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RESEARCH ARTICLE

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Effects of adolescent experience of food restriction and exercise on spatial learning and open field exploration of female rats

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Abstract

The hippocampus carries out multiple functions: spatial cognition dorsally (DH) and regulation of emotionality-driven behavior ventrally (VH). Previously, we showed that dendrites of DH and VH pyramidal neurons of female rats are still developing robustly during adolescence and are altered by the experience of food restriction and voluntary exercise on a wheel. We tested whether such anatomical changes during adolescence impact anxiety-like behavior and spatial cognition. Four groups of female rats were evaluated for these behaviors: those with wheel access in its cage from postnatal day (P) 36–44 (EX); those with food access restricted to 1 hr per day, from P40 to 44 (FR); those with EX from P36 to 44, combined with FR from P40 to 44, which we will refer to as EX + FR; and controls, CON (no EX, no FR). Open field test for anxiety-like behavior and active place avoidance test for spatial cognition were conducted at P47–49, the age when food restricted animals have restored body weight, or at P54–56, to identify more enduring effects. Anxiety-like behavior was elevated for the EX and FR groups at P47–49 but not for the EX + FR group. By P54–56, the EX + FR and EX groups exhibited less anxiety-like behavior, indicating a beneficial delayed main effect of exercise. There was a beneficial main effect of food restriction upon cognition, as the FR group showed cognition superior to CONs' at P44–46 and P54–56, while the EX + FR animals also showed enhanced spatial learning at P54–56. EX + FR animals with best adaptation to the feeding schedule showed the best spatial learning performance but with a delay. The EX group exhibited only a transient improvement. These findings indicate that FR, EX, and EX + FR in mid-adolescence are all beneficial in reducing anxiety-like behavior and improving spatial cognition but with subtle differences in the timing of their manifestation, possibly reflecting the protracted maturation of the hippocampus.

KEYWORDS

active place avoidance, activity-based anorexia, anxiety, cognition, exploratory behavior, female, hippocampus, hyperactivity, open field test, rats

1 | INTRODUCTION

There is abundance of evidence indicating that regular, structured, voluntary physical exercise is beneficial to the cardiovascular system as well as to mental health: it improves resilience to stress, can be anxiolytic and antidepressant (Chekroud et al., 2018; Mikkelsen, Stojanovska, Polenakovic, Bosevski, & Apostolopoulos, 2017; Salmon, 2001) and can also ameliorate drug addiction (Lynch, Peterson, Sanchez, Abel, & Smith, 2013). Improvement of mental health has also been measured based on self-esteem and mood (Barton & Pretty, 2010). While most human studies have not included adolescents or juveniles in their studies, those that have indicate that the associations between exercise and reduction of anxiety, depression and neuroticism hold across all age groups, ranging from 10 to >60 years of age (de Moore, Beem, Stubbe, Boomsma, & De Geus, 2006). Research using rodents has linked beneficial cognitive effects of exercise to synaptic plasticity in the hippocampus via elevation of neurotrophins there (Gomez-Pinilla, Vaynman, & Ying, 2008; Gomez-Pinilla, Ying, Roy, Molteni, & Edgerton, 2002; Kang & Schuman, 1995; Neeper, Gomez-Pinilla, Choi, & Cotman, 1996; Vaynman, Ying, & Gomez-Pinilla, 2003). A mitochondrial protein UCP2 is also up-regulated in the hippocampus by exercise and this is accompanied by dendritic spine synaptogenesis in hippocampus and dentate gyrus (Dietrich, Andrews, & Horvath, 2008). However, there are also reports that excessive exercise, such as the regimen followed by competitive athletes, can increase the chance of developing an infection, due to immune function depression and oxidative stress (Mikkelsen et al., 2017). Also, almost all patients diagnosed with anorexia nervosa exercise compulsively (Beumont, Arthur, Russell, & Touyz, 1994; Casper, Sullivan, & Tecott, 2008; Davis et al., 1997; Hebebrand et al., 2003; Kron, Katz, Gorzynski, & Weiner, 1978), greatly exacerbating their weight loss caused by severe dieting, which in turn increases their risk of cardiovascular diseases due to electrolyte imbalance and cardiac muscle loss (Oflaz et al., 2013). These reports indicate that that the benefit of physical exercise is dose-dependent.

Mental health can also be improved by dieting and this has been substantiated well in animal models. (Gomez-Pinilla, 2008; Gomez-Pinilla, 2011; Mattson, Duan, & Guo, 2003). For example, alternating feeding with food restriction reduces motor and cognitive decline associated with aging (Ingram, Young, & Mattison, 2007; Means, Higgins, & Fernandez, 1993). Like exercise, the effect of dieting is linked to elevation of BDNF (Lee, Duan, & Mattson, 2002) and reduction of oxidative stress within the brain (Bruce-Keller, Umberger, McFall, & Mattson, 1999). Ghrelin, which is secreted from the stomach during hunger, is produced centrally as well. Ghrelin enhances synaptic plasticity in the hippocampus, increases spine density of neurons in the hippocampus and enhances memory performance (Diano et al., 2006).

One aspect of research that is relatively unexplored is the impact of voluntary physical exercise and food restriction specifically during adolescence. Previous work showed that pyramidal neurons in this brain region exhibit robust extensions and retraction of dendritic arbors during adolescence (Chen, Akad, Aderogba, Chowdhury, & Aoki, 2018; Chowdhury et al., 2014). These changes are accompanied by spinogenesis, spine pruning (Chen et al., 2018; Chowdhury et al., 2014), and restructuring of GABAergic synapses on pyramidal neurons (Aoki et al., 2012). Moreover, these changes of dendritic morphology and GABAergic synapses are influenced significantly by voluntary exercise, food restriction, and the combination of the two during adolescence (Aoki et al., 2012; Chowdhury et al., 2019; Chowdhury, Wable, Sabaliauskas, & Aoki, 2013). Thus, the goals of this study were (1) to assess maturational changes to two hippocampus-dependent behaviors—anxiety-like behavior and spatial cognition—during adolescence and (2) to assess the influence of food restriction and/or exercise during mid-adolescence upon these behaviors.

To test how the experience of voluntary wheel running and food restriction during adolescence in female rats affects hippocampal-dependent behaviors, we measured spatial learning, memory retention, cognitive flexibility, and exploratory behavior in an open field using a protocol with an active place avoidance task. The active place avoidance task was previously demonstrated to require intact dorsal hippocampal function (Cimadevilla, Fenton, & Bures, 2000; Cimadevilla, Wesierska, Fenton, & Bures, 2001) and persistent protein-synthesis dependent (Tsokas et al., 2016) hippocampal synaptic plasticity (Pastalkova et al., 2006). We compared behavior of animals that underwent four contrasting environmental conditions: voluntary exercise as a result of wheel access within the cage (EX), food restriction (FR), whereby an unlimited amount of food was available for 1 hr per day, neither wheel access nor food restriction (CON) or both wheel access and food restriction (EX + FR). The environmental condition of EX + FR matched those of prior studies, which had shown that hunger evokes voluntary running so extreme as to cause many animals to continue running even during the 1 hr of food availability—in essence, voluntarily food-restricting. This condition is referred to in the literature as activity-based anorexia (Aoki, Chowdhury, Wable, & Chen, 2017; Gutierrez, 2013). These four groups were assessed for their hippocampus-dependent behaviors after short (3–5 days) or longer (−7 days) periods of recovery.

2 | EXPERIMENTAL PROCEDURES

2.1 | Experimental groups—adolescent experience

All procedures involving live rats were in accordance with the Institutional Animal Care and Use Committee of New York University (Animal Welfare Assurance No. A3317-01) and AAALAC (00–1,350).

Sixty-four Sprague–Dawley female rats were used for the active place avoidance and open field testing. All animals were delivered from Hilltop Lab Animals, Inc. to the NYU animal facility at age P28, specified to have average body weight of 100 g. We ensured that animals were from diverse litters by having them delivered to NYU on four different dates, 1 week to 4 weeks apart. In accordance with recommendations by the veterinary staff, animals were allowed to acclimate to the new facility for 3 days, while pair-housed (Obernier &

Baldwin, 2006), after which time they entered by experimental paradigm of single housing (P30). Single housing was required for measuring daily food consumption and for monitoring wheel activity of those with wheel access.. Initially, all animals were housed with ad libitum access to food (Purina Rodent Laboratory Chow 5,001, Neena, WI) 336 kcal/100 g) and water. Weight of food consumed was measured daily and used to calculate calories consumed, based on the 3.36 kcal/g caloric content as specified by the manufacturer. The room lights turned on every day at 7 a.m. and turned off at 7 p.m. Sixteen animals each were assigned to CON, FR, EX and EX + FR groups. The rearing conditions of these rats were identical to those described previously (Chowdhury, Wable, et al., 2019). These rats were delivered as eight cohorts of 8 animals each. Each cohort was divided into two groups, either CON and EX + FR or FR and EX. Thus, for each group, there were four cohorts of four animals, delivered on four different dates.

The schedule for the four groups of animals is as depicted in Figure 1a. On P36, EX and EX + FR animals were housed in a new cage (17″ *L* x 8.5″ *W* x 7.5″ *H*) with an attached running wheel of diameter 20.32 cm and grid rods spaced 1.6 cm (6°) apart (Model ENV-046, Med Associates, Inc., St. Albans, VT). CON and FR animals were given a fresh cage on P36 (11″ *L* x 11″ *W* x 7″ *H*), but without access to a running wheel. All animals were handled daily just before 7 p.m. (the onset of the dark period), and body weight and food weight were measured. Wheel activity was monitored over the entire period of wheel access, using the RotoRat software provided by Med Associates. Additionally, wheel counts were logged manually at the time of weighing, once per day at 7 p.m.

After 4 days of acclimation to the fresh cage with or without a running wheel, food was removed from the cages of EX + FR and FR animals at 8 p.m. on P40, beginning the 4-day period of food restriction. On days P41, P42, and P43, food was placed in the cage at 7 p.m. and removed at 8 p.m., allowing the animals unlimited access to food for 1 hr per day. At 7 p.m. on day P44, food access became ad libitum again to the cages of food-restricted animals, and all animals were housed in a fresh cage without access to a running wheel. All animals were kept in identical conditions of ad libitum access to food and without a running wheel from P44 onward, and for at least 2 days in order to allow food-restricted animals to recover from a state of hunger before beginning behavioral tests.

