

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

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 \pm 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	<i>n</i>	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.

^aData not shown

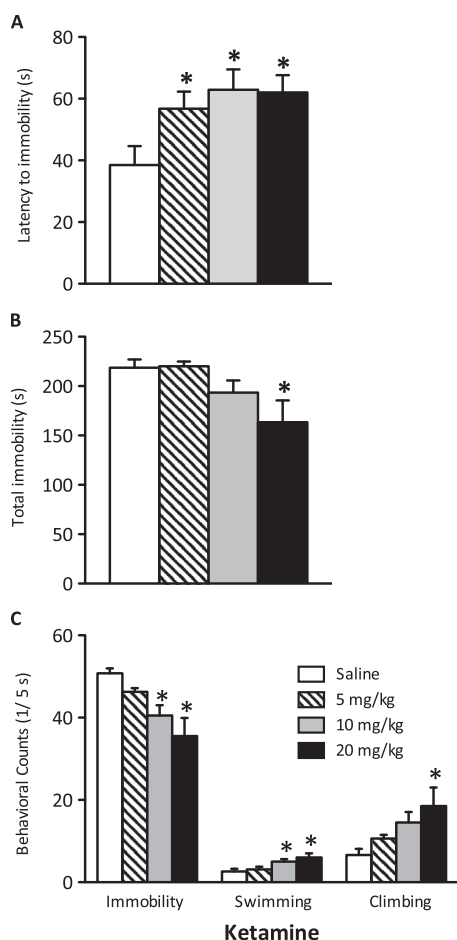


Figure 1. Effects of ketamine (0, 5, 10, or 20 mg/kg) on behavioral despair as measured in the forced swim test (FST) in adolescent (postnatal day 35) male rats 24 hours after 1 day (i.e., two injections, 4 hours apart) of ketamine ($n = 8/\text{dose}$). **(A)** Ketamine significantly increased latency to become immobile regardless of dose ($p < .05$, respectively). **(B)** Only the 20 mg/kg ketamine dose significantly reduced total immobility ($p < .05$). **(C)** Ketamine (10 and 20 mg/kg) significantly reduced immobility and increased swimming counts, whereas only 20 mg/kg significantly increased climbing counts compared with controls. Data are presented as latencies to become immobile and total immobility (in seconds) and as cumulative 5-second intervals of immobility, swimming, and climbing counts (mean \pm SEM). *Significantly different from saline-treated controls ($p < .05$).

24 hours before FST day 2, but this treatment failed to reliably induce escape-like behaviors (data not shown). Therefore, in a subsequent experiment, PD35 rats received two injections of ketamine (0, 5, 10, and 20 mg/kg), 4 hours apart, after day 1 of FST. Twenty-four hours later (PD36), their behavioral reactivity to swimming stress was assessed. Ketamine increased latency to immobility in adolescent rats ($F_{3,28} = 3.568$, $p < .05$; Figure 1A; $n = 32$). Analyses revealed that all three doses significantly increased latency to immobility when compared to controls ($p < .05$). Ketamine treatment reduced total immobility ($F_{3,28} = 3.873$, $p < .05$; Figure 1B) but only reliably at 20 mg/kg when compared with control subjects ($p < .05$). Treatment also influenced immobility ($F_{3,28} = 6.275$, $p < .005$), swimming ($F_{3,28} = 4.572$, $p < .05$), and climbing ($F_{3,28} = 3.411$, $p < .05$) counts (Figure 1C). Specifically, 10 and 20 mg/kg decreased immobility and increased swimming ($p < .05$), whereas only 20 mg/kg increased climbing counts ($p < .05$).

