while 2C-B elicited high HTR counts across a broad dose range, with maximal effects at 10 mg/kg (Fig. 1G).

To directly compare peak HTR across compounds, we selected the dose with the highest HTR for each drug: MDMA (1 mg/kg), methylone (0.5 mg/kg), MBDB (5 mg/kg), psilocybin (3 mg/kg), and 2C-B (10 mg/kg). When compared across different compounds, all entactogens showed low HTR counts that were statistically indistinguishable from saline, while psilocybin and 2C-B elicited significantly elevated responses (Fig. 1H). These findings confirm that 5-HT$_{2A}$R agonist psychedelics, but not entactogens, engage the head twitch circuit robustly, consistent with their differential receptor pharmacology.

MDMA increases the release of 5-HT and NE in PFC while psilocybin does not

To examine whether the behavioral differences between MDMA and psilocybin reflect distinct monoaminergic dynamics, we measured extracellular 5-HT and NE levels in the medial prefrontal cortex (mPFC) using fiber photometry. Mice expressing AAV-GRAB5-HT (Fig. 2A, B) or AAV-GRABNE (Fig. 2E, F) were recorded following i.p. administration of saline, MDMA (10 mg/kg), or psilocybin (1 mg/kg). Fiber placements are shown in Supplementary Fig. 1. MDMA produced a robust and sustained increase in mPFC 5-HT fluorescence relative to both saline and psilocybin (Fig. 2C, D). Parallel recordings using GRABNE revealed a similarly strong elevation in NE release (Fig. 2G, H). In contrast, psilocybin did not significantly alter either 5-HT or NE signals compared with saline, indicating minimal acute monoamine release in this region.

To confirm that these results were not driven by injection order or carryover effects, recordings were conducted in a counterbalanced crossover design. Sequence analyses revealed no significant effects or interactions between treatment order and response amplitude for either transmitter (Supplementary Fig. 2A, B), validating the reliability of the design and confirming that MDMA's effects reflect genuine pharmacological activation.

We next tested whether this monoamine release pattern generalizes to other entactogens. Both MBDB (10 mg/kg) and methylone (10 mg/kg) induced strong elevations in 5-HT and NE fluorescence in the mPFC, with methylone producing the largest NE increase (Supplementary Fig. 3A–D). These findings establish that robust serotonergic and noradrenergic release is a shared feature of entactogens, distinguishing them from serotonergic psychedelics such as psilocybin. Together, these results demonstrate that MDMA, but not psilocybin, promotes concurrent 5-HT and NE release in the mPFC. This dual monoaminergic activation may underlie MDMA's unique behavioral profile, with NE signaling potentially modulating 5-HT$_{2A}$R-driven responses such as the head twitch behavior (Fig. 2I).

NE suppresses 5-HT$_{2A}$R head twitch response

To test the hypothesis that NE release suppresses the HTR induced by serotonergic compounds, we first examined the effect of pharmacological attenuation of NE release during MDMA exposure. We performed fiber photometry recordings to assess NE dynamics after co-administration of a NET inhibitor (reboxetine, 10 mg/kg) with MDMA (10 mg/kg) in the same recording session (Fig. 3A, B). Co-administration of reboxetine one minute prior to MDMA significantly blunted NE release in the mPFC (Fig. 3C, D), confirming that a NET inhibition reduces the MDMA-induced NE release.

We next assessed whether suppression of NE release unmasks behavioral effects of MDMA using the HTR assay (Fig. 3E). Mice