Each animal was tested over 2 days (details described below, under "Active Place Avoidance Testing", APA in Figure 1). On the first day, animals were tested for open field exploration, to test innate anxiety-like behavior, and acquisition of a spatial memory, using an active place avoidance task to avoid a shock zone (Cimadevilla, Fenton, & Bures, 2000; Cimadevilla, Kaminsky, Fenton, & Bures, 2000). On the second day, 24-hr retention of the place avoidance memory was tested, followed by a test of cognitive flexibility by relocating the shock zone (Cimadevilla, Fenton, & Bures, 2000; Cimadevilla, Kaminsky, et al., 2000). Each cohort of eight animals underwent training together over a period of 3 days. Of the 16 animals assigned to each group of CON, FR, EX, and EX + FR, eight were trained soon after the end of the adolescent experience period and when body weight became restored (between P47 and 49) and eight were trained at the transition between late adolescence and early adulthood (P54–56), for identifying more enduring effects of the environment.

Each cohort that was trained between P47–49 was divided into two groups of four animals – trained on days P47–48 or from days P48–P49. These groups were combined for the purpose of analysis, and will be referred to as "P47–P49 group" (Figures 1a, 2, 3, and 4). Similarly, each cohort tested with longer recovery periods was divided into two groups of four animals and trained on days P54–55 or on days P55–56. These groups were also combined and will be referred to as the "P54–56 group" (Figures 1a, 2, 3, and 4).

2.2 | Testing active place avoidance and open field exploration

2.2.1 | The apparatus

The active place avoidance apparatus was set up in the NYU animal facility in a different room than the one in which the animals were housed. The apparatus consisted of an 82-cm-diameter circular arena that rotated counter-clockwise at a rate of 1 rpm. Visual cues were placed on the walls of the room surrounding the apparatus to help the animals orient within the room environment. A video camera above the arena monitored movement of the animal as well as the rotation of the arena by interfacing with a commercial computer-controlled interactive tracking system (Bio-Signal Group, Acton, MA). Tracker software (Bio-Signal Group) was used to monitor the movement of the animal as well as the rotation of the arena. The software also controlled a current generator to administer 0.3 mA 500 ms foot-shocks to the animal. The time series of tracked positions was analyzed offline using the TrackAnalysis software (Bio-Signal Group).

For delivery of the mild shocks, a low impedance, sterile conductive loop was pierced through the skin at the nape of the animal's neck at least 30 min before the first session on the arena. When the animal was placed on the arena, a cable was clipped to the loop to administer a current that passed through the animal and across the high impedance between the paws and the conductive arena surface, experienced by the animal as a shock to the paws. The location of the animal across the arena was tracked by monitoring the location of an infrared light-emitting diode on the clip at the nape of the neck. Clear plastic walls surrounded the floor of the arena to prevent the animal from falling from the platform. All other details of the active place avoidance paradigm were as described previously (Cimadevilla, Fenton, & Bures, 2000; Cimadevilla, Kaminsky, et al., 2000).

2.2.2 | Training and testing schedules

Each animal received four 10-min sessions on the arena per day, over 2 days. Each arena session was interleaved with a 10-min inter-trial interval, during which time the rat was returned to its home cage. Each cohort of 8 was divided into four pairs of animals from two experimental groups, to be tested on any 1 day (i.e., 1 CON paired

with 1 EX + FR; or 1 EX paired with 1 FR). Each pair was tested at the same time of day on each of the 2 days of testing. All testing occurred between 7 a.m. and 7 p.m., while the lights were on in the animal facility. The pairs were equalized for the experimental group that was tested first. For consistency in animal handling and to minimize the stress of handling on the animals, the same investigator handled the animals for daily weighing and during the testing sessions for all cohorts in this study.

2.2.3 | Pretraining and open-field test for quantifying exploratory behavior and anxiety-like behavior

On the first day of the behavioral testing, animals were allowed to rest in the home cage transported to the testing room for 30 min before testing sessions began. During the first 10-min "pretraining" session on the rotating arena, the animal was allowed to freely explore the arena and surroundings. No shocks were administered during the pretraining session.

Rodents in a novel open brightly lit space tend to express reduced locomotion and thigmotaxis to avoid the central space when they are unfamiliar with a space (Hall, 1934, 1936) and especially when treated with anxiogenic compounds (Felix-Ortiz, Burgos-Robles, Bhagat, Leppla, & Tye, 2016; Lund, Rovis, Chung, & Handa, 2005), while anxiolytic compounds such as diazepam increase locomotion in the center of unfamiliar space (Bahi, Schwed, Walter, Stark, & Sadek, 2014). Thus, exploration of the arena during the first 3 min in the area was analyzed as an open-field test for exploration and anxiety-like behavior.

For this analysis, the circular arena was divided into two regions of equal area: the circular 'center' region that contained the center of the platform and the ring-shaped 'surround' region that contained the remaining annulus. Exploratory behavior was determined by calculating the proportion of the total time that animals spent in the center region. Locomotor activity was also measured, as the total distance traveled on the arena.

2.2.4 | Training

Immediately following the pretraining trial, each animal was trained over three 10-min sessions, T1, T2, and T3, to avoid a 60° sector on the rotating arena. The sector was stationary with respect to the room. If the rat entered and remained in the shock zone for 500 ms, it received a 0.3 mA shock that repeated every 1,500 ms until the rat exited the shock zone. Each training session was interleaved by a 10-min rest period in the rat's home cage. After the third training session, the animal was allowed to rest in the home cage in the testing room for 30 min before being returned to its housing room for the night. For each animal, the number of entries into the shock zone during T1 and the total number of entries during T1, T2, and T3 were computed. The total distance travelled on the arena and the average number of shocks per entry into the shock zone were also computed.

2.2.5 | Retention

To measure 24-hr retention of the active place avoidance memory, the rat was returned to the training condition 24-hr after the pretraining trial, for a single 10-min session. For each animal, the number of entries into the shock zone, the total distance travelled on the arena and the average number of shocks per entry into the shock zone were measured.

2.2.6 | Conflict

After the retention test, three 10-min "conflict" sessions were given to evaluate cognitive flexibility. In the conflict sessions, the shock zone was relocated to 180° relative to where it had been for the training sessions. The same behavioral measurements were computed during each of the three conflict sessions.

2.3 | Euthanasia

Animals were deeply anesthetized by an intraperitoneal injection of urethane (34%; 0.85 ml/100 g body weight) prior to transcardial perfusion with phosphate-buffered saline and 4% paraformaldehyde in phosphate-buffer.

2.4 | Estrous cycle assessment

Manipulations required to assess the estrous cycle is a known stressor (Riddle et al., 2013). Pubescent female Sprague–Dawley rats have been shown to exhibit only partial cycling (Hodes & Shors, 2005). Moreover, calorically restricted rodents lose the cycling of ovarian hormones, thereby spending increased time in diestrus (Nelson, Gosden, & Felicio, 1985; Riddle et al., 2013). Based on these previous findings, we reasoned that knowledge gained from vaginal swabs would be limited, while adding an undesirable confound of increased stress. For these reasons, estrous cycle was not assessed for these animals, except after administration of anesthesia on the day of euthanasia. For data analysis, experimental groups were not subdivided according their phase of the estrous cycle on the day of euthanasia.

2.5 | Statistical analyses

The extent to which animals consumed food during the 1 hr of food availability on food-restricted days and during ad libitum food availability varied across individuals. The group mean average values of the FR, EX, and EX + FR groups were compared to the CON group's for each experimental day, using Student's *t*-test. Within each group, the mean average value of food consumption was compared across experimental days as well by repeated measures two-way ANOVA, with environmental treatment as one factor and time as another factor.

Wheel running activity of EX + FR and EX groups were compared by unpaired t-test for each of the eight experimental days that the two groups of animals were on a wheel. Repeated measures two-way ANOVA was also performed, with environmental treatment as one factor and experimental day as the other.

Anxiety/exploratory activity in the open field, performances in active place avoidance and daily body weights were compared across the four groups by one way ANOVA and by two-way ANOVA, the latter of which was to analyze the interaction of wheel access and food restriction. For outcomes that yielded no significant group difference by Tukey's multiple comparison's test, the *p*-value from the less stringent Uncorrected Fisher's LSD test is reported.

Pearson's correlational analysis was run to assess the relationship between anxiety-like/exploratory behavior or spatial cognition and wheel activity or food consumption.

3 | RESULTS

3.1 | Animal weight: 4 days of food restriction during mid-adolescence results in body weight reduction that persists for 10 days

Rats continue to grow during adolescence, and both food restriction and wheel running exercise are expected to alter body weight increases during this period (Aoki et al., 2012; Aoki et al., 2017; Gutierrez, 2013). In order to monitor the impact of food restriction and wheel running on growth, body weight was measured daily for all four groups (CON, EX, FR, and EX + FR) from P36 until the beginning of active place avoidance training, on P47 or P54.

Body weight of CON animals continued to increase throughout the experimental days (Figure 1b). The EX + FR and EX groups had slightly lower body weights than CON and FR groups after introduction of the running wheel. A main effect of reduced body weight by running wheel access was found starting on P38 and through P48. Starting on P40 (E5), when food restriction began, both EX + FR and FR groups began to lose weight relative to the CON and EX groups. A main effect of food restriction on body weight was found starting on P41 through the end of the experiment, P54. Additionally, an interaction between the factors of food restriction and wheel access was significant on days P44 and P45 (Figure 1b).

3.2 | Food consumption is reduced by food restriction and wheel access

Food consumption of the four groups was monitored daily. As expected, food consumption of the four groups was strongly influenced by the environment ($F_{(3,56)}$ = 73.11, p <.0001) and experimental day ($F_{(6,636)}$ = 311.5, p <.0001), with strong interactions of the two factors ($F_{(18,336)}$ = 83.50, p <.0001). Tukey's multiple comparisons revealed that the FR + EX groups decreased food consumption precipitously on days of food restriction (E5, E6, and E7), relative to the days just before (q ratios = 28.55, 28.05, and 26.79), and consumed significantly more than the days prior to food restriction on E9 (q ratio = 7.82). An identical pattern was observed for the FR group. The EX group exhibited no detectable change in food consumption across the experimental days but CON exhibited a small but significant decline in consumption from E2/E3 to E5/E6 (q ratio = 4.79–5.49). Somewhat surprising was the observation that the EX and EX + FR groups consumed significantly less than CONs on some of the days that they were acclimating to the wheel (E1 through E5) (unpaired t-test, Tables 1 and 2). Importantly, this group difference disappeared by E5, corresponding to the day that FR and EX + FR groups began to be food restricted.

