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Treatment with 8-OH-DPAT attenuates the weight loss associated with activity-based anorexia in female rats

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Abstract

Serotonin (5-HT) plays an important role in controlling food intake and regulating body weight. In addition, clinical studies suggest a possible role for 5-HT in the etiology of anorexia nervosa. Recently, we have examined the effects of pharmacological manipulation of the 5-HT system in female rats exposed to conditions that promote activity-based anorexia (ABA). In this animal model of anorexia nervosa, rats are food restricted (2 h access/day) while given the opportunity to exercise in running wheels. These conditions promote symptoms of anorexia nervosa including hypophagia, hyperactivity, progressive weight loss, and disruptions of the ovarian reproductive cycle. Previously, we demonstrated that increased 5-HT activity increased the weight loss associated with ABA in female rats. Here, we investigated whether decreased 5-HT activity would attenuate symptoms of ABA. Food-restricted female rats received injections of 8-OH-DPAT, a drug that reduces serotonergic neurotransmission, or saline vehicle 40 min prior to food access. During this restricted-feeding phase, food intake was similar between groups; however, 8-OH-DPAT prevented the hyperactivity observed in saline-treated rats. This resulted in less weight loss in 8-OH-DPAT-treated rats, suggesting that decreased activation of the 5-HT system attenuates the development of ABA.
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Keywords: Anorexia nervosa; Serotonin; Food intake; Body weight regulation

1. Introduction

Anorexia nervosa is a debilitating eating disorder characterized by caloric restriction, hyperactivity, an intense fear of becoming overweight, and extreme, often life-threatening, weight loss. This eating disorder, which has proven difficult to treat, is associated with frequent relapse and the highest mortality rate among psychiatric disorders (American Psychiatric Association, 1994). In recent years, there has been a renewed interest in identifying biological factors that may contribute to the development of anorexia nervosa.

Because prospective studies of anorexia nervosa are difficult to conduct in humans, animal models have been used to examine biological risk factors for anorexia nervosa. In the activity-based anorexia (ABA) model, rats are housed with access to running wheels and then placed on a restricted-feeding schedule consisting of 1–2 h access to food per day. Under these conditions rats develop a syndrome characterized by multiple symptoms of anorexia nervosa including hyperactivity, progressive weight loss, and estrous cycle disruptions (Atchley and Eckel, 2005; Dixon et al., 2003; Watanabe et al., 1992). This animal model allows researchers to directly manipulate neuronal and endocrine systems that may be dysregulated in anorexia nervosa. Recently, we reported that manipulation of the serotonin (5-HT) system can modulate the development of ABA in female rats (Atchley and Eckel, 2005). In this study, rats received daily injections of fenfluramine (FEN), a potent 5-HT releasing agent, concurrent with food restriction. Control groups received injections of vehicle alone or in combination with a pair-feeding protocol that limited food intake to the amount consumed by FEN-treated rats. Interestingly, FEN-treated rats displayed accelerated weight loss relative to both control groups. Because FEN-treated and pair-fed rats consumed comparable amounts of food, the more rapid weight loss in the former group could not simply be attributed to the anorectic effect of FEN.

Here, we determined whether a decrease in 5-HT activity would attenuate the weight loss associated with ABA. Female rats were placed on a chronic food-restriction schedule and injected daily with 8-hydroxy-2-(di-*n*-propylamino) tetralin

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(8-OH-DPAT), a drug that selectively activates 5-HT_{1A} autoreceptors and, thereby, decreases the release of 5-HT in brain regions innervated by serotonergic raphe neurons (Hjorth and Sharp, 1991). We hypothesized that if elevated 5-HT activity following FEN treatment increases the rate of weight loss associated with ABA, then suppression of 5-HT activity following 8-OH-DPAT treatment would have the opposite effect.

2. Methods

2.1. Animals and housing

Seventeen female Long–Evans rats (Charles River Breeding Laboratories, Raleigh, NC), weighing 221±3.5 g at study onset, were housed individually in wire-mesh bottom cages connected to Wahmann running wheels (35 cm in diameter). The running wheels were equipped with dipole magnets (DiLog Instruments, Tallahassee, FL) which signaled the occurrence of wheel revolutions. Outputs from the magnets were stored on a computer and custom-designed software (ESP 500; R. Henderson; Florida State University) was used to examine daily running wheel activity at specific intervals. The testing room was maintained at 20±2 °C on a 12:12 h light:dark schedule (dark onset=1300 h). Powdered rat chow (Purina 5001) was presented in food cups located in feeding niches that protruded from the cages. Papers were placed below the food cups to collect any food spillage. A computerized system was used to open and close a gate that limited access to the food cups to specific times. Throughout the experiment, food and water were freely available, except as noted below. Rats were adapted to the novel housing conditions prior to data collection. Animal usage and all procedures were in compliance with the Florida State University Institutional Animal Care and Use Committee.