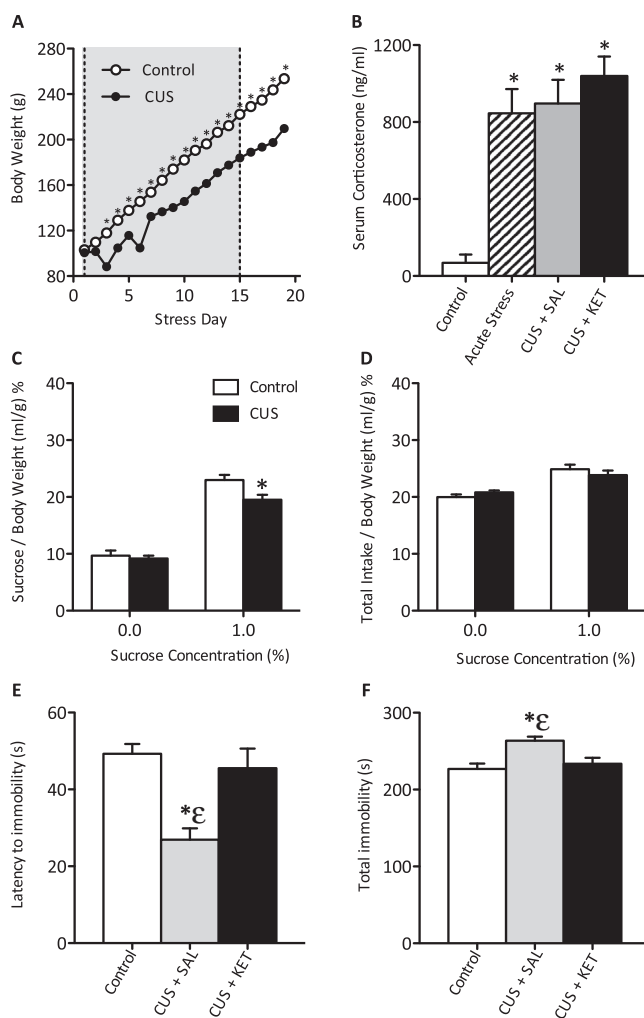


Figure 2. Effects of chronic unpredictable stress (CUS) and ketamine's (20 mg/kg) antidepressant efficacy in adolescent rats ($n = 8\text{--}12/\text{group}$). **(A)** Exposure to CUS reduced weight gain across days. **(B)** Levels of circulating serum corticosterone (CORT) were assessed in control (i.e., no stress) and CUS-exposed adolescent rats 72 hours after receiving saline (SAL) or ketamine (KET; 20 mg/kg) and in controls exposed to stress for 5 minutes immediately before blood collection. Serum CORT levels were significantly elevated by acute as well as CUS exposure and were not affected by ketamine treatment ($p < .05$). **(C)** Exposure to CUS significantly reduced adolescent rats' preference for sucrose ($p < .05$). **(D)** No changes in total liquid intake were observed. **(E)** CUS significantly reduced rats' latency to become immobile ($p < .05$), whereas a single ketamine injection (20 mg/kg) reversed the CUS-induced deficit back to control levels ($p > .05$). **(F)** Exposure to CUS significantly increased total immobility ($p < .05$), whereas a single ketamine injection (20 mg/kg) reduced total immobility back to control levels ($p > .05$). Data are presented as latencies to become immobile and total immobility (in seconds; mean \pm SEM). *Significantly different from saline-treated controls ($p < .05$). ^εSignificantly different from CUS + ketamine-treated group ($p < .05$).

Ketamine's Antidepressant Efficacy After CUS

Although ketamine increased escape-like behavioral reactivity, only the highest dose did so reliably on all measures (Figure 1). To confirm this finding, we exposed rats to a 15-day (PD31–46) CUS regimen to induce a depression-like phenotype to further assess ketamine's (20 mg/kg) antidepressant efficacy. Exposure to CUS affected weight gain in adolescent rats (Figure 2A; $N = 31$). Repeated measures ANOVA revealed that while rats gained

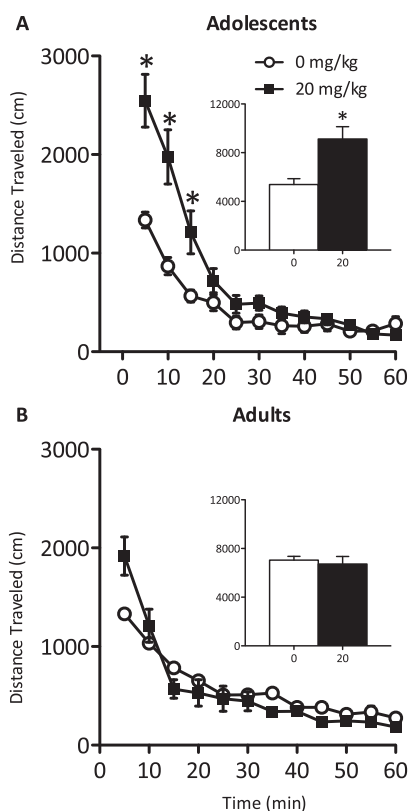


Figure 3. Immediate effects of a single injection of ketamine (20 mg/kg) on distance traveled in adolescent and adult rats. **(A)** Adolescent rats treated with ketamine ($n = 19$) had significantly increased locomotor activity during the first 15 minutes immediately after a single ketamine exposure compared with saline-treated adolescent controls ($n = 20$; $p < .05$). Their total distance traveled was also significantly higher than controls ($p < .05$). **(B)** Ketamine-treated adults ($n = 19$) exhibit no differences in distance traveled across time or total distance traveled compared with their respective saline-treated controls ($n = 20$). Data are represented as mean distance traveled (mean \pm SEM, in centimeters). *Significantly different from saline-treated controls ($p < .05$).

