ACS Chemical Neuroscience

pubs.acs.org/chemneuro Research Article

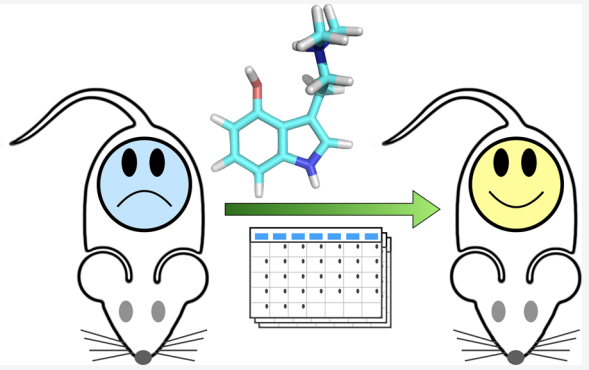
Psychedelics, but Not Ketamine, Produce Persistent Antidepressant-like Effects in a Rodent Experimental System for the Study of Depression

Meghan Hibicke, Alexus N. Landry, Hannah M. Kramer, Zoe K. Talman, and Charles D. Nichols*

Cite This: ACS Chem. Neurosci. 2020, 11, 864–871 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Psilocybin shows efficacy to alleviate depression in human clinical trials for six or more months after only one or two treatments. Another hallucinogenic drug, esketamine, has recently been U.S. Food and Drug Administration (FDA)-approved as a rapid-acting antidepressant. The mechanistic basis for the antidepressant effects of psilocybin and ketamine appear to be conserved. The efficacy of these two medications has not, however, been directly compared either clinically or preclinically. Further, whether or not a profound subjective existential experience is necessary for psilocybin to have antidepressant effects is unknown. To address these questions, we tested psilocybin, lysergic acid diethylamide (LSD), and ketamine in a rat model for depression. As in humans, a single administration of psilocybin or LSD produced persistent antidepressant-like effects in our model. In contrast, ketamine produced only a transient antidepressant-like effect. Our results indicate that classic psychedelics may have therapeutic efficacy that is more persistent than that of ketamine, and also suggest that a subjective existential experience may not be necessary for therapeutic effects.



KEYWORDS: *Psilocybin, lysergic acid diethylamide, ketamine, psychedelic, antidepressant, depression, forced swim test*

INTRODUCTION

Currently marketed antidepressants are poorly efficacious, requiring weeks of chronic dosing for symptomatic relief, and many of those who suffer from major depressive disorder fail to achieve complete remission.[1,2] First-line pharmacotherapies, frequently selective serotonin reuptake inhibitors, are poorly efficacious, and nonpharmacological approaches such as bright light therapy actually may have a greater antidepressant effect.[3] Alternative pharmacotherapies such as tricyclic antidepressants are associated with greater risk of adverse effects, including weight gain, gastrointestinal and urinary retention, sexual dysfunction, and cardiovascular problems. Furthermore, no currently available antidepressants produce long-term antidepressant effects without chronic administration.

Recently, ketamine has been shown to have extremely rapid antidepressant effects.[4−7] Ketamine is an NMDA receptor antagonist used as a sedative and anesthetic in human and veterinary medicine. Ketamine infusion can produce symptomatic relief for major depression and suicidal ideation within minutes,[6] and it has been used off-label and in clinics across the country to treat depression. Unfortunately, for patients with treatment-resistant depression, ketamine is an efficacious antidepressant only for approximately half of the people infused, and the mean time to relapse of another depressive episode is only 17 days.[8] To prolong the antidepressant effect, ketamine requires repeated clinical visits (typically 6 visits over 12 days),[6] but symptomatic relief lasts only for about 5 weeks before another round of infusion therapy is needed.

Significantly, in March 2019, the U.S. Food and Drug Administration (FDA) approved clinical administration of intranasal esketamine (the *S*-(+) enantiomer of ketamine) through a restricted distribution system, and in conjunction with standard antidepressants, for treatment-resistant depression.[9] Although shown to be efficacious for treatment-resistant depression, intranasal administration is similar to intravenous infusion in that it requires repeated clinical administration. Regardless, ketamine has provided a powerful context in which to study pharmacological mechanisms that contribute to rapid antidepressant effects of drugs.

Ketamine's antidepressant effects are associated with increased synaptic density in the hippocampus[7] and medial prefrontal cortex of rats[7,10] and in rat cortical cell culture.[11] These effects are consistent with neuroproliferation observed with traditional antidepressants[12−14] that has been associated

Received: September 7, 2019
Accepted: January 28, 2020
Published: March 5, 2020

https://dx.doi.org/10.1021/acschemneuro.9b00493
ACS Chem. Neurosci. 2020, 11, 864−871

with antidepressant effects. Additional recent studies indicate that ketamine-related neuroproliferation and plasticity are dependent on the mammalian target of rapamycin (mTOR) signaling cascade,[15,16] which is dysfunctional in humans suffering from major depressive disorder.[17] Like ketamine, certain psychedelic compounds such as lysergic acid diethylamide (LSD)[18] and *N*,*N*-dimethyltryptamine (DMT)[19] have been reported to have some antidepressant-like behavioral effects in rodents, as well as to promote neuroplasticity in rat cortical cell culture via a tropomyosin receptor kinase B (TrkB) and mTOR-dependent mechanism.[11] All classic psychedelic compounds activate the serotonin 5-HT_{2A} receptor,[20] several of which have been shown to activate the Akt/mTOR pathway.[21]

Psilocybin, a psychedelic prodrug long excluded from biomedical research due to legal restrictions and social stigma, has been shown in multiple clinical trials to have rapid, long lasting antidepressant[22,23] and anxiolytic[22,24,25] effects in humans after only one or two acute treatment sessions. These trials included strictly controlled and highly supervised sensory environments incorporating several psychotherapy sessions post drug administration to "integrate" the subjective experience. There has been significant debate in the field as to whether the therapeutic effects of psilocybin to treat depression and anxiety are purely dependent on the individuals' subjective "peak" experience,[26,27] or are physiological in nature with the peak experience merely serving as a biomarker for antidepressant efficacy.[28]

With psilocybin recently achieving Breakthrough Status by the FDA for human Phase III clinical trials in the United States, it is imperative that we gain a better understanding of the mechanisms through which psychedelics can, after only one or two treatments, produce positive and long lasting antidepressant and anxiolytic effects persisting for six or more months in patient populations. Further, in light of the recent approval of esketamine to treat major depression, it is unknown how the rapid and long lasting therapeutic effects of psilocybin directly compare with ketamine. Ketamine also has significant abuse liability, and there are certain safety issues associated with its long-term use. Therefore, it is important to determine comparative efficacy of psilocybin and ketamine toward developing safe and effective therapeutic strategies in the clinic for the treatment of depression.

