Olanzapine, but Not Fluoxetine, Treatment Increases Survival in Activity-Based Anorexia in Mice

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Anorexia nervosa (AN) is an eating disorder characterized by extreme hypophagia, hyperactivity, and fear of weight gain. No approved pharmacological treatments exist for AN despite high mortality rates. The activity-based anorexia (ABA) phenomenon models aspects of AN in rodents, including progressive weight loss, reduced food intake, and hyperactivity. First, we optimized the ABA paradigm for mice. We compared mouse strains (Balb/cJ, A/J) for susceptibility with ABA, and evaluated the effects of different food access durations (2, 4, 6, 8, and 10 h) on ABA parameters. Balb/cJ mice exhibited significantly shorter survival time (days until 25% bodyweight loss) in the ABA paradigm compared with A/J mice. Furthermore, 6 h of food access reduced survival in mice housed with wheels without reducing survival in mice housed without wheels. We then evaluated the effects of chronic treatment with fluoxetine (4 weeks) or subchronic treatment with olanzapine (OLZ) (1 week) on ABA in BALB/cJ mice. OLZ (12 mg/kg/day) significantly increased survival and reduced food anticipatory activity (FAA). However, OLZ did not alter food intake or running wheel activity during ad-lib feeding (baseline) or restriction conditions, or in mice housed without wheels. Fluoxetine (18 mg/kg/day) increased food intake and reduced FAA, but did not alter survival. Here, we report for the first time that OLZ, but not fluoxetine, reduces ABA in mice. Our findings indicate further need for clinical investigations into the effects of OLZ, but not selective serotonin reuptake inhibitors, on core features of AN.
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INTRODUCTION

Anorexia nervosa (AN) is an eating disorder characterized by profound hypophagia, refusal to maintain a normal body weight, and fear of weight gain. Moreover, AN patients often exhibit hyperactivity, manifesting as extreme exercise or general restlessness (Hebebrand *et al*, 2003; Kron *et al*, 1978). AN occurs primarily in females, and has the highest mortality rate of all psychiatric disorders (Arcelus *et al*, 2011; Sullivan, 1995). Despite the severity of AN, no approved pharmacological treatments currently exist.

Abnormalities of the serotonin (5-hydroxytryptamine, 5-HT) neurotransmitter system have been reported in both ill and recovered AN patients. The major metabolite of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), is reduced during illness and elevated after weight restoration (Kaye *et al*, 1988, 1991). Furthermore, several PET imaging studies

have found that patients ill or recovered from AN have increased 5-HT$_{1A}$ receptor (Bailer *et al*, 2005, 2007a) and serotonin transporter binding (Bailer *et al*, 2007a), and decreased 5-HT$_{2A}$ receptor binding (Audenaert *et al*, 2003; Frank *et al*, 2002). These alterations in the 5-HT system remain following weight restoration, and thus, are likely traits of AN. Genetic association studies have linked polymorphisms in the serotonin transporter and 5-HT$_{2A}$, 5-HT$_{1D}$, and 5-HT$_3$ receptor genes with AN (Bergen *et al*, 2003; Brown *et al*, 2007; Delorme *et al*, 2005; Monteleone and Maj, 2008; Ozaki *et al*, 2003). However, genome-wide association studies to date have not identified significant associations of any genes and AN (Wang *et al*, 2011).

Pharmacotherapy targeting the serotonergic system has been attempted in AN patients. Treatment with selective serotonin reuptake inhibitors (SSRIs) has been unsuccessful for malnourished patients (Attia *et al*, 1998; Ferguson *et al*, 1999; Holtkamp *et al*, 2005). One randomized-controlled trial (RCT) found that fluoxetine treatment reduces relapse (Kaye *et al*, 2001) in weight-restored patients; however, the largest RCT to date (Walsh *et al*, 2006) found fluoxetine to be ineffective in reducing relapse in AN. Thus, the effects of fluoxetine on AN remains unclear.

The dopamine (DA) system regulates reward processing, movement, and feeding behavior, and is also altered in AN.

Ill and recovered AN patients exhibit reductions in homovanillic acid, a major metabolite of DA (Kaye et al, 1999), and increased DA D_2 and DA D_3 receptor binding (Frank et al, 2005). Furthermore, polymorphisms in DA D_2 are associated with AN (Bulik et al, 2007; Burden et al, 1993; Monteleone and Maj, 2008). Recent findings suggest that drugs targeting both 5-HT and DA receptors may be effective in treating AN. Several open label studies have reported that the atypical antipsychotic olanzapine (OLZ) increases body weight, and reduces hyperactivity and anxiety about eating and body shape (Barbarich et al, 2004; Dennis et al, 2006; Leggero et al, 2010). Furthermore, two preliminary RCTs recently showed that OLZ significantly reduces obsessions about weight while increasing the rate of weight gain (Bissada et al, 2008), and reduces anorexic ruminations (Mondraty et al, 2005).

The activity-based anorexia (ABA) phenomenon provides a rodent model for aspects of AN. In the ABA paradigm, rodents subjected to restricted food access and housed with running wheels develop paradoxical hyperactivity, hypophagia, and extreme weight loss resulting in death. Conversely, rodents given either restricted food access or running wheels maintain body weight (Epling and Stefan 1983; Routtenberg and Kuznesof, 1967). ABA recapitulates several aspects of AN, including weight loss, reduced food intake, hyperactivity, increased hypothalamic–pituitary–adrenal (HPA) axis activity, and loss of estrus (Burden et al, 1993; Dixon et al, 2003; Watanabe et al, 1992). Furthermore, adolescent (Boakes et al, 1999; Pare, 1975; Woods and Routtenberg, 1971) and female rodents (Klenotich and Dulawa, in press; Paré et al, 1978) develop more severe ABA phenotypes. Recently, the effects of SSRIs and OLZ on ABA have been investigated in rats. FLX (5 mg/kg/day) (Altemus et al, 1996), fluvoxamine (50 mg/kg/day) (Yokoyama et al, 2007), and OLZ (7.5 mg/kg/day) (Hillebrand et al, 2005) were all reported to reduce ABA.

