Repeated Ketamine Exposure Induces an Enduring Resilient Phenotype in Adolescent and Adult Rats

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Background: Major depressive disorder afflicts up to 10% of adolescents. However, nearly 50% of those afflicted are considered nonresponsive to available treatments. Ketamine, a noncompetitive *N*-methyl-D-aspartate receptor antagonist has shown potential as a rapid-acting and long-lasting treatment for major depressive disorder in adults. Thus, the effectiveness and functional consequences of ketamine exposure during adolescence were explored.

Methods: Adolescent male rats (postnatal day [PD] 35) received two ketamine (0, 5, 10, or 20 mg/kg) injections, 4 hours apart, after exposure to day 1 of the forced swim test (FST). The next day, rats were reexposed to the FST to assess ketamine-induced antidepressant-like responses. Separate groups were exposed to chronic unpredictable stress to confirm findings from the FST. After these initial experiments, adolescent naive rats were exposed to either 1 or 15 consecutive days (PD35–49) of ketamine (20 mg/kg) twice daily. Ketamine's influence on behavioral reactivity to rewarding (i.e., sucrose preference) and aversive (i.e., elevated plus-maze, FST) circumstances was then assessed 2 months after treatment. To control for age-dependent effects, adult rats (PD75–89) were exposed to identical experimental conditions.

Results: Ketamine (20 mg/kg) reversed the chronic unpredictable stress–induced depression-like behaviors in the FST. Repeated ketamine exposure resulted in anxiolytic- and antidepressant-like responses 2 months after drug exposure. None of the ketamine doses used were capable of inducing drug-seeking behaviors as measured by place preference conditioning.

Conclusions: Repeated ketamine exposure induces enduring resilient-like responses regardless of age of exposure. These findings point to ketamine, and its repeated exposure, as a potentially useful antidepressant during adolescence.

Key Words: Adolescence, anxiety, depression, ketamine, rats, resilience, stress

Major depressive disorder (MDD) is a leading cause of disability (1–3), afflicting approximately 20% of the world's population (4–6), with annual costs of nearly $100 billion (7). MDD also affects approximately 10% of children and adolescents (8,9). Pediatric MDD can be highly debilitating, with negative consequences extending into adulthood, including increasing risk for conduct and substance abuse disorders, greater likelihood of relapse, and a disproportionate number of those affected do self-harm or attempt suicide (8,10). Although available treatments are generally effective and safe in adults, they are suboptimal, possessing low remission rates, delayed onset of efficacy, and unwanted side effects (1–5). Treatment options for youth are limited, with the selective serotonin reuptake inhibitor fluoxetine as the only pharmacotherapeutic currently approved for pediatric MDD (11,12). Despite emergence of studies on the efficacy and safety of treatment for childhood depression (12–14), reliable evidence-based indications for antidepressant use and potential long-term consequences in pediatric populations are lacking (12,15–17). Most troubling is that approximately 50% of adolescents with MDD are unresponsive to available treatments (18–20). Therefore, development of better, more effective treatment modalities for juvenile MDD is needed.

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Recently, the noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, ketamine, was identified as rapid-acting, long-lasting treatment for adult MDD, including those who are treatment resistant (21–26). Unfortunately, acute ketamine is not sufficient to maintain the antidepressant effects because patients return to the clinic for repeated treatment when experiencing relapse (25–27). Preclinical studies have paralleled clinical findings (28–30), focusing on acute ketamine exposure in adult rodents. Given the limited treatment options available, likelihood of treatment resistance, and higher risk for comorbidity later in life, ketamine's potential as a novel, efficacious treatment for adolescent MDD warrants assessment.

This study was designed to assess ketamine's antidepressant efficacy in adolescent male rats. We also examined enduring functional consequences of repeated ketamine exposure during adolescence by assessing subsequent behavioral reactivity to emotion-eliciting stimuli in adulthood.

Methods and Materials

Subjects

Male Sprague–Dawley rats obtained from our in-house breeding colony were used for this study. To avoid "oversampling" (31) or "within-litter effects" (32), one pup per litter was assigned to a particular condition. The age at the start of experimental manipulations in adolescent rats (postnatal day [PD] 35–49) was selected because it roughly approximates adolescence in humans (33–35). Rats were housed in clear polypropylene boxes containing wood shavings in an animal colony maintained at 23° to 25°C on a 12-hour light/dark cycle in which lights were on between 7:00 AM and 7:00 PM. Food and water were provided ad libitum.

Drug Treatment and Experimental Design

Ketamine was obtained from Butler Schein (Dublin, Ohio) in an injectable solution (100 mg/mL), diluted (5, 10, and 20 mg/kg) in

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sterile physiologic saline (.9% sodium chloride), and administered intraperitoneally (IP) at a volume of 1 mL/kg. Rats received ketamine (0, 20 mg/kg) twice daily for 1 or 15 consecutive days, and their behavioral reactivity to emotion-eliciting situations were assessed 2 months after treatment. Rats were exposed to only two behavioral assays and were never tested again after exposure to the forced swim test (FST) or the place preference conditioning (CPP) procedure (see Table 1 for experimental groups/testing sequence). There was a rest period of 48 hours between behavioral testing. All behaviors except sucrose preference and locomotor activity were recorded with a video camera. Behavioral observations and analyses were done by observers with no knowledge of the treatment conditions of each rat. All procedures were in strict accordance with the *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (National Research Council, 2003) and approved by the Florida State University Animal Care and Use Committee.