2.2. Behavioral measures, body weight, and estrous cycles

Food intake, wheel running, body weight, and stage of the estrous cycle were monitored daily. Between 1000 and 1100 h, food cups were weighed (±0.1 g) and any food spillage was subtracted from the daily food intake measurement, wheel running was recorded (±0.5 rev), rats were weighed (±0.1 g), and vaginal cytology samples were collected. Stage of the estrous cycle (diestrus 1, diestrus 2, proestrus, or estrus) was then determined by examining the appearance and abundance of cells within each sample, as described previously (Becker et al., 2005; Eckel et al., 2000). Using this strategy, proestrus included the light phase peak in estradiol secretion, and estrus included the subsequent dark phase when female rats ovulate and display increased sexual receptivity (Becker et al., 2005). At study onset, all rats had displayed a minimum of 2 regular, 4-day estrous cycles.

2.3. Procedure

Food intake, running wheel activity, body weight, and estrous cyclicity were monitored daily in all rats during baseline, restricted-feeding, and recovery test phases, as summarized in Fig. 1. Beginning on diestrus 1, baseline measurements of food intake, running wheel activity, and body weight were monitored in free-fed rats across one 4-day estrous cycle. At the start of the next estrous cycle, rats were assigned to one of two groups and daily access to food was restricted to 2 h, beginning 1 h after dark onset. Throughout this restricted-feeding phase, rats received daily subcutaneous injections of 0.5 mg/kg 8-OH-DPAT (Sigma Chemical, Natick, MA; $n=9$) or 1 ml/kg physiological saline vehicle ($n=8$) 40 min prior to food access. Food-restricted rats were maintained on this schedule of drug injections until they displayed a 25% body weight loss or after 10 days, whichever occurred first. These criteria for termination of the restricted-feeding schedule were based on previous studies in which the weight loss associated with ABA occurred at variable rates in female rats (Atchley and Eckel, 2005; Dixon et al., 2003). Because severe, often fatal, gastric lesions develop following a 30% weight loss in this paradigm (Doerries et al., 1991; Lambert and Kinsley, 1993; Tsuda et al., 1982), rats were not allowed to progress beyond a 25% weight loss in the present study. Upon reaching the 25% weight loss/10-day criterion, drug injections were terminated, rats were given free access to food, and recovery from ABA was assessed by monitoring daily food intake, wheel running, body weight, and vaginal cytology samples until individual rats displayed one regular 4-day estrous cycle.

2.4. Data analysis

Data are presented as means±SEM. During the 4-day baseline phase, group differences in mean daily food intake,

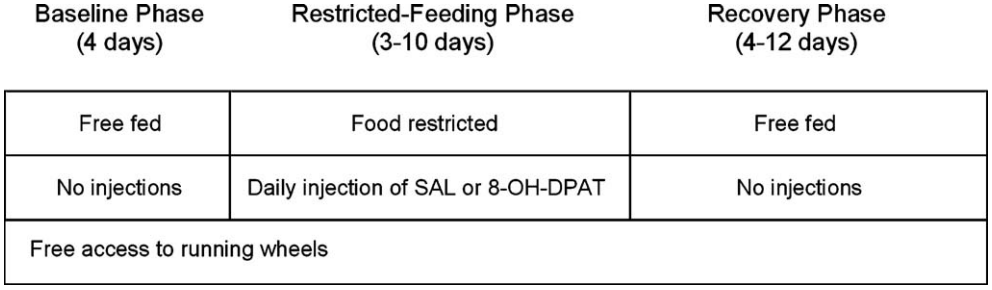

Fig. 1. Summary of the experimental paradigm. During the baseline phase, rats had free access to food and running wheels for 1 estrous cycle (4 days). During the restricted-feeding phase, rats had free access to running wheels but food was restricted to 2 h/day. Rats received injections of either saline or 0.5 mg/kg 8-OH-DPAT 40 min prior to food access. Food restriction was terminated when individual rats lost 25% of their baseline body weight or after 10 days, whichever occurred first. During the recovery phase, rats were given free access to food. This phase was terminated when individual rats displayed one 4-day estrous cycle. Abbreviations: SAL; saline.