The present studies investigated the effects of chronic treatment with the SSRI FLX or the atypical antipsychotic OLZ on ABA in mice. We first performed a strain comparison study to identify a mouse strain with high vulnerability to ABA, because mice show strain differences in ABA susceptibility (Gelegen et al, 2007, 2008, 2010). We then optimized ABA experimental parameters for investigating drug treatment effects in mice. For example, food access duration can alter the progression of ABA (Pare et al, 1985; Watanabe et al, 1992). Next, we identified doses of OLZ (12 mg/kg/day, 24 mg/kg/day) that alter ABA behavior. Finally, we determined the effects of chronic FLX (18 mg/kg/day) or subchronic OLZ (12 mg/kg/day) treatment on ABA behavior.

MATERIALS AND METHODS

Animals

Experimentally naive Balb/cJ and A/J female mice (Jackson Laboratories, Bar Harbor, ME) were aged 6–8 weeks at the beginning of all experimental procedures. Mice were given ad libitum access to standard chow and water, except during food restriction periods described below. Animals were euthanized, or 'dropped', from experiments when they lost 25% of their initial body weight, defined on the last day of baseline. All procedures were conducted in accord with the National Institutes of Health laboratory animal care guidelines and with the Institutional Animal Care and Use Committee approval at the University of Chicago.

Drugs

All drugs were administered in the drinking water in dark bottles. Fluoxetine hydrochloride (BIOTREND, Cologne, Germany) treatment was initiated 4 weeks before the restriction period since FLX has a therapeutic onset of 2–4 weeks in humans (Blier, 2003; Fineberg et al, 1992). FLX was dissolved in distilled water. In contrast to FLX, OLZ (Sequoia Research Products, Pangbourne, UK) has more rapid therapeutic onset (1–2 weeks) (Fulton and Goa, 1997); treatment began during the baseline period. OLZ was dissolved in a minimal quantity of glacial acetic acid, raised to volume with distilled water, and adjusted to a pH of 5.7–5.8. During baseline and restriction periods the same proportion of glacial acetic acid was added to FLX and vehicle control (VEH) solutions, which were also adjusted to a pH of 5.7–5.8. Drug concentrations were adjusted to account for changes in water intake and body weight twice weekly during baseline conditions. Drug solutions were changed daily from weekly stock solutions during food restriction conditions. Water intake values for drug-treated mice are shown in Supplementary Table 1.

Experimental Conditions

Animals were housed in a climate-controlled room maintained on a 12 : 12 light dark cycle (lights off at 1800 hours). Cages (19.56 × 34.70 × 14.41 cm) were equipped with wireless low-profile running wheels (Med Associates, St Albans, VT). Running wheels transmitted running data at 30-s intervals to a computer with Wheel Manager Software (Med Associates) 24 h a day throughout the experiment. Food was provided in a small glass jar (65 cm diameter × 50 cm height).

ABA Procedure

All animals were pseudo-randomly divided in experimental groups based on their body weights. During acclimation, baseline, and food restriction periods, mice were singly housed and given 24-h access to running wheels. For some experiments, additional groups of mice were treated identically, but were not provided running wheels. Daily body weight, food intake, running wheel activity, and food anticipatory activity (FAA) were recorded during baseline and food restriction conditions. FAA refers to running wheel activity during the 4 h preceding food delivery (Davidson and Stephan, 1999; Holmes and Mistlberger, 2000; Honma et al, 1983; Mistlberger, 1994). Days to dropout (loss of 25% baseline body weight) provided a measure of survival.

Experiment 1: Effect of Mouse Strain on ABA

Female Balb/cJ ($n = 15$) and A/J ($n = 15$) mice 6 weeks of age were acclimated to running wheels for 3 days before the baseline period. Following 4 days of baseline conditions, mice were given 2 h of food access per day beginning at 1000 hours.

Experiment 2: Effect of Food Access Duration on ABA

Eight-week-old female Balb/cJ mice ($n = 72$) were acclimated to wheel ($n = 36$) or non-wheel ($n = 36$) housing conditions for 2 days. Following 7 days of baseline conditions, mice housed with and without wheels received 2 h ($n = 7$ per group), 4 h ($n = 7$ per group), 6 h ($n = 8$ per group), 8 h ($n = 7$ per group), and 10 h ($n = 7$ per group) of food access during the restriction period. Food was given at 0800 hours each day. Mice were euthanized after losing 25% of baseline body weight, or following 12 days of restriction.

Experiment 3: Dose-Dependent Effects of Subchronic OLZ Treatment on ABA

Female Balb/cJ mice ($n = 15$) aged 8 weeks were acclimated to running wheels for 2 days. During baseline (7 days), mice received VEH ($n = 5$), OLZ 12 mg/kg/day ($n = 5$), or OLZ 24 mg/kg/day ($n = 5$). During the restriction period, food was given for 6 h per day beginning at 0800 hours. Mice were euthanized following either 25% loss of baseline body weight or 14 days of restriction.

Experiment 4: Effects of Chronic FLX and Subchronic OLZ Treatment on ABA

Female Balb/cJ mice ($n = 60$) 6 weeks of age were treated with either VEH (0 mg/kg/day, $n = 20$), OLZ (12 mg/kg/day, $n = 20$), or FLX (18 mg/kg/day, $n = 20$) and housed either with or without running wheels. Following the acclimation to single housing and running wheels (3 days) and the baseline period (7 days), mice received 6 h of food access a day beginning at 0800 hours. Mice were euthanized either after 25% baseline body weight loss, or 14 days had elapsed.

Statistical Analysis

For baseline data, ANOVAs assessed the effects of group (strain, drug, food access period, or wheel) as between subjects factors, and day as a within subjects factor for each dependent variable (body weight, food intake, running wheel activity, and FAA). When significant interactions were found, post hoc comparisons were made using the Student–Newman–Keuls test for between subjects factors or post hoc ANOVAs for within subjects factors. Bonferroni adjustments were made when post hoc ANOVAs were applied.

For restriction data, the linear mixed effects model (mixed ANOVA model) was used to assess differences in body weight, food intake, running wheel activity, and FAA. Mice continually drop out of the ABA paradigm, creating data sets with missing values. Post hoc analysis resolving 'treatment × day' interactions were adjusted for multiple

comparisons using the false discovery rate method. Survival analysis was performed using the Kaplan–Meier test with log-rank (Mantel–Cox) post hoc tests.