In order to investigate these issues, we have tested the ability of psilocybin, LSD, and ketamine to alleviate depressive-like symptoms and anxiety in a rat model for depression. Importantly, developing an animal model where psilocybin and LSD have therapeutic effects similar to the human demographic provides a context in which to investigate fundamental biological mechanism(s) to elucidate the robust and long lasting antidepressant and anxiolytic effects of psilocybin and related drugs.

RESULTS AND DISCUSSION

Psilocybin, LSD, and Ketamine Do Not Cause Persistent Stimulant or Sedative Effects. No differences in overt locomotor activity (LCA) were observed between treatment and control rats (Supplementary Figure S1).

Psychedelics Have Persistent Antidepressant-like Effects. We performed a number of experiments in which we administered intraperitoneal (IP) psilocybin (1 mg/kg), LSD (0.15 mg/kg), ketamine (5.0, 20, or 100 mg/kg), or saline, followed by evaluation for depressive-like behaviors at various intervals between 1 and 5 weeks following injection (Figure 1). Both psilocybin and LSD significantly reduced

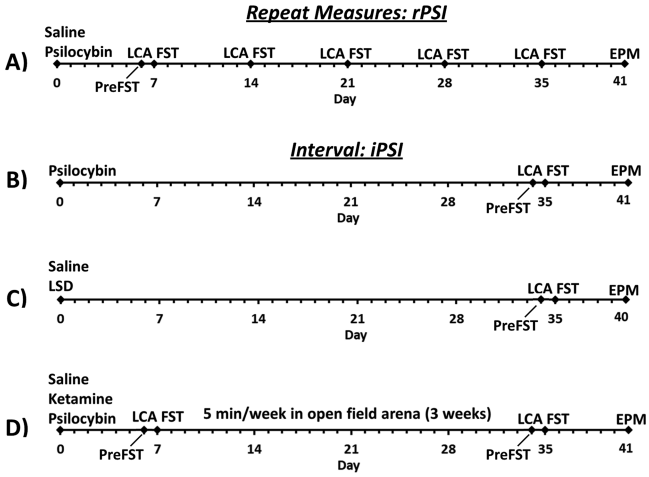


Figure 1. Experimental design. (A) Rats were injected with IP saline or 1.0 mg/kg psilocybin (PSI) on day 0. Locomotor activity (LCA) and forced swim test (FST) behaviors were assessed in SAL (n = 8) and rPSI (n = 8) rats on days 7, 14, 21, 28, and 35, and elevated plus maze (EPM) behaviors were assessed on day 41. (B) iPSI (n = 8) rats were injected with 1.0 mg/kg PSI on day 0. LCA and FST behaviors were assessed on day 35, and EPM behaviors were assessed on Day 41. The iPSI group was concurrent with SAL and rPSI shown above. (C) Rats were injected with IP saline (n = 6) or LSD (n = 6) on day 0. LCA and FST behaviors were assessed on day 35, and EPM behaviors were assessed on day 40. (D) Rats were injected with IP saline, 5.0 mg/kg ketamine, or 1.0 mg/kg PSI on day 0. LCA and FST behaviors were assessed on days 7 and 35, and EPM behaviors were assessed on day 41.

depressive-like behaviors 5 weeks after administration, although psilocybin's effect size (d = 4.985) was far greater than LSD's (d = 0.863) (Figure 2A–C,G) at the doses we used. Further, there was no indication of the effects decreasing over time, suggesting that the therapeutic effects of a single administration of psilocybin likely last far beyond the 5 weeks we tested. In our first experiment, a nonsignificant trend of reduced immobility by repeat measures psilocybin (rPSI, n = 8) rats in the forced swim test (FST) was observed 2 and 3 weeks following psilocybin administration, and rPSI rats were significantly less immobile in the FST than saline (SAL, n = 8) rats 4 weeks ($p < 0.05$) and 5 weeks ($p < 0.05$) following psilocybin administration (Figure 2A). Interval psilocybin (iPSI, n = 8) rats were significantly less immobile ($p < 0.0001$) 5 weeks following psilocybin treatment when compared to SAL rats in their first FST analysis (Figure 2B). Immobility reductions observed in iPSI rats were due to significantly increased swimming ($p < 0.0001$) and climbing ($p < 0.0001$) (Figure 2B). Consistent results were observed in our final experiment, where psilocybin (PSI, n = 6) rats were significantly less immobile than saline (SAL, n = 6) rats at 1 week ($p < 0.0001$) and 5 weeks ($p < 0.001$) following psilocybin administration, due to increased swimming ($p < 0.05$ both weeks) (Figure 2G). LSD (n = 6) rats were significantly less immobile in the FST 5 weeks following LSD ($p < 0.05$) treatment, due to increased swimming ($p < 0.05$) and climbing ($p < 0.05$) when compared to saline (SAL, n = 6) rats (Figure 2C).

https://dx.doi.org/10.1021/acschemneuro.9b00493
ACS Chem. Neurosci. 2020, 11, 864–871