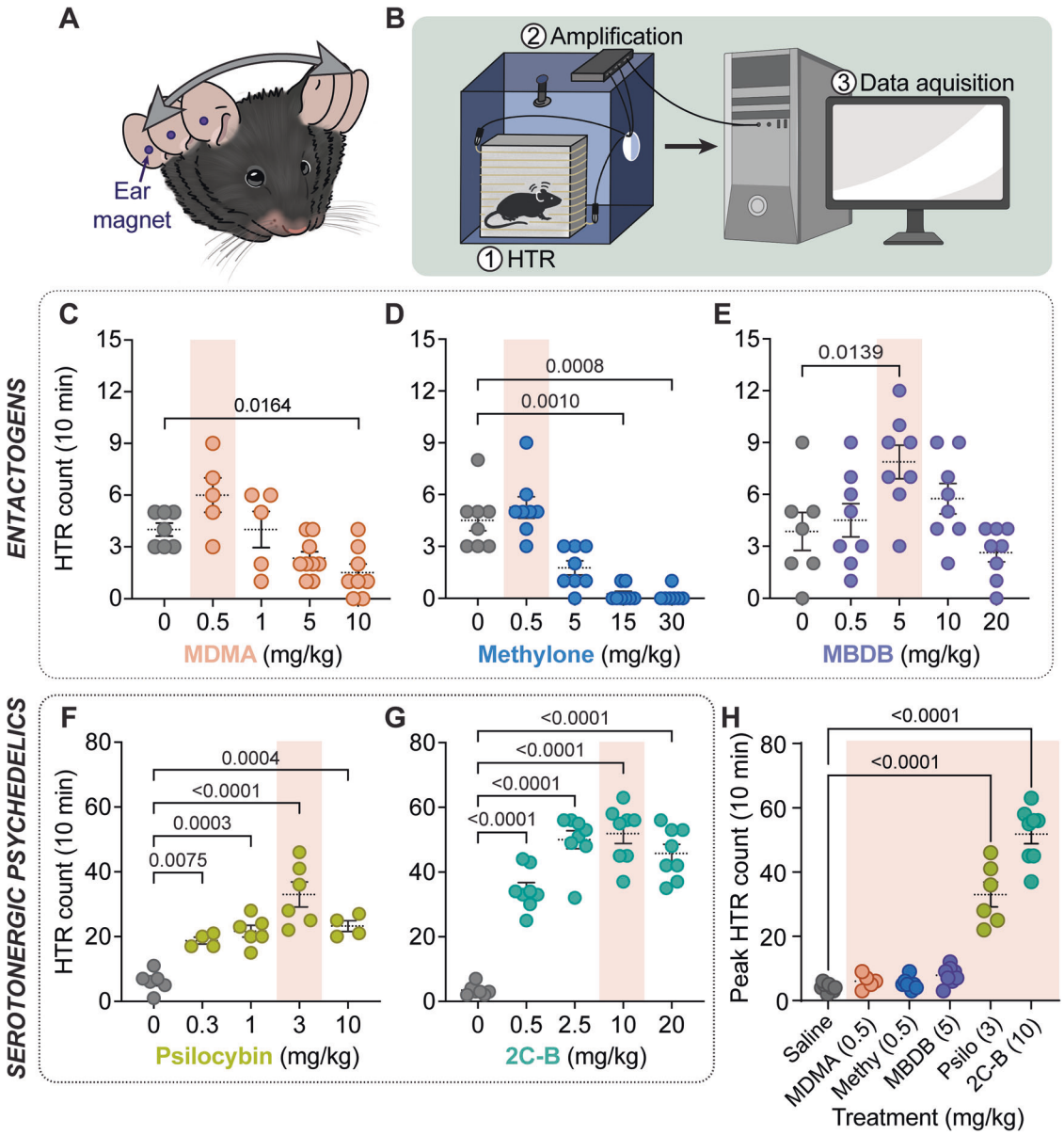

Fig. 1 Entactogens elicit fewer head-twitch responses than serotonergic psychedelics. **A** Cartoon of a mouse exhibiting a head-twitch response (HTR) detected via a magnetic ear tag. **B** Automated apparatus for HTR acquisition. **C–G** Total HTR counts over 10 min after i.p. drug administration: **C** MDMA: $F(4, 29) = 7.715$, $P = 0.0002$, 0: $N = 7$; 0.5: $N = 5$; 1: $N = 5$; 5: $N = 9$; 10: $N = 8$. **D** methylone: Kruskal-Wallis=31.49, $P < 0.0001$, 0: $N = 8$; 0.5: $N = 8$; 5: $N = 8$; 15: $N = 8$; 30: $N = 7$. **E** MBDB: $F(4, 34) = 5.010$, $P = 0.0028$, 0: $N = 7$; 0.5: $N = 8$; 5: $N = 8$; 10: $N = 8$; 20: $N = 8$. **F** psilocybin: $F(4, 21) = 18.00$, $P < 0.0001$, 0: $N = 6$; 0.3: $N = 4$; 1: $N = 6$; 3: $N = 6$; 10: $N = 4$. **G** 2C-B: $F(4, 34) = 58.59$, $P < 0.0001$, 0: $N = 7$; 0.5: $N = 8$; 2.5: $N = 8$; 10: $N = 8$; 20: $N = 8$. Shaded bands mark the dose carried forward to panel H. **H** Peak 10-min HTR counts extracted from C–G, $F(5, 39) = 110.4$, $P < 0.0001$ (MDMA 1 mg/kg; methylone 0.5 mg/kg; MBDB 5 mg/kg; psilocybin 3 mg/kg; 2C-B 10 mg/kg). Saline: $N = 10$. Statistical significance was determined using a one-way ANOVA with Dunnett's multiple comparisons for MDMA, MBDB, psilocybin, 2C-B, and Kruskal–Wallis for methylone. Data are mean ± SEM; points represent individual mice.

treated with reboxetine alone exhibited lower HTR counts compared to animals treated with saline. Mice receiving reboxetine + MDMA exhibited significantly higher HTR counts compared to animals treated with MDMA alone. Interestingly, the increase in HTR counts induced by reboxetine + MDMA was significantly greater in females than in males. This effect was driven primarily by females, with no significant differences observed in males relative to other treatment groups (Fig. 3F). However, post hoc analysis demonstrated that saline + saline versus reboxetine + MDMA was not significant in either sex, indicating that this effect reflects a larger potentiation in females rather than a female-specific increase above baseline (Fig. 3G).

To determine whether NE modulation similarly influences psilocybin's effects, we again used reboxetine to elevate NE levels. Unlike MDMA, psilocybin does not act on monoamine transporters and is not expected to alter NET activity (Fig. 3H). Administration of reboxetine alone led to a robust increase in NE levels 30 minutes post-injection (Fig. 3I–K), consistent with effective NET blockade. When reboxetine was administered 30 minutes prior to psilocybin (1 mg/kg), we observed a marked reduction in HTR compared to saline + psilocybin controls (Fig. 3L, M). No significant sex-specific differences were observed in any of the treatments (Supplementary Fig. 4). This finding supports a model in which elevated NE, whether pharmacologically or

