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Lysergic Acid Diethylamide Produces Anxiogenic Effects in the Rat Light/Dark Test and Elevated Plus Maze

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ABSTRACT. Recent clinical trials indicate favorable therapeutic outcomes of psychedelic-assisted psychotherapy for anxiety and treatment-resistant depression. Whereas the neurobiological systems underlying these effects are not well understood, animal behavioral models can serve to investigate these mechanisms. For the current study, we implemented 2 rodent models predictive of anxiolytic drug effects, a light/dark test and an elevated plus maze (EPM), to investigate the acute and subchronic effects of LSD, respectively. Forty-eight, adult male Sprague-Dawley rats were randomly assigned to receive LSD (0.00, 0.02, 0.04, 0.08 mg/kg) and assessed in the light/dark test 15 min after the first injection. Five additional injections were given, once every 48 hours, after which rats were assessed in the EPM either 48 (n = 24) or 72 hours (n = 24) after the last injection. A dose-dependent and statistically significant decrease was observed in number of entries into, $F(3, 44) = 12.79$, $p < .001$,$\eta^2 = .47$, and time spent, $F(3, 48) = 14.15$, $p < .001$, $\eta_p^2 = .47$, in the brightly lit compartment. In the EPM, closed arm entries, $F(1, 17) = 28.85$, $p < .001$, $\eta_p^2 = .36$, and time spent in closed arms, $F(1, 17) = 20.14$, $p < .001$, $\eta_p^2 = .99$, were significantly higher compared to entries or time in open arms by rats assessed 48 hours after the last LSD injection, but not by rats tested 72 hours after the last injection. These findings indicate that acute and subchronic LSD treatment produce transient anxiogenic effects. In consideration of positive therapeutic outcomes of psychedelic-assisted psychotherapy, alternative preclinical models may be warranted to discern the mechanisms underlying the putative therapeutic effects of serotonergic hallucinogens.

Keywords: Lysergic acid diethylamide, elevated plus maze, light/dark test, anxiety, rats

The psychedelic drug, *lysergic acid diethylamide* (LSD), has been used recreationally since the 1960s. Despite early indications of possible psychotherapeutic effects of various psychedelics (Liechti, 2017), negative public and political views of these substances stagnated clinical research for nearly 50 years. Consequently, controlled clinical investigations were not conducted until recently. For example, a recent investigation by Carhart-Harris et al. (2016) indicated that a single, acute dose of LSD (75 μg) can improve mood and increase cognitive flexibility among healthy participants. Additionally, anecdotal reports of psychedelic use indicate a popular trend in "microdosing" regular-interval use of LSD doses 10 to 20-fold lower than the typical hallucinogenic dose (Anderson et al., 2019; Fadiman & Korb, 2019). These self-reports have consistently indicated that microdosing of hallucinogens improved mood, enhanced sociability, and reduced symptoms of depression and anxiety among users (Johnstad, 2018; Polito & Stevenson, 2019). However, current trends in low-dose administration of psychedelics have been minimally researched. To consider the use of hallucinogens as an alternative therapy for treatment-resistant anxiety and depressive

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disorders, more substantial controlled clinical and preclinical analyses are warranted.

Similar to conventional antidepressant drugs, LSD and other classic hallucinogens exert their actions on brain serotonergic systems (Carhart-Harris & Goodwin, 2017). Specifically, LSD primarily binds to and activates the serotonin (5-HT) receptor subtypes, 5-HT_{1A} and 5-HT_{2A}, both of which are presumed to regulate symptoms associated with the onset of depression (Buchborn et al., 2014). Recent studies also suggest that LSD has a pleiotropic mechanism of action, affecting 5-HT, DA, and glutamatergic receptors (Gregorio et al., 2018). LSD is a partial agonist at 5-HT_{2A} receptors, with its main psychoactive effects thought to be mediated via 5-HT_{2A} receptors (Carhart-Harris & Goodwin, 2017).