wheel running, and body weight were assessed using independent *t*-tests. During the restricted-feeding phase, rats reached the 25% weight loss/10-day criterion at different times (range = 3–10 days). Thus, to permit statistical analysis of the restricted-feeding phase, daily food intake, running wheel activity, and weight loss were examined on the first, middle, and last days of food restriction. The middle day was defined as the median day of food restriction. To examine the pattern of running wheel activity in greater detail, wheel running was examined at 2-h intervals on the first, middle, and last days of food restriction. The effects of 8-OH-DPAT on food intake, wheel running, and weight loss were examined using mixed-design ANOVAs with drug treatment as the between-subject variable and day of food restriction as the within-subject variable. The effects of 8-OH-DPAT treatment on the pattern of running wheel activity were examined using a mixed-design ANOVA with group as the between-subject variable and day of food restriction and 2-h interval as the within-subject variables. The severity of ABA was determined by the mean latency (in days) to reach the 25% weight loss/10-day criterion for termination of the restricted-feeding schedule, the percentage of rats with estrous cycle disruptions at this time, and the total amount of body weight lost during food restriction. The number of days to reach the refeeding criterion was determined by either the actual number of restricted-feeding days (for those that reached the refeeding criterion) or 10 days (for those that failed to reach the refeeding criterion by day 10). Group differences in total weight loss and latency to reach the refeeding criterion were analyzed using independent *t*-tests. A Chi-square analysis was used to examine group differences in estrous cycle disruptions. During the recovery phase, group differences in mean daily food intake, wheel running, latency to regain body weight, and number of days to resume estrous cycles were analyzed using independent *t*-tests. Body weight gain on the first, middle, and last days of recovery was analyzed using a mixed-design ANOVA with day of recovery as the within-subject variable and drug treatment as the between-subject variable. Tukey's tests were used to investigate differences between means following significant ($p < 0.05$) main or interactive ANOVA effects.

3. Results

3.1. Baseline phase

Prior to food restriction and drug treatment, no significant differences in mean daily food intake, wheel running, or body weight were detected between groups, $t(15) = 0.01–0.67$, n.s. (Table 1). All rats displayed regular 4-day estrous cycles.

Table 1
Baseline measures of food intake, running wheel activity, and body weight

	Saline-treated rats	8-OH-DPAT-treated rats
Mean daily food intake (g)	23.4±0.9	23.2±0.4
Mean daily wheel running (rev)	7424±1388	7436±1483
Mean daily body weight (g)	228±6	223±3

Data are means±SEM. Food intake, running wheel activity, and body weight did not differ between saline- and 8-OH-DPAT-treated rats during the baseline phase. Abbreviations: rev; revolutions.

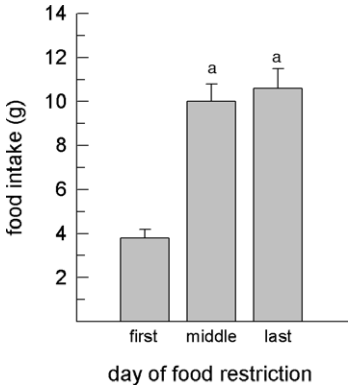

Fig. 2. Food intake on the first, middle, and last days of food restriction. Regardless of drug treatment, all rats displayed an increase in food intake on the middle and last days of food restriction, relative to the first day of food restriction. Drug treatment did not alter food intake during the restricted-feeding phase. [a]Greater than the first day ($p < 0.05$).

3.2. Restricted-feeding phase

An analysis of food intake on the first, middle, and last days of food restriction revealed a main effect of day, $F(2, 30) = 37.48$, $p < 0.00001$ (Fig. 2). Regardless of drug treatment, all rats consumed more food on the middle and last days of food restriction, relative to the first day of food restriction, $ps < 0.05$. Food intake was not influenced by either a main or an interactive effect of drug treatment.