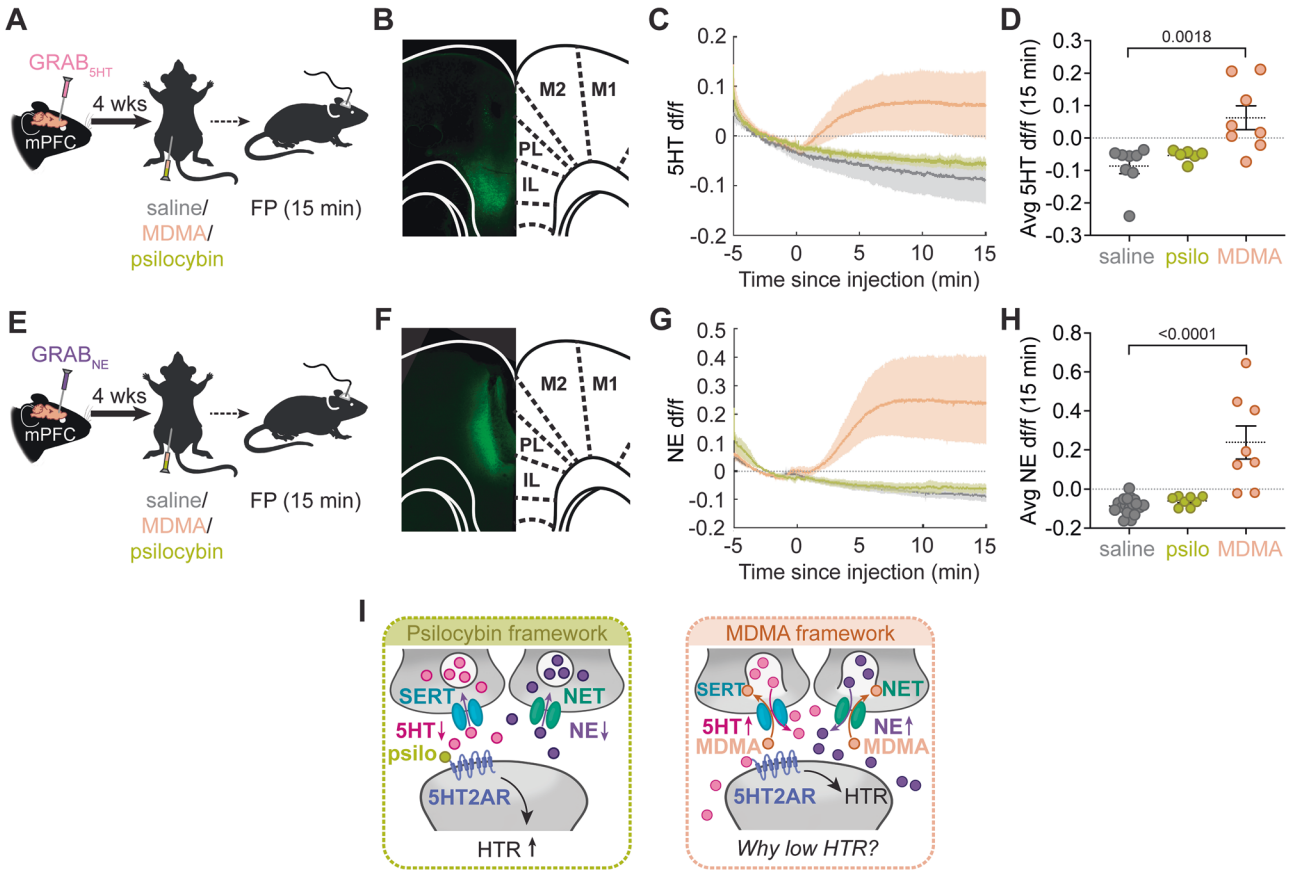

Fig. 2 MDMA and psilocybin produce dissociable 5-HT and NE dynamics in mPFC. A Schematic of fiber photometry recordings from mPFC in mice injected i.p. with saline, psilocybin (1 mg/kg), or MDMA (10 mg/kg). **B** Representative $GRAB_{5\text{-}HT}$ sensor expression and fiber placement in mPFC; outlines derived from the Allen Mouse Brain Atlas. **C** Group-averaged df/f traces aligned to injection (t = 0); mean ± 95% CI. **D** Mean df/f 5-HT florescence during the 15-minute post-injection period, quantified from (C), $F(2, 19) = 8.316$, $P = 0.0025$. Saline: N = 8; psilocybin: N = 6; MDMA: N = 8. **E** Same design as A using $GRAB_{NE}$. **F** Representative $GRAB_{NE}$ sensor expression and fiber placement in mPFC; atlas outlines as in B. **G** Group-averaged df/f traces aligned to injection (t = 0); mean ± 95% CI. **H** Mean df/f NE florescence during the 15-minute post-injection period, quantified from (G), $F(2, 31) = 22.39$, $P < 0.0001$. Saline: N = 18; psilocybin: N = 8; MDMA: N = 8. **I** Schematic frameworks illustrating distinct monoaminergic mechanisms underlying psilocybin- versus MDMA-induced HTRs. Statistical significance was determined using a one-way ANOVA with Dunnett's multiple comparisons versus saline for D and H. Data are mean ± SEM; points represent individual mice.

MDMA-induced, acts to suppress head twitch behavior (Fig. 3N).

α_2 receptor suppresses 5-HT_{2A}R head twitch response

To investigate whether α_2R modulate the suppressive effect of NE on HTR, we first administered the α_2R antagonist atipamezole (2 mg/kg) 20 minutes prior to MDMA (10 mg/kg) injection (Fig. 4A). Atipamezole + MDMA significantly increased HTRs compared to MDMA alone and atipamezole alone. More specifically, post hoc analysis demonstrated that females but not males significantly differed from saline controls, indicating a stronger female response to α_2R blockade during MDMA treatment. This supports a more robust female-selective unmasking of MDMA-induced head-twitch behavior by α_2R blockade. No significant HTRs were observed in atipamezole alone compared to saline (Fig. 4B). This indicates that the NE-mediated reduction in HTR is dependent on α_2R signaling in a sex-specific manner (Fig. 4C).

We next tested whether direct activation of α_2R is sufficient to suppress the HTR, even in the absence of MDMA-induced NE release. Pretreatment with the α_2R agonist guanfacine (0.15 mg/kg) 30 minutes prior to reboxetine + MDMA significantly reduced HTR counts compared to saline + reboxetine + MDMA (Fig. 4D, E). Notably, guanfacine alone also suppressed MDMA-induced HTRs, further supporting the sufficiency of α_2R activation in dampening this behavioral response (Fig. 4E, F). No significant sex-specific differences were observed in any of the treatments (Supplementary Fig. 5).

To determine whether this regulatory mechanism generalizes to serotonergic psychedelics, we examined the effect of α_2R activation on psilocybin induced HTRs. Guanfacine pretreatment significantly reduced psilocybin-evoked HTRs relative to saline-treated controls (Fig. 4G, H), and this suppressive effect was consistent across multiple psilocybin doses (0.3 mg/kg, 1 mg/kg, and 3 mg/kg, Supplementary Fig. 6), consistent with a non-competitive mechanism of action (Fig. 4I). No significant sex-specific differences were observed in any of the treatments (Supplementary Fig. 7A).

To test whether this suppression requires upstream NE signaling, we ablated LC neurons via bilateral injection of Cre-dependent AAV1-Flex-taCasp3 into DBH-Cre+ mice (Fig. 4J). Cre-dependent Casp3 injection induced near-complete ablation of tyrosine hydroxylase positive (TH+) neurons in LC (Fig. 4K). Four weeks after ablation, guanfacine pretreatment continued to reduce psilocybin-induced HTRs in both LC-ablated (Cre+) and control (Cre–) mice (Fig. 4L), indicating that α_2R modulation of HTR

