Received: 19 March 2018 | Revised: 12 July 2018 | Accepted: 14 July 2018

DOI: 10.1002/eat.22937

International Journal of EATING DISORDERS

BRIEF REPORT

Single injection of ketamine during mid-adolescence promotes long-lasting resilience to activity-based anorexia of female mice by increasing food intake and attenuating hyperactivity as well as anxiety-like behavior

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Funding information
Fulbright Scholarship, Grant/Award Number: no number; Klarman Family Foundation Grant Program in Eating Disorders Research, Grant/Award Number: R21 MH105846, R01NS066019-01A1, R01NS047557-07A1; National Eye Institute, Grant/Award Number: EY13079; National Institute of Mental Health, Grant/Award Number: R21 MH105846; National Institutes of Health, Grant/Award Number: R01NS047557-07A1, R01NS066019-01A1; National Science Foundation, Grant/Award Number: NSF-REU 1460880 ; NYU Research Challenge Fund, Grant/Award Number: NSF-REU 1460880

Abstract

Objective: This study tested the effects of ketamine on vulnerability of female adolescent mice to activity-based anorexia (ABA).

Method: Twenty-four female C57Bl/6 J mice underwent ABA induction, which involved exposing wheel-acclimated adolescent mice to two bouts of food restriction (FR)—the first ABA (P41–44, mid-adolescence) and the second ABA (P55–59, late adolescence), with recovery in between. Ketamine (3 or 30 mg/kg) or vehicle was given once, on the second day of FR of the first ABA (P42). Food consumption, body weight and wheel running activity were measured daily. Anxiety-like behaviors were accessed by elevated plus maze on P49 and P62, after weight restoration during the recovery phase.

Results: Ketamine (30 mg/kg) increased food intake during the first ABA (+38%, p = .015) and facilitated weight gain during recovery (+42%, p = .003). During the second ABA, the effect was manifested as increased food intake (+38%, p = .001) and weight gain (+47%, p = .001) while attenuating FR-induced wheel running activity (−24%, p = .09) and weight loss (−17%, p = .056). Ketamine also reduced anxiety-like behaviors.

Discussion: Thus, single injection of ketamine during mid-adolescence effectively attenuates vulnerability of female mice to repeated ABA exposures.

KEYWORDS

ABA, adolescence, anxiety-like behavior, body weight, C57BL6/J, EPM, food intake, hyperactivity, ketamine, rodent

1 | INTRODUCTION

There is at present no accepted pharmacological treatment for anorexia nervosa (AN) due, in part, to the limited knowledge of its etiology. Activity-based anorexia (ABA) is an animal model of AN that can provide clues regarding the neurobiological bases for vulnerability to AN. ABA captures four hallmarks of AN (American Psychiatric Association, 2013; Gutierrez, 2013): (a) excessive voluntary exercise (Wable, Min, Chen, & Aoki, 2015), (b) food restriction (FR), which appears to be voluntary, since animals continue to run on a wheel even during the hours of food availability (Chen, Wable, Chowdhury, & Aoki, 2016), which yield (c) weight loss so severe as to be lethal, even though equivalent degree of FR without a wheel is not lethal, and (d) heightened anxiety after weight recovery. Hyperactivity is readily evoked upon ~80% of wheel-acclimated wildtype adolescent female mice by restricting food access to 2 hr per day (Chowdhury, Chen, & Aoki, 2015; Chowdhury, Wable, Sabaliauskas, & Aoki, 2013). Previously, we showed that FR elevated anxiety-like behavior that was measurable during the recovery period, even though body weight had been restored 5 days prior (Chen, Surgent, Rana, Lee, & Aoki, 2017). Moreover, an individual's vulnerability to FR-evoked wheel running activity is highly correlated with individual's anxiety-like behaviors (Wable et al., 2015).