Related to LSD's potentially therapeutic pharmacological mechanisms of action, recent clinical investigations have explored psychedelic-assisted psychotherapy with psilocybin, a hallucinogen with similar actions to LSD. Psychedelic-assisted psychotherapy typically involves drug-free sessions before and after treatment, which aid in acclimating participants to the therapeutic environment and consolidating new information after each session. During treatment, subjects are supervised and assisted by a mental health professional and provided music to listen to while exploring introspective thoughts and memories (Schenberg, 2018). Recent studies have demonstrated clinically significant benefits of psilocybin-assisted psychotherapy among patients suffering from treatment-resistant anxiety and depressive disorders (Carhart-Harris et al., 2018; Griffiths et al., 2016). Furthermore, a report by Dolder et al. (2016) indicated that a single dose of LSD could increase trustfulness, suggestibility, reduce anxiety, and provide substantial mental health benefits during LSD-assisted psychotherapy in healthy volunteers. Carhart-Harris et al. (2016) also investigated the effects of a single, acute dose of LSD (75 μg) among healthy participants. They observed self-reported heightened mood among treatment groups and a lasting effect for at least two weeks. Although these studies provide encouraging results regarding reduced negative emotions and enhanced emotional empathy, the translational aspect of hallucinogens and its effects on negative affect in depressed and anxious populations is yet to be studied systematically in a controlled clinical setting. Therefore, research on the long-term behavioral consequences of LSD use is limited and requires a more substantial characterization before serious considerations can be made for widespread clinical use.

Although the aforementioned findings offer a compelling rationale for the consideration of hallucinogens for treatment-resistant mood disorders, current trends in low-dose administration of these substances have been minimally researched. Moreover, no controlled clinical studies have been implemented on the short and long-lasting outcomes of microdosing. Most published research on these effects have been obtained through online interviews, surveys, or private chats. Although limited research has been conducted using face-to-face participant involvement, a few articles have directly analyzed the acute effects of psychedelic microdosing among human subjects. Specifically, Prochazkova et al. (2018) recently assessed acute effects of ingested psychedelic truffles in an open-labeled setting and found a significant difference in divergent and convergent thinking among participants. In a separate within-subject study, volunteers were given varying low-doses of LSD (0 μg, 6.5 μg, 13 μg, or 26 μg) in one-week intervals (Bershad et al., 2019). Participants completed behavioral tasks and mood questionnaires around peak drug effect, with results indicating dose-related effects among higher LSD doses. Additionally, Bershad et al. (2019) reported a significant increase in reports of anxiety as LSD dose increased, with no effect on depression symptoms. However, participants were healthy volunteers with no previously diagnosed mental disorders, which may explain the lack of anxiolytic effect as compared to other studies involving self-reports.

Despite a resurgence in clinical investigations of the psychotherapeutic effects of hallucinogens, only a few published preclinical studies have evaluated the psychotherapeutic potential of these substances (Cameron et al., 2019; Hibicke et al., 2020; Horsley et al., 2018; Favaro et al., 2015). For example, a recent study conducted by Horsley et al. (2018) evaluated the persistent effects of intermittent microdosing with ketamine (0.5 or 3 mg/kg) or psilocin (0.05 or 0.075 mg/kg) in male rats. Over the course of six days, rats received three injections of one of the aforementioned drugs: ketamine, psilocin, or saline, with the last injection occurring 48 hours before behavioral assessment in an elevated plus maze (EPM). Ketamine (0.05 mg/kg) significantly decreased frequency of entries into the open arms and increased mean distance traveled per visit to the open arms, whereas psilocin (0.05 mg/kg) significantly reduced open arm entries. The main findings suggested that brief,

intermittent treatment with low doses of these substances induced a mild anxiogenic effect.

In a similar investigation, long-term administration of ayahuasca was used to examine memory and anxiety among male rats (Favaro et al., 2015). In that study, rats received daily oral treatments with water or ayahuasca (120 mg/kg, 240 mg/kg, 480 mg/kg) for 30 days and were assessed in the EPM 48 hours after the last treatment. No significant treatment effects were observed. These findings suggested that chronic oral administration of ayahuasca does not induce either anxiogenesis or anxiolysis in this rodent model (Favaro et al., 2015). Another study using the primary psychoactive agent contained within ayahuasca (DMT) evaluated the effects of chronic, intermittent injections on behavior in a battery of assessments predictive of anxiety and depression (Cameron et al., 2019). In this investigation, male and female rats were injected with DMT (1 mg/kg) every third day over an eight-week period. Rodents were assessed at various times over this period in a battery of assays predictive of anxiety. No significant treatment effects were evident in the novelty-induced locomotion or EPM paradigms. However, this drug regimen produced antidepressant-like effects in the forced swim test (Cameron et al., 2019). Collectively, these studies have produced marginally significant and varied results regarding the effects of intermittent psychedelic treatments.