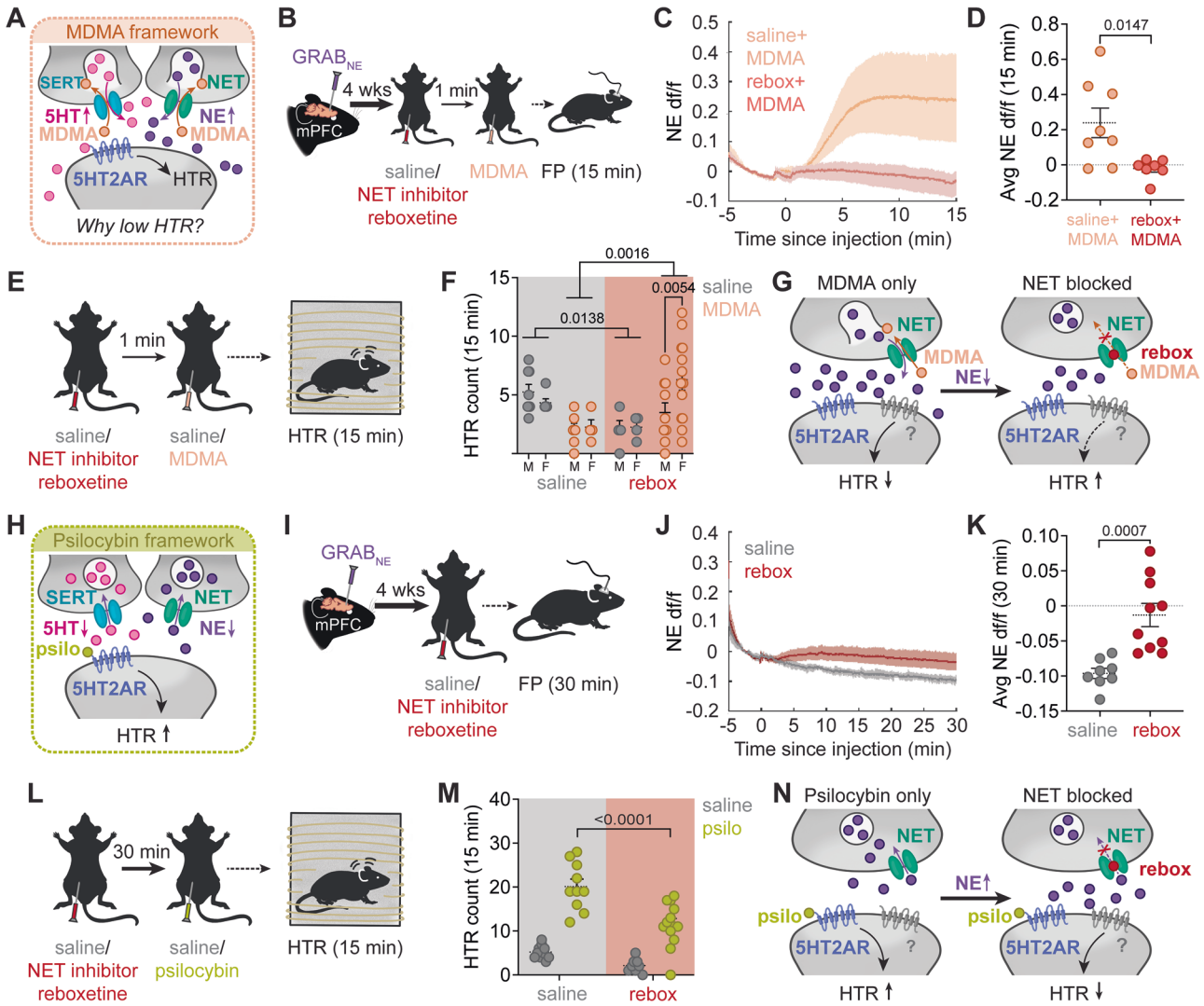

Fig. 3 Extracellular NE gates MDMA- and psilocybin-evoked head twitches. A MDMA Working model: MDMA elevates extracellular 5-HT and NE; NE can dampen HTR via downstream signaling. **B** GRAB$_{NE}$ fiber photometry in mPFC: saline or reboxetine (10 mg/kg, i.p.), then MDMA (10 mg/kg, i.p.) 1 min later; fiber photometry was recorded for 15 min post-injection. **C** Group-averaged NE df/f traces aligned to drug administration; mean ± 95% CI. **D** Mean df/f NE florescence during the 15-minute post-injection period using an unpaired t test, quantified from (C), t = 2.811, df=13, P = 0.0147. Saline + MDMA: N = 8; reboxetine + MDMA: N = 7. **E** HTR assay using the same injections as B, recorded for 15 min. **F** Total HTR counts two-way ANOVA (factor: sex, treatment): Interaction: F(3, 48) = 2.106, P = 0.1118; Sex: F(1, 48) = 0.7636, P = 0.3866; Treatment: F(3, 48) = 5.572, P = 0.0023. Tukey's post-hoc multiple comparisons (factors: reboxetine present/absent, MDMA present/absent): saline: saline vs MDMA: P = 0.0408; reboxetine: saline vs MDMA: P = 0.0259; saline: saline vs reboxetine: P = 0.0138; MDMA: saline vs reboxetine: P = 0.0016.Tukey's post-hoc multiple comparisons male vs female: Saline + saline: N = 14 (male N = 8, female N = 6): P = 0.4657; reboxetine + saline: N = 8 (male N = 4, female N = 4): P = 0.8674; saline + MDMA: N = 10 (male N = 6, female N = 4): P = 0.8789; reboxetine + MDMA: N = 24 (male N = 10, female N = 14): P = 0.0054. **G** Schematic summary: reducing NE unmasks MDMA-evoked HTR. **H** Psilocybin Working model: psilocybin increases HTR via 5-HT$_{2A}$ under low-NE conditions. **I** GRAB$_{NE}$ fiber photometry in mPFC: saline or reboxetine (10 mg/kg, i.p.) alone, fiber photometry was recorded for 30 min post-injection. **J** Group-averaged NE df/f traces aligned to drug administration; mean ± 95% CI. **K** Mean df/f NE florescence during the 30-minute post-injection period using an unpaired t test, quantified from (J), t = 4.213, df=16, P = 0.0007. Saline: N = 8; reboxetine: N = 10. **L** HTR assay: saline or reboxetine (10 mg/kg, i.p.), then psilocybin (1 mg/kg, i.p.) 30 min later, HTR recorded for 15 min. **M** Total HTR counts two-way ANOVA (factors: reboxetine present/absent, psilocybin present/absent): Interaction: F(1, 37) = 4.792, P = 0.0350; Reboxetine: F(1, 37) = 21.24, P < 0.0001; Psilocybin: F(1, 37) = 90.94, P < 0.0001. Tukey's post-hoc multiple comparisons: saline: saline vs psilocybin: P < 0.0001; reboxetine: saline vs psilocybin: P < 0.0001; saline: saline vs reboxetine: P = 0.3817; psilocybin: saline vs reboxetine: P < 0.0001. Saline + saline: N = 10 (male N = 5, female N = 5); reboxetine + saline: N = 8 (male N = 4, female N = 4); saline + psilocybin: N = 10 (male N = 6, female N = 4); reboxetine + psilocybin: N = 13 (male N = 7, female N = 6). **N** Schematic summary: high NE blunts psilocybin-evoked HTR. Data are mean ± SEM; points represent individual mice.

does not require LC-derived NE (Fig. 4M). No significant sex-specific differences were observed in any of the treatments (Supplementary Fig. 7B). Collectively, these results demonstrate that α_2R activation suppresses HTRs induced by both MDMA and psilocybin through a mechanism that is independent of NE release or LC activity, suggesting a direct postsynaptic modulatory role of α_2R on 5-HT$_{2A}$R-mediated behaviors.