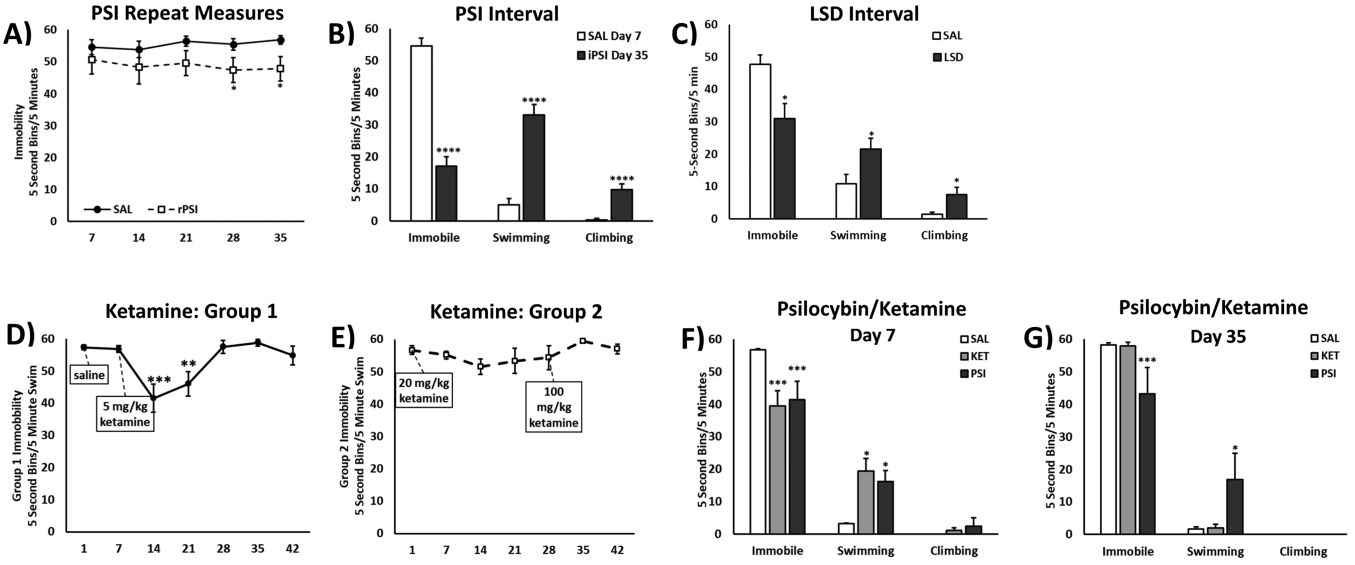


Figure 2. Forced swim test. (A) Immobility in the FST by SAL (n = 8) and rPSI (n = 8) did not change significantly over time. However, t tests found rPSI rats were less immobile than SAL rats on days 28 ($p < 0.05$) and 35 ($p < 0.05$). (B) iPSI (n = 8) rats were significantly less immobile than SAL rats during their first FST session ($p < 0.0001$) and significantly more likely to swim ($p < 0.0001$) and climb ($p < 0.0001$) than SAL rats. (C) LSD (n = 6) rats were significantly less immobile than SAL (n = 6, $p < 0.05$) rats, and significantly more likely to swim ($p < 0.05$) and climb ($p < 0.05$). (D) Group 1 (n = 6) rats were given saline on day 0, and LCA and FST behaviors were assessed day 1 to establish control behaviors for groups 1 and 2 (shown in E). 5.0 mg/kg KET did not alter immobility in the FST 1 day after injection (day 7), but it significantly reduced immobility in the FST 1 week ($p < 0.001$) and 2 weeks ($p < 0.01$) after injection (days 21 and 28). No differences in immobility were observed 3, 4, or 5 weeks after injection when compared to saline (day 1). (E) Group 2 (n = 6) was studied concurrently with group 1 (shown in D) and compared with group 1 saline (day 1) as control behaviors. No significant differences were observed within group 2 at 20 or 100 mg/kg, or between group 2 and group 1 saline (day 1). (F) 5.0 mg/kg IP KET (n = 6) significantly reduced immobility ($p < 0.0001$), and increased swimming ($p < 0.05$) but not climbing in the FST 1 week after injection vs SAL (n = 6). 1.0 mg/kg IP PSI (n = 6) significantly reduced immobility ($p < 0.0001$), and increased swimming ($p < 0.05$) but not climbing in the FST 1 week after injection vs SAL. (G) PSI significantly reduced immobility ($p < 0.001$), and increased swimming ($p < 0.05$) but not climbing in the FST 5 weeks (day 35) after injection vs SAL. No significant differences were observed between PSI day 7 (shown in F) and PSI day 35. KET day 35 was not different from SAL days 7 or 35.

The reduced effect size of LSD may simply be due to nonoptimization of dose size or to pharmacological differences between these two drugs. We selected the psilocybin dose (1.0 mg/kg) using guidance from previously published literature,[29] in which male rats given 1.0 mg/kg psilocybin displayed the greatest behavioral changes without impaired locomotor activity. Further, the results of a small pilot study we performed in male Sprague−Dawley rats supported the antidepressant-like efficacy of this dose. Our LSD dose (0.15 mg/kg) was determined by estimations of relative potency at the target receptor (5-HT_{2A}), and potential off-target effects of LSD at higher levels, with no pilot validating studies. All rats given psilocybin or LSD displayed acute behavioral changes within 30 min of administration, such as limb abduction and forepaw treading, that persisted less than 2 h. Both drugs elicited substantial reduction of depressive-like behavior in the FST several weeks after complete biological clearance of the drugs (Figure 2A−C,G).

Low-Dose Ketamine Has a Transient Antidepressant-like Effect. When comparing the antidepressant-like and anxiolytic effects of psilocybin with the established antidepressant ketamine in a dose−response experiment, we found only the lowest dose of ketamine (5.0 mg/kg) was efficacious in our experimental system (Figure 2D), as 5.0 mg/kg, but not 20 or 100 mg/kg, ketamine significantly reduced immobility in the FST 1 week ($p < 0.001$) and 2 weeks ($p < 0.01$) following ketamine administration (Figure 2E). These results are consistent with the human demographic,[30] correlate to generic allometric scaling of drug administration to rats,[31] and are consistent with literature reporting ketamine's antidepressant-like effect in WKY rats.[32] However, the low dose ketamine is inconsistent with the dosing strategy commonly used with healthy rat strains such as Sprague−Dawley.[10] In another divergence from the literature, we did not observe FST behavioral changes 24 h post ketamine treatment, but did 1 week and 2 weeks after, suggesting that the intrinsic behavioral differences of WKY rats are due, at least in part, to neurological structural deficiencies rather than simply reduced functionality. Although low-dose ketamine significantly reduced depressive-like behaviors, ketamine-related behavioral changes were transient (Figure 2D) compared to those observed in iPSI and LSD rats (Figure 2A,B). Immobility behaviors in the FST displayed by rats given 5.0 mg/kg ketamine increased to baseline by the third week following ketamine treatment (Figure 2D). Consistent results were observed during our final experiment, where ketamine (KET, n = 6) rats were significantly less immobile than saline (SAL, n = 6) rats 1 week following ketamine administration ($p < 0.0001$), due to increased swimming ($p < 0.05$) (Figure 2F), but 5 weeks after ketamine treatment KET and SAL rats were statistically indistinguishable from each other (Figure 2G). These results are consistent with the human demographic,[8,33] and they support further investigation of the therapeutic properties of drugs acting as 5-HT_{2A} receptor agonists using appropriate animal models.

Psilocybin Plus Repeated Open Field Exposure Has a Persistent Anxiolytic Effect. Clinical researchers have described the correlation between each individual's subjective

https://dx.doi.org/10.1021/acschemneuro.9b00493
ACS Chem. Neurosci. 2020, 11, 864−871

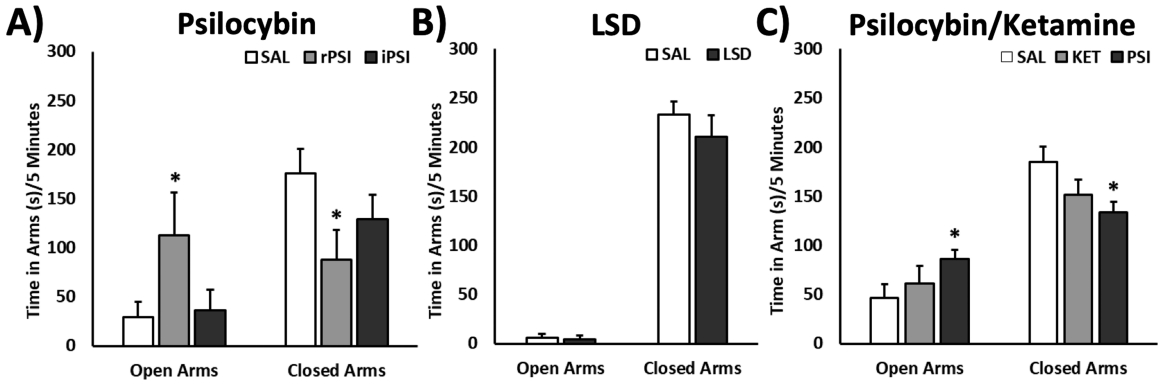


Figure 3. Elevated plus maze. (A) rPSI (n = 7 due to exclusion) spent significantly more time in the open arms of the EPM ($p < 0.05$) and significantly less time in the closed arms of the EPM ($p < 0.05$) than SAL (n = 8). No differences were observed between iPSI and SAL rats. (B) No differences were observed between LSD (n = 6) and SAL (n = 6) rats. C) PSI (n = 6) rats spent significantly more time in the open arms ($p < 0.05$) and significantly less time in the closed arms ($p < 0.05$) than SAL rats. KET (n = 6) rats were not statistically different from SAL (n = 6) rats.

