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Using Conditioned Place Preference to Investigate Changes in the Rewarding Effects of Running and Eating Following Activity-Based Anorexia

by

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We investigated whether activity-based anorexia would enhance the rewarding effects of running, causing running to be more rewarding than eating. This involved conditioning mice to associate one side of a conditioned place preference box with food and the other side with a running wheel. Following conditioning, mice were separated into the following three conditions: home cage control, food restricted control, and activity-based anorexia. On the last day of exposure to the activity-based anorexia model, hungry mice were tested a second time in the conditioned place preference box to determine if exposure to the model affected their preference for either the wheel- or the food-associated chamber. We found that home cage controls showed no preference for either side, while the food restricted control group preferred the food-associated chamber more than wheel-associated chamber. While a subset of activity-based anorexia mice showed the same preference as the food restricted control group, another subset preferred the wheel-associated chamber over the food-associated chamber. Interestingly, neither group showed a preference for either side following a 24-hour recovery period, during which they were allowed free-access to food and their weights returned to baseline.

Key words: activity-based anorexia, food restriction, conditioned place preference, reward
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Anorexia nervosa affects over 30 million people in the United States and has been associated with the highest mortality rate of all psychiatric disorders (ANAD, 2017). Anorexia also has a high rate of comorbidity with depression, anxiety, and substance abuse (Eating Disorder Hope, 2016). This disorder primarily affects females with an onset that usually occurs during adolescence. Individuals who suffer from anorexia nervosa have lost 15% or more of their original body weight, have an intense fear of gaining weight and lose their menstrual cycle. In addition, they tend to exhibit perfectionistic tendencies and often engage in excessive exercise (The Center for Eating Disorders, 2017). Anorexia is also associated with a high rate of relapse, with 30-50% of treated patients requiring hospitalization within one year. For these reasons, understanding the neural basis of anorexia nervosa is essential.

Activity-based anorexia is the most commonly used animal model of anorexia nervosa and is based on observations initially made in 1922 by C.P. Richter. He noticed that rats in his laboratory would increase their running wheel activity when deprived of food (Richter, 1922). A follow-up experiment conducted by Routtenberg and Kuznesof (1967) aimed to further investigate this observation by combining food restriction and wheel running in adult male rats. They found that rats given between 30 minutes and 1 hour to eat an unlimited amount of food would decrease their food intake and increase their wheel running with each day of food restriction, leading to significant weight loss. Remarkably, all of the rats in the experiment died. These findings demonstrate several similarities between the activity-based anorexia model and anorexia nervosa in humans. Both involve severe dietary restriction, extreme weight loss, and hyperactivity/excessive exercise. Similar to what is found in people with anorexia nervosa, the
activity-based anorexia model also leads to a loss of menstrual/estrous cycle and an enhanced stress response. In addition, female rats appear to be more vulnerable than males and adolescents are more vulnerable than adults.

It is currently not known why food restriction leads to an increase in running wheel activity in rodents, especially when conserving energy would increase their survival rate. Furthermore, it is not clear why rodents in captivity engage in wheel running activity when they are not food restricted. One possibility is that rodents find wheel running rewarding. Evidence in support of this idea is found in a study conducted by Meijer & Robbers, 2014. They placed a wheel in both urban and dune areas and lured animals to the wheel by placing a bowl of food nearby. They found that several different species used the wheel (mice, snails, shrews, etc.), but wild juvenile mice did the vast majority of wheel running. Levels of running increased during the night in both dune and urban areas, which is consistent with their nocturnal behavior. Interestingly, when food was removed from the wheel, visits to the wheel increased by 42% in mice and dropped in other animals. This study demonstrates that wheel running is not a behavior only demonstrated by rodents in captivity and indicates that it is intrinsically rewarding.

