Psychedelics

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RESEARCH REPORT

Single-dose psychedelic enhances cognitive flexibility and reversal learning in mice weeks after administration

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Psychedelic compounds have demonstrated remarkable therapeutic potential for treating neuropsychiatric disorders by promoting sustained neuroplasticity in the prefrontal cortex (PFC). Cognitive flexibility—the ability to adapt previously learned rules to novel situations—represents a critical PFC function that is frequently impaired in depression, PTSD, and neurodegenerative conditions. In this study, we demonstrate that a single administration of the selective serotonin 2A receptor agonist 25CN-NBOH produces significant, long-lasting improvements in cognitive flexibility in both male and female mice when measured 2–3 weeks posttreatment. Using a novel automated sequential learning paradigm, psychedelic-treated mice showed superior adaptability in rule reversal tasks compared to saline controls, as evidenced by enhanced poke efficiency, higher percentages of correct trials, and increased reward acquisition. These behavioral findings complement existing cellular research showing psychedelic-induced structural remodeling in the PFC and uniquely demonstrate sustained cognitive benefits persisting weeks after a single psychedelic dose. Our automated behavioral task provides a high-throughput method for evaluating cognitive flexibility effects of various psychedelic compounds, offering important implications for therapeutic applications in conditions characterized by cognitive rigidity, including depression, PTSD, and potentially Alzheimer's disease.

Keywords: Cognitive flexibility, neuroplasticity, psychedelic therapy, reversal learning, serotonin 2a receptor.

Introduction

Psychedelic drugs have been used to treat multiple neuropsychiatric disorders, including major depressive disorder, posttraumatic stress disorder (PTSD), and substance use disorders (1–14). These neuropsychiatric disorders are precipitated by chronic stress, which leads to both structural and functional changes in the prefrontal cortex (PFC) in humans and rodents (15–22). The therapeutic potential of psychedelics may be due to their ability to restore neural circuits damaged in these pathologies by boosting synaptic activity (23–31).

The PFC contributes to the control of many cognitive functions, including working memory, memory retrieval, decision-making, and executive function (32, 33). One key aspect of executive function is the ability to apply previously learned rules to novel situations, also known as cognitive flexibility (33, 34). Flexibility disruptions are associated with neuropsychiatric disorders, such as depression and PTSD, as well as neurodevelopmental and neurodegenerative disorders (34, 35). Cognitive flexibility has been examined using tasks such as the Flanker Task, Stroop Task, and the Wisconsin Card Sorting Task; however, these kinds of tasks are largely limited to humans (36). In contrast, most cognitive flexibility tasks for rodents can be classified as either attentional set-shifting paradigms, which involve the learning of two separate rules and associated cues, or reversal learning, which involves applying a learned rule to a reversed scenario (37, 38). Reversal learning is an effective method for studying cognitive flexibility in rodents, including mice (38–40). Reversal learning paradigms can be extremely diverse, varying in the kind of tasks being taught to the number and timing of reversals involved in the paradigm. These details are critical when evaluating the existing literature's examination of cognitive flexibility through reversal learning.

25CN-NBOH is a psychedelic agent with high affinity and selectivity for the serotonin 2A (5-HT_{2A}) receptor (41–43). It has demonstrated psychedelic-like effects and is commonly used in the study of psychedelic mechanisms in rodents (44–46). 25CN-NBOH has much stronger affinity for 5-HT_{2A} receptors (50–100x higher affinity) in comparison to the closely related 5-HT_{2B} and 5-HT_{2C} receptors. Compared to 25CN-NBOH, other psychedelic drugs have a lower ratio of 5-HT_{2A} to 5-HT_{2B} or 5-HT_{2C} affinity (47–49). As 5-HT_{2A} receptor activation, specifically, has previously been shown to be required for psychedelic-induced synaptogenesis that might contribute to behavioral changes, this high affinity and high selectivity 5-HT_{2A} receptor agonist was chosen as the psychedelic drug for the present study of psychedelic effects on cognitive flexibility.