3.3 | Wheel running is increased by food restriction

Wheel running is innately rewarding, even to feral rodents (Meijer & Robbers, 2014). Accordingly, rats of this cohort acclimated readily to the running wheel from P36–40 (E1 through E4), as assessed by their steady daily increases in mean 24-hr wheel count (Figure 1c). Individual differences in daily wheel running were also detected within both the EX and EX + FR groups: while some animals were very active on the wheel after the first day, others made only a few rotations per day (E1–E4 in Figure 1d,e).

Previous studies have shown that when food restriction is imposed, voluntary wheel running increases dramatically (Aoki et al., 2012; Aoki et al., 2017; Gutierrez, 2013). In order to assess the reactivity of this study's cohort of rats to food restriction, wheel running activity continued to be monitored for the EX + FR group of rats for the 4 days of food restriction (beginning on P40 and through the end of P44, corresponding to E5–E8) and compared to the EX group's wheel activity. Repeated measures two-way ANOVA indicated a significant main effect of the environment ($F_{(1,30)}$ = 11.50, p = .0020), a significant main effect of the experimental days ($F_{(6,180)}$ = 31.04, p <.0001) and a strong interaction of the two factors ($F_{(6,180)}$ = 7.894, p <.0001). All EX and EX + FR animals increased their amount of running wheel activity over the course of the 8 days, but the amount of activity during the latter 4 days of the experiment, corresponding to the days of food restriction for the EX + FR animals, was significantly greater for the EX + FR group of animals than the EX group (Table 3, Figure 1c) (q ratio of Tukey's multiple comparisons test = 5.16 comparing E2 versus E8 of EX; for EX + FR, q ratios of 6.93, 8.34, 12.66, and 16.01 comparing E2 versus E5, E6, E7, and E8, respectively). Individual differences in the extent to which EX + FR animals increased wheel running during the food-restricted days were also observed (Figure 1e).

3.4 | Open field behavior: Food restriction and exercise increase anxiety-like behavior transiently, while EX + FR animals exhibit no anxiety-like differences from controls

Our previous study had shown that the treatment of EX + FR ending on P44 accelerated the expansion and retraction of dendritic arbors in

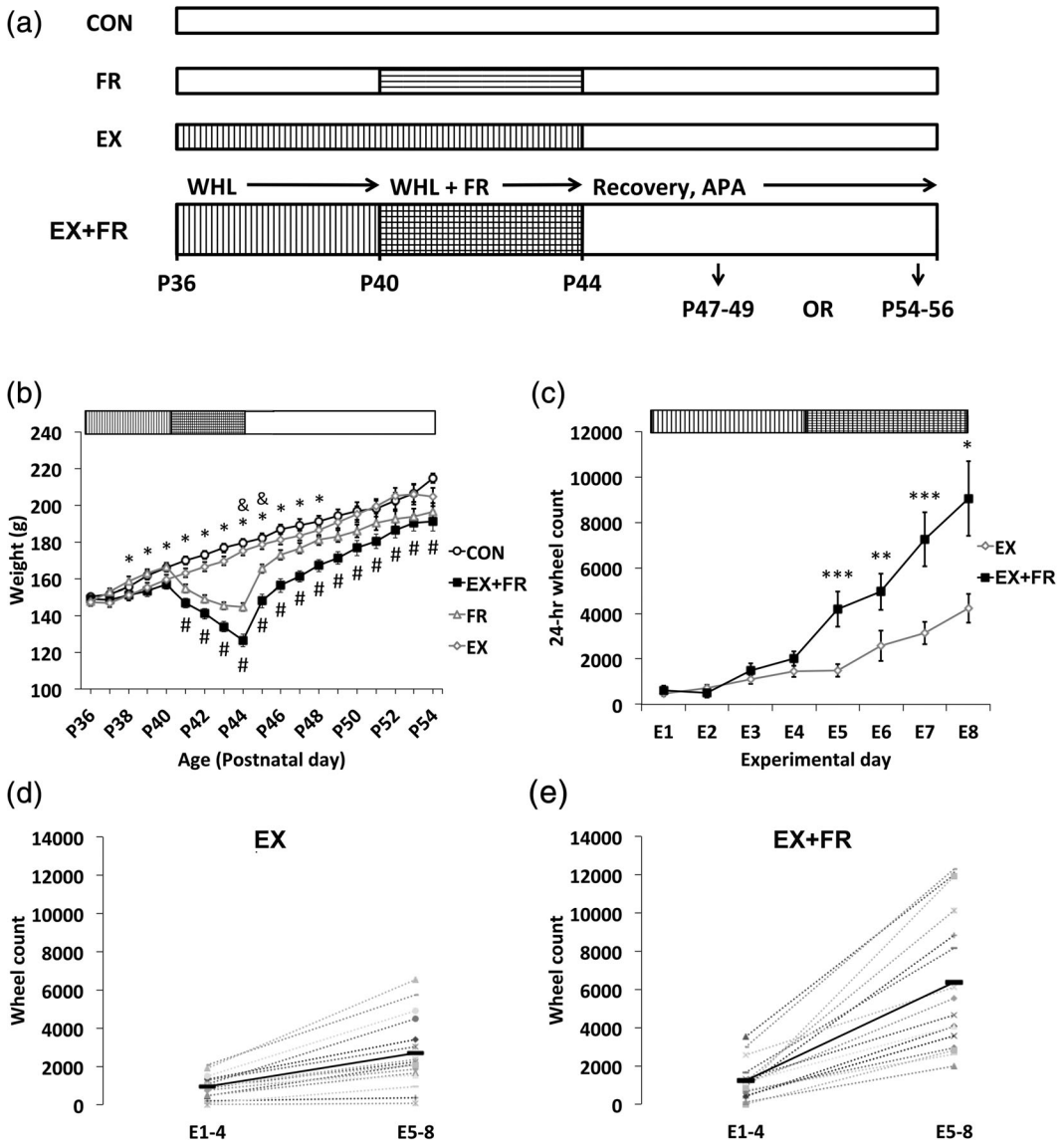

FIGURE 1 Body weight and wheel activity of CON, EX + FR, FR, and EX groups(a) A timeline of experimental groups indicates the experiences of wheel access (vertical hash) and food restriction (horizontal hash). Vertical arrows indicate the ages (in postnatal days) at which animals underwent testing for open field exploration and active place avoidance (APA). (b) Body weight of all groups is shown from age P36–P54. * indicates a main-effect of wheel access with p <.05; # indicates a main-effect of food restriction with p <.05; & indicates a significant interaction effect with p <.05. (c) Voluntary running wheel activity, measured at the end of each experimental day is displayed for EX and EX + FR groups for the 8 days of wheel access. * indicates significant difference between EX and EX + FR at p <.05, ** p <.01, *** p <.005. (d) The average activity per 24 hr during the first 4 days of wheel access was compared to the average activity during the last 4 days of wheel access in the EX group. The dashed lines each represent a single subject from the EX group. The first 4 days of wheel activity were averaged to give a single value for E1–4 (P35–P39), and the last 4 days were averaged to give a value for E5-8 (P40–P44). The solid black line represents the group average across all subjects. (e) The average activity during the first and last 4 days of wheel access was compared for the EX + FR group, exactly as in Panel d. In the EX + FR group, the latter 4 days of wheel access were accompanied by food restriction. 1 wheel count equals 0.64 m

the ventral hippocampus, relative to the dendritic arbors detected among CON brains, resulting in the dendritic arbors of ventral hippocampal pyramidal neurons of EX + FR animals being greater than CONs' at P44 but are less than those of CONs' by P55 (Chowdhury et al., 2014). If, as is widely accepted (Bannerman et al., 2002; Fanselow & Dong, 2010), the ventral hippocampus is more closely associated with emotionally driven behavior, and if dendritic expansion translates simplistically to enhanced excitability, the anxiety-like behavior might be expected to be greater for the EX + FR group at P47–48 and less at P54–55. This hypothesis was tested by measuring EX + FR animals' behavior in the open field compared to the FR, EX and CON groups. In an open field, time spent in the periphery indicates greater anxiety-like behavior levels in rodents, in contrast to their exploratory behavior in the center of the field (Prut & Belzung, 2003). Thus, for this cohort of rats, we measured animals' exploratory versus anxiety-like behavior quantified as the proportion of time