Behavioral Assays

All behavioral assays were conducted as described previously (see Methods in Supplement 1 for details).

Forced Swimming

Latency to immobility, total immobility, swimming, floating, and climbing counts were recorded (36).

Chronic Unpredictable Stress

Adolescent rats were subjected to a 15-day (PD31-46) chronic unpredictable stress (CUS) schedule with slight modifications (30).

Corticosterone Enzyme Immunoassay

Subgroups of control and CUS-exposed adolescent rats were subsequently used 72 hours after an injection of saline or ketamine to assess corticosterone levels. Half of the control group received an acute stressor (5 minutes of swimming stress) immediately before blood collection. A corticosterone enzyme immunoassay (Assay Designs, Ann Arbor, Michigan) was performed as previously described (37). See Methods in Supplement 1 for details.

Sucrose Preference

The sucrose preference test consisted of a two-bottle choice paradigm (38). Rats were exposed to either 1% under the CUS paradigm (Figure 2C,D) or to ascending concentrations of sucrose (.125%–1%; wt/vol) for 2 days per concentration after ketamine (see Figure S4A–4D in Supplement 1). The preference for sucrose over water was used as a measure for sensitivity to reward.

Locomotor Activity

Ketamine-induced locomotor activity was indexed as the distance traveled (centimeters) in an open-field apparatus immediately (Figure 3A,B), 1 hour after (Figure S1 in Supplement 1) a single injection or 60 days after repeated (1 or 15 days, twice daily) ketamine (0, 20 mg/kg) exposure (Figure S2A,C for adolescents and Figure S2B,D for adults in Supplement 1).

Elevated Plus Maze

Time spent in the open and closed arms of an elevated plus-maze (EPM) was assessed over 5 minutes (38).

Place Preference Conditioning

Conditioning trials occurred over 4 days. During conditioning, rats received saline (1.0 mL/kg, IP) and were confined to one of the side compartments of the apparatus for 30 minutes. After 3h, rats received ketamine (5, 10, or 20 mg/kg, IP) and were confined to the opposite side compartment for 30 minutes. On the test day (day 5), rats received saline (IP) and were allowed to explore the entire apparatus freely for 30 minutes (39,40).

Statistical Analyses

Behavioral data were analyzed using mixed-design analyses of variance (ANOVAs) followed by Fisher least significant difference (LSD) post hoc tests. The Nyholt correction was used to control for multiple comparisons (41). When appropriate, Student *t* tests were used to determine statistical significance of planned comparisons. Data are expressed as the mean ± SEM. Statistical significance was set at $p < .05$.

Results

Establishing Ketamine's Antidepressant Efficacy

An initial experiment was conducted to determine the antidepressant efficacy of ketamine in adolescent rats using the FST. Rats received a single injection of ketamine (0, 5, 10, or 20 mg/kg)

Table 1. Experimental Groups and Testing Sequence

Group	*n*	Treatment (mg/kg)	Age	Interval 1	Test 1	Interval 2	Test 2
1 (Figure 1)	8	1 day ketamine (0, 5, 10, 20)	Adolescent	24 hours	FST	-	-
2 (Figure 2)	8-12	CUS + single injection ketamine (0, 20)	Adolescent	1 hour	FST	-	-
3 (Figure 3)	19-20	Single injection ketamine (0, 20)	Adolescent	-	Locomotion	-	-
4 (Figure 3)	19-20	Single injection ketamine (0, 20)	Adult	-	Locomotion	-	-
5 (Figures 4, 5, S2 in Supplement 1)	10	15 days ketamine (0, 20; BID)	Adolescent	2 months	Locomotion	48 hours	EPM
6 (Figures 4, 5, S2 in Supplement 1)	10	15 days ketamine (0, 20; BID)	Adult	2 months	Locomotion	48 hours	EPM
7 (Figures 5, S2 in Supplement 1)	9-10	1 day ketamine (0, 20; BID)	Adolescent	2 months	Locomotion	48 hours	EPM
8 (Figures 5, S2 in Supplement 1)	10	1 day ketamine (0, 20 BID)	Adult	2 months	Locomotion	48 hours	EPM
9 (Figure S1 in Supplement 1)	9-10	Single injection ketamine (0, 20)	Adolescent	1 hour	Locomotion	-	-
10 (Figure S3 in Supplement 1)	10	1 day ketamine (0, 20; BID)	Adolescent	2 months	SP[a]	48 hours	FST
11 (Figure S3 in Supplement 1)	10	1 day ketamine (0, 20; BID)	Adult	2 months	SP[a]	48 hours	FST
12 (Figures 6, S4 in Supplement 1)	12	15 days ketamine (0, 20; BID)	Adolescent	2 months	SP	48 hours	FST
13 (Figures 6, S4 in Supplement 1)	11-12	15 days ketamine (0, 20; BID)	Adult	2 months	SP	48 hours	FST
14 (Figure 6)	6-10	Ketamine (0, 5,10, 20)	Adolescent	-	CPP	-	-

All injections administered intraperitoneally.
BID, twice daily; CPP, conditioned place preference; EPM, elevated plus maze; FST, forced swimming test; SP, sucrose preference.
[a]Data not shown