As single psychedelic administrations promote structural changes in the PFC that last for several weeks (24, 29, 31), here we asked whether a single psychedelic administration could also induce a weeks-long enhancement of flexible learning ability in mice. We conducted a reversal learning task in which female and male mice were administered a single dose of a psychedelic drug or saline and found enhanced performance on the reversal task which persists for at least 3 weeks after one psychedelic dose

Results

To determine whether psychedelic treatment induces long-lasting changes in flexible learning ability, we treated female and male mice with a single dose of the selective serotonin 2A (5-HT_{2A}) receptor agonist 25CN-NBOH (41–45) or saline via intraperitoneal injection. Following a waiting period of one day, light food restriction for 2 days, and 5 days of training with the Feeding Experimentation Device version 3 (FED3) device, we utilized a forward sequence learning protocol (Figure 1A). Mice learned to initiate a trial with a left poke and then had to poke right within the subsequent 30 s to receive a food pellet (Figure 1B). Following 6 days of 4 h/day forward protocol sessions, the required sequential poking pattern was reversed. For another 6 days of 4-h sessions, mice were then required to poke right and then poke left within 30 s to receive the food pellet (Figure 1C). This reversal of the experimental protocol is indicative of flexible learning: we measured the degree to which a mouse is able to adapt the previously learned 1 poke/hole sequence rule to a novel situation, which, in this case, was the reversed direction.

We found that psychedelic and saline-treated mice learned the forward task at similar rates, as reflected by the poke efficiency, which represents the proportion of pellets dispensed out of all pokes (Figure 2A), and the percentage of correct trials initiated out of all trials initiated (Figure 2B). While the change in forward learning poke efficiency and percentage correct was not affected by psychedelic treatment (poke efficiency: saline $R^2 = 0.25$, NBOH $R^2 = 0.20$; $F_{(1,557)} = .1721$, $P = 0.6784$; percent correct: saline $R^2 = 0.16$, NBOH $R^2 = 0.13$; $F_{(1,431)} = .7771$, $P = 0.3785$), the

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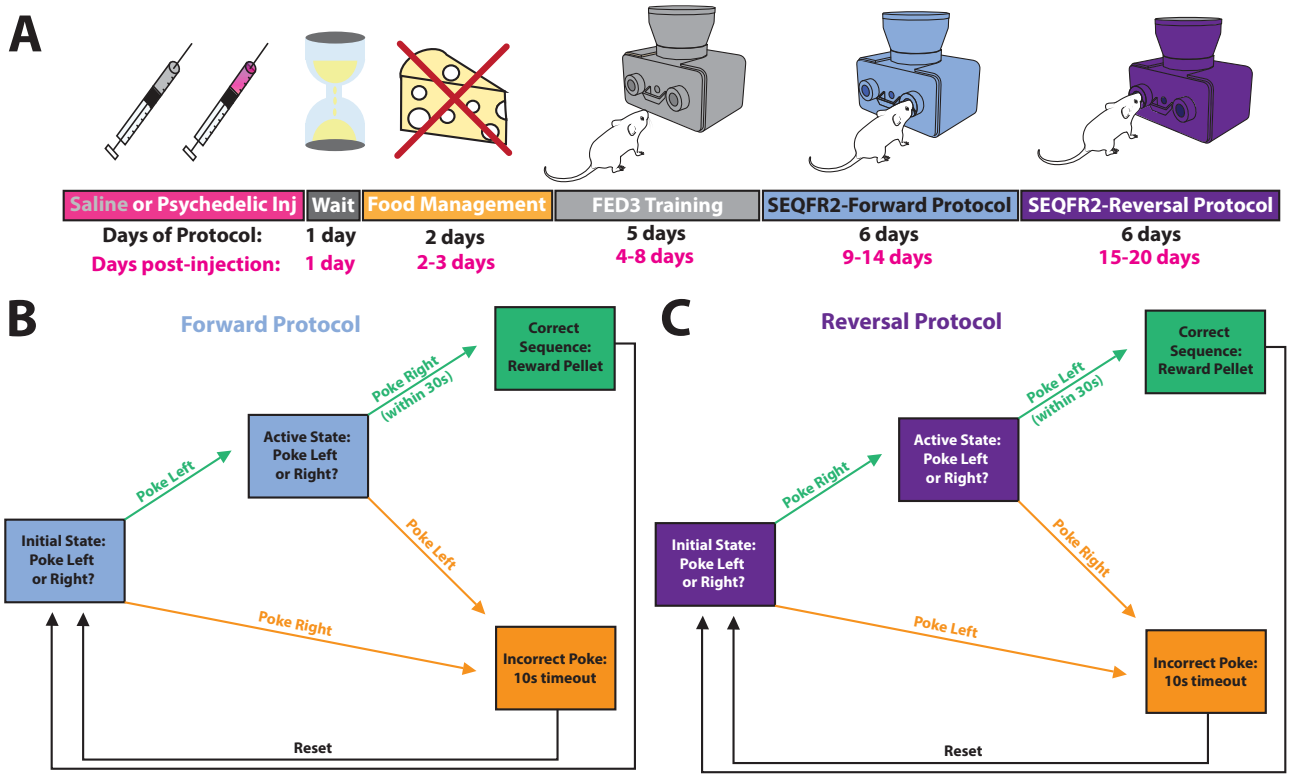