Fear extinction and locomotor effects of MDMA, psilocybin, and related entactogens

To assess whether MDMA and α_2R modulation produced fear extinction effects, mice underwent fear conditioning on Day 1, then received atipamezole (α_2R antagonist; 2 mg/kg) or saline 20 min before MDMA, followed by MDMA (7.8 mg/kg) or saline 30 min before extinction training on Day 2, with extinction recall

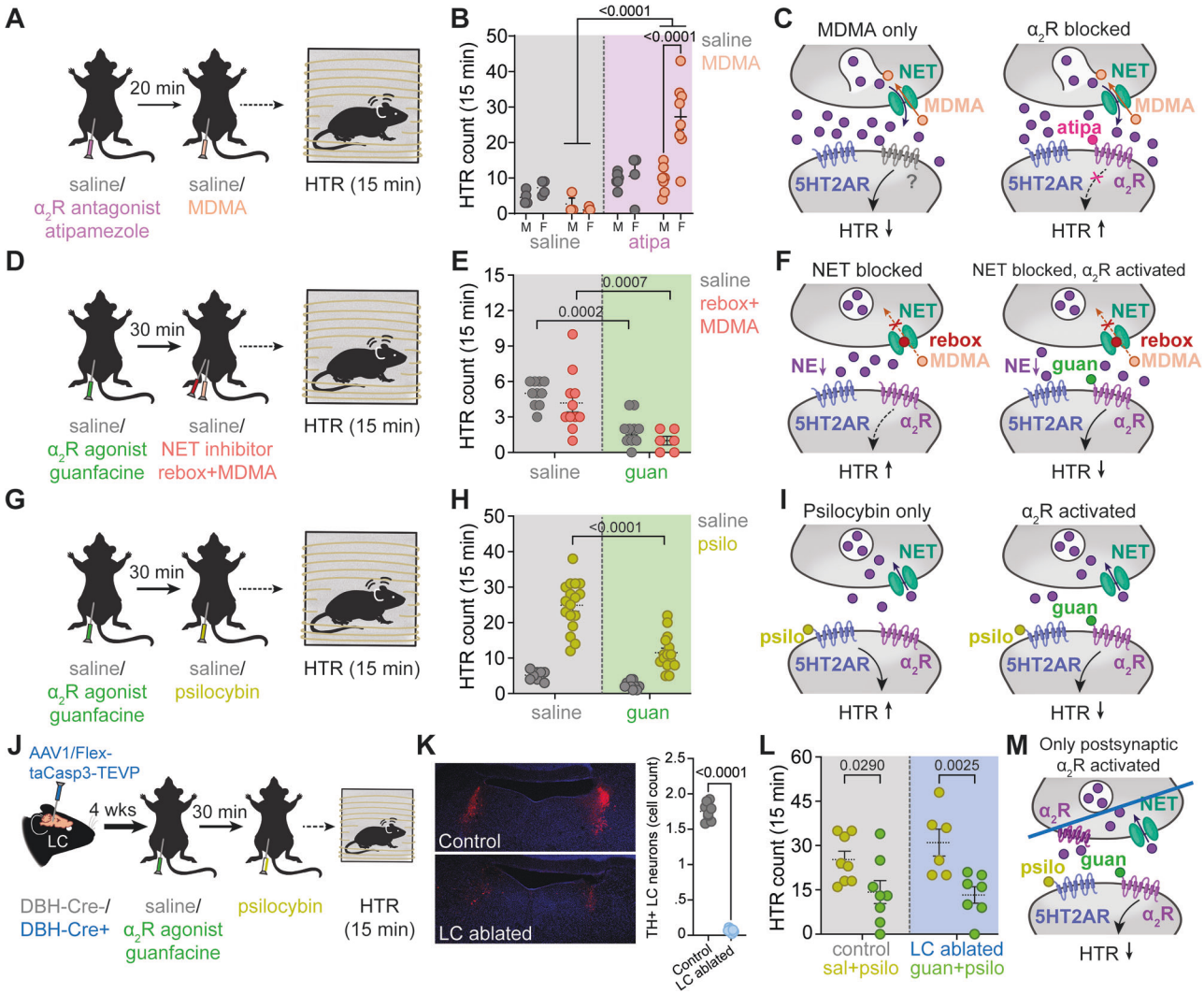

Fig. 4 Postsynaptic α_2 receptor activation suppresses head twitches. A HTR assay: saline or atipamezole (α_2R antagonist, 2 mg/kg, i.p.), then MDMA (10 mg/kg, i.p.) 20 min later, HTR recorded for 15 min. **B** Total HTR counts two-way ANOVA (factor: sex, treatment): Interaction: $F(3, 31) = 6.639$, $P = 0.0014$; Sex: $F(1, 31) = 6.373$, $P = 0.0169$; Treatment: $F(3, 31) = 15.54$, $P < 0.0001$. Tukey's post-hoc multiple comparisons (factors: atipamezole present/absent, MDMA present/absent): saline: saline vs MDMA: $P = 0.5694$; atipamezole: saline vs MDMA: $P = 0.0068$; saline: saline vs atipamezole: $P = 0.4510$; MDMA: saline vs atipamezole: $P < 0.0001$. Tukey's post-hoc multiple comparisons male vs female: Saline + saline: N = 8 (male N = 4, female N = 4): $P = 0.5041$; atipamezole + saline: N = 10 (male N = 6, female N = 4): $P = 0.7251$; saline + MDMA: N = 6 (male N = 3, female N = 3): $P = 0.7895$; atipamezole + MDMA: N = 15 (male N = 7, female N = 8): $P < 0.0001$. **C** Schematic: blocking α_2R relieves NE-dependent suppression of MDMA HTR. **D** HTR assay: saline or guanfacine (α_2R agonist, 0.15 mg/kg, i.p.), then reboxetine (10 mg/kg, i.p.) plus MDMA (10 mg/kg, i.p.) 1 min later, HTR recorded for 15 min. **E** Total HTR counts two-way ANOVA (factors: guanfacine present/absent, reboxetine+MDMA present/absent): Interaction: $F(1, 33) = 0.0052$, $P = 0.9429$; Guanfacine: $F(1, 33) = 30.75$, $P < 0.0001$; Reboxetine+MDMA: $F(1, 33) = 2.301$, $P = 0.1388$. Tukey's post-hoc multiple comparisons: saline: saline vs. rebox+MDMA: $P = 0.2715$; guanfacine: saline vs. rebox+MDMA: $P = 0.3055$; saline: saline vs. guanfacine: $P = 0.0002$; rebox+MDMA: saline vs. guanfacine: $P = 0.0007$. Saline + saline: N = 10 (male N = 5, female N = 5); guanfacine + saline: N = 10 (male N = 5, female N = 5); saline + [reboxetine + MDMA]: N = 11 (male N = 7, female N = 4); guanfacine + [reboxetine + MDMA]: N = 6 (male N = 3, female N = 3). **F** Schematic: activating α_2R restores suppression despite low-NE conditions. **G** HTR assay: saline or guanfacine (0.15 mg/kg, i.p.), then psilocybin (1 mg/kg, i.p.) 30 min later, HTR recorded for 15 min. **H** Total HTR counts two-way ANOVA (factors: guanfacine present/absent, psilocybin present/absent): Interaction: $F(1, 48) = 14.39$, $P = 0.0004$; Guanfacine: $F(1, 48) = 34.79$, $P < 0.0001$; Psilocybin: $F(1, 48) = 110.2$, $P < 0.0001$. Tukey's post-hoc multiple comparisons: saline: saline vs. psilocybin: $P < 0.0001$; guanfacine: saline vs. psilocybin, $P = 0.0001$; saline: saline vs. guanfacine: $P = 0.5407$; psilocybin: saline vs. guanfacine: $P < 0.0001$. Saline + saline: N = 10; guanfacine + saline: N = 10; saline + psilocybin: N = 18; guanfacine + psilocybin: N = 16. **I** Schematic: α_2R activation blunts psilocybin HTR. **J** Same design as G in DBH-Cre$^+$ mice with LC ablation and Cre$^-$ controls. **K** Left: Representative sections confirming LC ablation (Cre$^+$) vs intact LC (Cre$^-$). Right: LC TH+ cell count quantification (in thousands): $t = 34.71$, $df = 13$, $P < 0.0001$. **L** Total HTR counts two-way ANOVA (factors: Cre status, guanfacine present/absent with psilocybin): Interaction $F(1, 25) = 0.8935$, $P = 0.3536$; LC ablation $F(1, 25) = 0.4539$, $P = 0.5067$; Treatment $F(1, 25) = 16.34$, $P = 0.0004$. Tukey's post-hoc multiple comparisons: Cre-: saline + psilocybin vs. guanfacine + psilocybin: $P = 0.0290$; Cre+: saline + psilocybin vs. guanfacine + psilocybin: $P = 0.0025$; saline + psilocybin: Cre- vs. Cre+: $P = 0.2729$; guanfacine + psilocybin: Cre- vs. Cre+: $P = 0.8460$. DBH-Cre-, saline + psilocybin: N = 8; DBH-Cre-, guanfacine + psilocybin: N = 8; DBH-Cre+, saline + psilocybin: N = 7; DBH-Cre+, guanfacine + psilocybin: N = 7. **M** Schematic: suppression arises from postsynaptic α_2R activation. Data are mean ± SEM; points represent individual mice.