The dorsal and ventral hippocampus of adolescent females that have undergone ABA exhibit significant morphological and molecular changes (Aoki, Chowdhury, Wable, & Chen, 2017). Ventral hippocampus

is crucial for regulating anxiety responses (Bannerman et al., 2003; Jimenez et al., 2018). However, previous studies indicate that the dorsal hippocampus is also important for anxiety regulation (Huttunen & Myers, 1986; Kataoka et al., 1991), especially among adolescent females (Shen et al., 2007). In the dorsal hippocampus, individuals' ABA vulnerability, based on weight loss, correlates with elevated levels of NR2B subunits of NMDA receptors at synapses (Chen, Actor-Engel, et al., 2017). We reasoned that if elevation of NR2B-containing NMDARs are causal to ABA vulnerability, then blockade of NR2B-NMDARs may reduce ABA vulnerability. Ketamine is a non-competitive NMDAR antagonist, a single administration of which elicits fast and sustained antidepressant effects both in humans (Berman et al., 2000; Zarate et al., 2006) and animals (Autry et al., 2011; Li et al., 2010; Maeng et al., 2008; Zanos et al., 2016), possibly through antagonism of the NR2B-NMDARs (Miller et al., 2014). Ketamine is also anxiolytic in humans (aan het Rot et al., 2010; Krystal et al., 1994) and animals (Jiang et al., 2017). Thus, we asked whether a single systemic injection of ketamine in mid-adolescence could reduce ABA vulnerability through its anxiolytic action.

2 | MATERIALS AND METHODS

2.1 | Animals

Twenty-four female C57Bl/6 J littermates in this study were bred at New York University's animal facility (Wable et al., 2015). All procedures relating to the use of animals were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the IACUC of New York University (A3317–01). All animals were kept on a 12/12 light–dark cycle (lights on at 0700 h). Food and water were available ad libitum, except as noted below. After weaning at postnatal day (P) 25 (P25), animals of the same sex were group-housed 2–4 per cage (Figure 1a).

2.2 | ABA induction

ABA induction consisted of exposing wheel-acclimated singly-housed adolescent mice to two bouts of food restriction (FR)—the first and second ABA—with recovery in between (Figure 1a), as was described previously (Chen et al., 2016; Chowdhury et al., 2013; Wable et al., 2015; see Supporting Information). On P42, animals were pseudo-randomly assigned to one of three treatment groups—ketamine (3 and 30 mg/kg)-injected or vehicle (saline) controls, counter-balanced to ensure that the groups' average body weight and baseline running were not significantly different (Figure 1a). Ketamine (3 or 30 mg/ kg; Browne & Lucki, 2013; Henry Schein, Melville, NY), diluted with saline, was injected intraperitoneally (i.p.) 1 hr prior to the feeding time on P42, corresponding to the second day of the first ABA (Figure 1b). Vehicle control animals received i.p. saline at the same time. Further details are provided in Supporting Information.

2.3 | Elevated plus maze

Anxiety-like behaviors were measured by the elevated plus maze (EPM) test, conducted on P49 and P62 during the dark cycle. EPM duration was 10 min (Wable et al., 2015). The time spent in the open arms was recorded and analyzed using the EthoVision tracking system (version X12, Noldus Information Technology, Wageningen, The Netherlands).

2.4 | Statistical analysis

Normality of the distribution of measures was tested using the D'Agostino & Pearson omnibus normality test, Shapiro–Wilk normality test, and Kruskal-Wallis test. One-way analysis of variance (ANOVA) was used to evaluate significance of the differences among the three treatment groups, followed by Fisher's least significant difference (LSD) post hoc analysis. Repeated measures two-way ANOVA was used to evaluate significance of the differences among the three treatment groups across days, followed by Fisher's LSD post hoc analysis. All the results are expressed as mean ± *SEM*, with *p*-values <.05 considered statistically significant. GraphPad Prism Version 7.01 and IBM SPSS 24.0 were used.