Figure 1. Experimental timeline and overview. (A) Experimental timeline (65). (B) Schematic of the SEQFR2-forward protocol. Mice have to sequentially poke left and then right within 30 s to earn a reward pellet. (C) Schematic of the SEQFR2-reversal protocol. Mice now are required to poke right and then left within 30 s to get a reward pellet.

NBOH-treated group accumulated more reward pellets than the saline group (Figure 2C; saline: $R^2 = 0.18$, NBOH: $R^2 = 0.27$; $F_{(1,620)} = 7.513$, $P = 0.0063$), indicating an increased initiation of trials per hour with the FED3 (Figure 2C), as the baseline and learning rates were similar between groups (Supplemental Figure 1).

Importantly, during the reversal phase, measured 15–20 days after the single injection, psychedelic treatment resulted in significantly increased learning ability. This is indicated by the increased the efficiency of nose pokes (Supplemental Figure 2; Figure 2D; saline: $R^2 = 0.11$, NBOH: $R^2 = 0.32$; $F_{(1,528)} = 21.91$, $P < 0.0001$), the percent correct trials initiated (Figure 2E; saline: $R^2 = 0.11$, NBOH: $R^2 = 0.23$; $F_{(1,401)} = 6.629$, $P = 0.0104$), and again by the higher total number of pellets obtained (Figure 2F; saline: $R^2 = 0.10$, NBOH: $R^2 = 0.37$; $F_{(1,620)} = 20.74$, $P < 0.0001$).

We confirmed the robustness of these findings by conducting Welch's one-sided t-tests after calculating individual linear regression curves for each animal, and found that NBOH-treated mice (poke efficiency: $M = 0.036$, $SD = 0.026$; percent correct: $M = 0.084$, $SD = 0.052$), relative to saline treated mice (poke efficiency: $M = 0.016$, $SD = 0.015$; percent correct: $M = 0.043$, $SD = 0.049$) perform better on average in the reversal phase (reversal poke efficiency: $t(17.40) = 2.29$, $p = 0.017$, Cohen's $d = 0.928$; reversal percent correct: $t(22.92) = 2.07$, $p = 0.0252$, Cohen's $d = 0.814$). This was consistent with the aforementioned results. Also consistent with the above findings, the individual linear regression analysis showed that there were no significant differences in both the forward poke efficiency ($t(22.58) = 0.380$, $p = 0.354$, Cohen's $d = 0.152$) and forward percent correct ($t(23.78) = -0.729$, $p = 0.763$, Cohen's $d = -0.286$) metrics between NBOH (forward poke efficiency: $M = 0.035$, $SD = 0.026$; percent correct: $M = 0.049$, $SD = 0.050$) and saline-treated mice (forward poke efficiency: $M = 0.031$, $SD = 0.026$; percent correct: $M = 0.063$, $SD = 0.053$).

Finally, we considered sex as a biological variable to determine whether NBOH improved learning in both sexes. We found that, consistent with our sex-independent results (Figure 2), NBOH treatment did not affect poke efficiency during the forward phase (Female: $F_{(1,334)} = 0.986$, $P = .322$; Male: $F_{(1,219)} = 0.004$, $P = 0.952$), but significantly enhanced poke efficiency during the reversal phase (Figure 3; Female; $F_{(1,319)} = 16$, $P < .0001$; Male: $F_{(1,205)} = 8.3$, $P = 0.0044$). Thus, psychedelic treatment induced a weeks-long lasting enhancement of reversal learning in both male and female mice. When comparing male and female poke efficiency, we found that male mice treated with saline performed slightly better than female mice in both the forward and reversal phases ($P = .0011$; $P = 0.0218$), and male mice treated with NBOH performed better in the reversal phase than female mice ($P = 0.0206$).