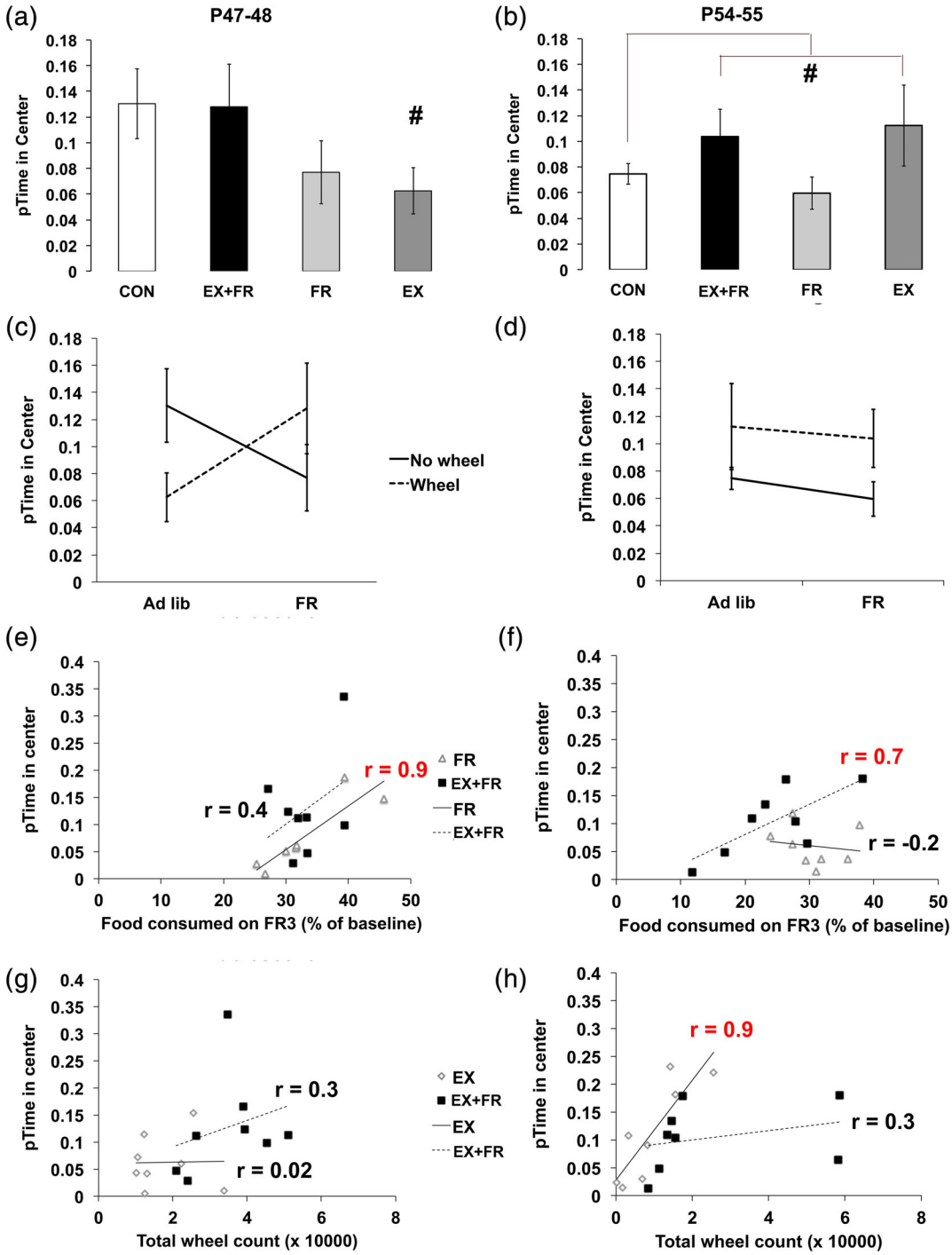

FIGURE 2 Open field test measuring exploratory behavior. (a) The proportion of total time spent in the center region of the open field during the first 3 min of the habituation trial (pTime in Center) is shown for the four experimental groups when tested during middle adolescence, at ages P47 or 48. Greater time spent in the center indicates greater exploratory behavior. (b) Proportion of time spent in the center region of the open field is shown for a second set of four experimental groups that were tested as late adolescent/young adults, at ages P54 or 55. # in a and b indicate marginally significant differences ($0.05 < p < .1$). (c) and (d) Two-way ANOVA interaction diagrams are shown to compare the proportion of the total time spent in the center across groups that had ad libitum access to food or 1-hr of food access per day (FR, *x*-axis) and groups that were housed without a wheel (solid line) or with wheel access (dashed line). Among the P47/48 groups of animals, two-way ANOVA indicates an interaction between the factors of wheel access and food restriction, with FR and EX groups showing increased anxiety while the EX + FR group is similar to CON. In contrast, among the P54/55 groups, no significant main effects of wheel access or food restriction nor a significant interaction were found. (e) and (f) The amount of food consumed on Day 3 of food restriction (FR3) as a percentage of the amount of food consumed on the day before food restriction is plotted against the proportion of time spent in the center of the open field by FR (open triangles and solid line) and EX + FR (solid squares and dashed line) individuals. Panel E shows data of the P47/48 animals, while Panel F shows data of the P55/56 animals. (g) and (h) The total wheel count after 8 days of wheel access is plotted against the proportion of time spent in the center of the open field by EX (open diamonds and solid lines) and EX + FR (solid squares and dashed lines) individuals. Panel g shows data of P47/48 animals and Panel h shows data of P55/56 animals [Color figure can be viewed at wileyonlinelibrary.com]

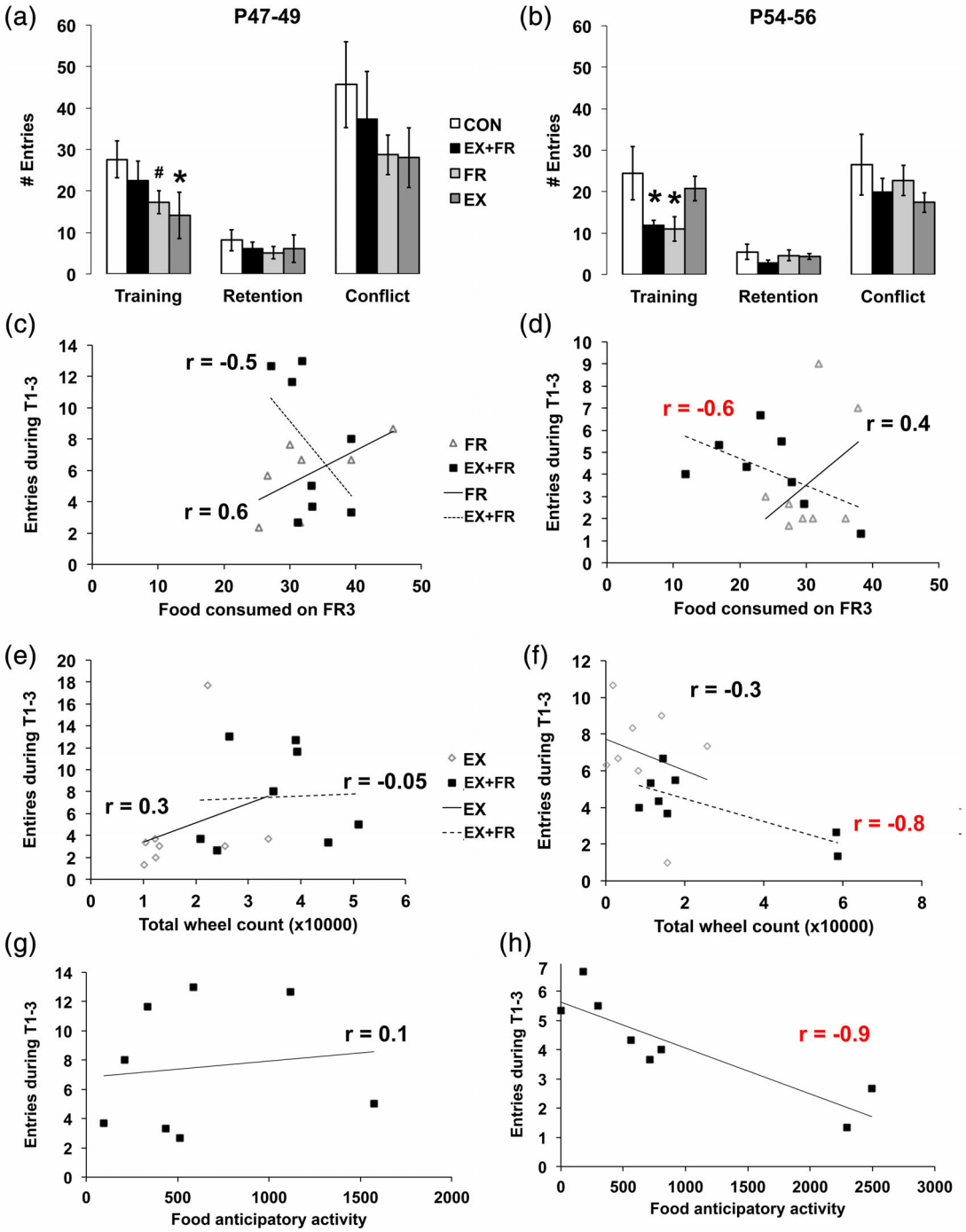

FIGURE 3 Active place avoidance measurements of spatial learning acquisition, retention and cognitive flexibility. (a) The number of entries into the shock zone (errors) is shown for the four experimental groups when tested at ages P47–49 (middle adolescents). Seven sessions were conducted over 2 days. Three training sessions were conducted on the first day (the average of the three sessions is shown), while one retention (R), and three conflict sessions were conducted on the following day (the average of the three conflict sessions is shown). Fewer entries indicate better performance. For each animal, the total number of entries across T1, T2, and T3 was determined. The bar represents the average of eight animals' total number of entries. (b) The number of entries into the shock zone (errors) is shown for a second set of four experimental groups that were tested at ages P54–56 (late adolescent/young adults). Asterisks in panels A and B indicate statistically significant differences (p <.05) while # indicates marginally significant differences (0.05 < p <.1). (c) and (d) The amount of food consumed on Day 3 of food restriction (FR3) as a percentage of the amount of food consumed on the day before food restriction is plotted against the number of errors during training (average of three sessions) of FR (open triangles and solid lines) and EX + FR (solid squares and dashed lines) individuals. Panel C shows data from middle adolescent individuals and Panel d shows data from late adolescent/young adult individuals. (e) and (f) The total wheel count after 8 days of wheel access is plotted against the number of errors during training by EX (open diamonds and solid lines) and EX + FR (solid squares and dashed lines) individuals. Panel e shows data from middle adolescent individuals and Panel f shows data from late adolescent/young adult individuals. (g) and (h) Food anticipatory activity (the wheel count during the 6 hr prior to feeding) is plotted against the number of errors during training. Panel g shows data from the middle adolescent individuals and Panel h shows data from the late adolescent/young adult individuals [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Comparison of food consumption between CON and EX + FR groups

	Mean + *SE* (*N*)			
	CON	Ex + FR	*t* ratio	*p*
E2	67.50 ± 1.84 (11)	63.88 ± 3.86 (8)	0.972651	.331
E3	65.77 ± 2.00 (16)	30.45 ± 2.57 (16)	1.87955	.061
E4	66.36 ± 1.75 (16)	58.55 ± 1.53 (16)	2.75376	.006
E5	64.47 ± 2.32 (16)	59.41 ± 1.71 (16)	1.78411	.075
E6	57.96 ± 1.85 (16)	13.84 ± 0.67 (16)	15.5646	<.0001
E7	58.38 ± 1.50 (16)	14.69 ± 0.90 (16)	15.415	<.0001
E8	60.06 ± 2.03 (16)	16.86 ± 1.04 (16)	15.2416	<.0001
E9	59.43 ± 1.70 (16)	71.19 ± 21.99 (15)	4.08113	<.0001
E10	60.48 ± 2.38 (12)	65.86 ± 3.60 (15)	1.73134	.084
E11	56.64 ± 3.55 (7)	77.62 ± 2.43 (10)	5.30905	<.0001
E12	64.26 ± 3.45 (8)	70.98 ± 3.89 (8)	1.67636	.095
E13	57.54 ± 2.57 (8)	77.28 ± 3.70 (8)	4.92432	<.0001
E14	57.54 ± 1.95 (8)	70.56 ± 3.02 (7)	3.13783	.0019
E15	57.60 ± 2.40 (7)	72.24 ± 4.45 (8)	3.52825	.0001