Based on evidence that wheel running is rewarding for rodents, we hypothesized that combining food restriction with wheel running in the activity-based anorexia model enhances the rewarding effects of running such that running becomes more rewarding than eating (Lett, Grant, Byrne, & Koh, 2004). To test this hypothesis, we conducted experiments that combined activity-based anorexia with conditioned place preference (CPP). Conditioned (or contextual) place preference is a form of Pavlovian conditioning that is often used in addiction studies to measure the rewarding effects of drugs of abuse. CPP generally involves training animals to associate one side of a two-chamber box with a drug and the other side with a neutral stimulus (e.g.
saline). After several days of conditioning, the animal is allowed to freely explore both sides of the CPP box, drug-free. If the animal spends more time on the drug-associated side than the saline-associated side, then the conclusion is that the drug was rewarding (Kelley & Rowan, 2004; Sora et. al., 1998; Zheng, Vaca, & Carr, 2012). Instead of using a drug, we conditioned animals to associate one side of the CPP box with a running wheel and the other side with food, so that we could evaluate the rewarding effects of running and eating. Then we tested whether exposure to the activity-based anorexia model alters preference for the food- or wheel-associated chamber. We hypothesized that a food-restricted control group would spend more time in the food-associated chamber than the wheel-associated chamber and that animals in the activity-based anorexia condition would show the opposite behavioral response.

**Method**

**Subjects**

Eight adolescent female 129Sv/Ev mice (Taconic Biosciences, Germantown, NY) were used for the pilot study (described below). Twenty-four adolescent female C57BL/6 mice (Taconic Biosciences, Germantown, NY) were used in the two subsequent experiments. Experiment 1 consisted of eight mice separated into two groups; half were in the food restricted group (N=4) and half were in the activity-based anorexia group (N=4). Experiment 2 consisted of sixteen mice that were separated into three groups: the home cage control group (N=6), the food restricted group (N=5), and the activity-based anorexia group (N=5). All mice were on a 12-hour light/dark schedule. With the exception of experiment 2, all mice were on a 5 a.m. / 5 p.m. light/dark schedule throughout the experiment. For experiment 2, mice were on a 6 a.m. / 6 p.m. light/dark schedule, due to daylight savings.
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Apparatus

The conditioned place preference box (Columbus Instruments, Columbus, OH) had two chambers adjacent to each other and an outside holding chamber. One chamber contained black walls and a black floor and the other chamber contained white walls a white floor. There was a clear holding chamber to the side that provided access to the white and black chambers. Each chamber measured 8.25” L x 12” H x 12” W. The holding chamber measured 3.5” H x 3.5” W x 5” deep. The conditioned place preference box also had a removable divider that was black on one side and white on the other that separated the white and black compartments.

Procedure

Mice arrived at the animal facility at Hunter College at postnatal day 21. They were housed in groups of four/cage. During the initial housing and conditioning phases, food and water were accessible *ad libitum*. Mice were housed with autoclaved beta bedding and were given Isopro food throughout the entire experiment. Prior to each experiment (Pilot Study, Experiment 1, Experiment 2), mice were brought to the behavior room at postnatal day 38 (mid-adolescence) where they were weighed and handled by the experimenter for two minutes. Mice had their tails marked with a colored Sharpie for identification purposes.

Pilot Study: In an effort to reduce innate preference for the dark chamber, we conducted a pilot study in which the conditioned place preference box was modified in six different ways and preference was tested. Preference was tested in the same 129Sv/Ev mice (N=8) following each modification and began postnatal day 40, after two days of handling. During each preference test, mice were placed in the clear holding chamber with access to the two chambers of the CPP box. If they did not leave the holding chamber within two minutes, the experimenter nudged them forward and closed the door to the holding chamber behind them. Mice were then
allowed free access to both sides of the CPP box for 30 minutes. Time spent on each side of the chamber was recorded and quantified with ANYMaze software.

Modifications of the CPP box: In the first condition (Figure 1), the CPP box had a red floor with white textured tape on it, surrounded by two white walls and a wall with a black insert. The adjacent side had a yellow floor with a black wall and two white inserts. In the second condition (Figure 2), the CPP box had a white floor surrounded by two white walls and a black insert. The adjacent side had a yellow floor surrounded by a black wall, a white insert, and a wall with black and white checkered wallpaper. In the third condition (Figure 3), the CPP box had a white floor surrounded by two white walls and a black insert with white vertical stripes. The adjacent side had a yellow floor with a black wall, a white insert, and a white insert with black horizontal stripes. In the fourth condition (Figure 4), the CPP box had a white floor surrounded by two white walls and a black insert with white vertical stripes. The adjacent side had a red floor with textured white tape on it, surrounded by a black wall, a white insert, and a white insert with black horizontal stripes. In the fifth condition (Figure 5), the CPP box was modified in the same way as the fourth condition, except both floors were red. In the sixth condition (Figure 6), the CPP box remained the same as the previous condition, except the floor was changed to a uniform white. The CPP box was modified as described in the sixth condition for Experiment 1.

