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Adolescent Activity-Based Anorexia Increases Anxiety-Like Behavior in Adulthood

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Abstract

Activity-based anorexia is a paradigm that induces increased physical activity, reduced food intake, and heightened activity of the hypothalamic-pituitary-adrenal axis in adult rats. To investigate whether experience with activity-based anorexia produced enduring effects on brain and behavior, female adolescent rats experienced activity-based anorexia during adolescence and were tested in adulthood for anxiety-like behavior on an elevated plus maze and in an open field. Analysis of elevated plus maze and open field behavior in adulthood revealed that rats that experienced activity-based anorexia during adolescence, but not rats that were simply food restricted, displayed increased anxiety-like behavior in adulthood. Plasma corticosterone and expression levels of corticotropin- releasing hormone mRNA in the hypothalamic paraventricular nucleus and in the central nucleus of the amygdala were significantly elevated in adult rats that had undergone activity-based anorexia in adolescence in response to the open field exposure, as compared to control rats. These data demonstrate enduring effects of adolescent activity-based anorexia on anxiety-like behavior and neuroendocrine factors critical in stress responsivity in adulthood. Furthermore, we demonstrate that activity-based anorexia during adolescence serves as a model whereby prolonged anxiety is induced, allowing for evaluation of the behavioral and neural correlates of mediating anxiety-like behaviors in adulthood.

Keywords

Anorexia nervosa; Stress; Development; HPA axis

1. Introduction

Anorexia nervosa (AN) is a highly morbid pathological condition, with the highest mortality rate of psychiatric disorders [1,4,5]. It is marked by refusal to maintain a body weight at or above a minimally normal weight for age and height, intense fear of gaining weight or becoming fat despite being underweight, disturbance in the way in which one's body weight is experienced, and amenorrhea in postmenarcheal females. AN is more prevalent in females than in males, affecting approximately 0.3% of the female population in the US [2,3], and is marked by a high rate of relapse, and a low rate of full recovery. Epidemiological studies

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indicate that only about half of patients diagnosed with AN will recover, while 20–30% remain chronically ill and > 10% will eventually die from the disorder [6–8].

Epidemiological studies indicate that the age of AN onset is typically in mid- to late adolescence [8,9], a period during which the CNS undergoes growth and modification that includes increased myelination, synaptogenesis, and neural pruning [10,11]. Importantly, areas of the CNS that are critically involved in mediation of stress and emotional responsivity continue to develop, such as the hippocampus, medial prefrontal cortex, and amygdala [12–16]. Development of the adolescent nervous system is associated with the increased prevalence of many psychiatric disorders, including AN [17].

Given the prevalence of AN in adolescent females, there is a paucity of research aimed at understanding the long-term consequences of experiencing AN during adolescence. While animal models of human psychiatric disorders are imperfect, they allow for elucidation of the effects of prolonged disordered eating as well as identification of neural and endocrine factors that may contribute to the maintenance of behaviors. The activity-based anorexia (ABA) model is popular because it includes both voluntary suppression of food intake coupled with increased physical activity [18,19]. In the ABA model, rats are allowed access to a running wheel, and access to food is limited to 1–2 h per day. Without access to a running wheel, rats with limited access to food adapt their feeding behavior to the shorter period of food availability; however rats with access to a running wheel *and* limited food access consume less food than sedentary controls and develop hyperactivity [19,20].

In addition to modeling behavioral characteristics of AN, the ABA paradigm induces elevated activity of the hypothalamic pituitary adrenal (HPA) axis. Corticotropin-releasing hormone (CRH) is elevated in cerebrospinal fluid of adult AN patients before treatment and prior to normalization of body weight, as are plasma cortisol levels and rates of cortisol production [21,22]. With weight gain, CRH is restored to levels comparable to control subjects [22]. Activity of the HPA axis has been measured in adult female rats immediately after 3 days of ABA [23] and in male or female rats subjected to ABA after a 25% loss of body weight [24]. It was demonstrated that ABA resulted in elevated plasma corticosterone, but no differences in PVN CRH mRNA were determined [23,24]. How ABA affects adolescent females and the long-tem effects of ABA on the HPA axis are currently unknown.

In these studies, we exposed animals to the ABA paradigm to test the hypothesis that ABA during adolescence would have enduring effects on adult anxiety-like behavior. We chose to expose animals to two bouts of ABA for a number of reasons. First, given that there is currently only one report of ABA in adolescent female rats [25], whether it could be implemented and whether it was repeatable in rats of this age required verification. Second, in young women the onset of AN often occurs during mid- to late- adolescence [8,9]. This is modeled in our paradigm by exposing animals to ABA at P38 and again at P46. Finally, AN is frequently recurrent and has a high relapse rate [26], therefore exposing animals to two periods of ABA better models this occurrence than does a single exposure.

Anxiety-like behavior was tested in adulthood by activity on an elevated plus maze and in an open field apparatus. Plasma levels of corticosterone and expression levels of CRH mRNA were assessed in the hypothalamic paraventricular nucleus (PVN) and the central nucleus of the amygdala (CeA). Finally, a naïve group of adult female rats was exposed to the ABA paradigm and subsequently tested for anxiety-like behavior, in order to determine whether the long-term effects of ABA were dependent upon the developmental state of the animal.

2. Material and methods

2.1 Animals and housing

Female Long Evans rats (Harlan, Indianapolis, IN) served as subjects. Rats arrived at the laboratory on postnatal day 25 (P25) or 26 (P26), were individually housed in plexiglass cages (26.7 × 48.3 × 20.3 cm), and were maintained at a constant temperature (25°C) on a 12:12 h light/dark cycle, with the dark cycle beginning at 4:00 PM. Individual stainless steel feeders were attached to the inside of the cage, and food was weighed daily. Because the running wheels are attached to tub cages and tub cages are lined with Aspen bedding (Harlan, Indianapolis, IN), bedding was sifted each day to collect spillage.

For 15 of the cages, running wheels (Lafayette Instruments, Lafayette, IN) were attached to the side, and running wheel activity was computer monitored and recorded hourly for the duration of the experiment. From arrival until P31, access to the running wheel was blocked and all rats were allowed *ad libitum* access to rodent chow (Harlan Teklad 2018 18% protein diet, Harlan, Indianapolis, IN) and water in order to acclimate to the laboratory. Food intake and body weights for all rats were recorded daily. Running wheel access was blocked again at P60 and for the duration of the study. All procedures were approved by the Purdue Animal Care and Use Committee.