weight as they matured (within-subject main effect: $F_{18,522} = 2707.444$, $p < .001$), CUS significantly reduced bodyweight (between-subject main effect: $F_{1,522} = 57.081$, $p < .001$) when compared with controls. There was a significant interaction between days and stress ($F_{18,522} = 50.181$, $p < .001$). CUS-exposed rats displayed lower bodyweights beginning on day 3, never returning to control levels ($p < .05$).

We also determined the effect of CUS and ketamine treatment on serum corticosterone (CORT) levels (Figure 2B). Serum CORT concentrations varied as a function of CUS ($F_{3,27} = 8.039$, $p < .001$). Acute stress, CUS + Saline (SAL), and the CUS + Ketamine groups had significantly elevated CORT levels when compared with No Stress controls ($p < .05$, respectively).

To verify that CUS produced a depressive-like phenotype in adolescents, we assessed sucrose preference. Exposure to CUS significantly reduced preference for sucrose ($t_{29} = 2.161$, $p < .05$) compared with controls (Figure 2C), indicating a decreased sensitivity to natural reward (i.e., anhedonia). No differences in total liquid intake were detected between groups in either condition (Figure 2D).

We also assessed ketamine's (20 mg/kg) ability to influence behavioral despair after CUS (Figure 2E,F). In addition to controls, rats were divided into groups receiving a single saline (CUS + SAL) or ketamine (CUS + KET) injection 60 minutes before FST

(day 2). CUS + SAL-treated rats displayed pro-depressive behaviors manifested in significantly reduced latencies to immobility ($F_{2,28} = 8.542$, $p < .005$) and increased total immobility ($F_{2,28} = 7.647$, $p < .005$) when compared with No Stress and the CUS + KET-treated groups. Ketamine reversed the effects of CUS (i.e., antidepressant effect), as escape-like behaviors of these rats did not differ from the No Stress controls ($p > .05$).

On the basis of these results (Figures 1, 2E,F), we chose the 20 mg/kg dose to assess the long-term functional consequences of repeated ketamine in adolescent and adult (PD75) rats.

Ketamine-Induced Locomotor Activity

Because forced swimming can be influenced by motor activity, we assessed the immediate effects of acute ketamine (20 mg/kg) on locomotor activity in adolescent and adult rats (Figure 3A,B). Repeated-measures ANOVA revealed drug-induced changes in locomotor activity across time ($F_{11,407} = 65.944$, $p < .001$) and as a function of time by drug ($F_{11,407} = 10.45$, $p < .001$). Post hoc analyses revealed adolescents with higher activity for the first 15 minutes following ketamine ($p < .05$) before returning to control levels. Ketamine-exposed adolescents traveled greater cumulative distance overall ($t_{37} = 3.303$, $p < .005$; Figure 3A, inset).

Ketamine did not influence adult rats' locomotor activity compared with controls (Figure 3B).

To control for novelty influencing ketamine-induced locomotion, distance traveled was assessed 1 hour after acute ketamine (20 mg/kg) in adolescent rats (Figure S1 in Supplement 1). Repeated-measures ANOVA revealed main effects indicating reduced activity across time ($F_{11,187} = 55.075$, $p < .001$) and drug treatment ($F_{1,187} = 6.896$, $p < .05$), without interaction between variables. These results indicate that novelty did not contribute to the ketamine-induced locomotion observed in Figure 3A.

Effects of Repeated Ketamine Exposure on Weight Gain and Food Intake

Adolescent and adult rats received ketamine (20 mg/kg, twice daily) for 1 or 15 consecutive days. One day of ketamine exposure had no effect on weight gain or food intake (data not shown).