experience and future outcomes as dependent upon "set and setting".[26] Set and setting contextually contribute to each individual's comfort or sense of vulnerability during their psychedelic experience and subsequent therapy sessions. Although our experimental design was unable to quantify the animals' subjective experience, animals from all groups that underwent weekly FST displayed progressively less locomotor activity immediately prior to FST testing, but animals placed in the open field arena weekly without subsequent FST evaluation did not. rPSI, but not iPSI, rats spent significantly more time in the open arms of the elevated plus maze (EPM) ($p < 0.05$) and significantly less time in the closed arms of the EPM ($p < 0.05$) than SAL rats (Figure 3A). LSD rats given a single exposure to the open field arena (during LCA analysis prior to FST) were indistinguishable from SAL rats given a single exposure (Figure 3B). It is possible that the animals that underwent weekly FST associated the open field arena with FST analysis, which they dislike, and changed their locomotor activity accordingly. Interestingly, weekly forays into the open field arena, with or without subsequent FST, resulted in psilocybin rats (rPSI and PSI) that displayed significantly less anxiety-like behavior in the EPM than control rats (Figure 3A,C), whereas drug alone (Figure 3A,B) and ketamine with weekly arena exposure did not (Figure 3C). PSI rats placed within the open field arena during weekly 5 min sessions (identical to LCA evaluation), but only those tested in the FST the first and the fifth weeks following psilocybin treatment spent significantly more time in the open arms ($p < 0.05$) and significantly less time in the closed arms ($p < 0.05$) than SAL rats. KET rats were not statistically different from SAL rats (Figure 3C).

Antidepressant-Like Effects of Psilocybin Appear Both Biological and Context-Dependent. Despite having a rudimentary default mode network,[34] and showing interest in mirrors, video feed, and still images of other rats,[35] rats are not commonly believed to be among the menagerie of self-aware animals. Thus, as far as modern science is able to determine, rats do not have a sense of self and are incapable of having existential anxiety, pondering the meaning behind their own existence or fearing a reality in which they, as individuals, do not exist. No one knows what the subjective experience of a rat is after being given a psychedelic, but it is highly unlikely that they are able to place that within the context of their life experiences and utilize that knowledge therapeutically to improve their affective state. Therefore, because psilocybin has robust effects similar to those of other antidepressants in rats that is very long lasting, which is similar to the long lasting antidepressant effects of psilocybin to alleviate the symptoms of depression in humans, we posit that the basis for the antidepressant effects in humans is at its core biological in nature, and that while *correlated* to antidepressant effect[27] peak ego dissolution subjective experiences following psilocybin administration is not *causational* to antidepressant effect. These biological processes could include cellular proliferation, increased synaptic connectivity, and anti-inflammatory effects. Drugs like LSD and psilocybin have been demonstrated to elicit these types of changes in preclinical models, and these types of physiological effects have each been associated with antidepressant-like behavioral outcomes.[32,36]

Although we propose that the antidepressant-like effects are primarily rooted in biological processes, our data suggest that post drug administration environmental factors also play a critical role in overall drug effects. Our results suggest that psilocybin facilitates a period of behavioral flexibility in which exploration of a non-home-cage environment reduces their anxiety during future exploration of a novel environment (the EPM, Figure 3A), but FST evaluation 1 week after psilocybin blunts the antidepressant-like effect in the FST (Figure 2A,F,G). In this context, psilocybin may open a window during which time certain experiences are salient to the development of new coping strategies, similar to MDMA-facilitated social learning reported in mice.[37] As the FST measures the coping strategies of rodents challenged with the threat of drowning, successfully surviving the first trial by floating may make rats given psilocybin more likely to use this coping strategy in the future, whereas rats given psilocybin and not subjected to FST for 5 weeks choose active coping strategies. Interestingly, that is not true of ketamine, as ketamine's antidepressant-like effect was independent of repeated FST, but transient (Figure 2D,G) and no anxiolytic effect was observed (Figure 3C). Therefore, overall efficacy in humans is likely a combination of both the acute neurological effects of the drug *and* subjective, contextual experiences during and/or immediately following the drug administration session.

https://dx.doi.org/10.1021/acschemneuro.9b00493
ACS Chem. Neurosci. 2020, 11, 864−871

CONCLUSIONS

The antidepressant-like and anxiolytic effects of psychedelics are measurable and significant in males of a rat experimental system that has been used by several other groups over many decades for the study of mood disorders in humans. These effects are evident many weeks after administration, are more persistent than those of ketamine, and are modulated by the rats' experiences in the first week following administration. The more persistent therapeutic effects of a single administration of psilocybin compared to ketamine in our experimental system support the notion that serotonin 5-HT_{2A} receptor directed therapeutic strategies may be superior to ketamine-based treatments in the clinic for depression. Finally, our experimental rodent system, which recapitulates the major features of psilocybin to treat depression in human patients, may represent a valuable system to utilize for the elucidation of molecular, cellular, and genetic mechanisms underlying the ability of psilocybin to produce the robust and long lasting antidepressant effects found in human clinical trials toward developing new and effective therapeutic strategies for treating depression.

METHODS

Animals. Results of a small pilot study we performed in healthy Sprague–Dawley rats indicated that although psilocybin's antidepressant-like effects persist for at least a month or longer after drug administration, they may not appear for a week or more after drug administration. This potential delayed efficacy excluded the use of chronic stress models of depression for evaluation in a model of depression because these models require testing *immediately* after 21 consecutive days of stress.[38,39] We chose to use a selectively bred rat strain, but we excluded the more commonly used Flinders Sensitive Line (FSL) rats, as FSL rats have abnormally low central $5HT_{2A}$ mRNA expression[40,41] and esketamine-related changes in the depressive-like behavior of that strain has been shown to be independent of the $5HT_{2A}$ receptor and instead be $5HT_{1B}$-dependent.[36] Given that the antidepressant effects of psychedelics are believed to be directly related to $5HT_{2A}$ agonism,[20] it is unsurprising that a recent study found no antidepressant-like effects of psilocybin in FSL rats.[42] Therefore, we chose to use the Wistar–Kyoto (WKY) rat, a less frequently used but well established and validated intrinsic model for the study of depression and anxiety vulnerability.[32,43−52] WKY rats display high immobility in the FST,[32,43,45−47,51,53] increased anxiety-like behaviors,[44,54] and an elevated stress response compared to control rats.[48,51,52] As very few publications have reported measures of baseline behaviors in female WKY rats, or their response to antidepressants, females were excluded from this study, but will be examined in future studies.