RESULTS

BALB/cJ Mice Exhibit Increased Vulnerability to ABA

During baseline, Balb/cJ mice had significantly higher body weights on day 1 ($F_{(7, 28)} = 3.735$; $p < 0.001$), and ran significantly more than A/J mice overall ($F_{(1, 28)} = 53.307$; $p < 0.0001$). There were no differences in food intake between strains during baseline (Table 1). During food restriction, Balb/cJ mice weighed significantly less than A/J mice on days 1 through 4 ($F_{(3, 66)} = 13.85$; $p < 0.0001$) (Figure 1a). Balb/cJ mice also ran significantly more than A/J mice during food restriction ($F_{(1, 66)} = 7.82$; $p < 0.01$) (Figure 1c). A trend was found for increased FAA in Balb/cJ mice ($F_{(1, 38)} = 3.01$; $p = 0.09$) (Figure 1d). Strain had no effect on food intake during restriction (Figure 1b). Overall, A/J mice were more resilient to the ABA paradigm, as they survived food restriction conditions significantly longer than Balb/cJ mice ($p < 0.0001$) (Figure 1e). As Balb/cJ mice exhibited a more severe ABA phenotype, this strain was used in subsequent studies.

Table 1 Baseline-Dependent Measures

Manipulation	Wheel	BW	FI	RWA
A/J	Y	17.08 ± 0.10^a	3.14 ± 0.10	2853.14 ± 869.02^a
Balb/cJ	Y	17.98 ± 0.08	3.12 ± 0.07	$23\,219.60 \pm 1392.58$
2 h	Y	19.15 ± 0.13	4.37 ± 0.09^b	$31\,234.89 \pm 1361.90$
2 h	N	19.53 ± 0.12	3.75 ± 0.07	—
4 h	Y	19.14 ± 0.11	4.25 ± 0.09^b	$34\,961.67 \pm 1544.30$
4 h	N	19.09 ± 0.12	3.41 ± 0.06	—
6 h	Y	18.75 ± 0.09^b	3.73 ± 0.09^b	$31\,573.98 \pm 1497.85$
6 h	N	20.04 ± 0.10	3.21 ± 0.06	—
8 h	Y	18.78 ± 0.15	3.73 ± 0.07^b	$34\,181.74 \pm 1671.04$
8 h	N	19.42 ± 0.10	3.22 ± 0.05	—
10 h	Y	18.66 ± 0.11	3.72 ± 0.07^b	$32\,972.25 \pm 2000.84$
10 h	N	19.49 ± 0.11	3.11 ± 0.06	—
0 mg/kg/day VEH	Y	19.16 ± 0.12	3.48 ± 0.09	$34\,572.89 \pm 2120.48$
12 mg/kg/day OLZ	Y	19.23 ± 0.15	3.27 ± 0.15	$27\,070.11 \pm 1930.15$
24 mg/kg/day OLZ	Y	19.02 ± 0.12	3.11 ± 0.10	$22\,398.69 \pm 1535.49$
0 mg/kg/day VEH	Y	19.30 ± 0.14	4.44 ± 0.11^c	$32\,547.43 \pm 1227.52$
0 mg/kg/day VEH	N	19.98 ± 0.11	3.85 ± 0.05	—
12 mg/kg/day OLZ	Y	19.05 ± 0.12	3.93 ± 0.07^c	$28\,575.06 \pm 925.39$
12 mg/kg/day OLZ	N	19.09 ± 0.12	3.60 ± 0.07	—
18 mg/kg/day FLX	Y	19.73 ± 0.15	4.61 ± 0.16^c	$30\,070.46 \pm 1429.23$
18 mg/kg/day FLX	N	19.79 ± 0.12	3.87 ± 0.05	—

Abbreviations: BW, daily body weight; FI, food intake; RWA, running wheel activity during baseline.
[a]Significant difference from Balb/cJ strain ($p < 0.01$).
[b]Significant difference between wheel groups within the food access duration group ($p < 0.01$).
[c]Overall significant effect of wheel ($p < 0.005$).
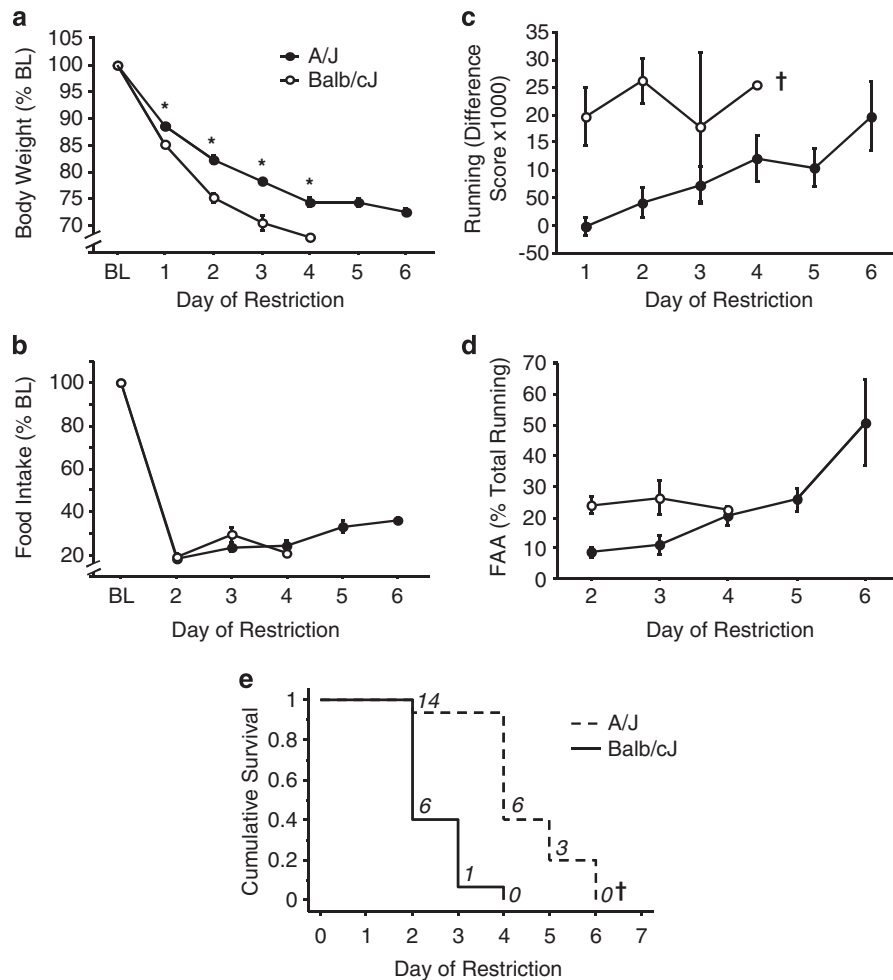Values are means ± SEM.