Fifteen days of ketamine treatment reduced weight gain in both adolescent (Figure 4A) and adult rats (Figure 4B). Repeated measures ANOVA revealed changes in bodyweight across days ($F_{34,612} = 2642.339$, $p < .001$), drug ($F_{1,612} = 5.932$, $p < .05$), and as a function of day by drug ($F_{34,612} = 6.907$, $p < .001$) in adolescents. Although ketamine-treated rats gained weight at a lower rate than controls, Nyholt corrections for multiple comparisons indicated significantly reduced bodyweights only on days 9 and 11 of treatment ($p < .05$). Nevertheless, adolescents' bodyweight remained lower and was accompanied by reduced daily food intake ($t_{18} = 4.933$, $p < .001$; Figure 4A, inset) throughout the experiment.

Ketamine reduced adult weight gain in a similar fashion as the adolescents (Figure 4B). The ANOVA revealed changes in weight across days ($F_{34,612} = 45887.454$, $p < .001$), drug ($F_{1,612} = 5.269$, $p < .05$), and as a function of day by drug ($F_{34,612} = 3.83$, $p < .001$). Nyholt corrections revealed lower bodyweights on days 4, 6, and 8 to 10 of treatment ($p < .05$), but similar to adolescents, adult rats displayed lower bodyweights and reduced daily food intake ($t_{18} = 4.476$, $p < .001$; Figure 4B, inset) throughout the experiment.

Long-Term Effects of Repeated Ketamine Exposure on Basal Locomotor Activity

Repeated ketamine had no effect on adolescent or adult rats' basal locomotor activity 2 months after drug exposure (Figure S2A-D in Supplement 1).

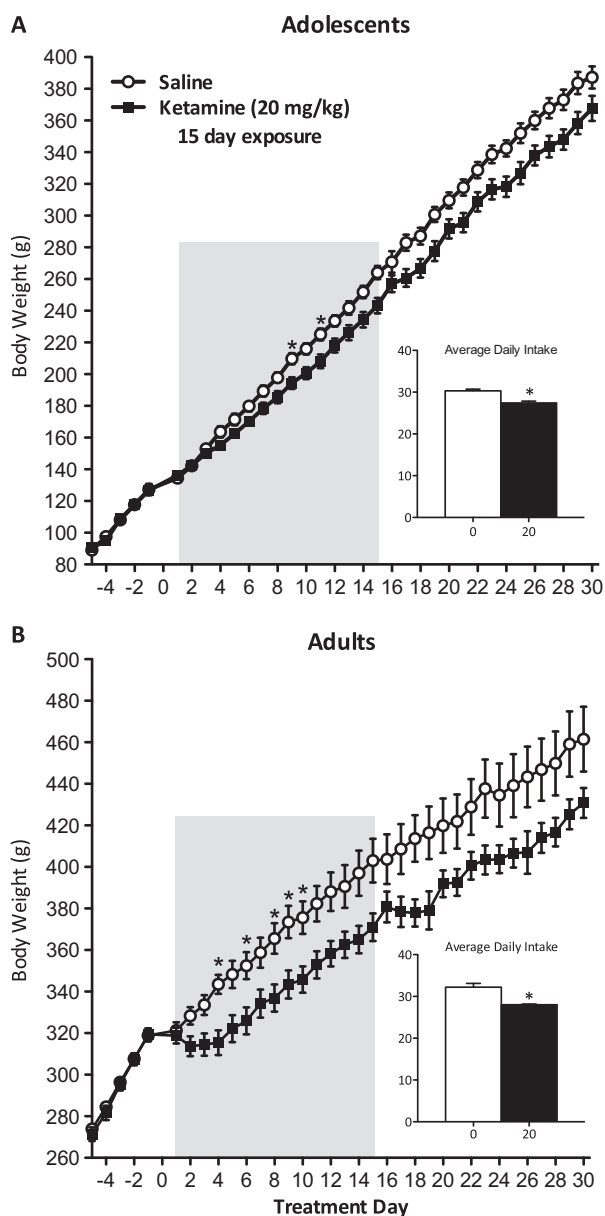


Figure 4. Repeated exposure to ketamine (20 mg/kg, twice daily) disrupts normal weight gain and average daily food intake of adolescent- and adult-treated rats ($n = 10/\text{group}$). **(A)** Repeated ketamine from postnatal days 35–49 reduced weight gain on days 9 and 11 of treatment ($p < .05$) in adolescent rats. Average daily chow intake during and 15 days after treatment was significantly reduced in ketamine-treated adolescents. **(B)** Adults (postnatal days 75+) repeatedly treated with ketamine also show reduced weight gain on days 4, 6, and 8 through 10 ($p < .05$). Ketamine-treated adults daily intake was also significantly reduced compared with saline-treated controls ($p < .05$). Data are represented as body weight in grams and average daily intake across days in grams (mean \pm SEM). Shaded area indicates ketamine treatment days. *Significantly different from saline-treated controls ($p < .05$).