Male WKY rats aged 50−56 days were obtained from Charles River and allowed to habituate to the colony room for at least 7 days prior to drug administration. Rats were pair-housed with a standard 12:12 light−dark cycle, handled daily, and given ad libitum access to water and food. All protocols were approved by the Institutional Animal Care and Use Committee and were consistent with the Guide, eighth edition.

Drugs. Psilocybin (PSI) and lysergic acid diethylamide tartrate (2:1) (LSD) were provided by the National Institute on Drug Abuse (Bethesda, MD), and ketamine (KET) was obtained from the LSUHSC Division of Animal Care and supplied by Henry Schein Animal Health (Dublin, OH). A single bolus injection of sterile saline or drug in saline vehicle was administered in a volume of 1 mL/kg intraperitoneally (IP) to each rat corresponding to their assigned treatment groups (day 0) (PSI = 1.0 mg/kg; LSD = 0.15 mg/kg; KET = 5.0, 20, or 100 mg/kg). Route of administration and dosing regimen were chosen using guidance from the literature,[10,29,32,55] a small pilot study, and in consultation with Dr. David E. Nichols (Purdue Univeristy).

Experimental Design. *Persistent Antidepressant-like and Anxiolytic Effects of Psilocybin.* To evaluate the antidepressant-like effects of a single injection of psilocybin in male WKY rats, three treatment groups were defined as saline (SAL, n = 8), repeat measures psilocybin (rPSI, n = 8), and interval psilocybin (iPSI, n = 8). Rats were treated on day 0. SAL and rPSI groups underwent a single FST pre-exposure on day 6 post-treatment and were tested in the FST on days 7, 14, 21, 28, and 35. The iPSI group was pre-exposed to the FST on day 34 post-treatment and was tested in the FST on day 35. All rats were tested for anxiety-like behaviors in the EPM on day 41. iPSI FST scores were compared with SAL FST scores from day 7 to avoid potential confounding by repeat measures. An interval-saline group was determined to be redundant, as there is no evidence that the slight age difference in these rats affects FST behavior, and previously published literature has established that FST behaviors throughout this age range is consistent.[56,57] Thus, we excluded the redundant control group for ethical animal resource use.[58] Further, our design allowed for within-subject comparison. LCA was assessed in the open field immediately prior to each FST (Figure 1A,B).

Persistent Antidepressant-like Effects of LSD. To evaluate the long-term antidepressant-like effects of a single injection of LSD in male WKY rats, two treatment groups were defined as saline (SAL, n = 6) and LSD (LSD, n = 6). Rats were treated on day 0, pre-exposed to the FST on day 34 post-treatment, and then tested for depressive-like behaviors in the FST on day 35. LCA was assessed in the open field immediately prior to the FST. Rats were tested for anxiety-like behaviors in the EPM on day 40 (Figure 1C).

Ketamine Dose–Response. To determine an antidepressant-like dose of ketamine in male WKY rats and to determine the persistence of ketamine's antidepressant-like effect, rats were given saline or ketamine (5, 20, or 100 mg/kg) IP and tested weekly in the FST. Group 1 rats (n = 6) were given saline 2 days prior to the first FST and 5 mg/kg a day prior to the second FST. Group 2 rats (n = 6) were given 20 mg/kg ketamine 2 days prior to the first FST and 100 mg/kg a day prior to the fifth FST (Figure 2D-E).

Salience of Experience to Behavioral End points of Psilocybin and Ketamine. To evaluate the contribution of experience to anxiolytic effect and apparent reduction in antidepressant-like effect of psilocybin observed in rPSI rats, three treatment groups of male WKY rats were defined as saline (SAL, n = 6), ketamine (KET, 5.0 mg/kg, n = 6), and psilocybin (PSI, n = 6). Rats were treated on day 0, pre-exposed to the FST on day 6, and tested for depressive-like behaviors in the FST on day 7. All rats were placed individually in the open field arena for 5 min sessions once per week for the next 3 weeks, pre-exposed to the FST for a second time on day 34, and tested for depressive-like behaviors at day 35. LCA was assessed in the open field arena immediately prior to each FST. Rats were tested for anxiety-like behaviors in the EPM on day 41 (Figure 1D).

Forced Swim Test. To test for the effects of psilocybin on depressive-like behavior, we used the FST as our primary outcome measure. The FST is a behavioral despair paradigm often used to measure depressive-like behavior and to screen for antidepressant-like effects in rats.[59] The FST interprets immobility, a passive coping strategy, as a depressive-like behavior, and reductions in immobility as evidence of antidepressant-like effect.[57,59] The FST measures coping strategies that are not solely mediated by neurocircuits known to be dysfunctional in the human demographic and thus lacks construct validity. However, it remains the most reliable measure of passive coping strategy, a depressive-like behavior, in both male and female rats,[60,61] as measures of anhedonia like the sucrose preference test have been shown to be inconsistent in females[60,61] and negatively correlated to behavioral despair independent of chronic stress paradigms.[62] The FST can be used to screen for antidepressant-like action of drugs given to otherwise healthy rats,[57,60] for depressive-like behaviors in animal models of depression,[63,64] or for antidepressant-like action of drugs given to animals modeling depression.[63−65] Our use of the term "antidepressant-like" when referring to the effects of psilocybin here are in reference to the published effects of antidepressants typically used to treat humans on rat behaviors, and not to the effects of antidepressants on human behaviors. As the FST

https://dx.doi.org/10.1021/acschemneuro.9b00493
ACS Chem. Neurosci. 2020, 11, 864−871

measures active behaviors, overall locomotor activity also must be assessed immediately prior to the FST to control for nonspecific sedative or stimulant effects that might otherwise lead to false positive or negative results.[57] Increased immobility or activity in the FST is only meaningful if overall locomotor activity is unchanged, which we found to be the case in each of our experiments. A 15 min pre-exposure to the paradigm is required to elicit measurable depressive-like behavior in otherwise healthy rats. It had been widely assumed that each subsequent/additional exposure to an FST would increase immobility.[66] This assumption was shown to be false in a 2011 study that used a single 15 min pre-exposure and weekly FST.[66] Multiple experiments were performed during our study, using both repeat measures and interval testing. While there is evidence of differences in FST behavior between distinct phases of a rat's life, prepubertal (<4 weeks), adult (2−18 months), and aged rats (18 months),[56] all tests in this study occurred during the same phase, young adulthood (9−15 weeks),[67] during which time FST behaviors are known to be consistent. Thus, we chose to reduce animal resources in studies with repeat measures by comparing our interval-tested treatment groups with the first FST of the repeatedly tested saline groups. Although our intent was not to confirm the validity of repeat measures FST, our results are consistent with those of Mezadri et al.,[66] in that we found repeated exposure to the FST did not increase or decrease immobility in control rats. However, antidepressant-like effects in animals given psilocybin and repeatedly exposed to the FST were not as robust as in those given the same drug, but only exposed to a single FST. We address this finding in the Results and Discussion section.