There were 5 groups in the first set of experiments. Group 1 was allowed access to the attached running wheel and *ad libitum* access to chow (Wheel/ad lib, n=7). Group 2 experienced two periods of ABA during adolescence (ABA, n=8). The third, fourth and fifth groups served as sedentary controls and did not have access to running wheels. Group 3 (Sed/time-restrict, n=8) was allowed one hour of access to food each day that the ABA rats were food restricted. Rats in this control group were not limited in the amount they could eat, only in the time during which they had access to food. This group of rats was only used to determine whether the ABA rats developed anorexia over the course of the restricted food access periods, and not in assessment of anxiety-like behavior or HPA activity. Group 4 was allowed access to the average amount of food consumed by the Wheel/ad lib group for that day (Sed/paired-ad lib, n=8) and the 5th received access to the average amount of food as was consumed by the ABA group on that day (Sed/paired-ABA, n=8). In order to match the food intake of the Sed/paired-ad lib and Sed/paired-ABA groups to that of the Wheel ad/lib and ABA, respectively, rats in these groups arrived at the laboratory on P25 and lagged behind the wheel groups by one day.

2.2 Activity-based anorexia during adolescence

The ABA group began the ABA paradigm on P38. For this group, food was removed from the cage 1 hour prior to the onset of the dark cycle on P38 and returned for 1 hour during the first hour of the dark cycle on P39, P40, and P41. We chose to allow food access during the first hour of the dark cycle as it has been demonstrated that this schedule results in a slower development of ABA [27]. This was important because adolescent rats have little body weight to lose and it was our aim to expose them to the paradigm for at least 4 days. The restriction phases were limited to 4 days each time to prevent weight loss beyond 20% of the body weight at the beginning of the restriction phase.

On P42, food was returned at the onset of the dark cycle and rats were allowed to feed *ad libitum* until the mean body weight for the group was no longer significantly lower than the Wheel/ad lib group (P46). At P46, the second period of ABA was implemented. Food was removed 1 hour prior to the onset of the dark cycle on P46, and access to food was allowed for one hour on each of P47, P48 and P49. Food was replaced at the onset of the dark cycle on P50 and ABA rats maintained *ad libitum* access to food for the duration of the

experiment. Access to the running wheels was blocked during the 1-hour feeding period, and water was available throughout the experiment.

The Sed/paired-ABA group received the same amount of food as was consumed by the ABA group each day beginning on P38 and continuing for the entirety of the experiment. On P38, food was removed from the cages of Sed/paired-ABA rats 1 hour prior to the onset of the dark cycle. Rats in this group were given the same amount of food as was consumed by the ABA rats daily, and on P39-P41 and P47-49, access to the food was limited to the first hour of the dark cycle. All Sed/paired-ABA rats consumed all of the allowed food each day. Sed/paired-ad lib rats were given food at the onset of the dark cycle each day and consumed all that was provided.

2.3 Elevated Plus Maze

After reaching adulthood, rats were tested for anxiety-like behavior on an elevated plus maze (EPM). The EPM consisted of 2 open arms (50 cm × 10 cm) and two enclosed arms (50 cm × 10 cm). On P90, each rat was removed from the home cage within the 2 hours prior to the onset of the dark cycle, placed on the EPM for 5 minutes, and behavior was recorded by video. An observer blinded to the group to which the rat belonged scored the amount of time spent in the closed or open arms of the EPM.

2.4 Open Field

Adult rats (P120) that had undergone the conditions described above (Wheel/ad lib, ABA during adolescence, Sed/paired-ad lib, and Sed/paired-ABA) were tested in an open field apparatus for anxiety-like behavior. The open field apparatus was 1 square meter, with walls that were 40 cm in height. All surfaces were a medium grey tone, and a grid with markings every 5 cm was painted in black on the base. The apparatus was placed inside a dark testing room, and illuminated with a single red light. Rats were individually placed inside the apparatus during the dark cycle, and were allowed to roam freely for 10 minutes. This activity was videotaped with a digital camera. The apparatus was cleaned completely with a mild soap and water, and then wiped dry.

Following testing, activity was scored using ImageJ (National Institutes of Health). Videos were rendered as image sequences, with images captured every second. These sequences were opened in ImageJ, and cropped to exactly 10 minutes (600 images) in length, beginning from the placement of the rat in the open field. To account for any changes in camera height or angle, the number of pixels per meter was calculated by measuring the length and width of the box in the frame, and averaging the two numbers. Activity was scored using the ImageJ plugins MTrackJ and ImageScience (Biomedical Imaging Group, Rotterdam). For each frame, a marker was placed on the nose of the rat; MTrackJ calculated the total number of pixels traveled by measuring the distance from one point to the next. This number was divided by the number of pixels in one meter to calculate the total distance in meters. Data are expressed as cumulative distance traveled.

Anxiety-like behavior in the open field apparatus was calculated by quantifying the time spent in the center, as opposed to the area near the walls of the arena. The same image sequences were used. A square exactly 15 cm from all sides of the arena was drawn on all images, and the number of seconds the rat’s nose was present in each region was calculated. Data are expressed as seconds spent in the center of the arena, with higher numbers indicating lower levels of anxiety-like behavior.

2.5 Blood and Tissue Collection

In order to determine baseline plasma corticosterone levels prior to open field testing, blood was collected from the rat via tail nick at 5 hours prior to the onset of the dark cycle. The tip of the tail was rapidly nicked with heat-sterilized stainless steel scissors while the rat was in its home cage. Approximately 100 μL of blood was collected into a chilled K+ EDTA vacutainer tube and immediately placed on ice. Blood samples were placed in a refrigerated centrifuged and spun at 3000 *g* for 15 minutes at 4° C. Plasma was removed and stored at −80° C until measurement of immunoreactive corticosterone by radioimmunoassay.

On the day of open field testing, two hours after open field exposure, rats were killed by decapitation under ether inhalation anesthesia. Brains were rapidly removed, submerged into iced isopentane for 25 seconds and immediately stored on dry ice. Trunk blood was collected into chilled K^+EDTA vacutainer tubes, briefly placed on ice, and then centrifuged at 4°C for 15 minutes at 3000 *g*. Plasma was aspirated into eppendorf tubes. Blood and brains were stored at −80°C until processing.

2.6 Radioimmunoassay

Radioimmunoassay (MP Biomedicals, Orangeburg, NY) was used to determine levels of plasma corticosterone. The upper and lower limits of the assay were 1000 and 25 ng/mL, and all samples fell within this range. The intra- and interassay coefficients of variation (CV) were below 6.5%, and the correlation coefficient for the standard curve was 0.93. Volumes of 10 μL of plasma were used in duplicate samples for each assay, as directed by the manufacturer.