Figure 1 Daily body weight (a), food intake (b), running wheel activity (c), FAA (d), and survival (e) curves for A/J and Balb/cJ mice during food restriction (2-h food access per day). Results expressed as mean ± SEM. Numbers in italics represent mice remaining in food restriction. *Significant difference from Balb/cJ $p < 0.001$. †Significant main effect of strain, $p < 0.01$.

Increasing Food Access Duration Reduces ABA Behavior

During the baseline period, body weight did not differ between animals housed with or without a wheel within the 2-, 4-, 8-, and 10-h food access duration. However, small but significant differences in body weight were found within the 6-h group ($F_{(1, 14)} = 12.854$; $p < 0.05$) (Table 1). Mice housed with wheels had significantly higher food intake during baseline in the 2-h ($F_{(1, 12)} = 25.810$; $p < 0.0005$), 4-h ($F_{(1, 12)} = 35.442$; $p < 0.0001$), 6-h ($F_{(1, 14)} = 10.724$; $p < 0.01$), 8-h ($F_{(1, 28)} = 19.184$; $p < 0.0005$), and 10-h ($F_{(1, 28)} = 27.001$; $p < 0.0001$) groups.

During the restriction phase, survival analyses found no difference between animals housed with or without wheels given 8 h ($p = 0.06$) or 10 h ($p > 0.05$) of food access (Figure 2). However, mice housed without wheels survived significantly longer than those housed with wheels in the 2-h ($p < 0.005$), 4-h ($p < 0.0005$), and 6-h ($p < 0.0001$) food access groups. Six hours was the only food access duration in which all mice under wheel conditions and none of the mice under non-wheel conditions met dropout criteria. Changes in daily bodyweight, food intake, wheel running, and FAA are shown in Supplemental Results and Supplementary Figures S1, S2.

Dose-Dependent Effect of Subchronic OLZ Treatment on ABA Behavior

During the baseline period, neither 12 mg/kg/day nor 24 mg/kg/day OLZ treatment altered body weight, food intake, or running wheel activity (Table 1). During the food restriction period, mice receiving 12 mg/kg/day OLZ weighed significantly more than those receiving VEH control ($F_{(1, 32)} = 6.43$; $p < 0.05$). Furthermore, mice receiving 24 mg/kg/day OLZ weighed significantly more than VEH-treated mice on days 3 and 4 of food restriction ($F_{(3, 32)} = 4.63$; $p < 0.05$) (Figure 3a). Neither 12 mg/kg/day nor 24 mg/kg/day OLZ altered food intake during restriction (Figure 3b). A trend for increased running wheel activity was found for mice treated with 12 mg/kg/day OLZ ($F_{(1, 32)} = 3.82$; $p = 0.06$) compared with VEH-treated mice, while mice treated with 24 mg/kg/day ran significantly more than VEH-treated mice ($F_{(1, 22)} = 14.23$; $p < 0.005$) (Figure 3c). Neither 12 mg/kg/day nor 24 mg/kg/day altered FAA (Figure 3d). Finally, both the 12 mg/kg/day ($p < 0.05$)
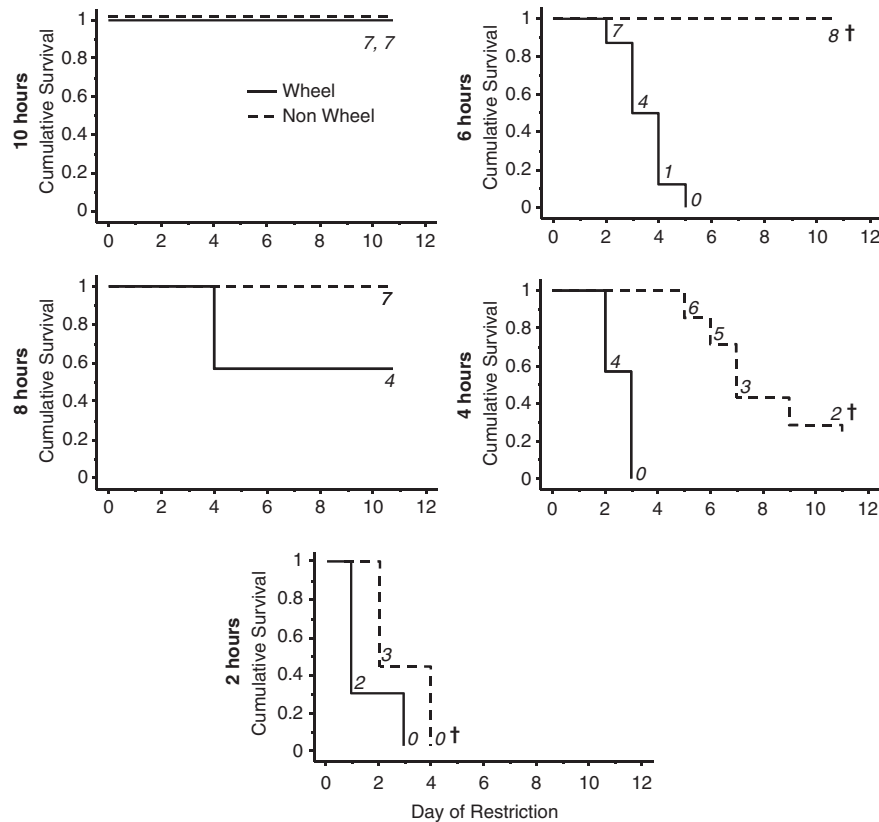
Figure 2 Survival curves for mice housed with and without wheels in each food access duration group. Results expressed as mean ± SEM. Numbers in italics represent mice remaining in food restriction. [†]Significant difference from Balb/cJ $p < 0.005$.

and the 24 mg/kg/day doses ($p < 0.05$) of OLZ significantly increased survival compared with VEH control treatment during food restriction (Figure 3e).