FST was conducted as previously described with some modifications.[57] During the pre-exposure, rats were placed into a plastic cylindrical tank (114 cm × 30.5 cm) that contained 30 cm of water at 28−30 °C. The water depth was such that the rats could not support themselves by touching the bottom of the tank with their hind paws, and their tails could not touch the bottom of the tank while keeping their noses above water. After a 15 min swim, the rats were removed, dried with paper towels, and replaced in their home cages. Fresh water was used for each animal. At the time of testing, a video camera was mounted to the side of the tank, and the rats were exposed to a 5 min swim under the conditions described above and then removed, dried with paper towels, and returned to their home cages. The 5 min swim was recorded for later scoring for immobility, swimming, climbing, or diving. FST scoring was performed by trained scorers blind to the treatment options and employed the modified sampling technique.[57] Immobility was defined as no active attempts to escape while maintaining a floating posture in which the rats make only the movements necessary to keep their heads above water. Swimming was defined as actively attempting escape with motions directed outward against the wall of the cylinder. Climbing was defined as actively attempting escape with motions directed upward against the wall of the cylinder. Diving was defined as actively attempting escape with motions directed downward below the water and the head of the rat submerged. Significantly greater immobility than control (SAL) rats indicates depressive-like behavior, and significantly less immobility indicates an antidepressant-like effect. Significantly increased swimming may suggest increased serotonergic signaling, and significantly increased climbing may suggest increased noradrenergic signaling. Diving is rarely observed and not correlated with specific neurotransmitter activity or drugs known to influence specific neurotransmitter activity.[57] No diving was observed during this study; thus, the behavior was not included in our results or analyses. Behaviors were manually scored by two independent observers blind to the treatments.

Locomotor Activity. Locomotor activity was assessed immediately prior to each FST in order to prevent confounding by nonspecific sedative or stimulant effects. LCA assessment was performed by placing each rat into a square open field arena (61 × 61 cm^2) with opaque walls 45 cm high and allowing the rats to explore freely for 5 min. The rats' movements within the arena were recorded and later scored for distance traveled (cm) using EthoVision XT 8.5 tracking software (Noldus Information Technology).

Elevated Plus Maze. To test for anxiety-like behavior we used the EPM, a widely used approach-avoidance assay that exploits the conflict between exploration behaviors and defensive thigmotaxis in a novel environment.[68] The EPM was conducted as previously described.[68] The maze apparatus consists of four equally sized arms, two of which are open runways and two of which are enclosed by walls. Each rat was allowed to habituate to the testing room for at least 15 min prior to exposure to the maze. After habituation, rats were placed at the junction (10 × 10 cm^2) of the four arms of the maze (114 × 114 × 114 cm^3 externally, runways 50 × 10 cm^2) facing an open arm and allowed to explore freely for 5 min during which time an overhead camera recorded their movements. Video recordings of the sessions were scored for time spent in the open arms, the closed arms, and the junction of the open and closed arms using EthoVision 8.5 (Noldus Information Technology). Although EPM data are traditionally presented as a ratio of open/closed arms, two rPSI rats never entered the closed arms. Thus, we chose to present EPM data as time spent in the open and closed arms during the 5 min (300 s) assay and to not report time spent in the hub/center of the EPM.

Statistical Analyses. Group sizes were determined using power analysis for a two-tailed t test, and conservative estimates based upon previously published literature[53] ($\mu 0 = 45$, $\mu 1 = 35$, $\sigma = 7$, $\alpha = 0.05$, power = 0.80). Data were compared by t test or two-way ANOVA with repeated measures and Holm−Sidak posthoc using Prism software (Graphpad, La Jolla, CA), and significant results from relevant comparisons are summarized in Supplementary Table 1. Data are expressed in figures as mean ± standard error of the mean.

Exclusions. During our first experiment, one rPSI rat was excluded from the EPM analysis due to confounding environmental disruptions during his trial. Thus, rPSI $n = 7$ for EPM behavior for that experiment. However, because FST and EPM behaviors are independent of each other, the rat excluded from EPM analysis was not excluded from the FST analysis, so that for FST $n = 8$ for all groups in that experiment. During the ketamine dose−response study, the first two LCA sessions suffered from equipment failure that resulted in exclusion of LCA data for those sessions. However, as no differences were observed in FST behavior corresponding to the excluded LCA sessions, the excluded LCA data were not needed for accurate FST analysis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acschemneuro.9b00493.

Open field locomotor activity (PDF)

Summary of significant results from relevant comparisons made during the four experiments presented (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Charles D. Nichols − *Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, New Orleans, Louisiana 70112, United States;* orcid.org/0000-0002-0615-0646; Email: Cnich1@lsuhsc.edu

Authors

Meghan Hibicke − *Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, New Orleans, Louisiana 70112, United States*

Alexus N. Landry − *Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, New Orleans, Louisiana 70112, United States*

Hannah M. Kramer − *Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, New Orleans, Louisiana 70112, United States*
Zoe K. Talman − *Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, New Orleans, Louisiana 70112, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acschemneuro.9b00493

Author Contributions

M.H. and C.D.N. designed the experiments. M.H., A.N.L., H.M.K., and Z.K.T performed the experiments. M.H. analyzed the data. M.H. and C.D.N. wrote the manuscript.

Funding

This work was supported by Eleusis, PBC.

Notes

The authors declare the following competing financial interest(s): C.D.N. has a sponsored research contract with Eleusis PBC. and is a member of its Scientific Advisory Board.

■ ACKNOWLEDGMENTS

The authors wish to thank Adam Levin and Alejandro Jose Molina for their time and effort scoring videos of rats in the FST, and Dr. D.E. Nichols for his assistance determining LSD dosing concentration.