2.7 In situ hybridization

Brains were coronally sectioned at 14 μm, mounted onto electrostatically charged Superfrost Plus slides (Fisher Scientific), and stored at −80°C. Brain slices were fixed with 4% paraformaldehyde and dehydrated with an ascending series of alcohols. Sections from each rat containing the PVN and the CeA were selected and stored at −80°C for future processing. A plasmid of CRH was linearized with the appropriate restriction enzyme. An antisense riboprobe was labeled with ^{35}S-labeled UTP (Perkin Elmer), using in vitro transcription systems with T3 polymerase, according to protocols provided by the manufacturer (Promega). Probes were then purified using Quick Spin RNA columns (Roche Diagnostics).

For processing, slides were warmed and rinsed in triethylamine (TEA) buffer (pH 8.0) and TEA with acetic anhydride. Sections were incubated in hybridization buffer comprised of 50% formamide, 0.3 M NaCl, 10 mM Tris HCl (pH 8.0), 1 nM EDTA (pH 8.0), 1× Denhardt's solution (Eppendorf), 10% dextran sulfate, 10 mM DTT, 500 μg/mL yeast tRNA, and 10^8 cpm/μL ^{35}S-UTP, and incubated overnight in a 56°C humid chamber. After hybridization, sections were washed three times in 2XSSC followed by one wash in 2XSSC + DTT at 56 C. Slides were then treated with 20 μg/mL RNase A (Sigma) in buffer containing 5 M NaCl, 0.5 M EDTA, 1 M Tris, pH 7.5 and dd$H_2$0. Sections were washed twice in 2XSSC + DTT, and then twice in 0.1XSSC + DTT, and dehydrated in an ascending series of alcohols. Slides were exposed to Kodak Biomax film for 2 days. Autoradiographic images were then scanned, and quantified with Scion Image software (National Institutes of Health), utilizing autoradiographic 14C-microscales (Amersham Pharmacia Biotech) as a standard. An experimentally blinded observer performed scanning and quantification. Data for each animal were means of the product of hybridization area × density, with the background density subtracted from the three sections, reflecting the region-specific levels of gene expression. Data for each animal were normalized to controls as 100%, and are expressed as mean ± standard error (SEM).

2.8 Activity-based anorexia during adulthood

In order to determine if the effects of experiencing ABA on anxiety-like behavior were specific to the age at which the experience occurred, a group of naïve, adult females that were 160 days old at the start of the experiment was exposed to the ABA paradigm for 2 bouts, following an identical schedule to that described for adolescent rats above. Three groups of rats served as subjects for this experiment. The first group had *ad libitum* access to food and a running wheel (Adult Wheel/ad lib, n=7), the second had access to a running wheel and was given two 4-day periods during which food was available for 1-hour only (Adult ABA, n=8), and the third served as a control to determine if the ABA rats consumed less food than sedentary rats with one hour of access to food but no wheel access (Adult Sed/time-restrict, n=7). This group was used only to verify that the Adult ABA rats developed anorexia in the ABA paradigm and was not used for behavioral testing. After the second bout of food restriction, Adult ABA and Adult Wheel/ad lib rats were given *ad libitum* access to food for 40 days and then tested, as described for the adolescent females, on the elevated plus maze. Thirty days later they were tested in the open field apparatus to determine anxiety-like behavior.

2.9 Statistical Analysis

For the effects of experiencing ABA in adolescence, repeated measures ANOVA followed by post hoc Bonferroni corrected t-tests were used to determine statistical differences between food intake during the two periods of restricted access to food, body weight changes, running wheel activity and amount of movement that occurred during open field testing. Total time spent in the open arm of the EPM, time spent in the center of the open field, and plasma corticosterone levels were analyzed by one-way ANOVA and post hoc Newman-Keul's Multiple Comparison Tests where appropriate. Expression of CRH mRNA was evaluated by independent *t* tests.

In the adult experiments, food intake during the two bouts of ABA, body weights, running wheel activity, and movement in the open field test were analyzed by repeated measures ANOVA and post hoc Bonferroni corrected t tests, when appropriate. Time spent in the open arm of the EPM and time spent in the center of the open field apparatus were analyzed by independent *t* tests. The level of significance was set at $p < 0.05$, and data are represented as mean standard error of the mean (SEM).

3. Results

3.1 Activity-based anorexia during adolescence

Adolescent rats exposed to the ABA paradigm consumed significantly less food than Sed/time-restrict that were allowed one hour of access to food for each of the days that the ABA rats were restricted. There were a main effects of group ($F_{(1,70)} = 20.95$, $p < 0.01$) and time ($F_{(7,70)} = 44.4$, $p < 0.001$), as well as a significant group × time interaction ($F_{(7,70)} = 7.99$, $p < 0.01$). As shown in Table 1, during the first bout of ABA, ABA rats consumed significantly less food on days 3 and 4 of both the first and second exposures to ABA than did the Sed/time-restrict rats ($p < 0.05$ for days 3 and 4, bouts 1 and 2).

As depicted in Figure 1, during the first period of activity-based anorexia, rats in the ABA group increased running wheel activity during the restriction period, as compared to running wheel activity by the unrestricted group with access to a running wheel ($F_{(1,168)} = 6.18$, $p < 0.05$). Post-hoc analysis revealed significant differences in distance traveled in the running wheels on each of days P39-P41 ($p < 0.05$ on each day). A similar pattern of increased running occurred during the second period of ABA, in which the ABA rats increased running wheel activity over the days of food restriction ($p < 0.05$ on each of days P47-P49).

On the unrestricted access to food days, there were no differences in running wheel activity between the ABA and Wheel/ad lib groups.

Body weight was measured daily and is depicted in Figure 2. Both bouts of ABA resulted in significantly lower body weights for the ABA and Sed/paired-ABA rats ($F_{(3,62)} = 13.75$, $p < 0.001$). Body weights for both food-restricted groups (ABA and Sed/paired-ABA) were restored to pre-restriction weights by the second day after food restriction had ended. For the second period of food restriction (P46-P50), the ABA and Sed/paired-ABA mean body weights were reduced from the pre-restriction body weight. Body weights for both groups (ABA and Sed/paired-ABA) were restored to pre-restriction level after two days of *ad libitum* access to food after restriction (P52). At P90, when behavior on the EPM was assessed, the Sed/paired-ad lib and Sed/paired-ABA groups weighed significantly more than the Wheel/ad lib and ABA groups ($p < 0.05$ for all comparisons). At P120, when open field behavior was evaluated, Wheel/ad lib and ABA body weights were significantly less than either of the groups without access to running wheels ($p < 0.05$ for all cases), but did not differ from one another.