3 | RESULTS

3.1 | Ketamine increases food intake during the first ABA in mid-adolescence

No difference in food consumption was detected among the groups prior to assignment of animals to the vehicle and two ketamine groups (Figure 1c, ketamine 3 and 30 mg/kg, $p = .167$ and $p = .999$, respectively), corresponding to the first day of ABA (P41), after 6 hr of FR and 23-hr before ketamine injection. The ketamine groups received ketamine (3 and 30 mg/kg) on the second FR day of the first ABA (P42), 1 hr before feeding. Repeated measures two-way ANOVA revealed a significant interaction between treatments and days ($F[4,42] = 3.214$, $p = .022$). Post hoc analysis revealed that food consumption by the ketamine (30 mg/kg) group during the feeding hours of the ketamine-injected evening (P42) was significantly greater than vehicle controls' on P42 (Figure 1c, $p = .005$, 37%) and significantly greater by 48%, compared to its food consumption under drug-free condition on P41 ($p = .001$). The vehicle group exhibited no change in food intake between P41 and P42 ($p = .624$). The enhancement of feeding behavior observed on P42 by the ketamine (30 mg/kg) group, compared to vehicle controls, persisted to P43 (Figure 1b, $p = .015$, 38%). Food consumptions by the lower dose of ketamine (3 mg/kg) on P42 and P43 were significantly greater, compared to its food consumption on P41 ($p = .008$ and $p = .0001$, respectively). On P43, ketamine (3 mg/kg) also enhanced feeding marginally, compared to vehicle group ($p = .051$).

No difference among ketamine and vehicle groups was found in food intake during baseline (P36–40) or of weight loss or wheel running activity induced by FR during the first ABA (Supporting Information, Table S1).

3.2 | Ketamine facilitates weight gain during recovery that follows the first ABA

At the end of the first ABA on P44, comprised of 3 days of FR, no difference was found in weight loss among the three groups (vehicle group: −27.072% ± 1.635%; ketamine (3 and 30 mg/kg): −23.71% ± 1.516%,