Running wheel activity during the restricted-feeding phase was influenced by an interactive effect of drug treatment and day, $F(2, 30) = 6.35$, $p < 0.005$ (Fig. 3). On the first day of food restriction, wheel running did not differ in 8-OH-DPAT- and saline-treated rats. On the middle and last days of food

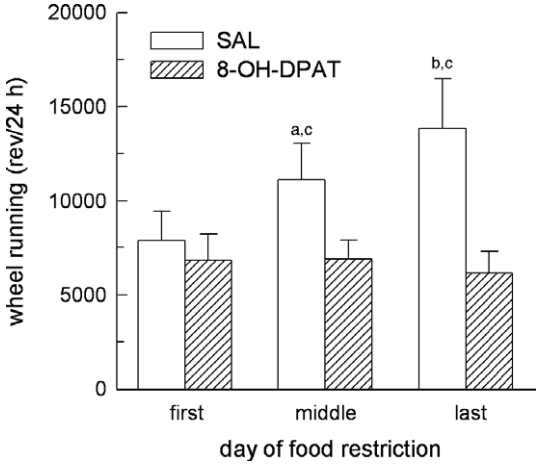

Fig. 3. Running wheel activity during the first, middle, and last days of food restriction. 8-OH-DPAT treatment blocked the progressive increase in wheel running that was observed in saline-treated rats during the restricted-feeding phase. [a]Greater than the first day ($p < 0.05$). [b]Greater than the first and middle days ($p < 0.05$). [c]Greater than the 8-OH-DPAT-treated group ($p < 0.05$). Abbreviations: rev; revolutions, SAL; saline.

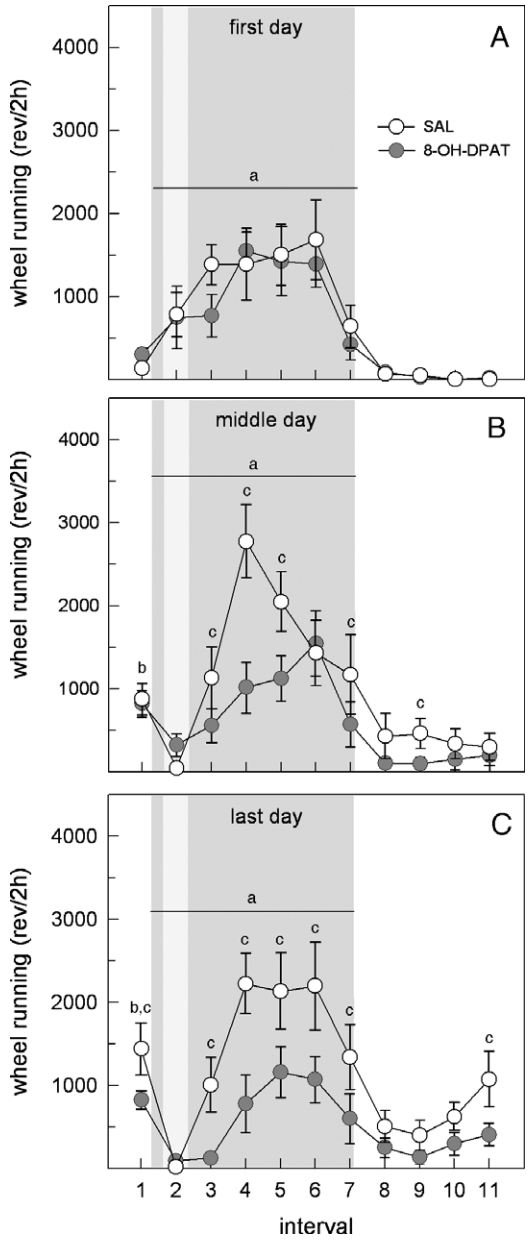

Fig. 4. Running wheel activity at 2-h intervals across the light:dark cycle during the first, middle, and last days of food restriction. The dark phase of the lighting cycle is indicated by dark grey shading. Food was available during the 2-h interval beginning 1 h after dark onset (interval 2; light grey shading). (A) On the first day of food restriction, the pattern of wheel running was similar in saline- and 8-OH-DPAT-treated rats. (B) On the middle day of food restriction, feeding anticipatory wheel running (during interval 1) was apparent in both groups. During 4 of the 6 dark-phase intervals, saline-treated rats ran more than 8-OH-DPAT-treated rats. (C) By the final day of food restriction, saline-treated rats displayed greater feeding anticipatory wheel running during intervals 1 and 11 (i.e., the two light phase intervals prior to food access) than 8-OH-DPAT-treated rats. Throughout the dark phase, saline-treated rats ran more than 8-OH-DPAT-treated rats. [a]Greater than light-phase running ($p<0.05$). [b]Greater than light-phase interval 1 on the first day of food restriction ($p<0.05$). [c]Greater than 8-OH-DPAT-treated rats ($p<0.05$). Abbreviations: rev; revolutions, SAL; saline.