3.2 Anxiety-like behavior in adulthood after adolescent activity-based anorexia

Anxiety-like behavior was assessed at P90. As shown in Figure 3A, rats that had experienced activity-based anorexia during adolescence spent significantly less time in the open arms of the EPM than any other group ($F_{(3,28)} = 6.24$, $p < 0.05$ for main effect of group). Analysis of open field activity also indicated increased anxiety-like behavior in the ABA rats. As shown in Figure 3B, total movement by the ABA group was significantly less than movement by the Wheel/ad lib, Sed/paired-ad lib, or Sed/paired-ABA groups (group ($F_{(3,23)} = 7.29$, $p < 0.01$, for main effect of group). Furthermore, the ABA rats spent significantly less time in the center of the open field ($F_{(3,27)} = 11.03$, $p < 0.05$). There were no differences in time spent in the center portion of the open field between Wheel/ad lib, Sed/paired-ad lib, and Sed/paired-ABA groups (Figure 3C).

3.3 Neuroendocrine effects of activity-based anorexia in adolescence

In order to evaluate corticosterone and levels of CRH mRNA in response to an anxiety-provoking environment, rats were sacrificed 2 hours after exposure to the open field test. The effects of open field exposure on plasma corticosterone differed among groups ($F_{(3,28)} = 9.18$, $p < 0.01$). As revealed by post hoc Newman-Keuls's test and depicted in Figure 4, the corticosterone response to the open field test was significantly affected by adolescent exposure to activity-based anorexia, ($p < 0.05$). Correspondingly, comparison of the percent expression level of CRH mRNA between Wheel/ad lib and ABA rats revealed that it was significantly elevated in both the PVN ($p < 0.01$, Figure 5A) and in the CeA ($p < 0.05$, Figure 5B). CRH mRNA was not assessed in Sed/paired-ad lib or Sed/paired-ABA groups because no differences were determined in behavior or corticosterone levels.

3.4 Activity-based anorexia in adulthood

Female rats that were exposed to the ABA paradigm during adulthood consumed significantly less food than female rats allowed access to food for one hour per day without access to a running wheel on the 3rd and 4th days of both bouts of restriction (Table 3). Repeated measures ANOVA revealed significant main effects of group ($F_{(1,70)} = 9.56$, $p < 0.05$) and time ($F_{(7,70)} = 84.80$, $p < 0.01$), as well as a significant interactive effect ($F_{(7,70)} = 12.06$, $p < 0.01$). Adult Wheel/restrict rats increased running wheel activity (total distance per day) during both periods of food restriction (Figure 6). Repeated measures ANOVA revealed a significant main effect of time ($F_{(13,156)} = 10.52$, $p < 0.001$) and a significant group × time interaction ($F_{(13, 156)} = 8.10$, $p < 0.001$), however there was no main effect of

group ($F_{(1,156)}$= 2.90, p = 0.11). Differences in body weight were abolished by the 6th day of gaining *ad libitum* access to food.

3.5 Anxiety-like behavior in adulthood after adult exposure to the ABA paradigm

Behavioral testing of rats that were exposed to the ABA paradigm in adulthood, rather than in adolescence, revealed no long-term effect on anxiety-like behavior. As depicted in Figure 8A, there were no differences between groups in the amount of time spent in the open arm of the elevated plus maze ($t_{(12)}$=0.37, p = 0.72). Further, as shown in Figures 8B and 8C, there were no differences in total distance traveled or time spent in the open portion of the open field (as opposed to the periphery). Because there were no differences in anxiety-like behavior, corticosterone and expression levels of CRH mRNA were not analyzed.

4. Discussion

Activity-based anorexia is an established animal model of AN that encompasses both decreased caloric intake and hyperactivity [28]. We first demonstrate that two brief bouts of ABA during adolescence did not result in long-term effects on body weight, as female rats that experienced activity-based anorexia during adolescence recover weight rapidly following cessation of the activity-based anorexia paradigm. However, this experience in adolescence resulted in profound differences in responsivity to novel, anxiety-provoking situations such as the elevated plus maze and open field test when the animals were adults.

When placed on an elevated plus maze as adults (P90), rats that had experienced ABA displayed more anxiety-like behavior than rats that had access to a running wheel during adolescence but no food restriction. The increased anxiety-like behavior following ABA in adolescence was also not present in sedentary rats or sedentary rats that were food-restricted to the same degree that the ABA rats were during adolescence. Furthermore, ABA during adolescence resulted in increased anxiety-like behavior at P120, when tested in an open field apparatus. Collectively, these data demonstrate persistent effects of activity-based anorexia during adolescence on anxiety-like behavior in the adult female rat. Finally, plasma corticosterone and levels of mRNA for CRH in the PVN and the CeA were significantly elevated after exposure to the open field in rats that had experienced ABA during adolescence, demonstrating that 70 days after ending the ABA, persistent effects on these parameters of stress responsivity remained. One caveat to these results is that the stage of estrous cycle was not determined at the time of testing or sacrifice. It has been demonstrated by others that the stage of estrous affects anxiety-like behavior and HPA axis activity [29–31]. Future studies will include evaluation of estrous cycle throughout testing.

Adolescence is a vulnerable period for the onset of an anxiety disorder, and stressful life experiences are established risk factors during this time [32]. The adolescent period in rats is ill-defined, but is generally accepted to occur between days 29 and 60 days of age [33]. In all species, including rats and humans, there is considerable brain development during adolescence, including remodeling and synaptic pruning in the prefrontal cortex, amygdala, and hippocampus [34–37]. The roles of these still developing brain areas in stress responsivity and emotionality suggest that exposure to stressors during adolescence may have lasting effects on stress responsivity and emotionality in adulthood. Here we demonstrate for the first time that the experience of ABA during adolescence produces profound effects on how the animal responds to a novel, anxiety-provoking stressor. Exposure to the same schedule of food restriction and wheel access in adult female rats did not result in increased anxiety-like behavior. It is likely that the age-related differences are due to differences in the developmental stage during which the food restriction was implemented. However, it is important to highlight two differences in behavior between the adolescent and adult females. First, the adolescent ABA females ran more than the adult

females, thereby raising the possibility that the long-term effects on behavior were induced by greater activity during the food restriction phases. Second, adolescent rats exposed to the ABA paradigm consumed significantly less food on the third and fourth days of timed feeding during both periods of ABA. Adults consumed less food than sedentary controls during the third and fourth days of the first ABA paradigm exposure; food intake, however, food intake was significantly lower than controls only on the last day of the second restriction phase. How these differences may have impacted long-term effects on behavior is not known.