Subchronic OLZ, but Not Chronic FLX, Increases Survival in ABA

Effects of chronic FLX on ABA. Chronic FLX treatment had no effect on baseline body weight, food intake, or running wheel activity in mice housed with or without wheels. Food intake was significantly increased for animals housed with wheels across treatments ($F_{(1, 36)} = 9.084$; $p < 0.005$) (Table 1).

During the restriction period, FLX had no effect on body weight for mice housed with or without wheels (Figure 4a, Supplementary Figure S3A). Chronic FLX treatment significantly increased food intake in mice housed with wheels ($F_{(1, 100)} = 5.24$; $p < 0.05$) (Figure 4b), but not in mice housed without wheels (Supplementary Figure S3B). Although FLX did not alter overall daily running wheel activity (Figure 4c), FAA was decreased in FLX-treated mice on days 7, 10, 12, 13, and 14 of restriction ($F_{(12, 82)} = 2.67$; $p < 0.005$) (Figure 4d). However, FLX had no effect on survival in the ABA paradigm ($p = 0.16$) (Figure 4e). FLX also had no effect on survival in mice housed without wheels (Supplementary Figure S3C).

Effects of subchronic OLZ on ABA. During the baseline period, subchronic OLZ treatment decreased body weight

on day 3 ($F_{(7, 36)} = 7.787$; $p < 0.0001$) regardless of wheel status. Although mice housed with wheels ate significantly more than mice without wheels across treatments ($F_{(1, 36)} = 10.807$; $p < 0.005$), OLZ treatment significantly decreased food intake on day 1 and day 2 of baseline ($F_{(6, 36)} = 4.314$; $p < 0.0005$). OLZ did not alter baseline running wheel activity (Table 1).

Subchronic OLZ treatment had no effect on body weight in mice housed with or without wheels during restriction (Figure 4a, Supplementary Figure S3A). A trend was found for OLZ to increase food intake in mice housed with wheels ($F_{(1, 114)} = 3.55$; $p = 0.06$) (Figure 4b), and OLZ had no effect on food intake in mice housed without wheels (Supplementary Figure S3B). Treatment with OLZ did not alter overall daily running wheel activity (Figure 4c). However, OLZ treatment significantly decreased FAA ($F_{(1, 96)} = 6.14$; $p < 0.05$) (Figure 4d). Finally, OLZ treatment significantly increased survival in mice housed with wheels ($p < 0.05$) (Figure 4e), and a trend for OLZ to increase survival in mice housed without wheels was found ($p = 0.06$) (Supplementary Figure S3C).

DISCUSSION

Here, we report for the first time that subchronic treatment with OLZ, but not chronic FLX, increases survival in the ABA paradigm in mice. First, we found that BALB/cJ mice exhibit shorter survival durations than A/J mice in the ABA
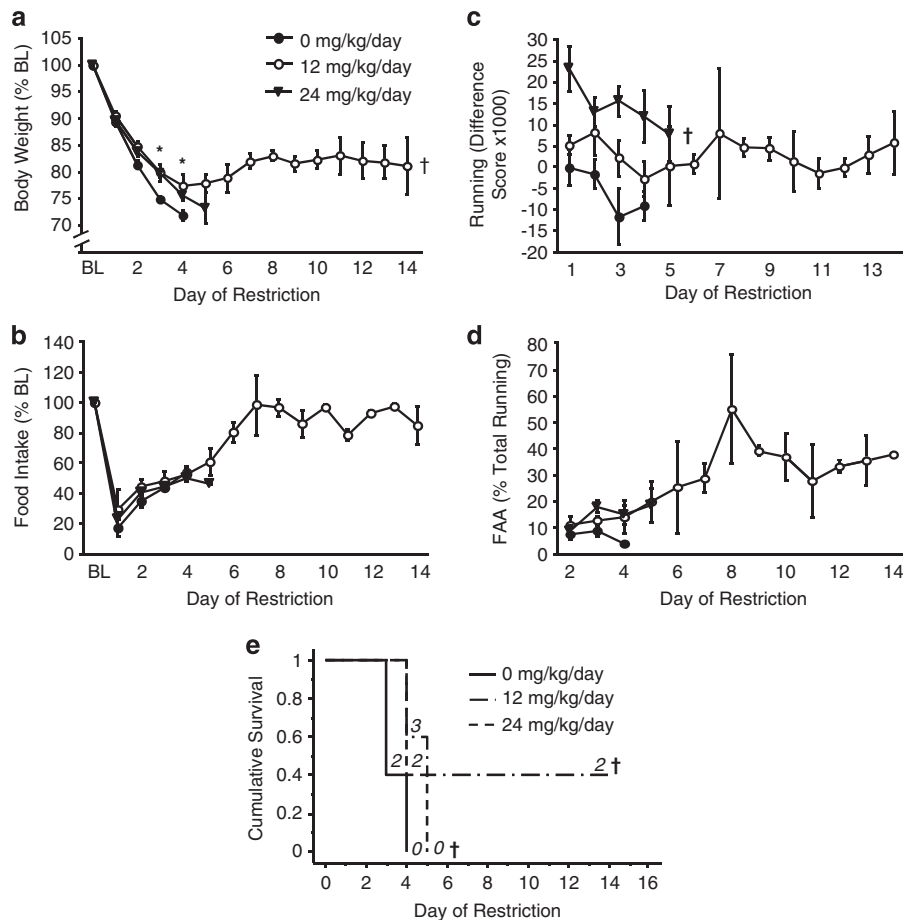
Figure 3 Daily body weight (a), food intake (b), running wheel activity (c), FAA (d), and survival (e) curves for mice treated with vehicle, 12 mg/kg/day, or 24 mg/kg/day OLZ during food restriction (6 h of food access per day). Results expressed as mean ± SEM. Numbers in italics represent mice remaining in food restriction. $*p < 0.05$ vs vehicle. $†$Significant main effect of treatment vs vehicle, $p < 0.05$.

paradigm; thus, BALB/cJ mice may provide a suitable mouse strain for modeling aspects of AN. Next, we determined the optimal food access duration for evaluating drug effects on ABA using mice. We found that a 6-h food access duration induced a moderate ABA phenotype, potentially allowing detection of either drug-induced increases or decreases in ABA. Finally, using these experimental conditions, we found that subchronic OLZ, but not chronic FLX, treatment increased survival in the ABA paradigm in BALB/cJ mice. Although FLX-treated mice showed increases in food intake and reductions in FAA, survival in ABA was not significantly extended compared with VEH-treated mice. Conversely, OLZ-treated mice exhibited significant increases in survival and reduced FAA. Our data support preliminary clinical findings on the efficacy of OLZ in AN (Bissada et al, 2008; Mondraty et al, 2005), and further validate the ABA mouse model as a tool for identifying potential treatments for AN.