Long-Term Effects of Repeated Ketamine Exposure on Anxiety-Like Behavior

One day of ketamine exposure did not affect time spent in the open arms of the EPM, regardless of age at time of treatment, 2 months after exposure (Figure 5A and 2B; $n = 19\text{--}20/\text{group}$). Conversely, 15 days of ketamine significantly increased time spent in the open arms of the EPM of both adolescent ($t_{18} =$

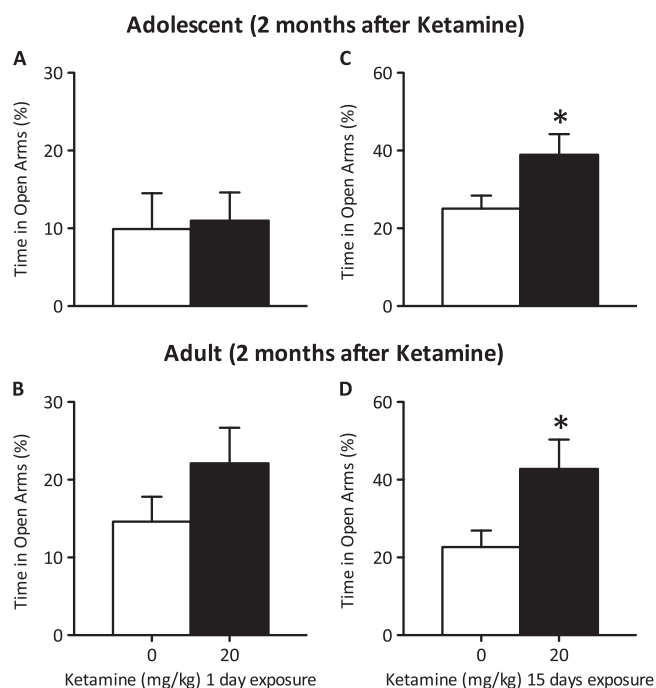


Figure 5. Effects of 1 and 15 days of ketamine exposure (20 mg/kg; twice daily) on anxiety-like behavior. **(A, B)** One day of ketamine treatment did not produce changes in anxietylike behaviors as measured in the elevated-plus maze (EPM) regardless of age of exposure ($n = 10/\text{group}$). **(C, D)** Conversely, repeated exposure to ketamine (20 mg/kg), twice daily, induced significant increases in time spent in the open arms of the EPM in both adolescent- and adult-treated rats ($p < .05$; $n = 10/\text{group}$). Data are presented as percent time spent (mean \pm SEM) in the open arms of the EPM. *Significantly different from saline-treated controls ($p < .05$).

2.205, $p < .05$) and adult ($t_{18} = 2.314$, $p < .05$) treated rats compared with controls (Figure 5C,D; $n = 20/\text{group}$) 2 months after drug exposure.

Long-Term Effects of Ketamine Exposure on Behavioral Despair

We used the FST to assess rats' responsiveness to stress 2 months after 1 day of ketamine exposure. No differences on any measures of the FST regardless of age at time of treatment were observed (adolescents: Figure S3A–C in Supplement 1; adults: Figure S3D–F in Supplement 1; $n = 20/\text{group}$).

Behavioral despair was also assessed 2 months after 15 days of treatment in adolescent ($N = 24$) and adult ($N = 20$) rats (Figure 6A–6F). Ketamine exposure during adolescence significantly increased latency to immobility ($t_{22} = 4.743$, $p < .005$) and decreased total immobility ($t_{22} = 3.684$, $p < .005$) compared with controls (Figure 6A,B). These rats also displayed less immobility ($t_{22} = 3.092$, $p < .05$) and higher swimming ($t_{22} = 4.364$, $p < .005$) counts than controls (Figure 6C).

Separate groups of adult rats were tested on the FST 2 months after 15 days of ketamine (matched treatment and testing schedule). Ketamine-treated adult rats displayed longer latencies to immobility ($t_{21} = 5.748$, $p < .005$) and decreased total immobility ($t_{21} = 4.247$, $p < .005$) compared with controls (Figure 6D,E). They also displayed lower immobility ($t_{21} = 3.995$, $p < .005$) and higher swimming ($t_{21} = 4.125$, $p < .005$) counts than controls (Figure 6F).