Indeed, stress exposure during adolescence has been demonstrated to have enduring effects in adulthood. For example, male rats exposed to repeated stressors during adolescence displayed blunted hormonal responses to novel stressors in adulthood [38]. While most studies of adolescent stress exposure have been conducted in male rats, there is increasing evidence of gender-specific effects. For example, only female rats that experienced chronic social stress in adolescence displayed increased anxiety-like behavior when tested on an elevated plus maze as adults [39]. Here, we provide evidence of persistent effects of ABA, rather than simply food restriction or increased activity independently, during adolescence on anxiety-like behavior in adults. In these studies, we evaluated whether rats that experienced food restriction to the same degree as ABA rats (Sed/paired-ABA) would result in similar behavioral and neuroendocrinological effects. Whereas food restriction in adolescence did not affect anxiety-like behavior in adulthood, it is possible that the increased anxiety-like behavior of the ABA rats was due to the greater degree of weight loss. Future studies are required to determine the effects of weight loss to the degree lost by the ABA rats on adult behavior, as it is feasible that severity of weight loss affects HPA axis activity.

Anxiety disorders and AN are highly comorbid [40–43], but the onset of the anxiety disorder frequently precedes the onset of the eating disorder [42–45]. This may be due to the earlier average age of onset of anxiety disorders [46] or presence of an anxiety disorder in adolescence may increase the risk for developing an eating disorder. Regardless, a number of studies demonstrate that the majority of individuals with AN experience one or more comorbid anxiety disorders that persist beyond recovery from this eating disorder [45,47–51], and individuals who have recovered from AN continue to show higher levels of anxiety [43]. Importantly, anxiety disorders persist and have a propensity to worsen in severity upon recovery from AN [52–55]. Our current data demonstrate that regardless of the pre-anorexia level of anxiety, severe food restriction coupled with hyperactivity induces long-term effects on brain and behavior.

Collectively, we demonstrate that brief exposure to ABA during adolescence produces lasting and profound effects on responsivity to anxiety-provoking external stimuli in adulthood. These effects are specific to rats that experience ABA during adolescence, as demonstrated by a lack of long-term effects in naïve adult females exposed to the same paradigm. Whether these effects are due to increased HPA axis activity in adolescence or are specific to the ABA paradigm is currently unknown, however other stressors, such as social isolation, unpredictable variable stress exposure in adolescence have been demonstrated to have persistent effects on adult behavior [39,56]. Given that adolescence is a period of developmental vulnerability and experience during this time has been demonstrated to have lasting effects of brain and behavior, the ABA model provides a tool to elucidate the neurobiological substrates mediating the relationship between adverse experience in adolescence and its role in persistent anxiety in the adult.

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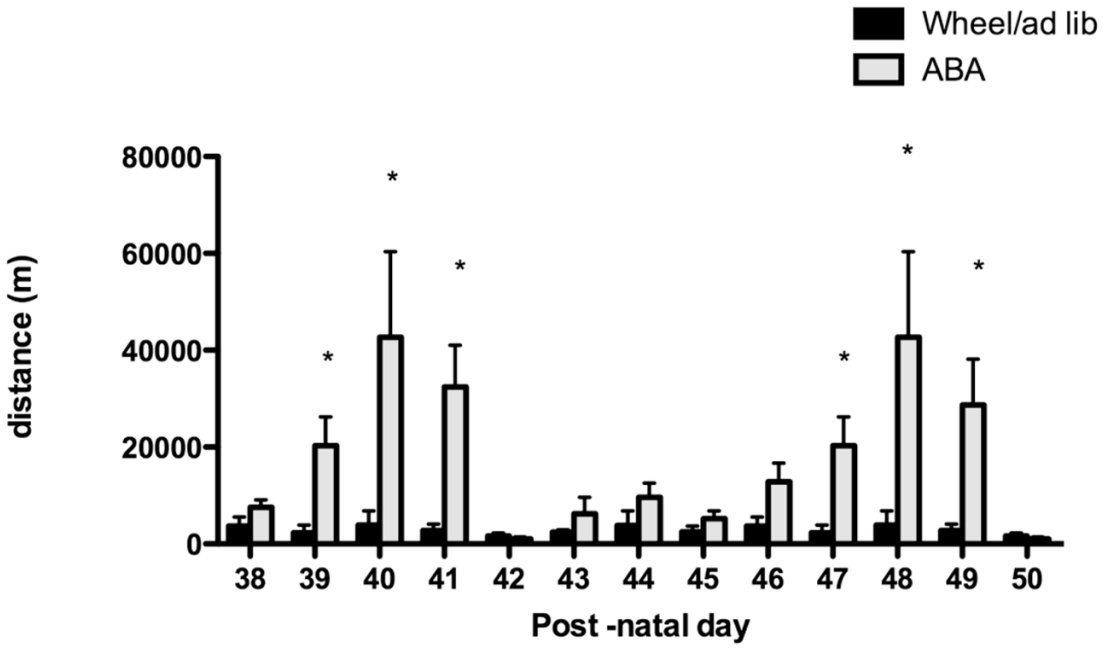

Figure 1.
Mean daily distance traveled in running wheels. Female adolescent rats increased running wheel activity over the course of food restriction in the ABA paradigm. Total distance (m) was significantly greater in the rats that experienced one hour of food exposure than it was in the unrestricted group (Wheel/ad lib: closed squares) on P39-41 and P47-49 ($p < 0.05$ on each day). Data are mean ± SEM and * denotes $p < 0.05$.