The vast majority of ABA studies have been performed using rats. However, recent advances in molecular genetics have established mice as the foremost mammalian model for basic studies of psychiatric disorders. We therefore identified an appropriate mouse strain with which to perform ABA studies. Mouse strain differences in

susceptibility to ABA have been reported previously, with the A/J and DBA/2J strains showing increased susceptibility to developing the ABA phenotype compared with C57BL/6J mice (Gelegen et al, 2007, 2008, 2010). In fact, C57BL/6J mice do not develop ABA, but instead show reductions in wheel running during food restriction. Our results show that Balb/cJ mice exhibit significant decreases in body weight (Figure 1a), increases in running wheel activity (Figure 1c), and reduced survival compared with A/J mice (Figure 1e). Interestingly, Balb/cJ mice have increased DA and DA metabolites in nucleus accumbens and prefrontal cortex, as well as decreased levels of 5-HT and 5-HIAA in comparison with A/J mice. Balb/cJ mice also show increased DA D_2 binding in nucleus accumbens in comparison with A/J mice (Couppis et al, 2008). These data are similar to findings in ill and recovered AN patients, whom exhibit increased D2 binding potentials (Frank et al, 2005), as well as decreases in 5-HIAA (Kaye et al, 1988; Kaye et al, 1991). These differences in 5-HT and DA neurochemistry may have a role in the significantly higher susceptibility of Balb/cJ mice for developing a severe ABA phenotype. Taken together, our findings indicate that Balb/cJ mice exhibit high susceptibility to developing ABA, and exhibit a neurochemical profile that supports use of this strain as an AN-like mouse. Therefore, the Balb/cJ mouse is an
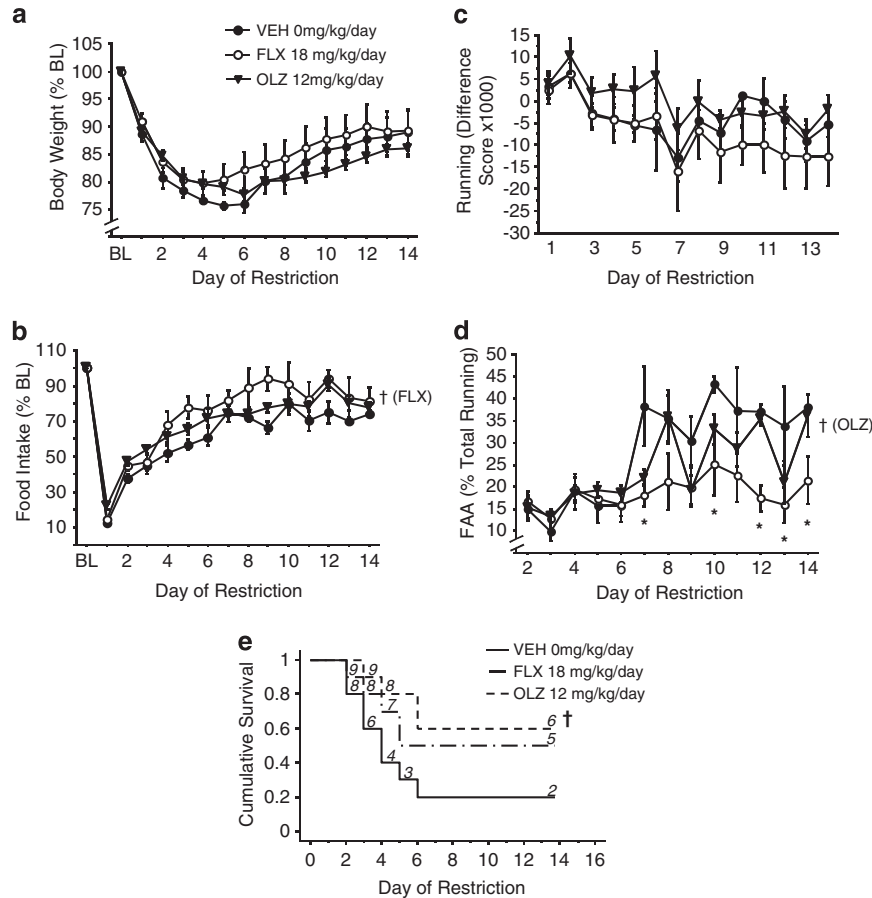
Figure 4 Daily body weight (a), food intake (b), running wheel activity (c), FAA (d), and survival (e) curves for mice treated with vehicle, 18 mg/kg/day FLX, or 12 mg/kg/day OLZ and housed with wheels during food restriction (6 h of food access per day). Results expressed as mean ± SEM. *$p < 0.05$ vs vehicle. Numbers in italics represent mice remaining in food restriction. †Significant main effect of treatment vs vehicle, $p < 0.05$.

appropriate strain for modeling AN-like behavior using the ABA paradigm.

We optimized the ABA paradigm for use with Balb/cJ mice. Food access duration can alter the progression of ABA, with longer food access periods reducing the phenotype (Pare et al, 1985; Watanabe et al, 1992). Furthermore, food access durations employed in rat ABA studies (2–4 h) (Pare et al, 1985; Watanabe et al, 1992) induced rapid dropout in mice housed without wheels (Figure 2), which should be able to adapt to daily food restriction and maintain body weight according to the definition of ABA (Routtenberg and Kuznesof, 1967). We sought to identify a food access duration that would extend the length of time mice survive during food restriction, allowing for the detection of decreases and increases in survival in response to various treatments. Our results indicate that 6 h of daily food access induces a length of survival (2–5 days) (Figure 2) that avoids both floor and ceiling effects. Mice given 6 h of food access showed significant reductions in body weight (see Supplementary Results, Supplementary Figure S1A), and food intake (see Supplementary Results, Supplementary Figure S1B) compared with mice without wheels. In addition, the 6-h food access condition induced dropout of all mice with wheels, whereas all mice without wheels maintained at least 75% of baseline body weight. Thus, this food access duration

permits the assessment of treatment effects during food restriction conditions alone. In addition, the 8- and 10-h food access duration times induced milder ABA phenotypes, and could be used to evaluate the effects of long-term treatments, or long-term ABA, on the brain.