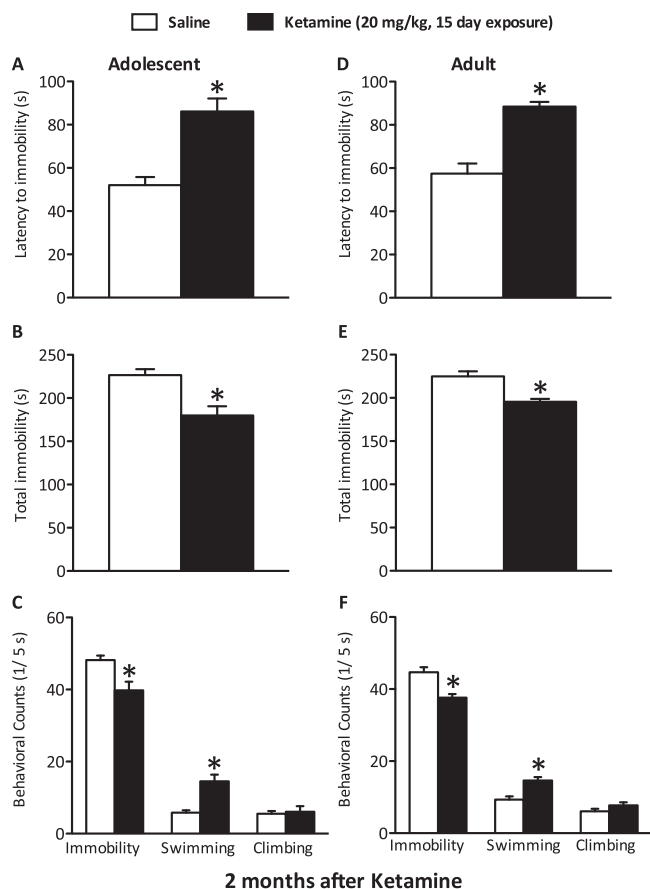


Figure 6. Lasting effects of repeated (15 days) exposure to ketamine (20 mg/kg, twice daily) on behavioral despair using the forced swim test (FST) paradigm, 2 months after drug exposure, in adolescent (A–C) and adult (D–F) rats. Adolescent (postnatal days 35–49; $n = 12/\text{group}$) rats show significantly increased latencies to immobility (A), lower total immobility (B), decreased immobility as well as higher swimming counts (C) compared with saline-treated rats 2 months after drug exposure ($p < .05$). Similarly treated adult rats (postnatal days 75–89; $n = 11\text{--}12/\text{group}$) also exhibited significantly increased latencies to immobility (D), lower total immobility (E), and decreased immobility and increased swimming counts (F) 2 months after drug treatment ($p < .05$). Data are presented as latencies to become immobile and total immobility (in seconds) and as cumulative 5-second intervals of swimming, climbing, and immobile counts (mean \pm SEM). *Significantly different from saline-treated rats ($p < .05$).

Effects of Ketamine on Reward-Related Behavior

Because stress can induce anhedonia and ketamine is a drug of abuse that interacts with brain reward circuits, we determined the effects of previous exposure to ketamine on sucrose preference in adolescent and adult rats. We also assessed whether ketamine would induce place preference using the CPP paradigm in drug-naïve adolescent rats. Adolescent and adult rats receiving 1 (data not shown) or 15 days of ketamine (Figure S4A,D in Supplement 1) did not show differences in sucrose preference 2 months after drug exposure.

Ketamine (0, 5, 10, and 20 mg/kg) failed to induce place conditioning in adolescent rats compared with controls (Figure S4E in Supplement 1).

Discussion

This study was designed to examine the effectiveness of ketamine's antidepressant properties in adolescent male rats and

assess the consequences of its repeated exposure during adolescence on functional reactivity to emotion-eliciting situations in adulthood. Here we report that ketamine yields rapid antidepressant-like effects in adolescent rats exposed to control and CUS conditions. Additionally, 15 days, but not 1 day, of twice-daily ketamine treatment results in an enduring stress-resistant phenotype, regardless of age at time of exposure.