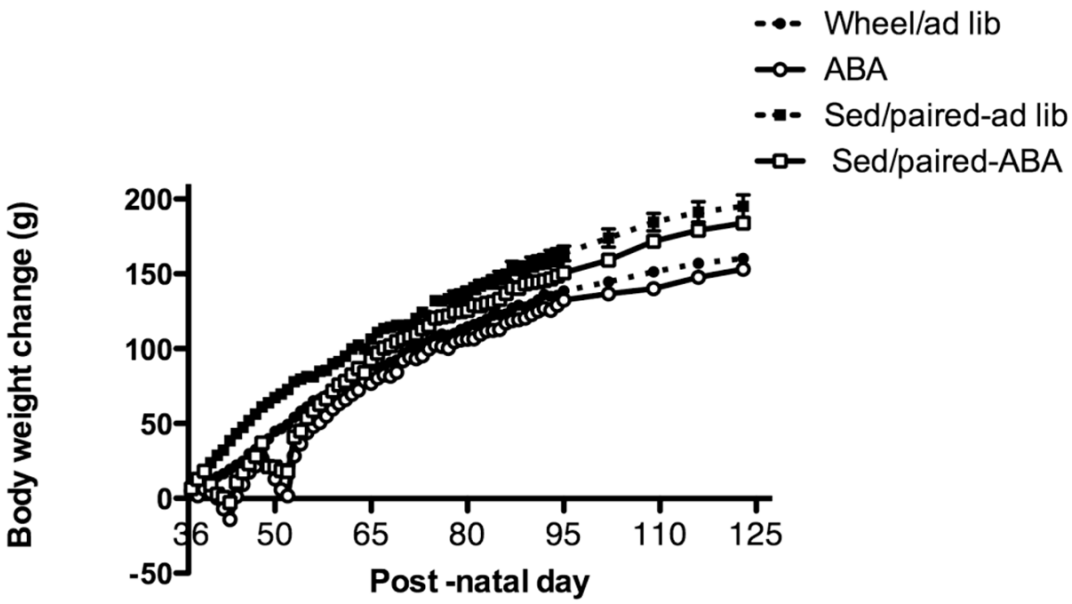

Figure 2.

Body weight during and after activity-based anorexia. In adolescence, body weight was significantly reduced in rats experiencing activity-based anorexia during adolescence and in sedentary rats that were pair-fed the amount of food consumed by the ABA group during activity-based anorexia, as compared to controls.

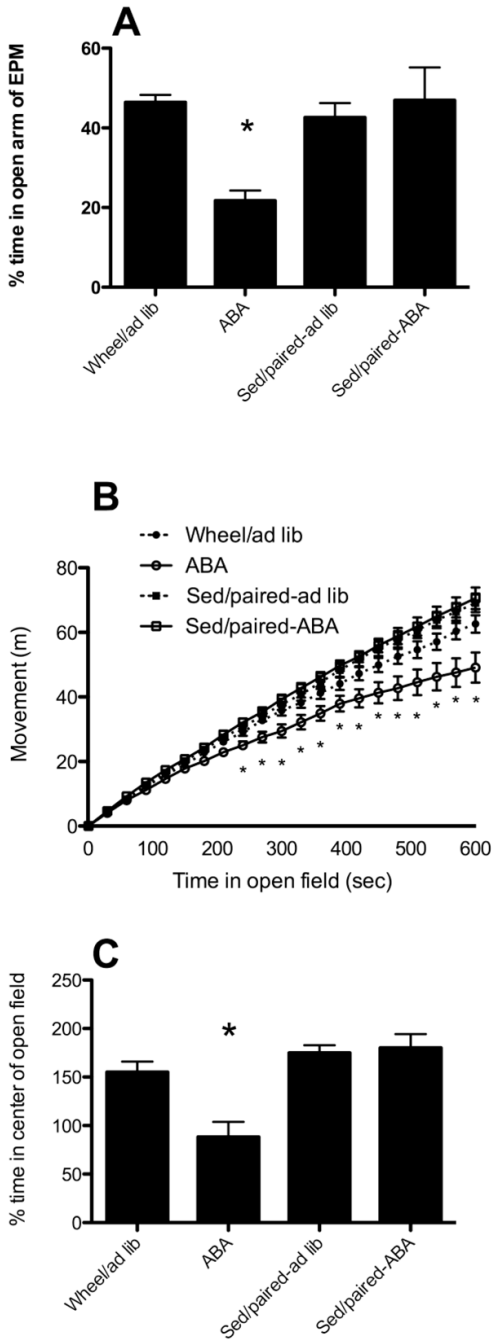

Figure 3.

Anxiety-like behavior. (A) At P90, ABA rats spent less time in the open arm of the elevated plus maze than did any other group ($p < 0.05$). (B) At P120, total movement in the open field demonstrated that ABA rats engaged in significantly less movement than any other group ($p < 0.05$). (C) ABA rats spent less time in the center portion of the open field than did controls ($p < 0.05$). * denotes $p < 0.05$, and bars represent mean ± SEM.