restriction, saline-treated rats displayed a progressive increase in wheel running, $ps<0.05$, that was not apparent in 8-OH-DPAT-treated rats. This resulted in greater wheel running in saline-treated rats, relative to 8-OH-DPAT-treated rats, on the middle and last days of food restriction, $ps<0.01$. Finally, 8-OH-DPAT failed to modulate running wheel activity across the restricted-feeding phase. On the first, middle and last days of food restriction, wheel running in 8-OH-DPAT-treated rats was similar to that observed in saline-treated rats on the first day of food restriction (Fig. 3) and in both groups during the baseline phase (see Table 1). To further investigate the pattern of running wheel activity in food-restricted rats, wheel running was examined at 2-h intervals across the light and dark phases of the first, middle, and last days of food restriction. This analysis revealed an interactive effect of drug treatment, day, and 2-h interval, $F(20, 299)=1.91$, $p<0.05$ (Fig. 4). As expected, all rats displayed greater running wheel activity during the dark phase, relative to the light phase, on each day, $ps<0.01$. On the first day of food restriction, the overall pattern of light- and dark-phase wheel running did not differ between saline- and 8-OH-DPAT-treated rats (Fig. 4A). However, by the middle day of food restriction, saline-treated rats ran more than 8-OH-DPAT-treated rats during 4 of the 6 dark-phase intervals, $ps<0.05$. By the last day of food restriction, saline-treated rats ran more than 8-OH-DPAT-treated rats throughout the entire dark phase, $ps<0.05$ (Fig. 4C). Group differences in light-phase wheel running also emerged. On the first day of food restriction, all rats displayed low levels of wheel running during the early segment of the light phase (i.e., interval 1) that preceded access to food (Fig. 4A). However, on the middle and last days, both groups displayed increased wheel running at this time, $ps<0.05$ (Fig. 4B,C). By the last day, this feeding anticipatory increase in wheel running, now evident during the two light-phase intervals preceding food access (i.e., intervals 1 and 11), was greater in saline-treated rats, relative to 8-OH-DPAT-treated rats, $ps<0.05$ (Fig. 4C).

Weight loss during the restricted-feeding phase was influenced by drug treatment and day, $F(1, 15)=8.10$, $p<0.01$ and $F(2, 30)=132.65$, $p<0.00001$, respectively (Fig. 5). While all rats

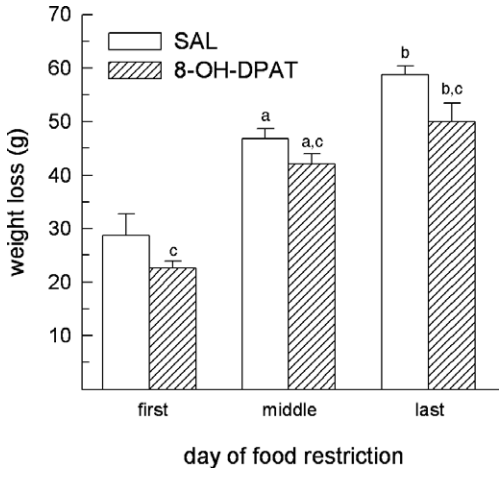

Fig. 5. Cumulative weight lost on the first, middle, and last days of food restriction. While progressive weight loss was observed in both groups, saline-treated rats had lost more weight than 8-OH-DPAT-treated rats on the first, middle and last days of food restriction. [a]Greater than the first day of food restriction ($p<0.05$). [b]Greater than the first and second days of food restriction ($p<0.05$). [c]Less than saline-treated rats ($p<0.05$). Abbreviations: SAL; saline.

displayed a progressive increase in weight loss from the first to the last day of food restriction, $ps < 0.05$, 8-OH-DPAT-treated rats lost less weight than saline-treated rats on the first, middle and last days of food restriction, $ps < 0.05$.