In consideration of the lack of preclinical investigation of LSD's psychotherapeutic potential, the objective of the current study was to aid in expanding the limited knowledge regarding the behavioral effects of LSD in preclinical models predictive of anxiolytic drug effects. Behavioral assessments predictive of anxiolytic drug action provide an important and necessary component for determining the potential uses of novel substances such as psychedelics. The current study implemented two rodent models predictive of anxiolytic drug effects, a *light/dark test* and an *elevated plus maze* (EPM) to assess the potential acute and subchronic behavioral effects of low-dose LSD treatment, respectively. The light/dark box assessment measures anxiety-like behaviors in rodents by using their natural aversion to bright light and novel environments, whereas the EPM evaluates anxiety in rodents by producing an approach-avoidance conflict (Crawley & Goodwin, 1980; Walf & Frye, 2007). It was hypothesized that acute treatment would produce anxiogenic effects, particularly at higher doses (LSD 0.08 mg/kg), and that intermittent treatment with low doses (LSD 0.02 mg/kg) might produce anxiolytic effects.

Methods

Subjects

Forty-eight, adult male Sprague-Dawley rats (Charles River Laboratories,Wilmington, MA, USA), weighing 420 to 600 grams, were used in this study. The rats were housed individually in polycarbonate cages lined with corncob bedding (Harlan Laboratories, Haslett, MI, USA) in animal facilities maintained at a constant temperature (20 ± 2 °C) and humidity (50 ± 5%). In addition, rodents were under a reverse 12:12 light/dark cycle with lights on from 19:00 to 07:00 h. Commercial rodent chow (Purina®, Richmond, IN, USA) and water was provided at all times in the home cages.

Apparatus

The light/dark test was conducted in six identical test chambers (MED-CPP2-RS; MED Associates Inc., St. Albans, VT, USA), each composed of two distinct compartments. The dark compartment had black walls and steel rod flooring. The light compartment consisted of white walls and steel grid flooring, with extra illumination provided by overhead standard desktop lamps. Each chamber was connected to an interface and computer running MED-PC version IV software (MED Associates Inc.). Infrared beam breaks were recorded to determine activity and time spent in each compartment.

A custom built EPM was used to evaluate the subchronic effects of LSD treatment. The apparatus was constructed of wood and painted black. The maze was elevated approximately 50 centimeters above the floor, and contained two enclosed arms (50 cm long, 10 cm wide, and walls 40 cm high) and two open arms (50 cm long, 10 cm wide, and walls 2 cm high). A video camera mounted above the maze was used to record each 5 min test session. Behavioral tracking software (ANY-maze®, Stoelting Co., Wood Dale, IL, USA) was used to analyze time spent and number of entries into each arm.

Drugs

Lysergic acid diethylamide was provided by the National Institute on Drug Abuse Drug Control Supply (Bethesda, MD). LSD was dissolved in 0.9% bacteriostatic saline and administered by intraperitoneal injections. Doses were determined based on the weight of the salt.

Procedures

Study procedures were reviewed and approved by the Western Michigan University Institutional Animal Care and Use Committee (IACUC Protocol

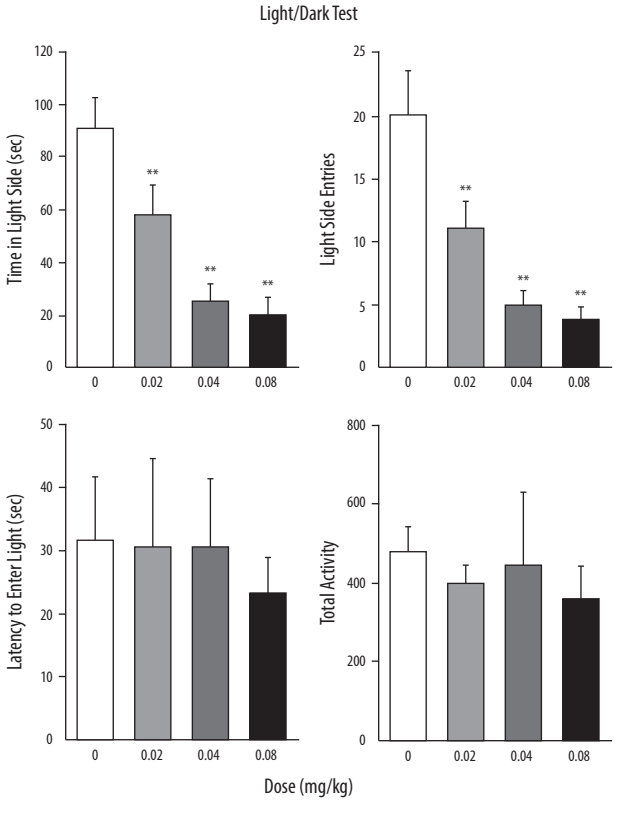
Number 19-05-04).