Discussion

This study sought to examine the effects of a single psychedelic dose on flexible learning. We found that even 2–3 weeks after a single dose, NBOH significantly enhanced reversal learning ability. Poke efficiency, percentage of correct trials, and cumulative pellets dispensed were all improved during the reversal phase in mice that received NBOH compared to mice that received saline. Both male and female mice displayed improved learning during the reversal phase with psychedelic treatment, highlighting the therapeutic potential of psychedelic medicine to boost cognitive flexibility in both sexes. The estrous cycle can influence cognitive flexibility performance in rodents (50). The task design utilized in our study (6 days for each of the forward and reversal phases) encompasses more than the full length of a mouse estrous cycle (4–5 days) and the analysis metric utilized here examines changes in the slope of learning across the full 6 days, thus calculating learning rates over the course of at least one full estrous cycle. Future work will help to better understand the precise influence of estrous cycle on higher temporal resolution changes in reversal learning properties.

This study contrasts with previous preclinical psychedelic reversal learning studies in terms of drug administration timepoints (46, 51–54). We administered the psychedelic or saline control 15 days before the

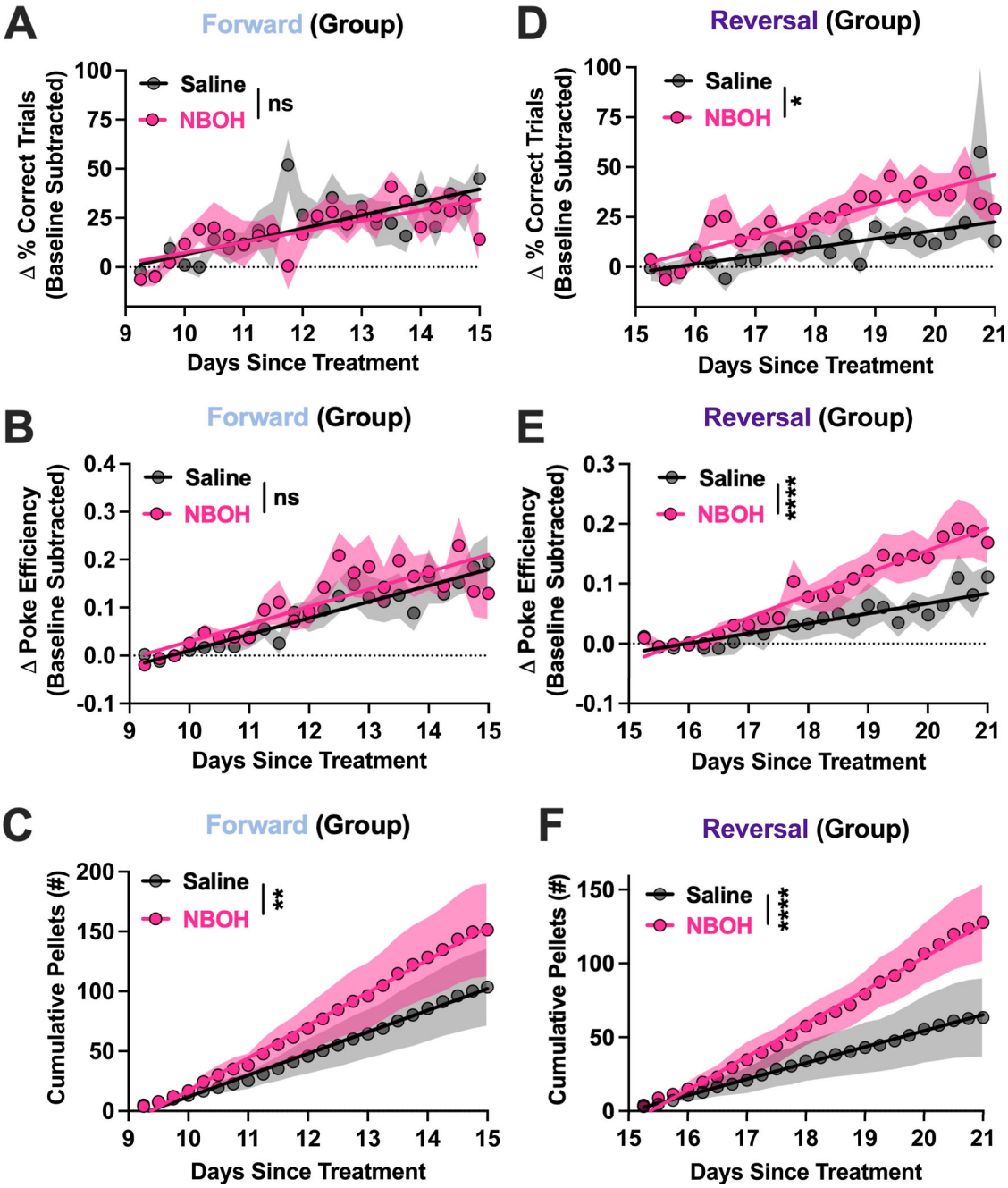

Figure 2. Single-dose psychedelic treatment induces a lasting reversal learning enhancement. (A) Group forward phase poke efficiency, with no significant effect of NBOH treatment. Each day has 4 points plotted corresponding to each hour of the 4-h per day sessions. (B) Group forward phase percentage of correct trials, indicating no significant effect of NBOH treatment. (C) NBOH treatment significantly