■ REFERENCES

(1) Sinyor, M., Schaffer, A., and Levitt, A. (2010) The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) Trial: A Review. *Can. J. Psychiatry 55*, 126−135.
(2) Warden, D., Rush, A. J., Trivedi, M. H., Fava, M., and Wisniewski, S. R. (2007) The STAR*D Project results: a comprehensive review of findings. *Curr. Psychiatry Rep. 9*, 449−459.
(3) Lam, R. W., et al. (2016) Efficacy of Bright Light Treatment, Fluoxetine, and the Combination in Patients With Nonseasonal Major Depressive Disorder: A Randomized Clinical Trial. *JAMA Psychiatry 73*, 56−63.
(4) Maeng, S., and Zarate, C. A. (2007) The role of glutamate in mood disorders: Results from the ketamine in major depression study and the presumed cellular mechanism underlying its antidepressant effects. *Curr. Psychiatry Rep. 9*, 467−474.
(5) Mathews, D. C., Henter, I. D., and Zarate, C. A. (2012) Targeting the Glutamatergic System to Treat Major Depressive Disorder. *Drugs 72*, 1313−1333.
(6) Murrough, J. W., et al. (2013) Rapid and Longer-Term Antidepressant Effects of Repeated Ketamine Infusions in Treatment-Resistant Major Depression. *Biol. Psychiatry 74*, 250−256.
(7) Dong, C., et al. (2016) Rapid and Sustained Antidepressant Action of the mGlu2/3 Receptor Antagonist MGS0039 in the Social Defeat Stress Model: Comparison with Ketamine. *Int. J. Neuropsychopharmacol.*, pyw089.
(8) Rong, C. (2018) Predictors of Response to Ketamine in Treatment Resistant Major Depressive Disorder and Bipolar Disorder. *Int. J. Environ. Res. Public Health 15*, 771.
(9) U.S. Food and Drug Administration (2019) FDA approves new nasal spray medication for treatment-resistant depression; available only at a certified doctor's office or clinic, U. S. Food and Drug Administration, http://www.fda.gov/news-events/press-announcements/fda-approves-new-nasal-spray-medication-treatment-resistant-depression-available-only-certified.
(10) Sarkar, A., and Kabbaj, M. (2016) Sex Differences in Effects of Ketamine on Behavior, Spine Density, and Synaptic Proteins in Socially Isolated Rats. *Biol. Psychiatry 80*, 448−456.
(11) Ly, C., et al. (2018) Psychedelics Promote Structural and Functional Neural Plasticity. *Cell Rep. 23*, 3170−3182.
(12) Ampuero, E., et al. (2010) Chronic fluoxetine treatment induces structural plasticity and selective changes in glutamate receptor subunits in the rat cerebral cortex. *Neuroscience 169*, 98−108.
(13) Andrade, C. (2014) Antidepressant augmentation with anti-inflammatory agents. *J. Clin. Psychiatry 75*, 975−977.
(14) Gałecki, P., Mossakowska-Wójcik, J., and Talarowska, M. (2018) The anti-inflammatory mechanism of antidepressants - SSRIs, SNRIs. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry 80*, 291−294.
(15) Abelaira, J. M., Reus, G. Z., Neotti, M. V., and Quevedo, J. (2014) The role of mTOR in depression and antidepressant responses. *Life Sci. 101*, 10−14.
(16) Li, N., et al. (2010) mTOR-Dependent Synapse Formation Underlies the Rapid Antidepressant Effects of NMDA Antagonists. *Science 329*, 959−964.
(17) Jernigan, C. S., et al. (2011) The mTOR signaling pathway in the prefrontal cortex is compromised in major depressive disorder. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry 35*, 1774−1779.
(18) Buchborn, T., Schröder, H., Höllt, V., and Grecksch, G. (2014) Repeated lysergic acid diethylamide in an animal model of depression: Normalisation of learning behaviour and hippocampal serotonin 5-HT2 signalling. *J. Psychopharmacol. (London, U. K.) 28*, 545−552.
(19) Cameron, L. P., Benson, C. J., Dunlap, L. E., and Olson, D. E. (2018) Effects of N,N-Dimethyltryptamine on Rat Behaviors Relevant to Anxiety and Depression. *ACS Chem. Neurosci. 9*, 1582.
(20) Nichols, D. E. (2012) Structure−activity relationships of serotonin 5-HT2A agonists. *Wiley Interdiscip. Rev. Membr. Transp. Signal. 1*, 559−579.
(21) Li, Q., Hosaka, T., Harada, N., Nakaya, Y., and Funaki, M. (2013) Activation of Akt through 5-HT2A receptor ameliorates serotonin-induced degradation of insulin receptor substrate-1 in adipocytes. *Mol. Cell. Endocrinol. 365*, 25−35.
(22) Grob, C. S., et al. (2011) Pilot Study of Psilocybin Treatment for Anxiety in Patients With Advanced-Stage Cancer. *Arch. Gen. Psychiatry 68*, 71−78.
(23) Carhart-Harris, R. L., et al. (2016) Psilocybin with psychological support for treatment-resistant depression: an open-label feasibility study. *Lancet Psychiatry 3*, 619−627.
(24) Ross, S., et al. (2016) Rapid and sustained symptom reduction following psilocybin treatment for anxiety and depression in patients with life-threatening cancer: a randomized controlled trial. *J. Psychopharmacol. 30*, 1165−1180.
(25) Reiche, S., et al. (2018) Serotonergic hallucinogens in the treatment of anxiety and depression in patients suffering from a life-threatening disease: A systematic review. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry 81*, 1−10.
(26) Hendricks, P. S. (2018) Awe: a putative mechanism underlying the effects of classic psychedelic-assisted psychotherapy. *Int. Rev. Psychiatry Abingdon Engl. 30*, 331−342.
(27) Roseman, L., Nutt, D. J., and Carhart-Harris, R. L. (2018) Quality of Acute Psychedelic Experience Predicts Therapeutic Efficacy of Psilocybin for Treatment-Resistant Depression. *Front. Pharmacol. 8*, 974.
(28) Nichols, D. E., Johnson, M. W., and Nichols, C. D. (2017) Psychedelics as Medicines: An Emerging New Paradigm. *Clin. Pharmacol. Ther. 101*, 209−219.
(29) Davis, M., and Walters, J. K. (1977) Psilocybin: Biphasic dose-response effects on the acoustic startle reflex in the rat. *Pharmacol., Biochem. Behav. 6*, 427−431.
(30) Archer, S., Chrenek, C., and Swainson, J. (2018) Maintenance Ketamine Therapy for Treatment-Resistant Depression. *J. Clin. Psychopharmacol. 1*, 380.
(31) Nair, A. B., and Jacob, S. (2016) A simple practice guide for dose conversion between animals and human. *J. Basic Clin. Pharm. 7*, 27−31.
(32) Tizabi, Y., Bhatti, B. H., Manaye, K. F., Das, J. R., and Akinfiresoye, L. (2012) Antidepressant-like effects of low ketamine dose is associated with increased hippocampal AMPA/NMDA receptor density ratio in female Wistar-Kyoto rats. *Neuroscience 213*, 72−80.