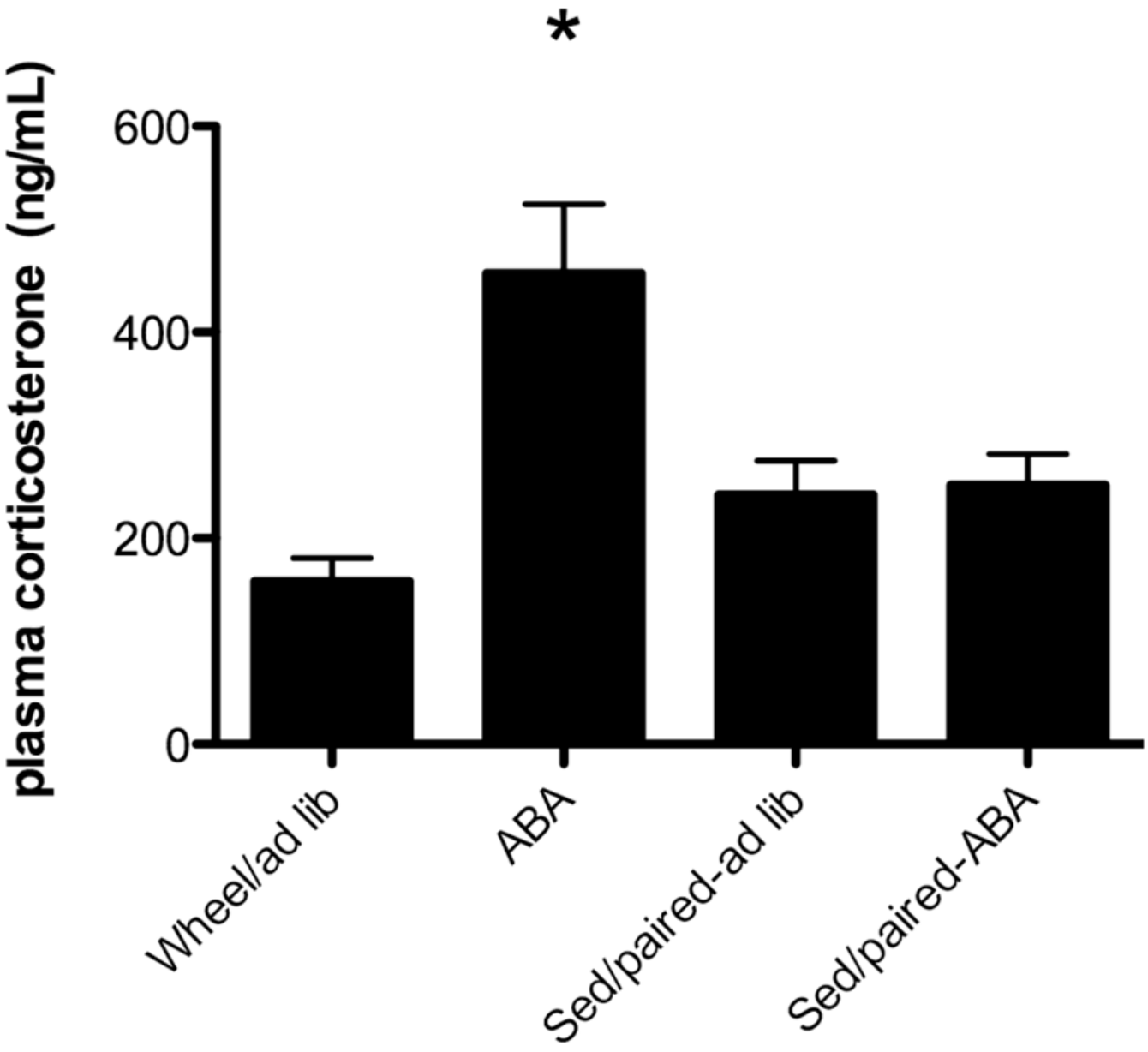

Figure 4.
Plasma corticosterone after open field exposure. Two hours after exposure to the open field, plasma corticosterone was significantly elevated in ABA rats (exposed to ABA in adolescence), as compared to controls. Bars represent mean ± SEM, * denotes $p < 0.05$.

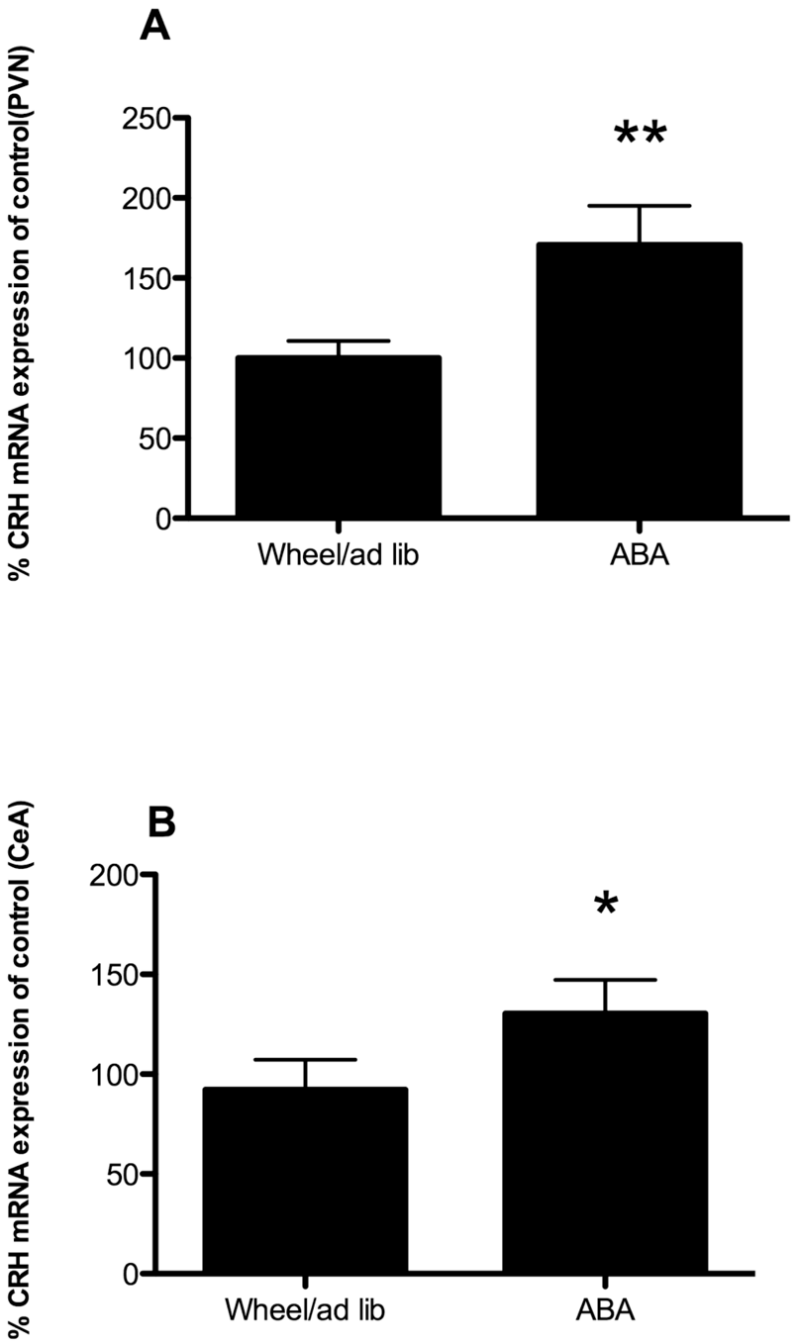

Figure 5.
Expression levels of CRH mRNA in PVN and amygdala after open field exposure. In response to open field exposure, expression levels of CRH mRNA were significantly greater in the PVN (A) and CeA (B) of adult rats that had experienced activity-based anorexia in adolescence. Bars represent mean ± SEM, * denotes $p < 0.05$, ** denotes $p < 0.01$).

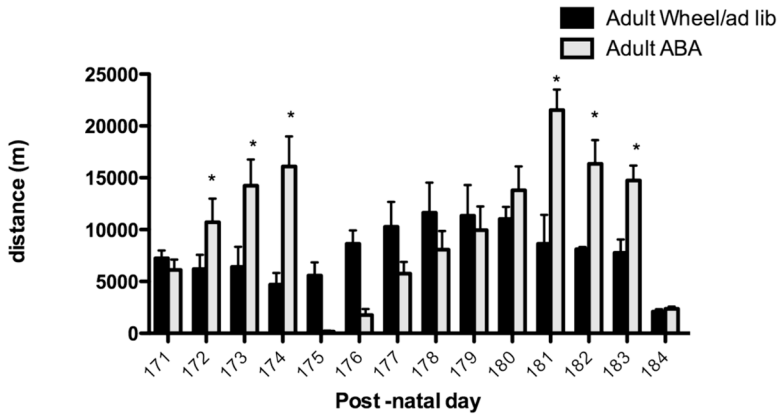

Figure 6.