increased the number of reward pellets dispensed during the forward phase. (D) NBOH treatment significantly increased poke efficiency during the reversal phase compared to saline injection, indicating enhanced cognitive flexibility. (E) NBOH increases the percentage of correct trials. (F) NBOH treatment significantly increased the number of reward pellets dispensed during the reversal phase. Shaded regions represent standard error of the mean (SEM), linear regressions shown in pink for NBOH and black for saline; ns, not significant; $^{**}p < 0.01$; $^{****}p < 0.0001$.

start of the reversal protocol. Thus, our study focuses on the longer-term therapeutic effects of the psychedelic drug. It is important to distinguish such longer-term effects from immediate or short-lasting acute effects, that may be more related to the mind-altering impact of psychedelics and not to their longer-term therapeutic effects. In a two-choice visual discrimination task, 25CN-NBOH (1–2 mg/kg) was found to have no significant effects on reversal learning in mice when administered acutely, immediately before testing (46). Other previous rodent studies using attentional set-shifting and T-maze paradigms found impairment of flexible learning with acute administration of the psychedelics DOI (1 mg/kg) or 25CN-NBOH (1 mg/kg) on cognitive flexibility (53, 54). However, one study found acutely enhanced cognitive flexibility with acute psilocybin (1 mg/kg) in the same attentional set shifting paradigm that found impairment following administration of DOI (54). The differences in the acute effects of psychedelics on reversal learning may be due to the study design, discussed below, as well as a combination of drug and dose. DOI and 25CN-NBOH are much more potent (>10x) than psilocybin (41, 42, 47–49). Concentration-dependent acute suppression of working memory