Conditioned Place Preference (preconditioning and postconditioning 1) (Experiments 1 and 2): After two days of handling, mice were placed in the clear holding chamber with access to the two modified chambers of the CPP box. If they did not leave the holding chamber within two minutes, the experimenter nudged them forward and closed the door to the holding chamber behind them. Mice were then allowed free access to both sides of the CPP box for a 30-minute preconditioning session. After preconditioning, the mice were assigned to their experimental
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groups (home cage control, food restricted, or activity-based anorexia), which were counterbalanced across the cages the mice were housed in, their weights, and any initial preference for a side in the conditioned place preference box. The next day, conditioning began and took place over eight days. During conditioning (days 1, 3, 5, 7) mice were confined to one side of the CPP box containing either a running wheel or a dish of food for 30 minutes. On alternating days (days 2, 4, 6, 8), mice were confined to the opposite side of the CPP box for 30 minutes, which contained the other stimulus (running wheel or food). During conditioning, one side of the chamber consistently contained one item (e.g. running wheel) and the other side of the chamber consistently contained the other item (e.g. food), so that animals would learn to associate each side with the corresponding object. After eight days of conditioning, the mice were tested for their preference, during which time they had free access to both sides of the chamber for 30 minutes (postconditioning 1). Food and running wheels were not present during preconditioning or postconditioning tests. Mouse behavior was recorded during each phase of CPP with an overhead video camera.

Activity-Based Anorexia: Immediately after postconditioning 1, each mouse was individually housed with a running wheel and unlimited access to food. The running wheel was locked for mice in the food restricted (FR) and home cage (HC) groups and was not locked for mice in the activity-based anorexia (ABA) group. Baseline measurements (body weight, food intake, water intake) were then recorded the three following days.

On Baseline Day 3, all food was removed from mice in the FR and ABA groups two hours after the onset of the dark cycle. The next day (ABA Day 1), all animals were weighed and an unlimited amount of food was provided to mice in the FR and ABA groups during the first two hours of the dark cycle. All mice remained in the activity-based anorexia model until
the ABA mice lost approximately 25% of their baseline body weight, at which point their preference was tested in the CPP box (see below).

**Conditioned Place Preference (postconditioning 2 and 3):** During postconditioning 2, ABA and the lowest weighing mice in the FR and HC conditions were placed in the CPP box for 30 minutes where they were free to explore both the food- and wheel-associated sides of the box. Following testing, running wheels were removed, mouse igloos were provided, and mice received unlimited access to food. Following one night of recovery, mice and food were weighed and preference was tested in a postconditioning 3 test. No food or wheels were present in the CPP box during postconditioning 2 or 3.

**Data Analysis and Statistics**

Videos were analyzed using ANY-maze software (Stoelting, Wood Dale, IL) to determine time spent on each side of the conditioned place preference box. Results were analyzed using a two-tail Student’s t-test.

**Results**

**Pilot Study**

During the first condition (no wallpaper_red floor), mice spent significantly more time on the modified light side ($\bar{x} = 1761.2$ seconds) than the modified dark side ($\bar{x} = 53.83$ side) ($p < 0.0001$). During the second condition (wallpaper_no red floor), mice spent significantly more time on the modified light side ($\bar{x} = 1412.94$ seconds) than the modified dark side ($\bar{x} = 394.99$ seconds) ($p = 0.009$). During the third condition (stripes_no red insert), mice spent an average of 1151.59 seconds on the modified light side and 629.89 seconds on the modified dark side ($p = 0.216$). During the fourth condition (stripes_red insert), mice spent significantly less time on the modified light side ($\bar{x} = 463.08$ seconds) than the modified dark side ($\bar{x} = 1336.59$ seconds) ($p =$
0.01). During the fifth condition (red insert_both sides), mice also spent significantly less time on the modified light side ($\bar{x}=163.21$ seconds) than the modified dark side ($\bar{x}=1631.21$) ($p < 0.0001$). During the final condition (white insert_both sides), mice spent an average of 612.99 seconds on the modified light side and an average of 1191.69 seconds on the modified dark side ($p = 0.147$) (Figure 7).