3.3. Severity of ABA

The restricted-feeding schedule was terminated when individual rats displayed a 25% weight loss, or after 10 days, whichever occurred first. At this time, individual rats were returned to a free-feeding schedule. All of the 8 saline-treated rats, but only 6 of the 9 8-OH-DPAT-treated rats, lost 25% of their body weight within 10 days. The number of days to reach the refeeding criterion was greater in 8-OH-DPAT-treated rats, relative to saline-treated rats, $t(15) = 1.75$, $p < 0.05$ (Fig. 6A). Despite being subjected to a longer period of food restriction, 8-OH-DPAT-treated rats lost less weight than saline-treated rats, $t(15) = 2.20$, $p < 0.05$ (Fig. 6B). No group differences in estrous cycle disruptions were detected (saline: 3 of 8 rats vs. 8-OH-DPAT: 5 of 9 rats disrupted), $\chi^2 = 1.9$, n.s.

3.4. Recovery phase

Following the return to a free-feeding schedule, no differences in either mean daily food intake or running wheel activity were detected between groups, $t(15) = 0.34–1.58$, n.s. (data not shown). Body weight gain was influenced by a main effect day, $F(2, 30) = 38.41$, $p < 0.00001$ (Fig. 7). Regardless of drug treatment, all rats displayed a progressive increase in weight gain through the first, middle, and last day of recovery, $ps < 0.01$. No main or interactive group differences in body weight gain were detected gain. Accordingly, the number of days to body weight recovery (saline: 4.5±0.5 vs. 8-OH-DPAT: 4.1±0.5) and the resumption of regular estrous cycles (saline: 8.8±0.3 vs. 8-OH-DPAT: 7.7±1.0) was similar in saline- and 8-OH-DPAT-treated rats, $t(15) = 0.56$ and 0.99, respectively, n.s. By the final day of recovery, no differences in body weight were observed between groups (saline: 236.5±4.2 vs. 8-OH-DPAT: 237.4±8.8), $t(15) = 0.09$, n.s.

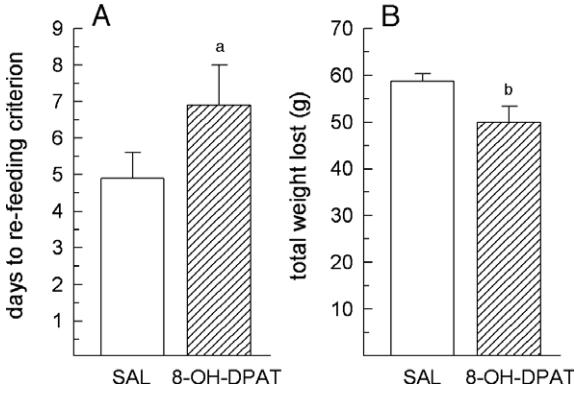

Fig. 6. The severity of ABA is attenuated by 8-OH-DPAT treatment. (A) Rats treated with 8-OH-DPAT took longer to reach the 25% weight loss criterion than saline-treated rats. (B) Rats treated with 8-OH-DPAT lost less weight than saline-treated rats. [a]Greater than saline-treated rats ($p < 0.05$). [b]Less than saline-treated rats ($p < 0.05$). Abbreviations: SAL; saline.

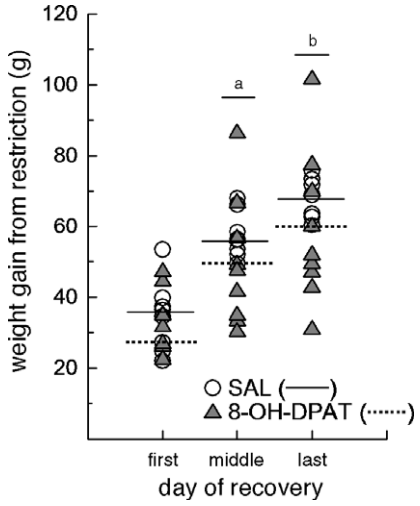

Fig. 7. Cumulative weight gain in individual rats on the first, middle, and last days of the recovery phase. Progressive weight gain was observed in all rats. Saline- and 8-OH-DPAT-treated rats gained similar amounts of weight on the first, middle and last days of recovery. The greater variability in weight gain on the middle and last days is likely related to individual differences in the latency to resume estrous cycles and, therefore, cyclic changes in body weight. Solid lines depict group means for saline-treated rats. Dotted lines depict group means for 8-OH-DPAT-treated rats. [a]Greater than the first day of recovery ($p < 0.01$). [b]Greater than the first and second days of recovery ($p < 0.01$). Abbreviations: SAL; saline.