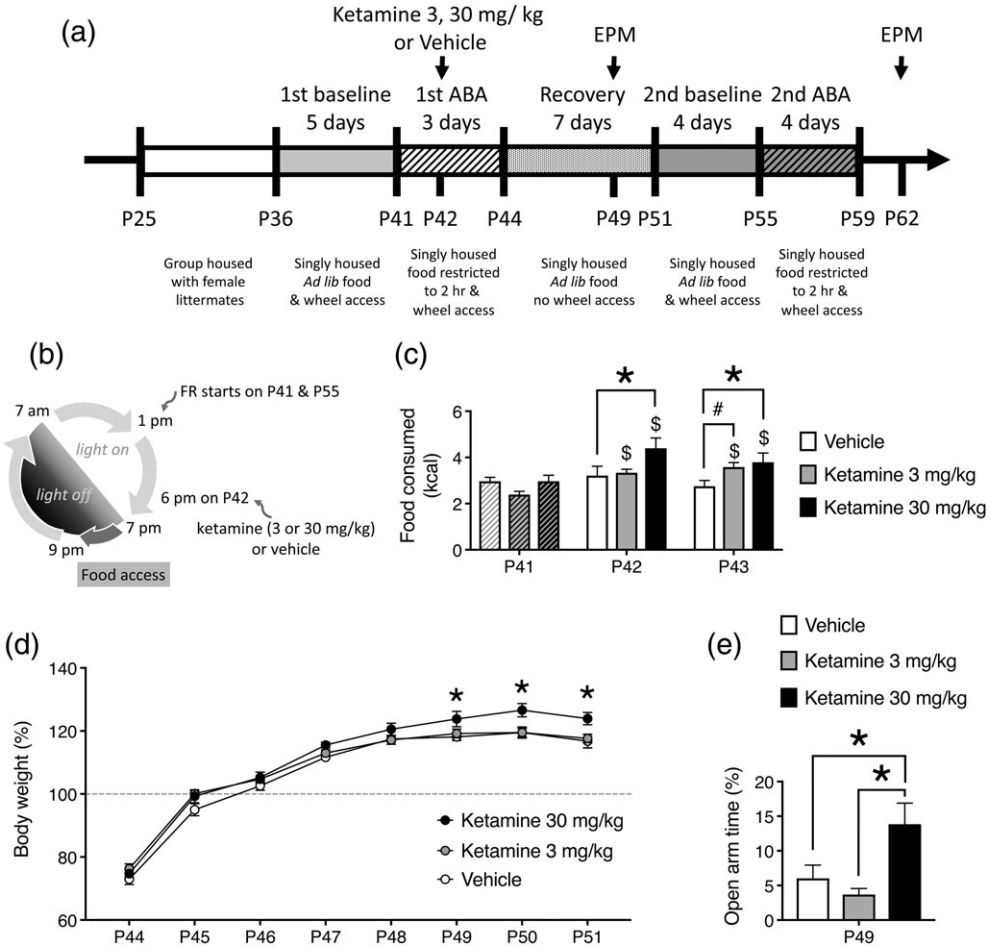

FIGURE 1 Single injection of ketamine increases food intake under FR during the first ABA and facilitates weight restoration during recovery and reduces anxiety-like behaviors panel a and b: Scheme of activity-based anorexia (ABA) design and timing of drug injections. All female C57Bl/6 J mice experienced repeated ABA exposures, the first ABA in mid-adolescence (P36–44) and the second one in late-adolescence (P51–59). On P42, the second day of the first ABA, female mice were assigned to either vehicle or ketamine groups that received vehicle or ketamine (3 and 30 mg/kg) injection at 6 p.m. 1 hr before the 2 hr of food access (7–9 p.m.). Anxiety-like behaviors were measured by the elevated plus maze (EPM) test, conducted on P49 and P62 panel c: ketamine (30 mg/kg) increases food intake under ABA during mid-adolescence. Before drug injection, on P41, no difference was found on food intake among three groups of animals that were assigned to the two groups (bars filled with stripes). On P42 and P43, the ketamine (30 mg/kg) group consumed more food compared to vehicle controls. * indicates $p < .05$. # indicates $p = .05$. $ indicates $p < .05$, compared to the food intake on P41 of the same group. Panel d: Ketamine (30 mg/kg) facilitates weight gain during recovery from ABA. After the end of the first ABA, repeated measures two-way ANOVA showed that the ketamine (30 mg/kg) group gained more weight during recovery period, especially on the last 3 days of recovery, P49–51. * indicates $p < .05$ revealed by Fisher's LSD test, comparing to the vehicle group. Panel e: ketamine (30 mg/kg) reduced anxiety-like behavior during recovery from the first ABA. Ketamine (30 mg/kg) group spent more time in the open arms. * indicates $p < .05$

−25.193% ± 0.944%, respectively; $F[2,21] = 1.449$, $p = .26$). All animals were returned to ad lib feeding without access to the wheel for 7 days (P44–51) to promote recovery from FR-stress. Repeated measures two-way ANOVA showed a significant main effect of treatment (Figure 1c, $F[2,20] = 5.442$, $p = .013$) and a significant main effect of days ($F[7,140] = 465.6$, $p < .0001$) on weight changes during recovery. Post hoc analysis further indicated that ketamine (30 mg/kg)-injected animals gained more weight during the recovery phase compared to the vehicle group ($p = .01$), especially during the last 3 days, P49 ($p = .02$), P50 ($p = .004$), and P51 ($p = .003$, +42%). No difference between ketamine (3 mg/kg) and vehicle groups was found in weight gain during recovery ($p = .417$). No difference among ketamine and vehicle groups was found in food intake during recovery (from P44–51, Supporting Information, Table S1).

3.3 | Ketamine increases food intake and weight gain during the feeding hours of the second ABA

We determined whether a single injection of ketamine in mid-adolescence has enduring effects on food intake under restricted food access in late adolescence. Higher dose of ketamine (30 mg/kg) enhanced food intake during food restriction, as indicated by Repeated measures two-way ANOVA showing a marginally significant main effect of treatment on food consumption during the 4 days of FR ($F[2,21] = 3.381$, $p = .05$), and revealed by post hoc analysis that the ketamine (30 mg/kg) group consumed more food, compared to vehicle group ($p = .02$). The ketamine (30 mg/kg) group showed no difference in food consumption, relative to the vehicle group on FR day 1 (P55), after 6 hr of FR (Figure 2a, $p = .73$), but showed enhancement of feeding behaviors on the subsequent 3 days of FR (P56,

p = .06, 23%; P57, p = .028, 24%; P58, p = .001, 38%). On the other hand, post hoc analysis revealed that lower dose of ketamine (3 mg/kg) had no effect on food intake compared to vehicle group (p = .5).