**Experiment 1**

**Conditioned Place Preference:** The CPP box was modified as described for the sixth condition. During preconditioning, mice spent significantly more time on the horizontal side ($\bar{x}=1222.53$ seconds) than the vertical side ($\bar{x}=577.46$ seconds) of the conditioned place preference box; $t(7) = 2.36$, $p < 0.01$ (Figure 8), indicating an innate preference for the chamber with horizontal stripes. During postconditioning 2, which was after mice were exposed to the activity-based anorexia model, both ABA and FR mice spent more time in the wheel-associated chamber than the food-associated chamber (ABA $t(3) = 3.18$, $p = 0.15$; FR $t(3) = 3.18$, $p = 0.13$) (Figure 9). Given the initial preference for the side of the CPP box with horizontal stripes, we next limited our analysis to the item (food or wheel) that was associated with the non-preferred side (vertical stripes) and compared time spent on that side before and after exposure to the ABA model. This involved calculating a CPP score for time spent on the non-preferred side (postconditioning 2 – postconditioning 1). To increase our N, we combined data from experiment 1 ($N=2$/group) with data collected in a previous CPP experiment conducted by Rachael Langa in which the CPP box was not modified and the food and wheel were paired with the light or dark compartments ($N=3$-$4$/group). We found a trend for ABA mice to spend more time on the wheel-associated side than the food-associated side ($p = 0.05$) and the FR group to spend more time on the food-associated side than the wheel-associated side ($p = 0.2$) (Figure 10).
Activity-Based Anorexia: All ABA mice reached criteria for removal (loss of 25% or more baseline body weight) by the third day of food restriction. We found that mice in the ABA condition lost significantly more weight than mice in the FR condition with each day of food restriction (Figure 11). When body weight was calculated as the percentage of baseline body weight, the ABA group weighed significantly less than the FR group on ABA days 2 ($t(3) = 3.18, p = 0.002$) and 3 ($t(3) = 3.18, p = 0.0006$) (Figures 11 and 12). While there was no significant difference between groups in food intake when mice were food restricted, there was a strong trend for ABA mice to eat less than FR mice on the last day of food restriction ($p = 0.056$). Furthermore, both groups ate significantly less when they were food restricted compared to when they were not food restricted during baseline days ($t(3) = 3.18, \text{FR and ABA } p < 0.001$) (Figure 13). Consistent with what has been previously reported in this model, we found that mice in the ABA condition ran significantly more when food restricted than when they had unlimited access to food during baseline. When average running across all three days of baseline was compared to average running across all three days of food restriction, we found that mice ran significantly more when food restricted during the first half of the light cycle ($t(3) = 3.18, p = 0.002$) and the second half of the light cycle ($t(3) = 3.18, p = 0.001$) (Figure 14). While there was a trend for running to increase during the dark cycle ($p = 0.10$), this increase was not significant during either the first half of the dark cycle ($p = 0.25$) or the second half of the dark cycle ($p = 0.07$) (Figure 15).

Experiment 2

Conditioned Place Preference: To reduce innate preference for either side of the CPP box, one white insert covered the remaining black wall of the CPP box (Figure 16). During preconditioning, all mice spent nearly an equal amount of time on the vertical ($\bar{x} = 859.06$
seconds) and horizontal ($\bar{x} = 940.94$ seconds) sides of the conditioned place preference box ($t(15) = 2.13, p = 0.36$) (Figure 17). During postconditioning 1, mice spent significantly more time on the food-associated side ($\bar{x} = 952.86$ seconds) than the wheel-associated side ($\bar{x} = 847.13$ seconds) ($t(15) = 2.13, p < 0.05$) (Figure 18). During postconditioning 2, which was after exposure to the activity-based anorexia model, animals in the FR groups spent significantly more time in the food-associated side ($\bar{x} = 1096.14$) than the wheel-associated side ($\bar{x} = 703.86$) ($t(4) = 2.77, p = 0.004$). In contrast, animals in the ABA group spent similar amounts of time on both sides (food $\bar{x} = 1061.58$ s, wheel $\bar{x} = 738.42$ s) ($t(4) = 2.77, p = 0.27$). Similarly, animals in the HC group showed no preference for either side (food $\bar{x} = 832.22$ s, wheel $\bar{x} = 967.78$ s) ($t(4) = 2.77, p = 0.40$) (Figure 19). However, there were two distinct responses to ABA, such that one subset of animals showed a preference for the food-associated side and the other subset of animals showed a preference for the wheel-associated side (Figure 20). When we calculated the percentage of time spent in each chamber during postconditioning 2, we found that a subset of ABA mice spent significantly more time on the food-associated (75.94%) than the wheel-associated (24.06%) sides ($t(2) = 4.30, p = 0.001$) and the other subset of ABA mice spent significantly more time on the wheel-associated (66.47%) than the food-associated (33.53%) sides ($t(1) = 12.70, p = 0.04$). As expected, the FR group spent significantly more time on the food-associated side (60.90%) than the wheel-associated (39.10%) side ($t(4) = 2.77, p = 0.004$) and the HC group spent a similar amount of time in the food- (46.23%) and wheel-associated sides (53.77%) ($t(5) = 2.57, p = 0.40$) (Figure 20).