Light/Dark Test

Subjects were randomly assigned to one of four treatment groups: saline, 0.02 mg/kg of LSD, 0.04 mg/kg of LSD, or 0.08 mg/kg of LSD. To be consistent with a previous published study by Horsley et al. (2018), rats were tested during the light phase (19:00–24:00). Before beginning the light/dark box test, rats were acclimated in their home cages to the laboratory environment for one hour before treatment. Injections were given 15 min prior to placement into the test apparatus for a 5 min period. Rats were individually placed into the dark compartment of each test chamber. Latency to enter the brightly lit compartment as well as activity and time spent in each compartment were recorded. Eight squads of six rats were tested at the same time, with treatment groups counterbalanced among test squads.

FIGURE 1

Acute Effects of LSD on the Light/Dark Box Test



Note. Acute LSD treatment effects on time spent, entries into, latency to enter the light, and activity counts during the light/dark test. Bars represent treatment group means (±S.E.M.). Statistically significant differences from saline-control are indicated by $^{**}(p < .001)$.

Elevated Plus Maze

The same rats used in the light/dark test continued to receive the aforementioned treatments (LSD 0, 0.02, 0.04, 0.08) every other day for a total of six injections. Following a two or three-day drug washout phase, each rat was individually placed in the EPM for a 5-minute test period during their light phase. Half of the animals in each treatment group were assessed 48 hours after the last injection and the remaining animals were assessed 72 hours after the last injection. Each rat was individually placed facing an open end of the arm, alternating the direction for every other animal. Activity was recorded by a camera mounted above the EPM. The behavioral tracking software, ANY-maze®, was used to record number of entries into the closed and open arms, latency to enter the closed and open arms, time spent in each arm of the maze, and distance travelled. If a rat fell or jumped off the maze, it was excluded from analysis.

Data Analysis

Light/Dark Test

A one-way ANOVA was used to determine any statistically significant effect of treatment on time spent in the lit chamber, light side entries, latency to enter the lit chamber, or total activity counts. This was followed by a Holm-Sidak multiple comparisons test to ascertain which treatment groups differed significantly from the saline-treated control group.

Elevated Plus Maze

EPM test results were analyzed using a two-way ANOVA (arm type, treatment group) to deduce if LSD had a statistically significant effect on latency to enter the closed or open arms, number of entries into the closed or open arms, or percentage of time spent in the closed or open arms. In addition, a two-way ANOVA was conducted to determine any statistically significant effects of treatment group or washout period (48 or 72 hours) on these dependent measures. Statistically significant findings were further evaluated with multiple comparisons using the Holm-Sidak multiple comparisons test.

Results

The main objective of this study was to evaluate acute and subchronic LSD treatment in behavioral tests predictive of anxiolytic drug effects, the light dark box and EPM, respectively. Of the 48 subjects, five were excluded from analysis due to experimenter error (3), failure to meet exclusionary criteria (1), or unexpected death (1) unrelated to the experiment.

Light/Dark Box

All dependent variables were assessed separately with a one-way ANOVA to determine any statistically significant differences among treatment groups. As shown in Figure 1, acute LSD treatment produced a dose-dependent and statistically significant decrease in time spent, $F(3, 48) = 14.15$, $p < .001$, $\eta^2 = .47$, and entries into, $F(3, 44) = 12.79$, $p < .001$, $\eta^2 = .47$, the brightly lit chamber. Additionally, Holm-Sidak multiple comparisons indicated statistically significant differences in time spent ($p < .01$) and entries into ($p < .001$) the lit chamber for all LSD treatment groups compared to the saline-control group. No statistically significant differences were observed among treatment groups in latency to enter the lit chamber or in total activity during the light/dark test.