Initially, adolescent rats received a single injection of ketamine (5, 10, or 20 mg/kg) 24 hours before reexposure to the FST (day 2) without yielding reliable results. Subsequently, the same experiment was conducted in separate groups, but this time receiving two injections of ketamine 4 hours apart. Exposure to ketamine induced antidepressant-like responses manifested in higher latencies to immobility and increased total immobility on day 2 of the FST (42–44). Interestingly, only the highest dose of ketamine (20 mg/kg) produced reliable antidepressant-like effects in adolescents, a dose higher than reported in adult rats (45). Because these findings were derived from drug-naïve rats, it was important to validate ketamine's antidepressant effects after exposure to CUS, a commonly used model of depression and antidepressant efficacy (46–48). Our data show that 15 days of CUS produces a robust depressive-like phenotype in adolescent rats, evidenced by reduced weight gain, elevated serum CORT levels, shorter latencies to immobility and greater total immobility in the FST, and anhedonia (i.e., reduced sucrose preference). Although reduced body weight in CUS-exposed rats was expected to influence sucrose preference, this was not the case because there were no significant changes in total liquid intake (sucrose+water) between the groups, and decreased preference for sucrose remained after controlling for body weight (48–50). Importantly, a single ketamine exposure (20 mg/kg) 1 hour before forced swimming successfully reversed the effects of CUS as indicated by coping patterns categorized as antidepressant-like behaviors (42–44). These effects were not due to ketamine-induced changes in locomotor activity because these rats were forced to swim long after ketamine's influence on motor behavior had dissipated (see Figures S1 and S3A in Supplement 1). These findings parallel those demonstrating acute ketamine's antidepressant effects in adults (51–53), and we now expand ketamine's antidepressant effects to adolescent rats.

Given ketamine's ability to ameliorate the effects of stress on CORT levels in adults (53), we assessed for similar effects in adolescent rats. Acute ketamine failed to influence serum CORT levels of CUS-exposed adolescents, yet it was capable of inducing antidepressant-like responses in the FST. The mechanism(s) underlying these effects are unknown, but ketamine's antidepressant-like effects may be independent of hypothalamic-pituitary-adrenal axis modulation in adolescents. The effects of NMDA antagonists on hypothalamic-pituitary-adrenal axis activity are equivocal, increasing or decreasing its functioning depending on the species and drug dose used (54–56), method, and duration of drug administration (57). Within the CUS context, it is conceivable that discrepancies of our findings and those by Garcia *et al.* (53) may be explained by differences in experimental design and methods (e.g., stress schedule), and/or by ontogenetic differences (i.e., age of exposure) that often emerge when manipulating the nervous system during maturational stages before adulthood (35,36,38), and more detailed studies are needed to assess these phenomena.

On the basis of these results, we assessed for enduring consequences after 1 or 15 days of twice-daily ketamine (20 mg/kg) exposure during adolescence on behavioral reactivity in adulthood. In the clinic, acute ketamine yields rapid and robust

antidepressant and anxiolytic effects (21–26,58), but patients experience relapse within days (25–27). Preclinical studies also report antidepressant- and anxiolytic-like responses lasting several days (28–30,52,59), but none have looked beyond 2 weeks. We report here that adolescent rats and their adult controls tested 2 months after 1 day of ketamine exposure show no significant changes in responsivity to anxiety- and stress-eliciting situations, as well as no changes in hedonic responses as measured by the sucrose preference paradigm. These results are in agreement with clinical and preclinical findings demonstrating that acute ketamine produces rapid antidepressant responses but that it is insufficient to sustain long-lasting antidepressant or anxiolytic effects.

Chronic ketamine exposure disrupts appetite and weight gain in adult humans and rats (60,61). Although 15 days of repeated ketamine significantly reduced food intake and weight gain in both adolescent and adult rats, it took longer for adolescents to show deficits than their adult counterparts (Figure 4), and both groups displayed lower body weights than controls throughout the experiment. Ketamine also induced age-dependent effects on locomotor activity as adolescents showed initial hyperlocomotion lasting 15 minutes, whereas adult-treated rats were unaffected. These results demonstrate that adolescents are more sensitive to the locomotor effects of ketamine, yet required higher doses than adults to elicit antidepressant-like responses. The underpinnings underlying these effects are unknown. NMDA receptor expression peaks during adolescence and steadily drops off thereafter (35,62,63). That adolescents have higher concentrations of NMDA receptors and typically metabolize drugs faster than adults (64) may explain why they require higher doses of ketamine to produce reliable antidepressant-like responses. Subanesthetic doses of ketamine are thought to enhance glutamatergic signaling and dopamine release within the prefrontal cortex by reducing excitatory input into gamma-aminobutyric acid-ergic neurons that subsequently leads to hyperactivity of corticolimbic pathways (59,65,66). Thus, it is conceivable that there is more gamma-aminobutyric acid-ergic inhibition of this pathway at rest during adolescence and that ketamine treatment results in increased hyperactivity compared with adults.