During postconditioning 3, which was while animals were weight recovered, we found that the subset of ABA mice that had previously spent more time on the food-associated side than the wheel-associated side continued to demonstrate this preference. Specifically, they spent
an average of 58.35% of their time on the food-associated side and an average of 41.65% of their
time on the wheel-associated side (t(2) = 4.3, p = 0.001). Similarly, the subset of ABA mice that
had previously spent more time on the wheel-associated side continued to spend significantly
more time on the wheel- (\(\bar{x} = 51.30\%\)) than the food-associated side (\(\bar{x} = 48.70\%\); t(1) = 12.71, p
= 0.001), although to a lesser extent. The FR group still spent significantly more time in the
food-associated side (\(\bar{x} = 64\%\)) than the wheel-associated side (\(\bar{x} = 36\%\); t(4) = 2.77, p = 0.01). As
expected, mice in the HC group spent a similar amount of time in the food- (\(\bar{x} = 50.17\%\)) and
wheel- (\(\bar{x} = 49.83\%\)) associated sides (t(5) = 2.57, p = 0.97) (Figure 28).

Activity-Based Anorexia: ABA mice reached criteria for removal (loss of 25% or more baseline
body weight) between days 3-6 of food restriction. While the ABA group did not weigh
significantly less than the FR group on any individual ABA day (Figure 21), a comparison of
groups on the last day each mouse was food restricted revealed that ABA mice weighed less (\(\bar{x} =
75.29\%\) baseline body weight) than the FR control group (\(\bar{x} = 81.47\%\) baseline body weight) (t(4)
= 2.77, p = 0.02) (Figures 22). During baseline, food intake was similar across groups (HC \(\bar{x} =
4.39g\), FR \(\bar{x} = 4.89g\), ABA \(\bar{x} = 4.39g\)), but during food restriction, both food restricted groups ate
significantly less than the HC group (t(4) = 2.77, HC vs. FR, p < 0.001; t(4) = 2.77, HC vs. ABA,
p < 0.001). We found no significant difference between the FR and ABA groups in food intake
during food restriction (t(4) = 2.77, FR \(\bar{x} = 1.47g\), t(4) = 2.77, ABA \(\bar{x} = 1.67g\)) (p = 0.26). (Figure
23). Consistent with what has been previously reported in this model, we found that mice in the
ABA condition ran significantly more when food restricted compared to when they were not
food restricted. Running was averaged across all three days of baseline and compared to the
average amount of running each animal exhibited while food restricted. We found that mice ran
significantly more when food restricted during the first half of the light cycle \((t(4) = 2.77, p = 0.01)\), the second half of the light cycle \((t(4) = 2.77, p = 0.0001)\), and the second half of the dark cycle \((t(4) = 2.77, p = 0.0004)\) (Figures 24 and 25).

Immediately following preference testing (postconditioning 2), wheels were removed and mice were given unlimited access to food. The next day, we found that body weight recovered and almost reached baseline levels \((ABA \bar{x} = 94.44\% \text{ baseline}; FR \bar{x} = 95.78\% \text{ baseline})\) (Figure 26). During this recovery period, animals in both the ABA and FR conditions ate significantly more than they did at baseline \((t(4) = 2.77, ABA \ p = 0.0007; t(4) = 2.77, FR \ p = 0.0002)\) (Figure 27).