TABLE 2 Comparison of food consumption of FR and EX groups relative to CON

	Mean ± *SEM* (*N*) FR	*t* ratio	*p*	Mean *SEM* (*N*) EX	*t* ratio	*p*
E2	61.56 ± 1.92 (14)	1.8776	.061	47.17 ± 2.88 (12)	5.94082	<.001
E3	62.52 ± 2.20 (14)	1.13142	.259	58.11 ± 2.30 (16)	2.64472	.0085
E4	61.15 ± 2.54 (15)	1.84384	.0666	52.75 ± 1.44 (16)	4.694	<.001
E5	61.73 ± 1.51 (15)	0.969836	.333	54.52 ± 1.50 (16)	3.43357	.0007
E6	12.21 ± 1.13 (15)	16.1981	<.0001	56.70 ± 1.58 (16)	0.433904	.665
E7	14.90 ± 1.12 (15)	15.3951	<.0001	55.04 ± 2.09 (16)	1.15177	.250
E8	19.488 ± 0.94 (15)	14.3641	<.0001	55.15 ± 2.25 (16)	1.66654	.0965
E9	78.18 ± 2.77 (15)	6.63685	<.0001	60.35 ± 1.84 (16)	0.318728	.750
E10	64.68 ± 2.68 (12)	1.30904	.191	61.11 ± 2.55 (16)	0.201195	.841
E11	66.75 ± 2.84 (15)	2.81092	.0052	60.9 ± 2.08 (16)	1.14646	.252
E12	66.75 ± 2.66 (15)	0.72475	.469	63.84 ± 4.26 (12)	0.112221	.911
E13	61.32 ± 1.88 (8)	4.92432	.337	53.76 ± 3.59 (8)	0.921988	.357
E14	64.68 ± 2.18 (8)	1.81701	.070	60.9 ± 3.01 (8)	0.819545	.413
E15	65.52 ± 3.86 (8)	1.94716	.052	61.74 ± 2.19 (8)	0.975556	.330

spent in the center during the first 3 min of the pretraining trial in the arena. Locomotor activity was also measured, as the total distance traveled on the arena.

Contrary to expectation, the combined exposure to wheel access and food restriction did not alter anxiety-like behavior among the short recovery groups (P47–48) that were tested 3–4 days after the last day in the EX + FR environment (Mean percent time in center ± *SEM* for CON = 0.13 ± 0.03; for EX + FR 0.13 ± 0.03). Exploration of the center of the open field trended toward reduction, when the experiences of wheel access and food restriction were presented separately (i.e., for the EX and FR groups) (Mean ± *SEM* was 0.08 ± 0.02 for FR; 0.06 ± 0.02 for EX; $F_{(3,27)}$ = 1.751; p = .1804 by one-way ANOVA, individual p = .17 comparing FR vs. CON; individual p = .07 comparing EX vs. CON by Uncorrected Fisher's LSD test). Two-way ANOVA indicated no significant main effect of food restriction or exercise but a significant interaction effect between wheel access and food restriction (Figure 2c; $F_{(1,27)}$ = 5.010, p = .03).

Accompanying the groups' changes in exploration of the center, there were significant group differences in the distance traveled within the entire arena (Table 4). Two-way ANOVA indicated a marginal main effect of wheel access ($F_{(1,27)}$ = 3.467, p = .07) and food restriction ($F_{(1,27)}$ = 3.695, p = .07) and a strong interaction of the two factors ($F_{(1,27)}$ = 12.62, p = .0014). According to Tukey's multiple comparisons tests, the distance traveled in the arena by the EX + FR group (40.3 ± 3.3 m) was marginally significantly different from CON (50.7 ± 2.9, adjusted p = .05) but significantly less than FR (55.2 ± 2.3, adjusted p = .004 by Tukey's multiple comparisons test) and EX (55.4

TABLE 3 Comparison of wheel counts between EX and EX + FR groups

	Mean ± *SEM* (*N*)			
	EX	EX + FR	*t* (df)	*p*
Total	13,448 ± 2,326 (16)	29,899 ± 4,235 (11)	−3.404 (30)	.002
E1	465 ± 115 (16)	630 ± 179 (16)	−0.808 (30)	.427
E2	703 ± 139 (16)	500 ± 199 (16)	0.838 (30)	.409
E3	1,092 ± 188 (16)	1,474 ± 317 (16)	−1.036 (30)	.308
E4	1,445 ± 243 (16)	2003 ± 314 (16)	−1.405 (30)	.170
E5	1,504 ± 285 (16)	4,196 ± 782 (16)	−3.233 (30)	.003
E6	1935 ± 580 (16)	4,952 ± 798 (16)	−3.058 (30)	.005
E7	3,138 ± 501 (16)	7,256 ± 1,194 (16)	−3.179 (30)	.003
E8	4,220 ± 638 (12)	9,045 ± 1,641 (16)	−2.435 (26)	.022

Note: The values show mean ± *SEM*, *t*-, and *p*-values.

± 2.2; adjusted $p = .003$ by Tukey's multiple comparisons test) groups' distance traveled in the arena.

When open field behavior was measured at P54–55, after an additional week of recovery from wheel access and/or food restriction, neither wheel access nor food restriction (Figure 2b) nor their combination (Figure 2d) showed a significant effect on the proportion of time spent in the center of the open field (Mean ± *SEM* was 0.07 ± 0.01 for CON; 0.10 ± 0.02 for EX + FR; 0.06 ± 0.01 for FR; 0.11 ± 0.03 for EX), although exercise showed a marginal main effect ($F_{(1,28)} = 4.010$; $p = .055$) that was independent of the distance run in the arena ($F_{(1,28)} = 0.5646$; $p = .46$) (Table 4).

In order to determine whether maturation during adolescence is reflected by a change in open field behavior, we compared the open field exploratory behavior of the CON group of animals of ages P47–48 versus P54–55. Results indicate a trend for a decrease in open field exploration with maturation (mean of proportion of time in Center 0.13 ± 0.03 for P47–48; 0.07 ± 0.01 for P54–55; $p = .08$) without accompanying changes in the distance traveled (Table 5; $p = .37$, $t = 0.9339$). This observation indicates a trend toward a rise of anxiety-like behavior with maturation (Prut & Belzung, 2003).

3.5 | Individual differences in exploratory behavior are related to their food consumption behavior and voluntary exercise

As was noted earlier, individuals within the EX + FR and EX groups varied widely in terms of wheel activity (Figures 1d,e). The EX + FR and FR-only groups of individuals also varied widely in the amount of food that they consumed during the 1 hr of food access, ranging from 10 to 46% of the prefood restriction (i.e., baseline) calorie amount. We surmised that the lack of difference in the exploratory behavior among the four P54–56 groups might be due to the dominance of these individual differences. In order to test this possibility, correlation analyses of open field behavior to the individual animals' food consumption and wheel activity were performed. Indeed, when open field behavior was measured for the EX + FR group that was P54–56, a positive correlation was found between the amount of food consumed on the third day of food restriction (as a percentage of baseline 24-hr food consumption) and the proportion of time spent in the center of the open field (Pearson's $r = .7$; $p = .04$; Figure 2f). In other words, those animals that managed to consume relatively more food during the 1 hr of food access were the ones more willing to explore the center of the open field. This correlation did not hold for the FR group of individuals tested at P54–56 (Pearson's $r = -.2$; $p = .7$; Figure 2f), even though FR individuals that were tested shortly after food restriction, at P47–49, had exhibited strong correlation ($r = .9$; $p < .01$; Figure 2e).

A positive correlation was also found between the total wheel count (8 days of wheel activity) and the proportion of time spent in the center of the open field among the EX individuals at P54–56 ($r = .9$; $p = .006$; Figure 2h), but not at P47–49 ($r = .02$; Figure 2g). This positive correlation between wheel count and time spent in the center was also observed for six animals of the EX + FR group at P54–56. However, two individuals in the EX + FR group that ran maximally before food restriction and excessively after food restriction reduced the overall correlation for the EX + FR group ($r = .3$, $p = .5$) (Figure 2h). As was found for the EX group, the EX + FR group of individuals that was tested on the open field at P47–49 did not show a correlation between the proportion of time spent in the center of the open field and their total wheel activity ($r = .3$, $p = .51$).

3.6 | Enhanced acquisition of spatial memory in EX + FR animals after recovery

Since the dorsal hippocampus is required for active place avoidance (Cimadevilla, Fenton, & Bures, 2000; Cimadevilla, Kaminsky, et al., 2000), retraction of dendritic arbors of dorsal hippocampal neurons might be expected to impair active place avoidance. We used the number of errors, that is, entries into the shock zone during the active place avoidance task during T1, T2, and T3 to measure memory acquisition, and the next day, to estimate memory retention, while performance during the conflict trials measured flexibility in spatial cognition (Burghardt, Park, Hen, & Fenton, 2012).

As animals learned the position of the shock zone, they progressively entered it less. All animals acquired the spatial memory.

TABLE 4 Comparison of the distance run on the active place avoidance arena

	CON	EX + FR	FR	EX	EX main effect *p*-value	FR main effect *p*-value	Interaction *p*-value
Distance run P47–49 (m)							
OFT	50.7 ± 2.9	40.3 ± 3.3	55.2 ± 2.3	55.4 ± 2.2	.07	.07	.001
Training trials							
Trial 1	42.5 ± 1.9	41.9 ± 1.6	44.4 ± 2.1	41.6 ± 1.4	.33	.53	.67
Trial 2	39.2 ± 1.6	37.7 ± 0.9	38.5 ± 1.9	38.6 ± 1.0	.61	.56	.91
Trial 3	38.3 ± 1.4	37.5 ± 0.6	38.2 ± 1.3	37.1 ± 1.1	.39	.89	.82
Retention	43.6 ± 1.9	41.7 ± 1.3	51.1 ± 4.0	43.9 ± 2.4	.08	.28	.07
Conflicts							
Conflict 1	43.9 ± 2.8	44.7 ± 1.7	47.0 ± 3.2	39.9 ± 0.9	.18	.09	.72
Conflict 2	41.0 ± 1.4	37.7 ± 1.1	43.9 ± 3.3	34.9 ± 2.0	.005	.17	.96
Conflict 3	37.9 ± 1.6	38.5 ± 1.1	45.5 ± 3.9	36.4 ± 1.8	.06	.04	.24
Distance run P54–56 (m)							
OFT	54.9 ± 3.4	54.4 ± 3.4	54.2 ± 2.3	59.8 ± 4.1	.46	.375	.50
Training trials							
Trial 1	42.8 ± 0.9	43.8 ± 1.4	42.5 ± 1.0	48.6 ± 2.4	.03	.11	.15
Trial 2	42.5 ± 0.9	40.4 ± 2.4	38.5 ± 0.7	40.9 ± 1.6	.91	.14	.25
Trial 3	40.8 ± 1.4	37.8 ± 1.5	36.9 ± 0.7	38.3 ± 0.9	.51	.075	.17
Retention	44.3 ± 1.0	44.9 ± 2.5	46.8 ± 1.6	45.3 ± 1.9	.79	.57	.44
Conflicts							
Conflict 1	46.9 ± 1.8	47.0 ± 1.7	46.1 ± 1.5	43.0 ± 1.6	.36	.33	.15
Conflict 2	43.2 ± 2.1	41.9 ± 0.9	41.45 ± 1.6	38.3 ± 0.7	.14	.54	.08
Conflict 3	39.3 ± 3.5	43.7 ± 2.4	36.4 ± 3.6	40.1 ± 2.5	.19	.89	.28

Note: Mean ± *SEM* and the *p*-values of 2-way ANOVA indicating the main effects of EX, FR, and their interactions.