Weight gain during the 2 hr of food availability also differed among the three groups. Repeated measures two-way ANOVA of this measure revealed a marginally significant main effect of treatment (F[2,21] = 3.162, p = .06), and revealed by post hoc analysis that the ketamine (30 mg/kg) group gained more weight, compared to vehicle group (p = .02). On FR day 1 (P55), after 6 hr of FR, no difference was found in weight gain between the ketamine (30 mg/kg) injected and vehicle groups (Figure 2b, p = .81). On the last 2 days of FR, the ketamine (30 mg/kg) group showed more efficient weight gain during the feeding hours, compared to the vehicle controls (P57, p = .09, 20%; P58, p = .001, 47%). Post hoc analysis revealed that the lower dose of ketamine (3 mg/kg) had no effect on weight gain during 2 hr of food availability compared to vehicle group (p = .26).

3.4 | Ketamine attenuates food restriction-induced wheel running and weight loss during the second ABA

During the second ABA in late adolescence, one-way ANOVA revealed a significant main effect of treatment (F[2,21] = 3.869, p = .037) on wheel running. Post hoc analysis revealed that both doses of ketamine (3 and 30 mg/kg) that were injected during mid-adolescence lowered the averaged wheel running activity under FR, compared to the vehicle group (Figure 2c, 3 mg/kg, p = .012, −37%; 30 mg/kg, p = .09, −24%).

At the end of the second ABA, one-way ANOVA revealed a marginally significant main effect of treatment (F[2,21] = 3.13, p = .065) on weight change. Post hoc analysis showed that ketamine (30 mg/kg)-injected animals showed marginally less weight loss compared with the vehicle group (Figure 2d, p = .056, −17%). No difference in food restriction evoked weight change was found between the lower dose of ketamine (3 mg/kg)-treated animals and vehicle controls (p = .79).