Varying the food access period provided the most practical means for adjusting ABA severity. However, many other variables have been shown to affect ABA severity. For example, the adaptation theory of ABA is based on findings that food access during the light cycle and pre-exposure to running wheels increases the ABA phenotype (Dwyer and Boakes, 1997; Ratnovsky and Neuman, 2011). Thus, adaptation to a feeding schedule that is more similar to species-typical behaviors reduces ABA behavior. We found that acclimation and baseline exposure to running wheels before restriction, coupled with 6-h food access during restriction, produced a moderate ABA phenotype in Balb/cJ mice, which serves as a tool for investigating the effects of drug treatment on ABA. In addition to varying food access duration, our design also varies circadian feeding patterns between groups, and the effects of these two factors on ABA cannot be dissociated.

In an initial dose-finding study, we found that both 12 and 24 mg/kg/day of OLZ significantly increased survival in ABA. A previous report suggests that, OLZ reduces ABA in rats (Hillebrand et al, 2005), although the dose of OLZ used

caused robust reductions in running wheel activity in rats fed *ad libitum* and food restricted rats (1-h daily food access). These findings suggest that the dose of OLZ used induced sedation. We therefore aimed to identify a non-sedative dose that would significantly reduce ABA behavior. Both the 12 and 24 mg/kg/day doses of OLZ significantly increased body weight during food restriction (Figure 3a), and increased survival in the ABA paradigm (Figure 3e). Importantly, OLZ did not significantly decrease running wheel activity or increase food intake during baseline (Table 1). Although not significant, mice treated with the highest dose (24 mg/kg/day) did show about a 35% decreases in running wheel activity during baseline, illustrating that this high dose approached a sedative effect. Although only subchronic OLZ treatment (1 week) is sufficient to increase survival in ABA (Figures 3e and 4e) without significant sedation or hyperphagia, an intermediate, chronic dose of OLZ (4 weeks; 16 mg/kg/day) also produces significant increases in survival (Supplementary Figure S6A) without significant increases in pre-treatment or baseline body weight (Supplementary Figure S6B) or sedation (Supplementary Figure S6D) (see Supplementary Results and Methods). In fact, chronic OLZ (16 mg/kg/day) treatment produced significant hypophagia in mice housed with wheels during baseline (Supplementary Figure S6C) (see Supplementary Results). These data indicate that chronic OLZ treatment does not induce significant increases in body weight, metabolism, or general activity levels in Balb/cJ mice. Overall, these results indicate that subchronic (1 week) and chronic (4 weeks) OLZ treatment increases survival in ABA without inducing sedation or hyperphagia.

Our results indicate that subchronic OLZ, but not chronic FLX, treatment increases murine survival in the ABA paradigm (Figure 4e). Although both treatments appeared to increase survival under the experimental conditions employed, this effect only achieved significance in OLZ-treated mice. Under food restriction conditions, neither treatment significantly altered body weight (Figure 4a, Supplementary Figure S3A). Although 12 and 24 mg/kg/day of OLZ induced small but significant increases in body weight during food restriction in the dose-response study (Figure 3a), 12 mg/kg/day OLZ only induced small, non-significant increases in body weight during the first 6 days of food restriction in the drug comparison study (Figure 4a). Both OLZ and FLX significantly reduced FAA (Figure 4d), which contributes substantially to progressive weight loss in the ABA paradigm (Beneke et al, 1995; Dwyer and Boakes, 1997). During restriction, OLZ only produced a trend for increased food intake, while FLX significantly increased food intake during restriction (Figure 4b). However, increases in food intake did not result in significant increases in body weight or survival for FLX-treated mice. Although both OLZ and FLX reduced aspects of ABA behavior, only OLZ produced significant increases in survival (Figure 4e).

Subchronic OLZ treatment (12 mg/kg/day) and chronic OLZ treatment (8 mg/kg/day, 16 mg/kg/day) produced OLZ plasma levels (Supplementary Figure S7, S8) that are above the threshold, which predicts a therapeutic response in patients with schizophrenia (Perry et al, 2001). Only two studies, with few patients ($n = 14$, $n = 13$) have measured OLZ plasma level in AN (Powers et al, 2002; Theisen et al,

2006), and more work needs to be done to evaluate the OLZ serum level required for therapeutic response in AN. Although the OLZ plasma levels reported here are higher than levels seen in patients with AN, it is unclear whether these differences are due to species differences or how the ABA phenotype affect OLZ metabolism as animals approach 75% of baseline body weight. We additionally found that under conditions of 3-h daily food access, subchronic OLZ treatment (30 mg/kg/day) significantly increased survival, whereas chronic FLX treatment (25 mg/kg/day) was ineffective (Supplementary Figure S4). Furthermore, we also found that subchronic OLZ treatment (25 mg/kg/day, 60 mg/kg/day) increased the survival of female adolescent (4–6 weeks) BALB/cJ mice in the ABA paradigm (Supplementary Figure S5). Our data suggest that OLZ treatment at doses ranging from approximately 12–25 mg/kg/day in Balb/cJ mice given 6 h of food access per day significantly increase survival in ABA. These findings suggest that OLZ treatment may reduce AN symptomology in patients.

SSRI treatment has previously been reported to reduce ABA in rats (Altemus et al, 1996; Yokoyama et al, 2007). The discrepancy between findings in rats and our present findings in mice may result from species differences, or differences in methodology. For example, we performed studies using BALB/cJ, a mouse strain that exhibits a robust ABA phenotype. However, the rat studies reporting effects of SSRI treatment on ABA employed commonly used rat strains, which have not been evaluated phenotypically for ABA severity. Methodological differences include the sex of rodents used and treatment duration. For example, Yokoyama et al (2007) treated male rats with a very high dose SSRI (50 mg/kg/day fluvoxamine) for only 7 days during restriction. Our negative findings with FLX are unlikely to have resulted from using a non-therapeutic dose because in BALB/cJ mice, as 18 mg/kg/day FLX has been shown to produce plasma FLX levels that fall in the middle of the range observed in depressed patients taking therapeutic doses (Dulawa et al, 2004). Mice receiving 18 mg/mg/day FLX in the present studies had somewhat higher plasma FLX levels than previously reported using this dose (Supplementary Figure S7) (Dulawa et al, 2004). The significant decreases in body temperature that develops during ABA could have reduced systemic clearance of FLX leading to higher plasma levels (Tortorici et al, 2007), since samples were collected as mice reached dropout criteria. Current reports conflict regarding the influence of serotonergic tone on ABA. For example, both decreasing (Atchley and Eckel, 2006), and increasing (Altemus et al, 1996) serotonergic tone have suggested to ameliorate ABA. Our results suggest that chronic FLX treatment with doses producing similar plasma levels in patients do not reduce ABA in mice.