At P47–48, two-way ANOVA on the average number of entries during T1, T2, and T3 showed no main-effect of exercise ($F_{(1,27)}$ = 0.81, p = .38) or food restriction ($F_{(1,27)}$ = 0.05, p = .83), but a marginally significant interaction between food restriction and exercise ($F_{(1,27)}$ = 4.1; p = .05) (Figure 3a). This is because both the FR and EX groups performed marginally better than CON (9.21 ± 1.50 for CON; 5.76 ± 0.91 for FR; 4.71 ± 1.87 for EX; p = .04 comparing CON vs. EX; p = .13 comparing CON vs. FR), but the combination of the two treatments as EX + FR minimized this effect (7.50 ± 1.56 for EX + FR, p = .43 comparing CON vs. EX + FR). There were no significant main-effects of exercise or food restriction during retention or conflict trials that were run on P48/49, nor were there significant main effects of exercise or food restriction upon the average number of shocks per entry for any of the training, retention or conflict trials (Table 5).

At P54–55, two-way ANOVA indicated a significant main effect of food restriction. Animals that had experienced food restriction (i.e., EX + FR and FR) made fewer entries during training ($F_{(1,27)}$ = 7.5; p = .01), indicating superior acquisition of spatial memory, compared to CON. However, there was no effect of food restriction on retention or conflict performance that were run on P55/56 (Figure 3b; $F_{(1,28)}$ = 0.94, p = .34 for Retention; $_{(1,28)}$ = 0.02, p = .89 for Conflict performance). There was also no main effect of wheel access (i.e., EX + FR and EX) during training, retention, or conflict (Figure 3b; $F_{(1,28)}$ = 0.07, p = .78 for Training; $F_{(1,28)}$ = 1.383, p = .25 for Retention; $F_{(1,28)}$ = 1.69, p = .20 for Conflict). The number of shocks per entry during the training sessions on P54/55 was uniform across all groups and trials (<1.4) (Table 5).

In order to determine whether cognitive ability exhibits maturation during adolescence, we compared the acquisition, retention and conflict performance of the CON animals across the two ages: P47–49 versus P55–57. This comparison revealed no change (Mean ± *SEM* for Training 9.2 ± 1.5 for P47–48, 8.2 ± 2.2 for P54–55, p = .70; for Retention 8.125 ± 2.5 at P48–49, 5.50 ± 1.9 for P55–56, p = .41; for Conflict 15.21 ± 3.4 for P48–9, 8.8 ± 2.5 for P55–56 and p = .15).

3.7 | Individual differences among the EX + FR group in spatial learning at P54–56 correlates with adaptation to the restricted food access schedule

3.7.1 | Correlation between spatial cognition and food consumption

The main effect of food restriction on performance during training, when tested at P54–56, suggested that the experience of food restriction related to a delayed cognitive enhancement. To determine whether the entrainment to the 1 hr/day food access schedule related to enhanced performance in active place avoidance training, we tested for a correlation between average entries into the shock zone during training and the amount of food consumed on day 3 of food restriction

TABLE 5 Comparison of the number of shocks per entry across the four groups of animals

	CON	EX + FR	FR	EX	EX main effect *p*-value	FR main effect *p*-value	Interaction *p*-value
Number of shocks per entry P47–49							
OFT[a]	2.1 ± 0.2	3.0 ± 0.4	2.1 ± 0.12	2.1 ± 0.2	.11	.07	.07
Avg of trainings 1, 2 & 3	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	.79	.56	029.
Trial 1	1.1 ± 0.06	1.2 ± 0.1	1.2 ± 0.12	1.1 ± 0.06	.98	.37	..64
Trial 2	1.3 ± 0.1	1.15 ± 0.1	1.0 ± 0.0	1.4 ± 0.3	.48	.09	.89
Trial 3	1.3 ± 0.1	1.2 ± 0.1	1.0 ± 0.0	1.0 ± 0.0	.38	.46	.02
Retention	1.1 ± 0.1	1.0 ± 0.2	1.1 ± 0.0	1.0 ± 0.0	.75	.89	.93
Avg. of conflicts 1, 2, & 3	1.6 ± 0.3	1.3 ± 0.1	1.2 ± 10.0	1.3 ± 0.1	.70	.20	.31
Conflict 1	1.65 ± 0.3	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	.45	.30	.34
Conflict 2	1.4 ± 0.3	1.2 ± 0.2	1.1 ± 0.05	1.4 ± 0.1	.96	.28	.74
Conflict 3	1.6 ± 0.3	1.2 ± 0.1	1.1 ± 0.0	1.5 ± 0.2	.98	.07	.59
Number of shocks per entry P54–56							
OFT[a]	2.3 ± 1.3	2.0 ± 0.1	2.1 ± 0.1	1.9 ± 0.2	.11	.80	.30
Avg. of trainings 1, 2, & 3 PT	1.2 ± 0.1	1.1 ± 0.0	1.2 ± 0.1	1.2 ± 0.0	.07	.46	.62
Trial 1	1.3 ± 0.1	1.1 ± 0.0	1.4 ± 0.1	1.3 ± 0.1	.10	.40	.17
Trial 2	1.1 ± 0.1	1.0 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	.17	.3	.81
Trial 3	1.1 ± 0.05	1.1 ± 0.1	1.0 ± 0.0	1.0 ± 0.0	.56	.94	.28
Retention	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.0	.76	.50	.76
Avg. of conflicts 1, 2, & 3	1.4 ± 0.14	1.2 ± 0.4	1.1 ± 0.4	1.0 ± 0.2	.04	.40	.04
Conflict 1	1.4 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.05 ± 0.0	.05	.57	.15
Conflict 2	1.3 ± 0.1	1.0 ± 0.0	1.0 ± 0.1	1.1 ± 0.4	.11	.25	.38
Conflict 3	1.35 ± 0.2	1.1 ± 0.1	1.0 ± 0.0	1.0 ± 0.0	.20	.28	.04

Note: Mean ± *SEM* and the *p*-values of 2-way ANOVA indicating the main effects of EX, FR, and their interactions.
[a]Number of shocks an animal would have received, had the current been delivered.

(E7 in Figure 1c, corresponding to P43), relative to the baseline 24-hr food consumption before food restriction (E3 in Figure 1c, corresponding to P39). Indeed, in the EX + FR group, we found a negative correlation between food consumed (as a percentage of baseline 24-hr food consumption) and number of entries ($r = -.6$; $p = .1$)—those individuals that consumed relatively more food were the ones that made fewer errors 10–11 days later (Figure 3d). In comparison, the correlation between food consumed and the number of entries for the EX + FR group tested at P47–49 was much weaker ($r = -.5$, $p = .2$; Figure 3c). In contrast to the EX + FR group, the correlation for the FR group was positive at P47–49 ($r = .6$; $p = .1$) (Figure 3c) and nonexistent at P54–56 ($r = .4$; $p = .3$). This suggests that the presence of the running wheel potently alters the impact of food restriction upon performance on active place avoidance training—from a positive to a negative correlation—and especially with a delay.

3.7.2 | Correlation between spatial cognition and total wheel running

Although comparisons of the group mean values revealed no main effect of wheel access on the animals' performance on active place avoidance training (Figure 3a,b), analysis of individual differences in wheel running revealed an effect of wheel access. A negative correlation between the total wheel count and the average number of entries during training was found in the EX + FR group at P54–56 ($r = -.8$; $p = .02$; Figure 3f), while no correlation was found in the EX group tested at that age ($r = -.3$; $p = .5$). In contrast, no correlation was found between total wheel count and average entries during training among either EX or EX + FR individuals tested at P47–49 (EX: $r = .3$, $p = .5$; EX + FR: $r = .05$, $p = .91$; Figure 3e).

Further analysis of the animals' acquisition of spatial memory revealed a negative correlation between the total wheel count and the number of entries during T1 in the EX ($r = -.6$; $p = .10$) and a stronger negative correlation in the EX + FR ($r = -.7$; $p = .06$) groups tested at P54–55, but not at P47–48 (EX: $r = .4$, $p = .3$; EX + FR: $r = .09$, $p = .8$). This suggests that animals that ran more made fewer errors during the earliest phase of acquisition of spatial memory, but only when spatial memory training occurred with a delay of 10–11 days after food restriction and wheel access.

3.7.3 | Strongest correlation between food anticipatory activity and spatial cognition

Previous work had shown that the increase in total running following EX + FR is due, largely, to the increase in food anticipatory activity

(Chowdhury, Wable, et al., 2013). It is widely recognized that food anticipatory activity emerges when animals are put on scheduled food access (Gallardo et al., 2014; Gelegen et al., 2008). Food anticipatory activity was calculated as the number of wheel rotations during the 6 hr prior to feeding (i.e., 1 p.m. to 7 p.m.), as we have calculated previously (Chowdhury, Wable, et al., 2013). The strongest correlation was found between the amount of food anticipatory activity exhibited by the EX + FR animals and their cognitive performance. The average of the food anticipatory activity on the last 3 days of food restriction correlated negatively with the average number of entries during training sessions T1 through T3 ($r = -.9$; $p = .004$) (Figure 3h). In other words, those EX + FR individuals that exhibited the strongest food anticipatory wheel activity were the ones that made the least number of errors during training. This correlation emerged with a delay of 10–11 days, indicating that EX + FR is likely to have altered the trajectory of the protracted development of the hippocampus during adolescence.

4 | DISCUSSION

For the purpose of discussion, the main findings of this study have been drawn in schematic form (Figure 4). There are three parts to the Discussion: Food restriction effects, Exercise effects and Anatomical considerations, including synaptic mechanisms.