assessed on Days 3–5 (Supplementary Fig. 8A). MDMA reduced cue-elicited freezing during the earliest recall session (Day 3), but this effect was not observed when mice were pretreated with atipamezole, indicating that α_2R blockade prevented the early reduction in freezing produced by MDMA (Supplementary Fig. 8B). On Day 4, a significant MDMA × atipamezole interaction was detected, although post hoc comparisons were not significant, and by Day 5 no treatment effects were observed (Supplementary Fig. 8C, D). Across recall days, freezing declined significantly, consistent with extinction, and a significant MDMA × atipamezole interaction was maintained in the overall analysis (Supplementary Fig. 8E).

Pre-cue freezing did not differ across days or treatment groups, indicating that baseline or contextual freezing was not affected by MDMA, atipamezole, or their combination (Supplementary Fig. 9A). Average motion also showed no day-dependent or pairwise treatment differences during recall, arguing against locomotor confounds as an explanation for the Day 3 cue-freezing effect (Supplementary Fig. 9B).

To assess whether psilocybin and α_2R modulation produced comparable effects, mice received psilocybin (1 mg/kg) with or without guanfacine (α_2R agonist; 0.15 mg/kg) 30 min before extinction training, with extinction recall assessed on Day 3 (Supplementary Fig. 10A). Psilocybin, alone or combined with guanfacine, did not significantly affect freezing or locomotor activity during extinction training on Day 2 or extinction recall on Day 3 (Supplementary Fig. 10B–E). These data indicate that, unlike MDMA, psilocybin did not measurably alter conditioned freezing behavior in this fear extinction paradigm.

We next tested whether other entactogens produced similar effects. 2C-B, methylone, and MBDB were administered before extinction training at varying doses. 2C-B, methylone, and MBDB showed treatment effects on conditioned freezing during extinction training and recall, with the strongest acute effects observed at higher doses of methylone and MBDB (Supplementary Fig. 11A, B). Locomotor activity was increased during extinction training by methylone (30 mg/kg) and MBDB (10 mg/kg), but did not differ during recall on Day 3, indicating that acute stimulant-like effects were restricted to the training session (Supplementary Fig. 11C, D). Overall, these compounds did not reproduce the selective early recall effect observed with MDMA. Analysis of pre-cue and inter-tone freezing confirmed generally low baseline/contextual freezing across groups (Supplementary Fig. 12A, B). Aside from a modest increase in pre-cue freezing in the MBDB 5 mg/kg group, treatment effects were largely expressed during cue presentation or inter-trial periods rather than as generalized contextual freezing, supporting the interpretation that the observed drug effects were primarily cue-related (Supplementary Fig. 12). Together, these findings indicate that MDMA uniquely produces an early, α_2R-sensitive reduction in cue-elicited freezing during extinction recall, whereas psilocybin and related entactogens do not reproduce this profile.

Antidepressive effect of psilocybin is not blocked by α_2R activation

We next tested whether α_2R activation alters psilocybin's behavioral effects after CMMS (Fig. 5A). Treatments were administered on Day 20, and the FST was conducted on Day 21, approximately 24 h after a single dose. To avoid group bias and ensure equal baseline behavioral variation across treatment conditions, animals were first assessed in the TST and post-stress sucrose preference test before assignment into treatment groups. There were no significant baseline differences across the three planned treatment conditions in either the TST (Supplementary Fig. 13A) or in baseline sucrose preference (Supplementary Fig. 13B).

CMMS significantly reduced sucrose preference relative to baseline (Fig. 5B). Although psilocybin-treated mice showed a modest numerical increase in sucrose preference following CMMS, this effect was significant only relative to their own stressed baseline and did not translate into significant differences from saline controls at the post-treatment time point. Guanfacine co-treatment likewise did not modify this outcome. In contrast, psilocybin significantly reduced immobility in the FST compared with saline controls (Fig. 5C), and this reduction was preserved in the presence of guanfacine, indicating that α_2R activation did not block psilocybin's active/passive coping effects in this assay.