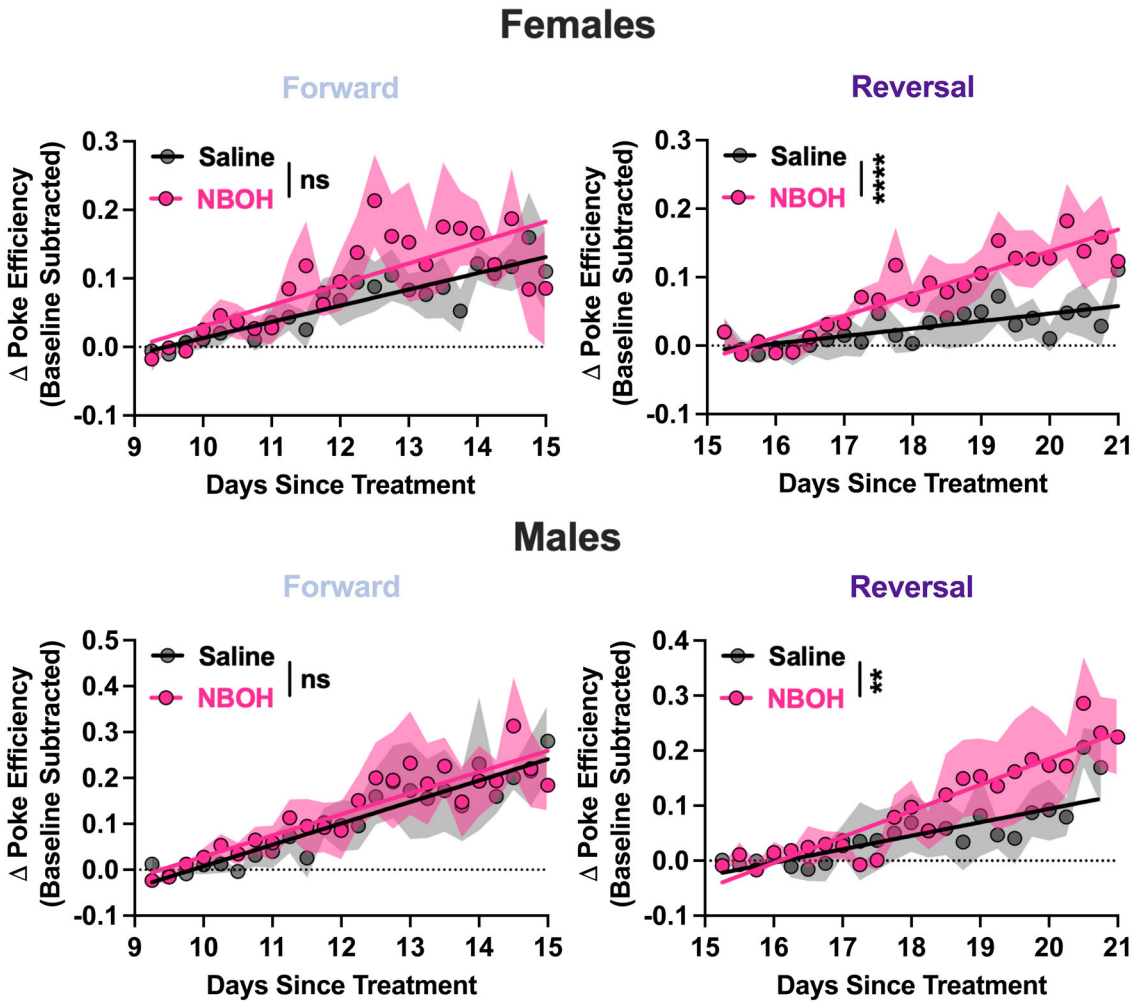

Figure 3. Lasting psychedelic enhancement of reversal learning ability in male and female mice. (Top) Forward and reversal phase changes to poke efficiency indicating NBOH treatment significantly improves reversal learning in female mice weeks after a single dose. (Bottom) Forward and reversal phase changes to poke efficiency in male mice indicating NBOH treatment significantly improves reversal learning weeks after a single dose. Shaded regions represent standard error of the mean (SEM), linear regressions shown in pink for NBOH and black for saline; ns, not significant; $^{**}p < 0.01$; $^{****}p < 0.0001$.

(27, 55–57) likely explains why the relatively less potent psychedelic psilocybin doesn't acutely impair behavioral performance at this dose. As the long-term effects of psychedelics on cognition are the effects that are more relevant therapeutically, it is important that future work continues to examine the sustained, in addition to acute, effects of psychedelics.

The current study also contrasts with previous studies in protocol design. We selected a reversal learning paradigm that is sufficiently complicated and somewhat easier to interpret compared to attentional set-shifting or T-maze paradigms. Two recent studies have also made use of reversal learning paradigms and have found long-term (study 1: 3 days; study 2: 14 days) positive effects of psychedelics on cognitive flexibility in female rats, but there are a few notable differences in behavioral protocols compared to the current study (51, 52). As opposed to conducting a sequential FR2-style task with only one reversal of the task, the 14-day long reversal study implemented an FR1 task that repeatedly reversed every 10 successful trials (51). While this study demonstrated that psilocybin increases the number of successes over time, it did not show if psilocybin improves accuracy, or if this is a function of increased trial initiations after psilocybin (51). Our FR2 style task with a single reversal after many days of training highlights the different effects psychedelics have on initial (forward) learning and reversal learning separately, which we would have otherwise been unable to do in a paradigm that frequently reverses. Our paradigm also likely results in fewer random successes that could inflate an animal's actual performance as we require precisely two sequential pokes in two separate holes. In addition, we conducted the task in both female and male mice. A similar study testing the long-term effects of DOI on reversal learning found that, depending on task structure, DOI has mixed effects on reversal learning ability (58). A week-long evaluation of initial learning after dosing appeared to assist in the enhancement of flexible learning, but if the animals were not exposed again to the task prior to reversal after dosing, DOI appears to have a negative effect on reversal learning ability. This finding suggests that further work needs to be done to evaluate what role practice following dosing has on cognitive flexibility.