4.1 | Food restriction effects—improvement of cognitive function with a delay

Our main finding is that the experience of food restriction during adolescence in female rats results in an improvement in spatial learning acquisition on an active place avoidance task that requires activation and long-term plasticity of synapses in the hippocampus (Cimadevilla et al., 2001; Cimadevilla, Fenton, & Bures, 2000; Hsieh et al., 2017; Pastalkova et al., 2006). This group difference is not likely to have arisen from differences in pain sensitivity, since there was also no group difference in the number of shocks per entry.

Cognitive benefits of moderate food restriction have been reported before. For example, caloric restriction has been shown to increase BDNF expression and neurogenesis in the hippocampus (Lee et al., 2002); protect against excitotoxicity (Bruce-Keller et al., 1999) and ischemia (Yu & Mattson, 1999); improve cognitive function and protect against amyloid-β accumulation and cell death in Alzheimer's disease models (Halagappa et al., 2007; Patel et al., 2005; Zhu, Guo, & Mattson, 1999). Our paradigm results in an 80% calorie reduction as opposed to the other cited studies, which imposed a less severe 40% calorie reduction on their animals. Despite the severe caloric reduction animals experience during the 1-hr food access schedule, the lack of detrimental cognitive effects suggests that malnutrition did not have immediate or delayed negative effects on the function of the hippocampus during adolescence to young adulthood. Indeed, we have previously shown

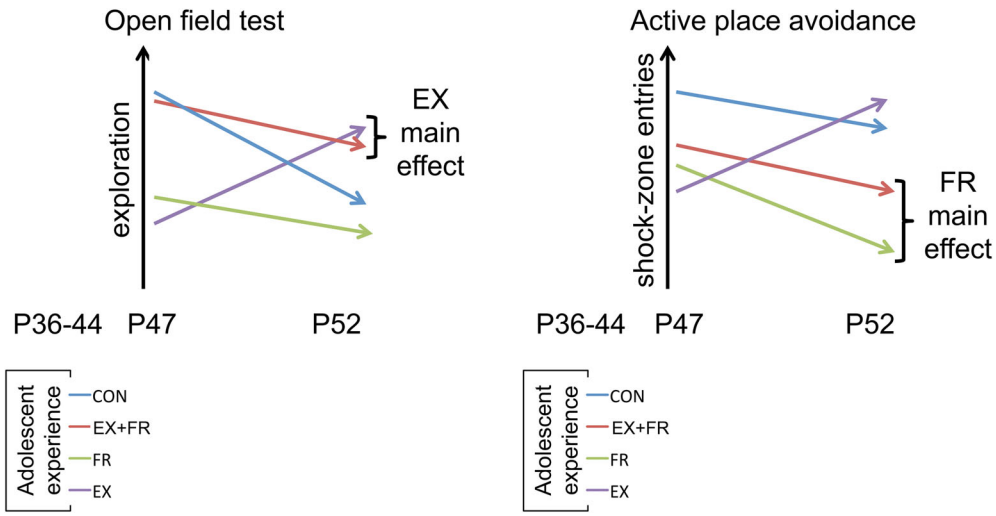

FIGURE 4 Summary of the effects of environmental manipulations on measurements exploration in the open field and spatial cognition at time points differing in recovery. The left panel compares animals' exploration in an open field, indicative of anxiety-like behavior. The right panel compares animals' acquisition of active place avoidance, indicative of cognitive function. The behavioral measurements were assessed within 3–4 days after the last day in the experimental environment, at P47–48 or after 10–11 days, at P54–55. For both behavioral measurements, the four groups of animals exhibit differences within each time point and across time points. Exploratory behavior in an open field is also reflective inversely of an animal's anxiety-like behavior. The experience of exercise has a main effect of increasing EX + FR and EX rats' exploratory behavior. However, this change is evident only after a longer delay. Exploratory behavior diminished with maturation for the CON group but was enhanced over the same period for the EX group. Food restriction has a main effect of improving spatial cognition upon EX + FR and FR groups of animals, but with a delay. In contrast, the benefit of exercise alone (EX) is transient, since it is evident shortly after the days of exercise but not when the assessment is delayed after the wheel has been removed from the cage for 10 days. Both the main effects of exercise and food restriction emerge with a delay of 10–11 days. This delay suggests that a cellular event, such as synaptogenesis and changes in circuitry are evoked following alterations in neurogenesis due to environmental factors [Color figure can be viewed at wileyonlinelibrary.com]

that food restriction caused by EX + FR does not result in widespread atrophy of dendritic arbors in the hippocampus (Aoki et al., 2017; Chowdhury et al., 2014; Chowdhury, Barbarich-Marsteller, Chan, & Aoki, 2013). The lack of detrimental effects could be attributed at least in part to a decrease in metabolic rate via homeostatic mechanisms induced by the hypothalamus (Roh, Song, & Kim, 2016).

4.1.1 | Food restriction, without running wheel access, results in minimal anxiogenesis

Comparisons of the group mean values of animals that experienced food restriction without wheel access (FR) showed no significant change in exploration of the center of an open field (Figure 2a). This lack of change in anxiety-like behavior contradicts previous literature that showed a decrease in anxiety following food restriction in adolescent female rats (Genn, Tucci, Thomas, Edwards, & File, 2003) and contrasts our data on anxiety-like behavior measured among adolescent female mice during the food—restricted period (Wable, Min, Chen, & Aoki, 2015). Perhaps the most salient difference is that in our study, the open field area rotates, which may be more anxiogenic than the stationary open fields of the other studies. Furthermore, compared to our current experiment, the study by Genn et al. (2003) imposed a less severe food restriction and used Hooded Lister rats. The peri-adolescent group that they used (P28–35) were younger than the age at which rats in our study underwent food restriction (P41–44). Moreover, their study measured anxiety-like behavior immediately after the 7 days of mild food deprivation, while ours were analyzed for axiety-like behavior following 2–3 days of weight restoration. These procedural differences may be the reason for the different results. Indeed, literature is emerging to indicate that environmental effects can differ, depending on their imposition at early (P24–35) versus middle (P37–48) versus late (P50–61) adolescence (Adriani, Macri, Pacifici, & Laviola, 2002; Varlinskaya & Spear, 2006).

Our results also indicate a trend for a decrease in open field exploration with maturation. This observation indicates a trend toward a rise of anxiety-like behavior with maturation (Prut & Belzung, 2003). This rise in anxiety-like behavior is consistent with a report of increased anxiety-like behavior as measured by the elevated plus maze seen in female, but not male, rats after experiencing a reward- and punishment-based learning paradigm in young adulthood—suggesting that females are particularly sensitive to generalized anxiety-like behavior after behavioral task experience (Chowdhury et al., 2019). It is important to note that the current study is limited to describing behavioral changes in female Sprague–Dawley rats, and cannot be extrapolated to males as well. These behavioral data indicate that the precocious dendritic arbors and spine maturity that was detected previously in the ventral hippocampus of another cohort of the EX + FR group or the retraction of dendritic arbors detected between P51–55 for another cohort of CON animals (Chen et al., 2018; Chowdhury et al., 2014) do not translate to alterations in anxiety-like behavior at P47–48 or P54–55.

In our study, a positive correlation was found between food consumed and exploratory behavior among the rats in the FR group that were tested soon after food restriction (at P47–48; Figure 2e). This equates to a positive correlation between food restriction and anxiety-like behavior, meaning that the more severely food-deprived animals showed more anxiety-like behavior. While this may not be surprising, it is remarkable that the EX + FR group did not show increased anxiety-like behavior, relative to the CON group or a correlation between anxiety-like behavior and food consumption as adolescents. However, when tested 10–11 days later, a positive correlation between exploratory behavior and food consumption during the food restricted days emerged in the EX + FR group, while this correlation disappeared in the FR group (Figure 2f). In other words, food restriction impacted anxiety-like behavior for both the FR and EX + FREX + FR groups of animals: the expression of this anxiety-like behavior is transient without wheel access (FR) but is delayed when given access to the wheel (EX + FR). Voluntary exercise as measured by running wheel activity shows a delayed correlation with reduced anxiety-like behavior (Figure 2h), and in the EX + FR group, wheel access may occlude the anxiety-like behavior that is induced by food restriction.

The combined treatment of EX + FR is the same manipulation that has been used as an animal model of anorexia nervosa, called activity-based anorexia, to investigate the neurobiological underpinnings of this mental illness (Aoki et al., 2012; Aoki et al., 2017; Casper et al., 2008; Gutierrez, 2013). As such, it is remarkable that the positive relationship between high levels of wheel running and percent time in the center (i.e., less anxiety-like behavior) supports explanations by physicians that patients diagnosed with anorexic nervosa are often be found to be excessive exercisers (Beumont et al., 1994; Casper et al., 2008; Davis et al., 1997; Kron et al., 1978), because anxiety is a co-morbidity (Dellava et al., 2010; Kaye, Bulik, Thornton, Barbarich, & Masters, 2004) and exercise is anxiolytic (Schoenfeld, Rada, Pieruzzini, Hsueh, & Gould, 2013; Sciolino & Holmes, 2012).

4.1.2 | Food restriction results in improvement of cognitive function, but improvement is delayed if food restriction is accompanied by wheel access

We found that food restriction improves spatial learning (Figure 3) when animals were tested at P54–55, but not when tested at P47–48. While the FR group showed improvement when tested at P47–48, the EX + FR group did not show this improvement until P54–55. The improvement observed by the two groups that experienced food restriction (FR and EX + FR) exceeds the slight improvement that occurs with maturation, alone, for the CON group (Figure 4) and reported in earlier studies on the ontogeny of spatial cognition (Rossier & Schenk, 2003; Schenk, 1985). Food restriction may improve performance on the active place avoidance task by acting as a form of adolescent cognitive training. Since food is presented for 1 hr per day during the same hour every day, it may benefit animals to be able to anticipate the presentation of food in order to eat as much as possible during the limited hour of availability. Thus, we predicted that animals that had learned to eat more during the days of food restriction would also have learned to avoid the shock zone best. We measured EX + FR animals' ability to anticipate the food access schedule by

measuring their voluntary wheel activity during the 6 hr preceding the feeding time as well as their consumption of food during that 1 hr. Indeed, the EX + FR animals' food anticipatory activity correlated strongly with their active place avoidance performance (Figure 3h), while their food consumption also correlated marginally with this performance (Figure 3d). Surprisingly, the FR animals showed an opposite direction of the correlation between active place avoidance and food consumption. These observations indicate that (1) food restriction interacts with wheel activity to improve cognitive performance; (2) the effect of food restriction in the EX + FR group is distinct from changes associated with maturation, alone, as is seen for the CON group (Figure 4).