Elevated Plus Maze

Half the animals in each treatment group were assessed in the EPM 48 hours after the last injection, and the remaining animals were assessed 72 hours after the last injection. Therefore, each EPM dependent variable was statistically analyzed for the 48-hour and the 72-hour washout period separately. These results are displayed in Figure 2.

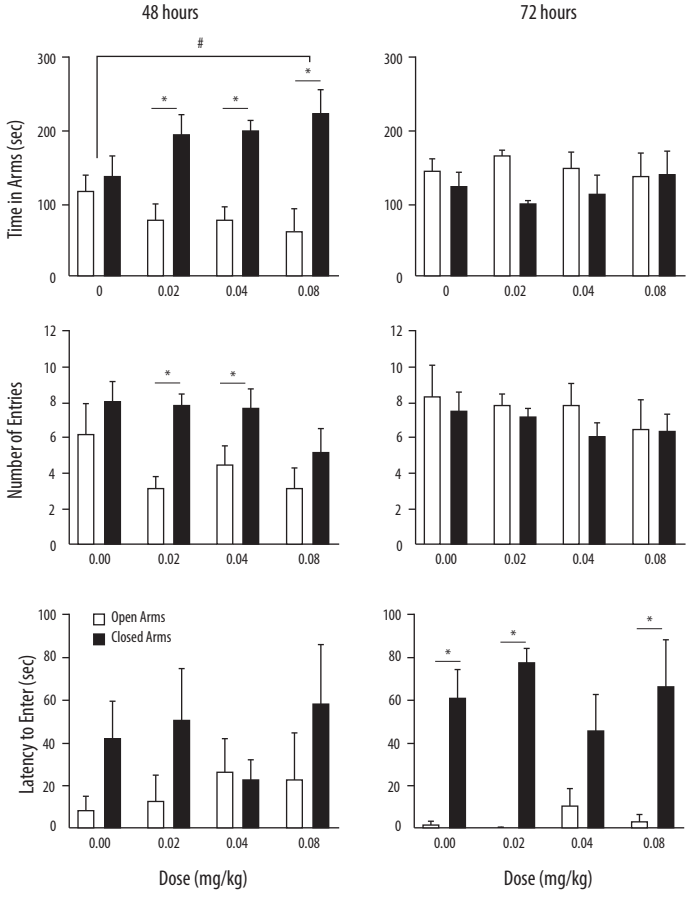
Among subjects tested after a 48-hour washout phase, a two-way ANOVA (dose, arm type) of time spent in each arm type indicated a statistically significant effect of dose, $F(3, 17) = 4.59$, $p = .016$, $\eta_p^2 = .01$, and arm type, $F(1, 17) = 20.14$, $p = .001$, $\eta_p^2 = .99$, but no dose by arm type interaction. A Holm-Sidak multiple comparisons analysis indicated that all LSD doses produced a statistically significant difference in time spent in open versus closed arms ($p < .05$), whereas saline-treated controls displayed no significant difference between time spent in open and closed arms. Additionally, multiple comparisons indicated a statistically significant difference ($p < .05$) in time spent in either arm type only between the LSD 0.08 mg/kg treated animals and the saline-treated animals. A similar analysis of these results from animals assessed after a 72-hour washout period indicated no statistically significant effect of dose, arm type, or dose by arm type interaction.

A two-way ANOVA (dose, arm type) on the number of arm entries by the 48 hour washout group yielded statistical significance of arm type, $F(1, 17) = 28.85$, $p < .001$, $\eta_p^2 = .36$, but no statistical significance of dose. In addition, multiple comparisons revealed statistically significant differences between open and closed arm entries for subjects assessed 48 hours after 0.02 mg/kg LSD ($p < .01$) or 0.04 mg/kg LSD ($p < .05$). A similar analysis revealed no statistically significant differences among treatment groups or between closed and open arm entries among subjects assessed 72 hours after the last injection.