In humans, psilocybin treatment has been found to improve cognitive flexibility up to 1 month after dosing (13, 14). However, these studies utilized a within-subject repeated measures design with no non-psilocybin control group (13), or with low dose psilocybin (1 mg) as the control group (14). Although promising, it is possible that behavioral performance was improved through familiarity with the task design rather than a direct result of the psychedelic treatment. It is currently unknown whether a single psychedelic dose would improve cognitive flexibility measured in a human study using independent measures.

PFC neurons have been shown to undergo spinogenesis and synaptogenesis after a single psychedelic administration through a pathway requiring 5-HT_{2A} receptor activation (23–31), but the precise mechanisms of 5-HT_{2A} receptor induced flexible learning and how long these benefits can last are still unknown. Here, we use 25CN-NBOH, which is 50–100x more selective for 5-HT_{2A} receptors over 5-HT_{2B} and 5-HT_{2C} receptors and has even weaker affinity for other 5-HT receptors (41, 42, 49). Future research into the long-term effects of other psychedelic drugs on cognitive flexibility will need to be conducted to examine whether psychedelics that target additional 5-HT receptor subtypes have similar long-lasting effects, or to determine whether the interaction with other 5-HT receptors abolishes the ability to enhance long-lasting flexibility. In addition, it remains unknown if non-hallucinogenic 5-HT_{2A} receptor agonists such as 2-bromo-LSD, lisuride, and 6-fluoro-diethyltryptamine (59–61), are also able to induce a lasting enhancement of flexible learning.

Psychedelic-mediated weeks-long enhancement of reversal learning ability allows for many further directions of research. Future studies will examine the effects that psychedelics have on mice across different ages. Additional studies will also determine the effects of different psychedelic drugs, dose levels, number of doses, or dose timing in this behavioral paradigm. While we did find an enhancement in a mouse model of cognitive flexibility with a 5-HT_{2A} receptor agonist, we did not use 5-HT_{2A} knockout mice to see if the absence of 5-HT_{2A} receptors would cause any deficits in this behavior, or if psychedelics would improve this behavior without the engagement of 5-HT_{2A} receptors.

A long-term positive psychedelic-induced enhancement of cognitive flexibility has several implications for future human psychedelic medicine, specifically for pathologies that involve deficits in executive function or synaptic loss. Cognitive flexibility is impaired in many disorders, including depression, PTSD, and Alzheimer's disease (AD) (62–64). While clinical trials evaluating the impact of psychedelic medicine on depression and PTSD are already underway and have shown promising results (13, 14), psychedelics have not yet been used to try to treat cognitive flexibility in AD and related neurodegenerative diseases. Additional research using mouse models of AD would be important to mechanistically demonstrate that psychedelics can indeed boost flexibility in these models and to confirm that psychedelics can also boost long-term synaptic activity in brain regions related to cognitive flexibility, such as the PFC in these same preclinical models. The task design presented here will facilitate future studies that can address these and other questions. This will allow for an even greater mechanistic understanding of the relationship between psychedelic treatment and cognitive flexibility.

Materials and Methods

Animals and Behavioral Apparatus

The open source, programmable FED3 device was used for all behavioral experiments. We programmed the FED3 via Arduino to deliver a 10 mg reward pellet if an animal successfully pressed the correct sequence of nose poke holes (left-then-right or right-then-left within 30 s, depending on the forward or reversal phase of the task). The reward pellets used in this study were 10 mg Bio-Serv Dustless Precision Pellets in the chocolate flavor. The cages used with the FED3 devices were modified standard mouse housing cages, with holes drilled into the front of the cage and magnets affixed to the cage's front to allow the animal to interact with the reward well and nose poke holes and ensure the device stays flush to the side of the cage during data collection. Data were collected within the animals' vivarium on a static shelf to minimize any effect of changing locations on stress and ensure ample room for both the cage and the device on the shelf. Each animal had their own experimentation cage and FED3 used for the duration of the experiment to minimize the stress of unfamiliar environment and odors. The animals' vivarium ran on a reversed light cycle with lights off (dark phase) from 7:00 AM to 7:00 PM. Each day, data were collected from approximately 10:00 AM to 2:00 PM, within the vivarium's dark phase. After each 4-h session, the animals were returned to their home cages until the next day. A total of 27 adult male and female C57BL/6 mice with a mean age of ~6 months were used in experiments, but the data from one mouse were excluded because the mouse did not interact with the FED3 device. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals.