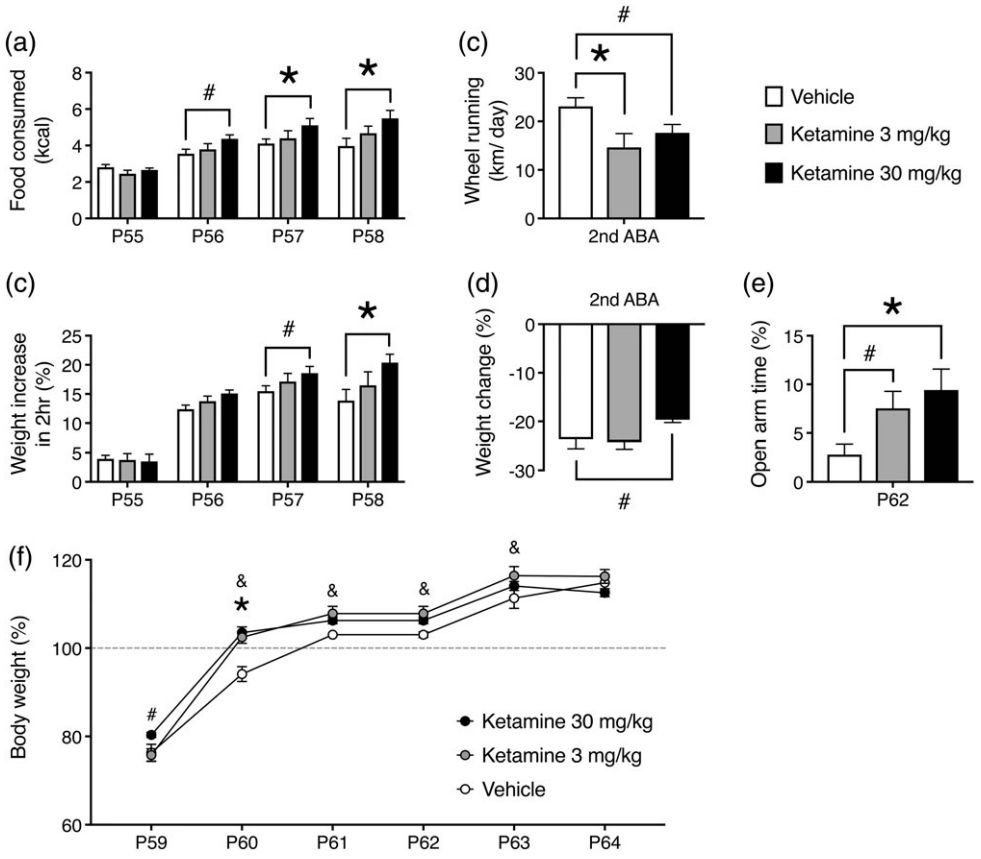

FIGURE 2 Single injection of ketamine in mid-adolescence has enduring protective effects that are evident during the second ABA and recovery. Panels a and b: Single injection of ketamine (30 mg/kg) in mid-adolescence increases food intake (a) and weight gain (b) under second ABA exposure during late-adolescence. On the first day of FR during the second ABA (P55), no difference was found on food intake (a) or weight increase during the 2-hr feeding period (b) among the three groups. On the second (P56), third (P57), and last day (P58) of FR, the ketamine (30 mg/kg) group increased food intake and gained more weight during the 2-hr feeding period compared with the vehicle controls. * indicates p < .05, comparing to the vehicle group. # indicates p < .1, comparing to the vehicle group. Panel c and d: Single injection of ketamine in mid-adolescence attenuates FR-induced hyperactivity (c) and weight loss (d) under the second ABA exposure during late-adolescence. Ketamine (3 and 30 mg/kg) groups showed less averaged wheel running activity during 4 days of FR of the ABA, compared to vehicle controls. The ketamine (30 mg/kg) group also lost less weight at the end of the second ABA, compared with vehicle group. * indicates p < .05. # indicates p < .1. Panel e: ketamine reduced anxiety-like behavior during recovery from the second ABA. Ketamine groups spent more time in the open arms. * indicates p < .05. # indicates p = .067. Panel f: Ketamine facilitates weight gain during recovery from ABA. After the end of the second ABA, repeated measures two-way ANOVA showed that the ketamine (3 and 30 mg/kg) group gained more weight during recovery period. * indicates p < .05, # indicates p = .057, of ketamine (30 mg/kg) group revealed by Fisher's LSD test, comparing to the vehicle group. "&" indicates p < .05 of ketamine (3 mg/kg) group revealed by Fisher's LSD test, comparing to the vehicle group

3.5 | Ketamine facilitates weight gain during recovery after second ABA

At the end of the second ABA on P59, all animals were returned to ad lib feeding for 5 days (P59–64), without access to the wheel, to promote recovery from FR-stress. Repeated measures two-way ANOVA showed a significant main effect of treatment (Figure 2f, $F[2,21] = 8.049$, $p = .003$) and a significant main effect of days ($F[5,105] = 314.7$, $p < .0001$) on weight changes during recovery. Post hoc analysis further indicated that both the higher (30 mg/kg) and lower (3 mg/kg) dose groups of ketamine-injected animals gained more weight during the recovery phase compared to the vehicle group ($p = .013$ for 30 mg/kg, $p = .004$ for 3 mg/kg). No difference among ketamine and vehicle groups was found in food intake during recovery (from P59–64, Supporting Information, Table S1).

3.6 | Ketamine reduces anxiety-like behavior after recovery from the first and second ABA

Previously, we showed that FR elevated anxiety-like behavior that was measurable during the recovery period, even though body weight had been restored 5 days prior (Chen, Surgent, Rana, Lee, & Aoki, 2017). We investigated whether ketamine ameliorates this post-FR elevation of anxiety-like behavior. On P49, by which time animals' body weights were fully recovered from the first ABA, one-way ANOVA revealed significant main effect of treatment ($F[2,21] = 6.168$, $p = .008$) on time spent in open arms of the EPM. Post hoc analysis revealed that ketamine (30 mg/kg)-treated animals showed significantly more time spent in open arms, compared with vehicle controls (Figure 1e; $p = .017$) and animals that had received lower dose of ketamine (3 mg/kg; Figure 1e, $p = .003$), reflecting reduction of anxiety-like behavior. On P62, after animals' body weights were fully recovered from the second ABA, one-way ANOVA again revealed a significant main effect of treatment ($F[2,21] = 3.895$, $p = .036$) on time spent in open arms of the EPM. Both lower (3 mg/kg) and higher (30 mg/kg) dosed ketamine-groups showed significantly more time spent in open arms (Figure 2e, $p = .067$ for 3 mg/kg, $p = .013$ for 30 mg/kg), compared to vehicle controls.