The mechanisms by which chronic OLZ treatment increases survival in the ABA paradigm have not been identified. OLZ might increase survival in ABA by decreasing HPA axis reactivity or altering metabolism. HPA axis activity is increased during ABA as indicated by increased corticosterone levels (Burden et al, 1993), and corticosterone is required for the development of hyperactivity in ABA (Duclos et al, 2009). Chronic treatment with atypical antipsychotics reduces markers of HPA axis activity in patients with psychosis (Zhang et al, 2005). Thus, OLZ may

reduce ABA by reducing HPA axis activity. Chronic OLZ treatment has also been associated with increases in body weight and metabolic syndrome (Pramyothin and Khaodhiar, 2010) in clinical populations with psychosis, suggesting alterations in metabolism (Allison *et al*, 1999; Newcomer, 2007). However, any OLZ-induced metabolic changes in our study are unlikely, because 12 and 24 mg/kg/day OLZ did not increase baseline body weight or food intake (Table 1).

OLZ is an antagonist at multiple neurotransmitter receptors, including DA D_1, D_2, D_4, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, $\alpha 1$-adrenergic, histamine H_1, and several muscarinic receptors (Bymaster *et al*, 1996). OLZ may increase survival in the ABA paradigm by blocking one or more 5-HT and/or DA receptors. Recently, Verhagen *et al* (2009) found that the nonselective DA antagonist *cis*-flupenthixol increased body weight, food intake, and body temperature during ABA, but also reduced general locomotor activity in a sedative manner. Patients with AN likely have disruptions in 5-HT and DA signaling, because levels of DA (Kaye *et al*, 1999) and 5-HT (Kaye *et al*, 1988, 1991) metabolites are altered in ill and recovered AN patients. Furthermore, AN patients also show altered expression of specific 5-HT and DA receptors and transporters. Binding potentials for the 5-HT_{1A} receptor and serotonin transporter are increased (Bailer *et al*, 2005, 2007a, 2007b) and binding potentials for the 5-HT_{2A} receptor are decreased (Audenaert *et al*, 2003; Frank *et al*, 2002) in ill and recovered AN patients. Increases in DA D_2/D_3 receptor binding in the ventral striatum are also seen following recovery (Frank *et al*, 2005). Future work should examine the receptor subtypes at which OLZ acts to reduce ABA.

Patients with AN find little rewarding in life beyond their drive for thinness (APA, 2000; Davis and Woodside, 2002), and abnormalities in dopaminergic signaling in AN may alter reward processing. Indeed, AN patients are more sensitive to reward and punishment (Harrison *et al*, 2010; Jappe *et al*, 2011). However, AN patients do not show the differential activation in the anterior ventral striatum distinguishing wins and losses following a reward task that control subjects exhibit (Wagner *et al*, 2008). Thus, AN patients may not differentiate positive and negative feedback normally. They also do not find palatable foods as rewarding (Fernstrom *et al*, 1994; Santel *et al*, 2006) have reduced novelty seeking (Harrison *et al*, 2010), and exhibit altered responses to taste stimuli in insular and striatal regions (Wagner *et al*, 2008). The suppression theory of ABA suggests that reward processes may also be altered in ABA because rodents lever press more for access to a running wheel when food deprived, and find food less salient as activity levels increase (Pierce *et al*, 1986).

The effects of OLZ on reward processes may contribute to its ability to reduce ABA. OLZ has variable effects on reward anticipation in the ventral striatum (Abler *et al*, 2007; Juckel *et al*, 2006; Schlagenhauf *et al*, 2008), and has been suggested to reduce substance abuse (Petrakis *et al*, 2006; Sayers *et al*, 2005; Smelson *et al*, 2006). OLZ may alter the reward saliency of wheel running, demonstrated by reductions in FAA (Figure 4d). Furthermore, OLZ has been shown to improve cognitive functioning in patients with schizophrenia (McGurk *et al*, 2004; Meltzer and McGurk, 1999). It is unclear whether OLZ improves cognitive function in mice. Several studies provide evidence for improvements in learning and memory (Hou *et al*, 2006; Mutlu *et al*, 2011; Wolff and Leander, 2003), while others show no effect (Rosengarten and Quartermain, 2002) or deficits (Arnt and Skarsfeldt, 1998; Mutlu *et al*, 2011; Skarsfeldt, 1996; Terry *et al*, 2002). This discrepancy may be due to differences in methodology, including duration of treatment, learning tasks, and species used. Although it is unclear whether OLZ may enhance learning in ABA, OLZ-treated mice housed with wheels exhibited increased adaptation to the food restriction paradigm, as illustrated by a trend for increases food intake in comparison with vehicle-treated mice (Figure 4b). Overall, both FLX and OLZ treatment reduced FAA and increased food intake in ABA in comparison with vehicle-treated mice, possibly through alterations in the reward saliency of the wheel and/or improved adaption to the food restriction paradigm via enhanced cognitive function. Nevertheless, these improvements in ABA behavior only produced significant increases in survival in OLZ-treated mice. The therapeutic mechanism by which OLZ increases survival needs further investigation.

In summary, chronic OLZ treatment reduces FAA and increases survival in the ABA paradigm in mice, without inducing sedation or hyperphagia. Furthermore, chronic FLX treatment was ineffective in increasing survival in ABA. Given the high mortality rate in AN (Arcelus *et al*, 2011; Sullivan, 1995), approved pharmacological treatments for the disorder are sorely needed. Our findings strongly suggest that OLZ, but not FLX, may provide treatment for AN. Future work will investigate the mechanisms by which OLZ reduces ABA.

DISCLOSURE

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)