(33) Carhart-Harris, R. L., et al. (2018) Psilocybin with psychological support for treatment-resistant depression: six-month follow-up. *Psychopharmacology (Berl.) 235*, 399−408.
(34) Lu, H., et al. (2012) Rat brains also have a default mode network. *Proc. Natl. Acad. Sci. U. S. A. 109*, 3979−3984.
(35) Yakura, T. (2018) Visual recognition of mirror, video-recorded, and still images in rats. *PLoS One 13*, e0194215.
(36) du Jardin, K. G., et al. (2018) S-Ketamine Mediates Its Acute and Sustained Antidepressant-Like Activity through a 5-HT1B Receptor Dependent Mechanism in a Genetic Rat Model of Depression. *Front. Pharmacol. 8*, 978.
(37) Nardou, R., et al. (2019) Oxytocin-dependent reopening of a social reward learning critical period with MDMA. *Nature 569*, 116.
(38) Chiba, S., et al. (2012) Chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry 39*, 112−119.
(39) McLaughlin, K. J., Gomez, J. L., Baran, S. E., and Conrad, C. D. (2007) The effects of chronic stress on hippocampal morphology and function: An evaluation of chronic restraint paradigms. *Brain Res. 1161*, 56−64.
(40) Österlund, M. K., Overstreet, D. H., and Hurd, Y. L. (1999) The Flinders Sensitive Line rats, a genetic model of depression, show abnormal serotonin receptor mRNA expression in the brain that is reversed by 17β-estradiol. *Mol. Brain Res. 74*, 158−166.
(41) Du Jardin, K. G., Müller, H. K., Sanchez, C., Wegener, G., and Elfving, B. (2017) Gene expression related to serotonergic and glutamatergic neurotransmission is altered in the flinders sensitive line rat model of depression: Effect of ketamine. *Synapse 71*, 37−45.
(42) Jefsen, O. (2019) Psilocybin lacks antidepressant-like effect in the Flinders Sensitive Line rat. *Acta Neuropsychiatr. 31*, 213.
(43) Lahmame, A., del Arco, C., Pazos, A., Yritia, M., and Armario, A. (1997) Are Wistar-Kyoto rats a genetic animal model of depression resistant to antidepressants? *Eur. J. Pharmacol. 337*, 115−123.
(44) Paré, W. P., and Redei, E. (1993) Sex differences and stress response of WKY rats. *Physiol. Behav. 54*, 1179−1185.
(45) Yamada, M., et al. (2013) Upregulation of the dorsal raphe nucleus-prefrontal cortex serotonin system by chronic treatment with escitalopram in hyposerotonergic Wistar-Kyoto rats. *Neuropharmacology 72*, 169−178.
(46) Overstreet, D. H. (2012) Modeling Depression in Animal Models. In *Psychiatric Disorders* (Kobeissy, F. H., Ed.), Vol. *829*, pp 125−144, Humana Press.
(47) López-Rubalcava, C., and Lucki, I. (2000) Strain differences in the behavioral effects of antidepressant drugs in the rat forced swimming test. *Neuropsychopharmacology 22*, 191.
(48) De La Garza, R., and Mahoney, J. J. (2004) A distinct neurochemical profile in WKY rats at baseline and in response to acute stress: implications for animal models of anxiety and depression. *Brain Res. 1021*, 209−218.
(49) Hurley, L. L., et al. (2013) Antidepressant-like effects of curcumin in WKY rat model of depression is associated with an increase in hippocampal BDNF. *Behav. Brain Res. 239*, 27−30.
(50) Paré, W. P., and Tejani-Butt, S. M. (1996) Effect of stress on the behavior and 5-HT system in Sprague-Dawley and Wistar Kyoto rat strains. *Integr. Physiol. Behav. Sci. 31*, 112−121.
(51) Rittenhouse, P. A., López-Rubalcava, C., Stanwood, G. D., and Lucki, I. (2002) Amplified behavioral and endocrine responses to forced swim stress in the Wistar−Kyoto rat. *Psychoneuroendocrinology 27*, 303−318.
(52) Solberg, L. C., Olson, S. L., Turek, F. W., and Redei, E. (2001) Altered hormone levels and circadian rhythm of activity in the WKY rat, a putative animal model of depression. *Am. J. Physiol. - Regul. Integr. Comp. Physiol. 281*, R786−R794.
(53) Carr, G. V., et al. (2010) Antidepressant-Like Effects of κ-Opioid Receptor Antagonists in Wistar Kyoto Rats. *Neuropsychopharmacology 35*, 752−763.
(54) McAuley, J. D., et al. (2009) Wistar-Kyoto rats as an animal model of anxiety vulnerability: support for a hypervigilance hypothesis. *Behav. Brain Res. 204*, 162−168.
(55) Pálenícek, T., Hlinák, Z., Bubeníková-Valesová, V., Novák, T., and Horácek, J. (2010) Sex differences in the effects of N,N-diethyllysergamide (LSD) on behavioural activity and prepulse inhibition. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry 34*, 588−596.
(56) Turner, R. C., et al. (2012) Effects of aging on behavioral assessment performance: implications for clinically relevant models of neurological disease: Laboratory investigation. *J. Neurosurg. 117*, 629−637.
(57) Slattery, D. A., and Cryan, J. F. (2012) Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nat. Protoc. 7*, 1009−14.
(58) Russell, W. M. S., Burch, R. L., and Balls, M. (2009) *The three Rs and the humanity criterion*, FRAME.
(59) Porsolt, R. D., Le Pichon, M., and Jalfre, M. (1977) Depression: a new animal model sensitive to antidepressant treatments. *Nature 266*, 730−732.
(60) Kokras, N., and Dalla, C. (2014) Sex differences in animal models of psychiatric disorders. *Br. J. Pharmacol. 171*, 4595−4619.
(61) Dalla, C., Pitychoutis, P. M., Kokras, N., and Papadopoulou-Daifoti, Z. (2010) Sex Differences in Animal Models of Depression and Antidepressant Response. *Basic Clin. Pharmacol. Toxicol. 106*, 226−233.
(62) Brenes Sáenz, J. C., Villagra, O. R., and Fornaguera Trías, J. (2006) Factor analysis of Forced Swimming test, Sucrose Preference test and Open Field test on enriched, social and isolated reared rats. *Behav. Brain Res. 169*, 57−65.
(63) Hibicke, M., Graham, M. A., and Hayslett, R. L. (2017) Adolescent chronic restraint stress (aCRS) elicits robust depressive-like behavior in freely cycling, adult female rats without increasing anxiety-like behaviors. *Exp. Clin. Psychopharmacol. 25*, 74−83.
(64) Hibicke, M. (2017) Development and Evaluation of an Adolescent Chronic Restraint Stress (ACRS) Protocol to Model Adult Depression in Female Rats. Ph.D. Thesis, Mercer University.
(65) Kandi, P., Hibicke, M., and Hayslett, R. L. (2015) Acute Cytisine and Diarylpropionitrile Independently and Additively Reduce Depressive-Like Behavior in Female Ovariectomized Rats. *J. Pharm. Sci. Pharmacol 2*, 250−258.
(66) Mezadri, T. J., Batista, G. M., Portes, A. C., Marino-Neto, J., and Lino-de-Oliveira, C. (2011) Repeated rat-forced swim test: reducing the number of animals to evaluate gradual effects of antidepressants. *J. Neurosci. Methods 195*, 200−205.
(67) Sengupta, P. (2013) The Laboratory Rat: Relating Its Age With Human's. *Int. J. Prev. Med. 4*, 624−630.
(68) Walf, A. A., and Frye, C. A. (2007) The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat. Protoc. 2*, 322−328.

https://dx.doi.org/10.1021/acschemneuro.9b00493
ACS Chem. Neurosci. 2020, 11, 864−871