Repeated ketamine is being explored as a treatment modality for the long-lasting maintenance of antidepressant response (27,67,68). Repeated ketamine exposure in adult rats is effective (69), but the longevity of these effects in adult or adolescent rats is unknown. It is well documented that drug exposure during development can yield mood-related perturbations later in life (36,38,39,70–72); therefore, we expected repeated ketamine to induce deficits in coping reactivity to anxiety- and stress-eliciting challenges. Surprisingly, repeated ketamine exposure yields long-lasting (i.e., at least 2 months) anxiolytic- and antidepressant-like responses in both adolescent and adult rats. These effects cannot be explained by ketamine-induced changes in basal locomotor activity because no differences were detected 2 months after drug exposure (Figure S2 in Supplement 1). Our results provide support for repeated ketamine's improved efficacy in maintaining antidepressant-like effects and demonstrate its effectiveness in adolescent rats.

Despite ketamine's therapeutic potential, this drug possess abuse liability (73–75), and its repeated exposure has the capacity to induce behavioral sensitization in rodents (76,77), a known mechanism implicated in drug addiction (78,79). Therefore, we further evaluated whether the doses used in these studies could produce place preference conditioning, as measured by the CPP

paradigm, in adolescent rats. CPP is a well-established behavioral assay used to assess drug reward (40,80–83). Ketamine failed to produce place preference at any of the doses tested (Figure S4E in Supplement 1). These results suggest that the range of ketamine doses, specifically the 20 mg/kg, do not induce rewarding effects in adolescent rats. Few studies have successfully reported ketamine-induced CPP in adult rats (84–86). Although unlikely, given our experience with this behavioral assay (40,72,87,88), it is possible that our drug-conditioning procedure was insufficient to induce CPP. However, to our knowledge, there is no literature assessing ketamine-induced rewarding effects in juvenile rodents. It is therefore conceivable that low doses of ketamine in adolescents could serve as a relatively safe antidepressant with low abuse potential. This assumption is supported, at least partially, by findings demonstrating lack of behavioral sensitization in healthy human subjects after repeated ketamine (89). Furthermore, no long-term changes in hedonic responses were observed, as measured by sucrose preference, following repeated exposure to ketamine in adolescent or adult treated rats (Figure S4A–D in Supplement 1). Nevertheless, the adolescent rats used here were ketamine-naïve, and different results could be observed in rats previously treated with ketamine during adolescence. Future studies should assess this possibility.

The mechanism(s) underlying ketamine's antidepressant effects have only recently begun to be elucidated, with most research centering on understanding its rapid actions. Current evidence suggests that ketamine rapidly enhances the structure and function of cortical synapses known to play a role in mood (45,64). Studies demonstrate that ketamine's antidepressant effects may depend on rapid activation of the mammalian target of rapamycin pathway, including increases in extracellular signal-regulated kinase, protein kinase B, and brain-derived neurotrophic factor in the hippocampus, and number of new spines in the prefrontal cortex (28,64). The mechanism(s) involved in the sustained effects observed with ketamine are unclear, although mounting evidence points to changes in alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor density and their intracellular signaling cascades (52,90). Although speculative, our data suggest that repeated ketamine might lead to robust, perhaps permanent, changes to cortical synapses thus maintaining longer-lasting antidepressant effects. Nevertheless, ketamine's actions on the nervous system are complex, and much more detailed assessments of its effects on other behavioral outputs, signaling pathways, and brain areas are clearly needed to understand the mechanism(s) involved in its rapid and sustained antidepressant effects.

Although this study provides evidence that repeated ketamine induces long-lasting antidepressant effects, there are some limitations. Our experiments were conducted on male rats, and given higher prevalence of MDD in women (91), it will be important to replicate these findings using female rats. NMDA receptors play a role in learning and memory (92,93), and ketamine can affect memory (94,95). Therefore, the effects of repeated sub-anesthetic exposure to ketamine on learning and memory must be determined because findings in the FST could have been influenced by disruption in memory retention in ketamine-treated rats. In addition, the role of ketamine-induced impulsivity within the context of these findings must be explored.

The overall results from our study demonstrate that repeated exposure to ketamine results in an enduring resilient phenotype. To our knowledge, we show for the first time that ketamine is capable of inducing antidepressant-like responses in adolescent

rats. Furthermore, we show that repeated, but not acute, ketamine produces lasting anxiolytic- and antidepressant-like responses independent of age at time of exposure. This supports the view that repeated ketamine is more efficacious and that it could serve as a novel antidepressant for pediatric MDD.

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