Procedure

Animals were injected intraperitoneally with either saline to function as a control ($N = 14$), or 25CN-NBOH ($N = 12$ mice) a 5-HT_{2A} receptor agonist, purchased from Tocris Bioscience, at a dose of 10 mg/kg (a dose previously shown to induce psychedelic-like effects in mice (44)), dissolved in sterile saline and brought up to a total volume less than 1% of the mouse weight. To allow the blinding of the main experimenter, these injections were done by another experimenter, and the main experimenter remained blinded until after the protocol had been completed. After injection, the animals were left to rest for 24 h in their home cage before beginning an 85% free feeding weight schedule for 2 days. During those 2 days, a few reward pellets were dropped into each animal's cage. This was done to ensure proper food motivation and acclimation to the pellets.

After 2 days of food restriction, animals were introduced to a training period to acclimatize to the FED3. After this point, most of the daily food was obtained through the FED3 device; chow was added to home cages supplementally as needed to maintain at least 85% free-feeding weight. However, most mice returned to free-feeding weight over the course of the full protocol. For 2 days, the animals underwent the habituation phase of the protocol, in which the animals were introduced to the FED3 and experimentation cages. Over the course of two separate 4-h sessions, the FED3 automatically delivered a reward pellet every 4 min and in response to any pokes to either nose poke hole. After the 2 days of habituation, the animals began fixed-ratio 1 (FR1) training phase in which a reward pellet would be delivered any time the mouse poked the left nose poke hole. Similarly, these sessions were 4 h long each and took place over the course of 3 days.

After the 5 days of training were completed, the mouse was then introduced to the sequential fixed-ratio 2 (SEQFR2)-forward and SEQFR2-reversal phases. To receive a reward pellet in the SEQFR2-forward phase, the animal must poke the left nose poke hole followed by the right nose poke hole within 30 s. Should the animal poke the right hole in isolation, the left hole twice in a row, or not follow a left poke with a right poke, the device entered a 10-s timeout phase in which no further pokes would be registered and any nose pokes during this timeout period were ignored. Like all other sessions, these sessions lasted for 4 h each over the course of 6 days. After those 6 days elapsed, the SEQFR2 rule was reversed, meaning the animal had to poke the right nose poke hole first followed by the left to receive a reward pellet. Like the forward phase, the reversal phase lasted for 6 days. After the protocol was completed, mice were returned to their normal feeding schedules, cages were cleaned and sanitized with Liquinox lab detergent, and FED3s were sanitized with 70% ethanol. The experimental timeline and protocol overviews are summarized in Figure 1.

Behavioral Analysis

In the SEQFR2 task, poke efficiency is the main measure of performance. This was calculated by finding the proportion of pellets dispensed out of all pokes carried out by the animal in each hour. As additional metrics of behavioral performance, we examined cumulative pellets dispensed over the course of each phase, as well as the proportion of correct trials initiated out of all trials ("percent correct"). Together, these three metrics reflect the absolute performance animals over the course of the task (cumulative pellets), as well performance relative to the amount of engagement with the device (poke efficiency and percent correct).

Statistics

Statistical procedures were performed with Prism GraphPad (version 10.3.0) and R. We conducted multiple linear regression analyses as our primary statistical method, which has also been used as the analysis method in other reversal learning paradigms (66–68). To ensure the robustness of our results, we conducted additional analyses by calculating each individual animal's regression curve and conducting Welch's one-sided t-tests to compare the saline and NBOH cohorts. Further statistical test information and significance are provided in the results section and figure legends.

Data Availability

Data generated in this study is available from the corresponding author upon reasonable request.

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Author Contributions

Elizabeth Brouns acted as an investigator for this study. She conducted all the data collection, developed the task, contributed significantly to the writing of the final manuscript, generated figures, conducted preliminary analyses, managed data, and planned all animals run on this task. Tyler Ekins acted as an investigator for this study. He injected all mice to keep the main experimenter blinded, ran final statistical analyses, generated figures, and contributed significantly to the writing of this manuscript. Omar Ahmed was the principal investigator and senior author of this study, designed the study, developed the task, helped with planning the animals run on the task, contributed to figure design, and contributed significantly to the writing and editing of this manuscript.

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Author Disclosures

The authors declare no competing financial interest.

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