4 | DISCUSSION

Vulnerability of adolescent female mice to ABA can be quantified by (a) the FR-evoked heightened wheel running activity which (b) exacerbates weight loss (Chowdhury et al., 2013), together with (c) the enduring effect of stress-induced anxiety-like behavior, evident among recovered animals with fully restored body weight (Chen, Surgent, et al., 2017). We show that a single systemic ketamine (30 mg/kg, i.p.) injection effectively attenuates all three of these measures of ABA vulnerability, in addition to promoting increased food intake immediately and for prolonged period (at least 16 days), ultimately contributing to the suppression of body weight loss during the second ABA. Although the weight loss effect was marginal ($p = .056$), it shows promise that perhaps multiple dosing of ketamine, such as another during the second ABA, would have yielded suppression that reached statistical significance. We also show that a single ketamine injection in mid-adolescence has long-lasting effects of suppressing FR-induced anxiety, evident 16 days later. Reduction of anxiety-like behavior by ketamine is already evident during the recovery phase from the first ABA, 7 days later. This may have contributed to the suppression of FR-induced hyperactivity throughout the second ABA. These findings add to another recent study showing that ketamine decreases anxiety-like levels in rats under chronic stress (Jiang et al., 2017). These multiple effects of ketamine are all promising features for enhancing resilience of individuals to recurrence of ABA.

This finding is consistent with prior studies (Sarrau, Jourdan, Dupuis-Soyris, & Verwaerde, 2007; Treece, Ritter, & Burns, 2000) reporting that ketamine and other NMDAR antagonists increase feeding behaviors but reveals additionally that ketamine increases food intake specifically under food restriction (FR), since ketamine did not alter food intake following just 6 hr of FR on the first day of ABA or during the recovery phase.

We do not know how ketamine exerts its prolonged protective effect against ABA but underlying mechanisms have been hypothesized for ketamine's prolonged effect as an antidepressant: the reduction of eukaryotic elongation factor (eEF2) phosphorylation and desuppression of the translation of brain-derived neurotrophic factor (BDNF) in the hippocampus (Autry et al., 2011; Kavalali & Monteggia, 2015). Ketamine may have an addictive potential and is not free of additional unwanted side effects (Short et al., 2018). As alternative anti-depressant drugs with glutamate receptor binding property emerge in the future, it would be useful to test their potential for reducing ABA. Future studies that access the effects of local infusion of ketamine in the hippocampus for investigating ketamine-mediated blockade of NR2B-NMDARs and synaptic translational machinery should help to elucidate the underlying mechanisms of the effects of ketamine on attenuating the vulnerability to ABA and AN, especially during adolescence.

ACKNOWLEDGMENTS

This study was supported by The Klarman Family Foundation Grant Program in Eating Disorders Research, Grant number: R21 MH105846; Grant number: R01NS066019-01A1; Grant number: R01NS047557-07A1; Grant number: NEI Core grant EY13079; NYU's Research Challenge Fund, Grant number: NSF-REU 1460880 to CA, YWC, and the Fulbright Scholarship to YWC.

CONFLICT OF INTEREST

We declare no conflict of interest in relation with the work described.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Chen Y-W, Sherpa AD, Aoki C. Single injection of ketamine during mid-adolescence promotes long-lasting resilience to activity-based anorexia of female mice by increasing food intake and attenuating hyperactivity as well as anxiety-like behavior. *Int J Eat Disord*. 2018;51:1020–1025. https://doi.org/10.1002/eat.22937