Statistical analysis of latency to enter arms among the 48-hour washout group revealed no statistically significant differences among treatment group or between open and closed arms. In contrast, a two-way ANOVA (dose, arm type) on latency to enter arms for the 72-hour washout group indicated statistical significance of arm type, $F(1, 18) = 43.97$, $p < .0001$, $\eta_p^2 = .77$, but no statistically significant dose effect. A Holm-Sidak multiple comparisons analysis revealed statistically significant

FIGURE 2

Subchronic Effects of LSD on the Elevated Plus Maze Assessment



Note. Subchronic LSD treatment effects on latency to enter, number of entries, and time spent in open (white bars) and closed arms (black bars) in animals tested either 48 hours (left) or 72 hours (right) after the last injection. Bars represent treatment group means (± S.E.M.). Statistically significant differences between open and closed arms are indicated by * ($p < .05$); # indicates a statistically significant difference from saline control group ($p < .05$).

differences ($p < .01$) in latency to enter the closed versus open arms for subjects assessed 72 hours after saline, 0.02 mg/kg, and 0.08 mg/kg LSD.

Additional statistical analyses were conducted to determine any statistically significant effects of washout period. A two-way ANOVA (dose, washout period) on time spent in the open arms indicated a statistically significant main effect of washout period, $F(1, 35) = 18.69$, $p = .001$, $\eta_p^2 = .35$, but no statistically significant dose effect or dose by washout interaction. A similar statistical analysis on time spent in the closed arms indicated a statistically significant main effect of washout period, $F(1, 35) = 16.45$, $p = .001$, $\eta_p^2 = .32$, but no significant dose effect or dose by washout period interaction. Holm-Sidak multiple comparisons indicated that these differences between the 48- and 72-hour washout period were statistically significant only for the 0.02 mg/kg LSD treated animals ($p < .05$).

A two-way ANOVA (dose, washout) revealed a statistically significant main effect of washout period on open arm entries, $F(1, 35) = 12.95$, $p = .001$, $\eta_p^2 = .27$, but no significant dose effect and/or dose by washout period interaction. Although the main effect of washout period was statistically significant, Holm-Sidak multiple comparisons revealed no significant differences between 48- and 72-hour washout tests for any particular treatment group. A similar analysis of closed arm entries indicated no statistically significant effects of dose or washout period. Finally, no statistically significant effects of dose or washout period were observed for latency to enter either open arms or closed arms.

Discussion

The current findings indicate that acute LSD produces dose-dependent anxiogenic effects in male rodents. Specifically, LSD treatment decreased time spent and number of entries into the brightly lit chamber in the light/dark test. An extensive search of the research literature revealed few published preclinical studies on the acute effects of psychedelics on behavioral measures of anxiety. However, a few clinical reports have indicated that moderate, single-dose treatments of LSD produce anxiolytic effects (Carhart-Harris et al., 2016; Santos et al., 2016). In contrast, a recent study assessing the acute effects of low-dose LSD in healthy subjects found increases in self-reported anxiety as dosage increased (Bershad et al., 2019).

Statistical analyses of EPM results accounting for washout period revealed that animals assessed 48 hours after the last injection exhibited statistically significant differences in the number of closed arm entries and time spent in closed arms, whereas animals assessed 72 hours after the last injection did not exhibit these differences. These findings indicate mild anxiogenic effects of brief, intermittent LSD treatment, but these effects do not appear to persist beyond 48 hours.

Although no previous studies were found assessing the subchronic effects of LSD on anxiety in a preclinical research paradigm, a few published studies have evaluated the effects of other psychedelic drugs, including psilocin, ketamine, or DMT. However, the few reports found were inconsistent regarding anxiolytic or anxiogenic effects of these substances. The current findings are consistent with those of Horsley et al. (2018) who reported mild anxiogenic effects following brief, intermittent dosing with psilocin or ketamine in the EPM test. Oppositely, Cameron et al. (2019) reported that chronic, intermittent DMT administration in rodents resulted in antidepressant-like effects in a battery of behavioral assessments. Perhaps differences in pharmacological mechanisms of action across different subtypes of hallucinogens account for these conflicting findings. Moreover, the aforementioned studies implemented different dosing regimens. Across clinical research, numerous self-reports have suggested that regular interval ingestion of low-dose hallucinogens relieves anxiety symptoms and improves mood (Cameron et al., 2020; Fadiman & Korb, 2019; Johnstad, 2018; Polito & Stevenson, 2019). However, few studies have assessed the long-term effects of low-dose administration of psychedelic substances on mood in a controlled clinical setting.