4. Discussion

The goal of the present study was to determine whether pharmacological manipulation of the 5-HT system modulates the development of ABA in female rats. Previously, administration of a 5-HT releasing agent, concurrent with food restriction, increased the weight loss associated with ABA (Atchley and Eckel, 2005; Rieg et al., 1994). Here, rats were treated with 8-OH-DPAT, a drug that decreases the firing rate of 5-HT neurons, to investigate whether reduced 5-HT activity attenuates the weight loss associated with ABA. Despite similar food intake throughout the restricted-feeding phase, 8-OH-DPAT-treated rats lost weight more slowly and thus lost less weight than saline-treated rats. This attenuated weight loss appears to be mediated by 8-OH-DPAT's ability to abolish the hyperactivity that is critical for the development of ABA.

In the present study, 8-OH-DPAT attenuated the weight loss associated with ABA. Although 8-OH-DPAT increases food intake in male rats (Dourish et al., 1985a,b), we saw no evidence of an orexigenic effect of the drug here. Throughout the restricted-feeding phase, food intake did not differ between saline- and 8-OH-DPAT-treated rats. It is possible that restricting food access to 2 h per day may have introduced a ceiling effect that would prevent any treatment from increasing food intake at this time. Indeed, there are reports that the orexigenic effect of 8-OH-DPAT may be limited to free-fed rats (Bendotti and Samanin, 1986; Dourish et al., 1985b, 1986). However, another

treatment involving chronic clonidine administration increased food intake during the last three days of food restriction in an ABA paradigm similar to ours (Rieg and Aravich, 1994). This suggests that a ceiling effect may not have limited our ability to detect an orexigenic effect of 8-OH-DPAT. Rather, we contend that a diurnal variation in 8-OH-DPAT's ability to increase food intake may account for our findings. In two previous studies, the orexigenic effect of 8-OH-DPAT was limited to feeding tests conducted during the light phase (Shimizu et al., 2000). Regardless of the mechanism that prevented 8-OH-DPAT from increasing food intake in the present study, our observation that saline- and 8-OH-DPAT-treated rats consumed similar amounts of food throughout the restricted-feeding phase suggests that the reduction in weight loss in the former group cannot simply be attributed to an orexigenic effect of 8-OH-DPAT.

During the restricted-feeding phase, 8-OH-DPAT prevented the increase in daily running wheel activity that was observed in food restricted, saline-treated rats (Fig. 3). In addition, 8-OH-DPAT-treated rats did not display any changes in daily wheel running during the restricted-feeding phase, relative to the baseline phase. Thus, 8-OH-DPAT did not appear to influence daily running wheel activity in this study. While a general increase in wheel running is important for the development of ABA (e.g., Dixon et al., 2003), an increase in feeding anticipatory wheel running appears crucial. Male rats do not develop ABA when wheel access is blocked during the 4 h preceding food access, and limiting wheel access to this 4-h period is sufficient for the development of ABA (Beneke et al., 1995; Dwyer and Boakes, 1997). In the present study, 8-OH-DPAT not only prevented the rapid increase in dark-phase wheel running observed in saline-treated rats, but it also attenuated feeding anticipatory wheel running (Fig. 4). Thus, the blockade of excessive daily wheel running in general, and feeding anticipatory wheel running in particular, likely contributed to the attenuated weight loss of 8-OH-DPAT-treated rats. In previous studies, 8-OH-DPAT produced dose-related effects on general locomotor activity (i.e., ambulatory behavior) in free-fed rats. In both open field and home cage tests, low doses of 8-OH-DPAT (0.01–0.05 mg/kg) decreased ambulatory behavior (Carey et al., 2004), whereas higher doses of 8-OH-DPAT (0.2–0.4 mg/kg), similar to that used in the present study, increased ambulatory behavior (Blanchard et al., 1993; Carey et al., 2004). The latter studies rule out the possibility that a decrease in ambulatory behavior contributed to 8-OH-DPAT's ability to attenuate the weight loss associated with ABA. Although previous studies suggest that ambulatory behavior within the home cage may have been increased in the present study, we believe this to be an unlikely outcome. Our rats were housed in custom-designed cages that were equipped with relatively small home cages connected to running wheels. Under these housing conditions, the majority of ambulatory behavior is expressed within the running wheels because of size constraints of the home cages. Taken together, our findings suggest that an attenuation of the excessive running associated with chronic food restriction, rather than an overall decrease in ambulatory behavior, contributed to the lower rate of weight loss in 8-OH-DPAT-treated rats.