Notably, guanfacine alone did not alter baseline tail suspension test performance, sucrose preference, or FST immobility in a separate control cohort (Supplementary Fig. 14A–C), indicating that the maintained effect of psilocybin during co-treatment was not attributable to an independent action of guanfacine itself. Together, these findings show that psilocybin's antidepressant-like effect in the FST remains intact during α_2R activation, whereas its effect on sucrose preference after chronic stress is modest and not significantly different from controls.

DISCUSSION

Our findings clarify how serotonergic and noradrenergic systems interact to shape the behavioral effects of psychedelics. The HTR remains a reliable behavioral proxy of cortical 5-HT_{2A}R activation [4, 47, 48]. While classic psychedelics such as psilocybin and 2C-B robustly engage this pathway, entactogens like MDMA, methylone, and MBDB produce minimal HTR despite strong 5-HT release, indicating that increased extracellular serotonin alone is insufficient to drive 5-HT_{2A}R-mediated behavioral output. Instead, noradrenergic signaling appears to be a key modulatory factor, consistent with prior work showing that elevated NE tone can suppress 5-HT_{2A}R-mediated activity through α_2-adrenergic and 5-HT_{1A}R pathways [32, 49].

Our data extend this model by showing that reducing NE tone through NET blockade unmasks MDMA-induced HTRs, whereas increasing NE suppresses psilocybin-evoked HTRs, confirming a bidirectional inhibitory role of NE in 5-HT_{2A}R-linked behaviors. We further identify α_2R signaling as a key component of this interaction, α_2R blockade enhanced MDMA-induced HTRs, whereas α_2R activation suppressed both MDMA- and psilocybin-evoked responses, even after LC ablation, supporting a post-synaptic, NE-independent inhibitory control of 5-HT_{2A}R output. This mechanism may operate within cortico-serotonergic circuits and complement broader LC–DRN noradrenergic regulation of 5-HT neuron activity [50].

These neuromodulatory dynamics may also help explain why MDMA, but not psilocybin, enhances fear extinction. By co-releasing NE and 5-HT, MDMA may engage arousal and attentional processes that support adaptive learning, consistent with phase 3 clinical findings in PTSD [51, 52]. Human studies further support this interpretation, where reboxetine reduced MDMA-induced subjective drug high, stimulation, emotional excitation, and cardiovascular activation [53], duloxetine similarly blunted subjective and autonomic effects [54], and citalopram attenuated acute psychological and vegetative responses [55, 56]. These findings support an important role for transporter-mediated monoamine release in shaping MDMA's effects. In contrast, psilocybin's primarily serotonergic profile lacks this noradrenergic contribution. Comparisons with ketamine, which also elevates NE signaling and facilitates extinction [57, 58], suggest that balanced serotonergic and noradrenergic engagement may represent a shared mechanism for rapid emotional learning. In the chronic stress model, psilocybin's antidepressant-like effects were differentially modulated by α_2R activation, suggesting that α_2R signaling may selectively gate motivational and affective components of antidepressant responses.

Although mice are nocturnal, behavioral testing during the light phase is common in behavioral pharmacology because it supports

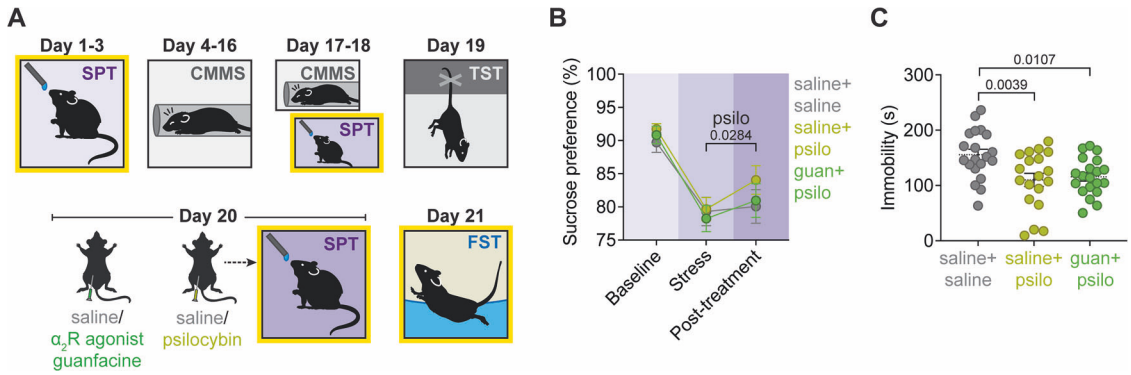

Fig. 5 α_2R activation does not block the antidepressant-like effect of psilocybin after chronic multimodal stress. A Schematic representation of the experimental timeline, with chronic multimodal stress (CMMS; days 4–18), tail suspension test (TST; day 19), sucrose preference tests (SPT; baseline: days 1–3; stress: days 17–18; post-treatment: day 20) and forced-swim tests (FST; day 21). Treatments administered on Day 20. **B** Percent sucrose preference at baseline, during CMMS, and post-treatment using two-way ANOVA with repeated measures (factor: time, treatment): time x treatment $F(3.566, 99.86) = 0.3521$, $P = 0.8210$; time $F(1.783, 99.86) = 39.84$, $P < 0.0001$; treatment $F(2, 56) = 0.6856$, $P = 0.5080$; subject $F(56, 112) = 1.990$, $P = 0.0010$. Tukey's multiple comparison: Vehicle: stress vs. post-treatment $P = 0.9471$; Psilocybin: stress vs. post-treatment $P = 0.0284$; Psilocybin + Guanfacine: stress vs. post-treatment $P = 0.3017$. Post-treatment comparisons: Vehicle vs. Psilocybin $P = 0.4606$; Vehicle vs. Psilocybin + Guanfacine $P = 0.9665$; Psilocybin vs. Psilocybin + Guanfacine $P = 0.6268$. **C** FST immobility on Day 21 using one-way ANOVA: $F(2, 56) = 6.338$, $P = 0.0033$. Dunnett's post-hoc multiple comparisons versus saline: saline + psilocybin $P = 0.0039$; guanfacine + psilocybin $P = 0.0107$. Saline + saline: $N = 20$; saline + psilocybin: $N = 19$; guanfacine + psilocybin: $N = 20$. Data are mean ± SEM; points represent individual mice.

standardization and comparability across studies [59, 60]. Available evidence indicates that circadian phase does not uniformly determine behavioral outcomes across commonly used mouse assays [59, 61], although this remains a limitation that may reduce ethological relevance and should be considered when interpreting the present findings [62].