Adult running wheel activity during exposure to the ABA paradigm. Adult female rats increased running wheel activity during restricted access to food, as compared to unrestricted control rats. Data represented are mean distance per 24-hour period, ± SEM. * denotes $p < 0.05$.

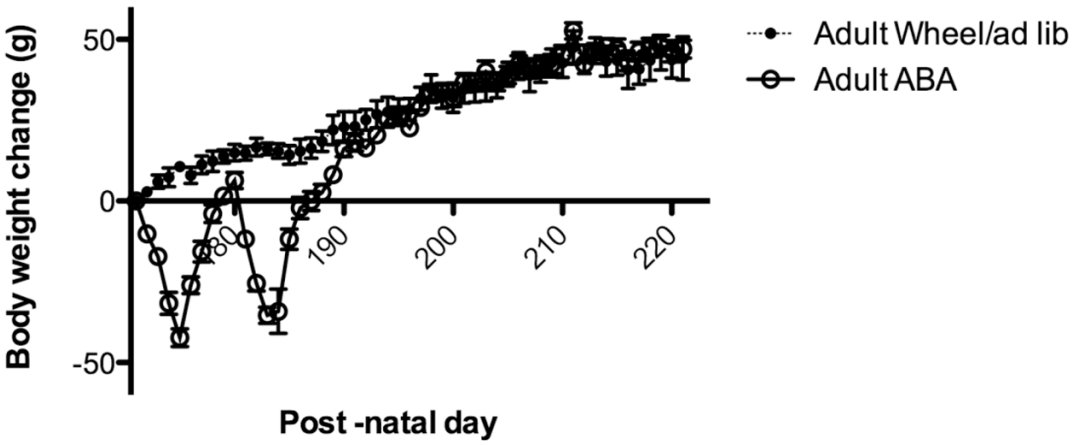

Figure 7.

Adult body weight during and after exposure to the ABA paradigm. In adult female rats, body weight was significantly reduced in response to activity-based anorexia, as compared to controls. After the second exposure to the ABA paradigm, body weights were no longer different from one another by the 6th day of *ad libitum* access to food.

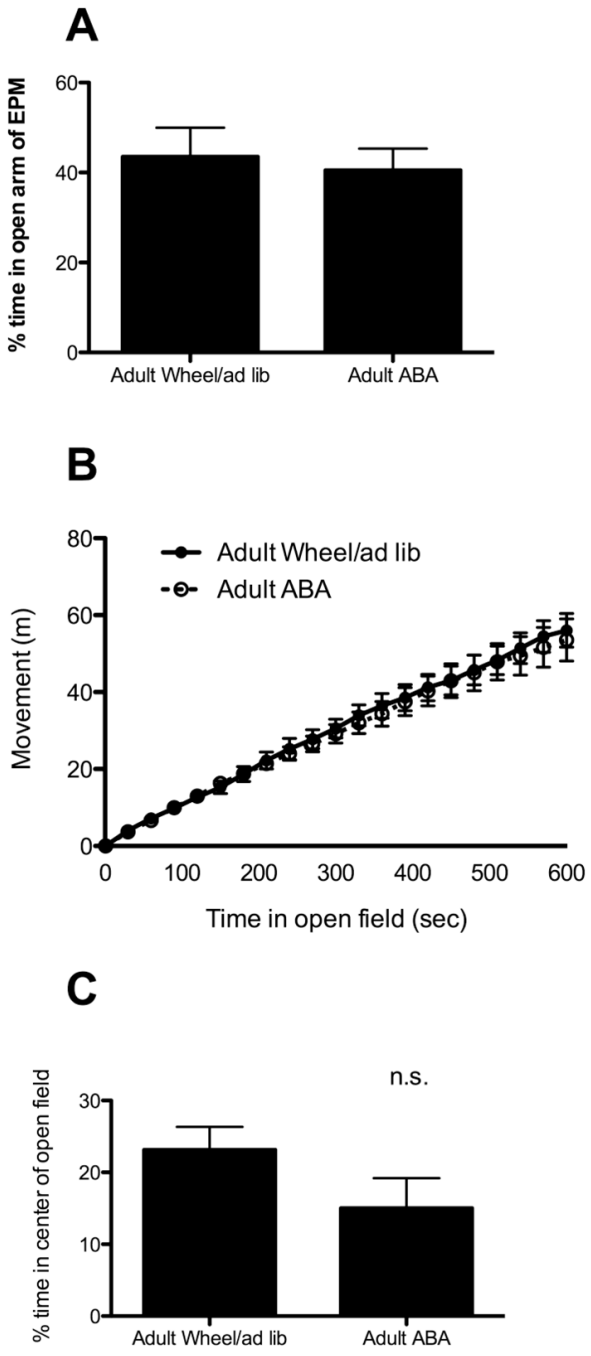

Figure 8.

EPM and open field behavior in adult females exposed to the ABA paradigm in adulthood. (A) There were no differences in the % time spent in the open arms of the EPM, as compared to control rats. Similarly, no differences were measured in movement in the open field 70 days after exposure to the ABA paradigm (B), or in the time (seconds) spent in the center portion of the open field (C). Data are mean ± SEM.

Table 1

Food intake during 2 bouts of ABA by adolescent female rats.

	Sed/time-restrict	ABA
P38	3.3 ± 0.2	3.6 ± 0.6
P39	4.7 ± 0.2	5.4 ± 0.8
P40	6.4 ± 0.2	4.0 ± 0.1*
P41	7.7 ± 0.3	4.7 ± 0.2*
P46	4.5 ± 0.5	5.0 ± 0.5
P47	6.5 ± 0.5	5.6 ± 0.6
P48	8.2 ± 0.4	6.5 ± 0.5*
P49	8.3 ± 0.5	7.0 ± 0.5*

Data are mean intake (g) ± SEM.

* denotes $p < 0.05$.

Table 2

Food intake during exposure to the ABA paradigm by adult female rats.

	Sed/time-restrict	Adult ABA
P171	3.3 ± 0.3	4.1 ± 0.4
P172	4.5 ± 0.4	4.1 ± 0.4
P173	7.9 ± 0.8	5.1 ± 0.3*
P174	7.9 ± 0.8	6.0 ± 0.4*
P180	6.1 ± 0.7	4.6 ± 0.3
P181	7.1 ± 0.8	7.2 ± 0.4
P182	10.5 ± 0.9	8.1 ± 0.6*
P183	11.4 ± 0.7	9.8 ± 0.6*

Data are mean intake (g) SEM.

* denotes $p < 0.05$.