It remains to be determined what common factor, if any, may be related to both entrainment during food restriction and enhanced cognitive performance 10 days later. Food anticipatory activity and circadian entrainment to the time of food access demonstrate learning of the time-dependence of feeding. While it has been shown that a lateral entorhinal cortex → hippocampus circuit involving dopamine D2 receptor-expressing hippocampal cells regulates feeding behavior (Azevedo et al., 2019), this anticipatory behavior has been linked to the dopamine D1 receptor expression in the dorsal striatum (Gallardo et al., 2014). Furthermore, neuroanatomical and pharmacological evidence points to the joint role of the two structures—striatum and hippocampus—both of which are modulated by dopamine to guide hippocampus-dependent memory in decision making (Shohamy & Wimmer, 2013). In our paradigm, there were two components to decision making: (1) to eat versus run during the 1 hr of food access and (2) to explore an open field versus stay safe in the periphery of an area. It is possible that entrainment to a restrictive food schedule represents hippocampus-dependent learning and serves as a form of cognitive enrichment of the adolescent environment. The delay of the food restriction effect by 10–11 days may reflect neurogenesis in the dentate gyrus, then of synaptogenesis that is stimulated by the release of BDNF in the hippocampus during the food-restricted days (Lee et al., 2002). If cognitive improvement requires synaptic as well as dendritic arbor re-organization in the CA1, then this type of change may very well require more than 3–4 days, since synapses in CA1 are removed from those in the dentate gyrus by at least two synaptic relays.

4.2 | Exercise effects

4.2.1 | Exercise, in the absence of food restriction, results in short-term enhancement of cognitive performance

We found that the experience of exercise alone (EX), but not EX + FR, improved spatial learning on the active place avoidance task when tested at P47–48. In the EX group, this effect did not persist to P54–55, and in the EX + FR group, it emerged with a delay. A correlation between increased running and improved performance did not exist in P47–49 adolescents in either EX or EX + FR groups, but emerged with a delay for the EX + FR group for the acquisition phase (Figures 3f,h). Exercise has been shown to increase BDNF expression in the hippocampus (Gomez-Pinilla, Dao, & So, 1997; Neeper et al., 1996) and improve cognitive performance. Our results suggest that the beneficial effects of exercise are long-lasting only for the highly active individuals, while the more moderately active individuals do not exhibit effects that are measurably different from sedentary animals', once the running wheel is removed. Neurogenesis is already high for adolescent dentate gyrus, even without exercise (Curlik 2nd, Difeo, & Shors, 2014; DiFeo, Curlik 2nd, & Shors, 2015). Perhaps it is only at the highest levels of wheel activity that BDNF is released at levels that are sufficiently high to augment neurogenesis beyond the basal levels that are already characteristically high during adolescence. This, in turn, may support increased synaptogenesis in the dorsal hippocampus, which would require a delay of more than 3–4 days, due to it being at least two synapses removed from the dentate gyrus, that then becomes manifest as improved cognition. The high level of activity may also have interfered with cognitive performance of the EX + FR group at P47–48, due to the temporal proximity to the extensive weight loss.

4.2.2 | Exercise results in short-term anxiogenesis but delayed anxiolysis

Exercise is widely believed to have anxiolytic effects (Binder, Droste, Ohl, & Reul, 2004; Salim et al., 2010). We were, therefore, surprised to find that the EX group showed increased anxiety-like behavior when tested at P47–48. Earlier reports indicated that exercise can be anxiogenic, specifically when animals are housed in isolation, due to increased corticosterone levels in isolated animals (Fuss et al., 2010; Stranahan, Khalil, & Gould, 2006). We found that a correlation between greater total wheel count and reduced anxiety-like behavior emerges by P54–55 in both EX and EX + FR (Figure 2h), even though the animals continued to be housed in isolation. The reduced anxiety-like behavior of the EX and EX + FR group of animals at P54–55 contrast the ontogenetic change observed for the CON group from P47–48 to P54–55, which is the emergence, albeit of marginal degree, in anxiety-like behavior (Figure 4). This difference across the CON versus EX and EX + FR groups together indicates that the experience of exercise during early adolescence results in apparent anxiolysis but with a delay. Whether this result reflects a delayed effect of exercise or an effect of continued neuroanatomical maturation during adolescence remains to be resolved by future anatomical experiments.

4.3 | Neuroanatomical considerations of the findings, including synaptic mechanisms

4.3.1 | Dorsal hippocampus

We previously showed that running wheel access decreased dendritic length in *stratum radiatum* of dorsal hippocampus while food restriction decreased *stratum lacunosum-moleculare* dendritic length in dorsal hippocampus at P44 (Chowdhury et al., 2014; Chowdhury, Barbarich-Marsteller, et al., 2013). The dorsal hippocampus connects with regions involved in cognitive processing of spatial information,

memory, exploration, and navigation (Fanselow & Dong, 2010). Based on these known connections and our anatomical findings, we had predicted that the dendritic retraction effects would result in impaired performance on a dorsal hippocampus-dependent cognitive task. However, both EX and FR groups showed improved performance on the active place avoidance task at P47/49. This suggests that reduced branching at P44 is not predictive of reduced functionality in the dorsal hippocampus CA1. In fact, reduced dendritic length may imply a higher input resistance and therefore increase the efficacy of individual synaptic inputs. Dorsal hippocampus, like the ventral hippocampus, exhibits protracted maturation during adolescence between P50 and P55 among sedentary animals like the CON group of this study (Chen et al., 2018). It is possible that FR and EX during P40–44 has a long-lasting influence on the developmental trajectory of the dorsal hippocampus through P55.

In the EX + FR group at P47–48, the beneficial effects of food restriction and exercise on cognitive performance may have been occluded by the increased GABAergic innervation of pyramidal neurons, which has been shown in the hippocampus of adolescent female mice (Chowdhury, Wable, et al., 2013) and rats (Chowdhury, Wable, et al., 2019), based on electron microscopic analyses performed immediately following the end of the food restriction period (P44). Rats and mice that have undergone EX + FR also exhibit increased expression of $\alpha4/\delta$-containing $GABA_A$ receptors at excitatory axo-spinous synapses of the hippocampus (Aoki et al., 2012; Aoki et al., 2014; Aoki et al., 2017; Chen, Wable, Chowdhury, & Aoki, 2016). These receptors are activated by tonically ambient GABA, as opposed to the phasically released GABA at GABAergic synapses. It has been shown previously that the location of the $\alpha4/\delta$-containing $GABA_A$ receptors at excitatory axo-spinous synapses causes tonic shunting of excitatory inputs, thereby suppressing excitability of pyramidal neurons and especially of the NMDA receptor currents, leading to impairment in the active place avoidance task (Shen et al., 2010). Another study that analyzed NMDA receptor expression at excitatory synapses of the dorsal hippocampus indicated that the experience of FR + EX increases the expression level of both NR2A- and NR2B-containing NMDA receptors at pre- and postsynaptic sites (Chen et al., 2017). While this change would have predicted that cognitive performance would be enhanced, GABAergic occlusion described above may be at play. It remains to be determined whether these changes in the GABA system recedes during the 10–11 days that follows food restriction and wheel access.

By comparison, food restriction, imposed exactly as described in this report, increases NR2B subunit expression nearly 100% at presynaptic locations, relative to CONs (Chen et al., 2017) without increases in the expression of GABAergic axon terminals (Chowdhury, Wable, et al., 2019) or $\alpha4\beta\delta$-$GABA_A$Rs (Aoki et al., 2012). Increases in presynaptic NMDARs have been implicated to enhance glutamate release (Bidoret, Ayon, Barbour, & Casado, 2009; Dore et al., 2017), thereby adding to the postsynaptic mechanism of boosting excitatory synaptic transmission. This mechanism for enhanced excitatory synaptic transmission, without the occlusion by the GABAergic system, may underlie the improved cognitive performance of the FR group.

4.3.2 | Ventral Hippocampus

FR, EX, and EX + FR all cause increased branching in *stratum radiatum* of the ventral hippocampus at P44 (Chowdhury et al., 2014). Based on the known connections of the ventral hippocampus with structures mediating motivated behaviors with strong emotional components (Fanselow & Dong, 2010), we had predicted that all three groups would show increased anxiety-like behavior. However, only the FR and EX groups showed this effect. By P55, the dendritic branching of the ventral hippocampus of the EX + FR group normalizes to that seen for the P55 CON (Chowdhury et al., 2014). This neuroanatomical normalization fits with the lack of difference in anxiety-like behavior of the EX + FR group, relative to CON.

4.4 | The interaction of food restriction and wheel access during mid-adolescence with the protracted maturation of the hippocampus during adolescence

We previously showed that the hippocampus of CON animals exhibits protracted maturation, evident as dynamic remodeling of dendrites during late adolescence (P50–60; Chen et al., 2018; Chowdhury et al., 2014). Our current result strengthens the hypothesis that adolescence is a period of particular sensitivity of the hippocampus to experience-dependent changes that become manifest with a delay of 10–11 days, extending into late adolescence and early adulthood. A number of other studies have also suggested that the hippocampus interacts with adolescent development and stress to mediate anxiety-like behavior during adulthood. For example, neonatal lesion of the ventral hippocampus results in the delayed effects of anxiety-like behavior and impaired active place avoidance in adulthood (Lee et al., 2012; Lipska, Jaskiw, & Weinberger, 1993), and early stress results in delayed effects on dorsal hippocampal development—reducing synaptic input to CA1 and CA3 at P60 (Andersen & Teicher, 2004).

4.5 | Future directions

The current study did not determine the stage of estrous cycle at the time of testing. Thus, it is possible that a larger sample size that accounts for the stages of the estrous cycle will uncover additional effects of food restriction and exercise.

We previously showed that the experiences of food restriction and exercise during adolescence do not cause atrophy but induce layer-specific changes in dendritic branching of CA1 pyramidal cells. To understand how the anatomical changes in the *stratum radiatum* and *stratum lacunosum-moleculare* of dorsal and ventral CA1 are contributing to the behaviors of anxiety-like behavior and spatial cognition, it is necessary to examine how these neuroanatomical changes functionally affect the various inputs that are converging at the hippocampal CA1 as well as the efferent targets, including the amygdala and hypothalamus that also regulate anxiety-like behavior. Future studies would benefit from incorporating electrophysiology and

anatomical analyses with behavior to strengthen a causal link between anatomical and synaptic circuit changes that are involved in the behaviors of wheel running, food anticipation, anxiety-like behavior, and learning.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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