Previous experiments using the ABA model suggest that thermoregulation plays an important role in the development of ABA. If rats are housed at temperatures that exceed standard housing temperatures they do not develop ABA (Gutierrez et al., 2002), and access to a warm plate attenuates the weight loss associated with ABA (Hillebrand et al., 2005). These studies have led to the hypothesis that rats in the ABA paradigm display excessive wheel running in order to increase core body temperature (Gutierrez et al., 2002; Lambert and Porter, 1992). Interestingly, 8-OH-DPAT decreases body temperature in male rats (Eltayb et al., 2001; Hedlund et al., 2004; Maswood and Uphouse, 1997), although this effect appears to be limited to the first 2 h following drug treatment (Hedlund et al., 2004). Thus, it is possible that 8-OH-DPAT, which was administered 40 min prior to food access, may have contributed to the feeding anticipatory wheel running that was observed by the middle day of food restriction. Any such effect must have been quite small, as 8-OH-DPAT-treated rats displayed less feeding anticipatory wheel running than saline-treated rats on the last day of food restriction. In addition, 8-OH-DPAT-treated rats failed to display any hypothermia-related decrease in food intake during the restricted-feeding phase. Thus, it is unlikely that our regimen of 8-OH-DPAT treatment produced any long-term hypothermic effects that could have modulated the development of ABA.

In female rats, significant weight loss is often associated with disruptions in estrous cyclicity. Thus, it is not surprising that many of the rats in the present study became acyclic during the restricted-feeding phase. In rodents, maintenance of reproductive function appears to be partially dependent upon plasma leptin concentration (Ahima et al., 1997; Chehab et al., 1996; Gruaz et al., 1998). Because leptin is secreted from adipocytes in proportion to body adiposity (Fredrich et al., 1995), the progressive weight loss associated with the development of ABA undoubtedly results in reduced plasma leptin concentration. Thus, loss of estrous cyclicity in the present study may have been secondary to low plasma leptin concentration following weight loss. Although 8-OH-DPAT-treated rats lost less weight than control rats, we failed to detect any group differences in the prevalence of estrous cycle disruptions. This lack of association between total weight loss and prevalence of estrous cycle disruptions may be related to the minimal weight loss required to induce estrous cycle disruptions under our test conditions. That is, the weight loss of 8-OH-DPAT-treated rats, though less than the weight loss of control rats, may have been sufficient to induce estrous cycle disruptions. This hypothesis is supported by previous studies in which female rats displayed estrous cycle disruptions following weight loss between 13% and 17% of their baseline body weight (Knuth and Friesen, 1983; Tropp and Markus, 2001).

Our regimen of 8-OH-DPAT treatment throughout the restricted-feeding phase failed to modulate recovery from ABA. Both 8-OH-DPAT- and saline-treated groups displayed similar mean daily food intake and running wheel activity during recovery. Weight gain, assessed on the first, middle, and last days of recovery was similar between groups. In addition, latency to regain body weight to baseline values and latency to

resume estrous cycles were similar between groups. Thus, treatment with 8-OH-DPAT during food restriction does not interfere with recovery from ABA. This finding is consistent with the short half-life of 8-OH-DPAT (Perry and Fuller, 1989).

Here, we demonstrated that decreased serotonergic neurotransmission induced by chronic administration of 8-OH-DPAT attenuated the weight loss associated with ABA. This finding supports the hypothesis that reduced 5-HT activity, following chronic activation of presynaptic raphe 5-HT_{1A} autoreceptors, decreases susceptibility to ABA. Because modulation of the serotonergic system affects development of ABA in female rats, and this system is implicated in the etiology of anorexia nervosa (Kaye et al., 1991, 2003), further examination of 5-HT's role in the development of eating disorders is critical. In future studies, it will be important to determine the precise mechanism by which reduced 5-HT neurotransmission attenuates the weight loss associated with ABA.

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