In consideration of the full scope of available literature on the subjective effects of psychedelics, the current results reflect a clear translational boundary between preclinical behavioral assessments and clinical applications. Rodent models of unconditioned anxiety have a long history of inconsistent findings with antidepressant and antianxiety medications (Ennaceur, 2014). The construct validity of these assessments depends mainly on their ability to detect substances that have anxiolytic effects, which have been primarily upheld with benzodiazepines. However, mixed results have been found among 5-HT_{1A} agonists, selective serotonin reuptake inhibitors, and tricyclic antidepressants (Ennaceur, 2014). Although preclinical investigations are a necessary component for determining the potential psychotherapeutic effects of novel treatments, standardized animal models

have failed to provide consistency in determining the full therapeutic potential of hallucinogens in clinical settings.

Two common issues, construct and face validity, arise when determining the accuracy of behavioral assessments predictive of anxiety. As defined by Ennaceur (2014, p.56), "anxiety is a negative emotional state associated with the perception of potential or ambiguous threat," which is typically modeled with aversion or avoidance-learning in preclinical tests. These models utilize unconditioned aversive stimuli, such as bright light or open spaces, to predict treatment responses to various anxiety-altering drugs. However, animal assessments fail to accurately model or measure elements of human anxiety involving emotional processing and reactivity. Hence, the construct validity of these tests has repeatedly come into question as preclinical findings fail to address the emotional aspect of anxiety in addition to behavior.

The face validity of unconditioned anxiety assessments in rodents is also commonly disputed. In particular, changes in behavioral responses to aversive stimuli may be due to drug-induced sensory distortion, perception, or motor functioning rather than anxiety-like behavior. For example, benzodiazepines have consistently produced "anxiolytic" responses in rodents, but these changes in behavior are largely due to their sedative and cognitive/motor impairing effects (Ennaceur, 2014). Thus, sensitivity to external stimuli, like light or open spaces, is reduced. Oppositely, the current study found acute doses of LSD to decrease time spent in the lit chamber of the light/dark box. The sensory and perceptual changes from psychedelic-induced activation of cerebral cortex regions dense in 5-HT_{2A} receptors may have enhanced sensitivity to bright light in the light/dark box test. This in turn implies that psychedelics stimulate sensory systems but may not accurately measure how they affect anxiety in terms of higher-order emotional processing. Therefore, these models may reflect simpler behavioral characteristics of anxiety that may be unrelated to introspective processing associated with psychedelic effects reported by humans.

The psychoactive effects of serotonergic hallucinogens vary greatly from typical antidepressants. As noted by Nutt and colleagues (2020), the current understanding of psychedelics is that they reduce anxiety symptoms by altering networks associated with high emotional reactivity, which in turn increases introspectiveness and openness among users. Perhaps the beneficial effects of hallucinogens are not directly measurable in animal models that rely solely on behavioral modification in the presence of novel stimuli.

The current findings provide preliminary indications of the anxiety-increasing effects of low-dose LSD in animal models. Due to the limited knowledge of psychedelic effects on anxiety, this study assessed both acute and subchronic effects of LSD. However, the limited number of behavioral assessments and the brief subchronic dose regimen limit the generalizability of the current findings. To fully characterize the potential psychotherapeutic effects of hallucinogens, more substantial preclinical research is needed to examine the mechanisms underlying their putative treatment efficacy. Subsequent preclinical analyses could evaluate various hallucinogens and their effects in a multitude of behavioral models predictive of anxiety. In addition, wider dose variations and longer dose regimens are recommended to elucidate the dose-length and threshold for potential therapeutic effects. The inclusion of female rodents in future preclinical studies would also add to the generalizability of findings.

This study is the first to assess the anxiety-altering effects of low-dose LSD treatment in a preclinical behavioral paradigm. In addition, our procedures were intended to propose a standardized method for screening the acute and subchronic effects of psychedelics. Although the current study results are comparable to those of Horsley et al. (2018), who assessed psilocin and ketamine in the EPM, additional preclinical research is needed to fully discern the impact of LSD on anxiety and its potential clinical uses. Moreover, continued development of appropriate preclinical models is essential to understanding the behavioral and neurobiological mechanisms underlying their putative therapeutic effects.

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