HTR was assessed within 10 min post-injection, consistent with established protocols [48, 63]. Photometry recordings were extended to 20 min to include a 5 min baseline and a 15 min post-injection period that captured both the behavioral epoch and evolving monoamine dynamics [36, 37]. All subsequent HTR manipulations were aligned to this same 15 min post-administration window for consistency. Dose selection and pretreatment timing were based on known pharmacology and prior studies of MDMA, psilocybin, and α_2-adrenergic manipulations [25, 36, 40, 42, 43]. The MBDB HTR profile is biologically relevant because MBDB retains serotonergic activity while showing weaker NET inhibition than MDMA or methylone [64, 65], consistent with our supplementary GRAB data showing lower NE release.

Similarly, psilocybin can exhibit an inverted-U HTR dose-response in mice [66–68], and higher doses may recruit additional serotonergic mechanisms, including 5-HT_{1A}R signaling [20, 68]. For GRABNE/5HT recordings, MDMA and psilocybin doses were chosen to generate robust acute monoaminergic and behavioral responses within the photometry window [37, 68], whereas the 7.8 mg/kg MDMA dose used in fear conditioning was selected to match prior extinction studies [25, 26]. The reboxetine pretreatment intervals were similarly tailored to the mechanistic question being tested, with short pretreatment before MDMA to block NET during acute drug exposure [53], and longer pretreatment before psilocybin to allow NE accumulation before psilocybin administration [38, 39]. Although these choices improve pharmacological precision, they also limit direct comparison across assays.

Both males and females were included to improve translational relevance, although the study was not powered to test sex as a primary biological variable. In HTR experiments, the enhancement of MDMA-induced HTR by noradrenergic manipulations was greater in females, particularly after α_2R blockade. This is notable given prior reports of sex differences in acute MDMA responses, LC–NE organization, and 5-HT_{2A}R-mediated behaviors such as HTR and fear extinction [69–75], although such effects are not always consistent across studies [74–76]. These findings suggest that α_2R-dependent noradrenergic gating of 5-HT_{2A}R output may differ by sex and demand dedicated investigation.

All pharmacological manipulations were systemic, preventing causal assignment of the behavioral effects to any single brain region or receptor population. However, photometry measurements were restricted to the mPFC, a region strongly linked to 5-HT_{2A}R-dependent HTR [77]. Although this approach captured key cortical 5-HT and NE dynamics, it did not address regional heterogeneity, possible dopaminergic contributions, or distinguish local mPFC actions from effects in other circuits involved in HTR, fear extinction, and stress-related behaviors. Future studies should define the role of DA in 5-HT_{2A}R/α_2R interactions, extend monoamine mapping beyond mPFC, and use local manipulations to identify the relevant anatomical substrates.

In summary, α_2R blockade enhanced entactogen-induced HTRs, whereas postsynaptic α_2R activation was sufficient to suppress 5-HT_{2A}R-mediated HTR even in the absence of LC NE input. Psilocybin-induced effects in the forced swim test persisted in the presence of α_2R activation. Together, these findings identify a noradrenergic gating mechanism via α_2Rs that constrains overt 5-HT_{2A}R signaling and suggest that α_2R-targeted modulation may be used to fine-tune psychedelic therapies by enhancing or suppressing specific downstream effects.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

CODE AVAILABILITY

All code will be made available at https://github.com/kayelab/a2-5ht2a.

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AUTHOR CONTRIBUTIONS

AFR, ALY, and APK designed the experiments. AFR, ALY, JHY, JR, GF, AB, and SS performed the experiments. ALY, AB, and MD assembled the experiments apparatus. JF and YL were responsible for resources. JWS, BK, JHK, CP, and ACK were responsible for intellectual contributions and manuscript feedback. AFR, ALY, and APK were responsible for writing the manuscript.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All experiments were performed in accordance with the protocols approved by the Yale Institutional Animal Care and Use Committee (Approval No. 20381). Written informed consent was not required, as no human participant was involved in this study. All methods were performed in accordance with the relevant guidelines and regulations.

COMPETING INTERESTS

Research funding from Freedom Biosciences and Transend Therapeutics (APK, CP). A patent application relevant to this manuscript has been filed by APK, ALY, AFR, JHK, CP. ACK has been a scientific advisor or consultant for Boehringer Ingelheim, Eli Lilly, Empyrean Neuroscience, Freedom Biosciences, Otsuka, and Xylo Bio. ACK has received research support from Intra-Cellular Therapies. BK serves as the Chief Scientific Advisor for Transcend Therapeutics, and he's a co-founder and serves as a consultant for Transcend Therapeutics. He served as a consultant in the past year for Ceruvia Lifesciences and Lobe Sciences. JK (consultations less than $5000/yr) Aptinyx, Inc., BioXcel, Biogen, Idec, MA, Bionomics, Limited (Australia), Boehringer Ingelheim International, Cerevel Pharmaceuticals, Epiodyne, Inc., Esai, Inc., Janssen Research & Development, Jazz Pharmaceuticals, Inc., Otsuka America Pharmaceutical, Inc., PsychoGenics, Inc., Sunovion Pharmaceuticals, Inc., Takeda Pharmaceuticals (Stock/Options) Biohaven Pharmaceuticals, Cartego Therapeutics, Damona Pharmaceuticals, Delix Therapeutics, Inc., EpiVario, Inc., Freedom Biosciences, Neumora Therapeutics, Inc., Response Pharmaceuticals, Rest Therapeutics, Spring Health, Inc, Tempero Bio, Inc, Terran Biosciences, Tetricus Inc (non-monetary support, i.e., provision of drug) Cerevel Pharmaceuticals, Novartis. Dr. Krystal stands to benefit from patents held by Yale University that were licensed to Janssen Pharmaceuticals (ketamine), Biohaven Pharmaceuticals (riluzole), Freedom Biosciences (NMDA-R antagonist plus MTORC inhibitor), and Spring Health (precision medicine strategy). PRC is Co-founder and Board Member of Tetricus Labs, a precision-psychiatry company who did not fund this work. CP consults for Biohaven Pharmaceuticals, Ceruvia Lifesciences, Transcend Therapeutics, Freedom Biosciences, Nobilis Therapeutics, UCB Biopharma, and F-Prime Capital Partners and receives research funding from Biohaven, Transcend, and Freedom. He has filed patents for psychedelics in the treatment of obsessive-compulsive disorder and the identification of autoantibodies in neuropsychiatric disease, both unrelated to the current work. He owns equity in Alco Therapeutics for drug development unrelated to this work. JWS is a full-time employee with equity in Transcend Therapeutics.

ADDITIONAL INFORMATION

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