**Discussion**

Consistent with previous studies conducted in rat, our results demonstrate that combining unlimited access to a wheel with limited access to food leads to significantly more weight loss in adolescence female mice than food restriction alone. This weight loss was accompanied by increases in running wheel activity that was most pronounced during the light cycle, which is when mice are normally sleeping. This shift in circadian rhythm may contribute to changes in the stress response previously reported with this model. Unlike what has been reported in rat, we did not find a consistent difference in food intake between mice in the ABA and FR conditions. Although there was a trend for ABA mice to eat less than FR mice in Experiment 1, this was not replicated in Experiment 2, indicating that additional weight loss exhibited by mice in the ABA group can primarily be attributed to running behavior.

In Experiment 1, we found that ABA and FR groups exhibited a slight and non-significant preference for the wheel-associated side over the food-associated side of the CPP box (Figure 9). This is surprising given that animals were hungry when they were tested and it is
expected that the FR control group would demonstrate a strong preference for the food-associated side over the wheel-associated side. However, these results are confounded by innate preference for the horizontal side prior to conditioning. As a result, two mice learned to associate the wheel with the preferred side and two mice learned to associate food with the preferred side. This pre-existing preference limits the ability to detect increases in time spent on the preferred side during postconditioning sessions. We therefore excluded these 4 mice from the analysis and instead included data collected by Rachael Langa in a previous study. In that study, the methods were identical to those used in our study, but the CPP box was not modified and mice showed a clear innate preference for the dark chamber during preconditioning. After combining the data, we limited our analysis to the item (food or wheel) that was associated with the non-preferred side (vertical stripes or light chamber) and compared time spent on that side before and after exposure to the ABA model. Consistent with our hypothesis, the results revealed a trend for the FR group to spend more time on the food-associated side than the wheel-associated side. In contrast, there was a non-significant trend for ABA mice to spend more time on the wheel-associated side than the food-associated side, indicating that exposure to the activity-based anorexia model may make running more rewarding than eating.

Additional modification of the CPP box in Experiment 2 prevented innate preference for either side of the CPP box, which allowed us to directly test whether exposure to the activity-based anorexia causes animals to spend more time in the wheel-associated chamber than the food-associated chamber. We found that animals that had not been food restricted (HC group) spent a similar amount of time in both chambers, indicating no preference for either side. As expected, food restricted animals that were not housed with a running wheel (FR group) spent more time in the food-associated chamber than the wheel-associated chamber, consistent with
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foraging behavior. Interestingly, there was a subset of food restricted animals that were housed with a wheel (ABA group) that behaved liked animals in the FR group and spent more time on the food-associated side than the wheel-associated side, indicating a preference for eating over running. However, there was a separate subset of ABA mice that spent more time on the wheel-associated side than the food-associated side, indicating a preference from running over eating. These data are suggestive of alterations in the reward pathway following exposure to the activity-based anorexia model and are consistent with what has been documented in a subset of patients with anorexia nervosa who are consumed with activity-based behaviors.

Following the second postconditioning test, wheels were removed, food was provided ad libitum, and mice were allowed to recover overnight. We found that after one night of unlimited access to food, both the food restricted and activity-based anorexia groups reached their baseline weights (~96%). Interestingly, recovery did not alter which side was preferred, but the strength of the preference was lessened. Although the subset of ABA mice that had a significant preference for the food-associated side maintained that preference, they spent 18% less time on that side following recovery. Similarly, the subset of ABA mice that had previously spent significantly more time on the wheel-associated side, continued to prefer that side during recovery, but spent 15% less time there. These results indicate that preference persists when animals have recovered from the ABA model, although it is possible that this would not be the case if given a longer recovery time.

Further research is required to identify what molecular changes in the brain correlate with activity-based anorexia-induced alterations in reward circuitry. For example, this could be explored by repeating Experiment 2 and perfusing mice 90 minutes after post-conditioning 2 so that changes in immediate early gene protein expression can be visualized in the nucleus.
accumbens. Additional analyses of running behavior during the 2-hour period when food is available are also required to see if there is a correlation between running and subsequent preference during postconditioning 2. Such analyses may reveal a clear distinction between the subset of ABA animals that show the same preference as FR animals and the subset of ABA animals that show preference for the wheel-associated side over the food-associated side. A final additional experiment would involve testing the effects of activity-based anorexia on conditioned place preference when one side is associated with a wheel and the other side is not associated with food, in which case there would be no conflict between the two sides. It is possible that once the conflict is removed, exposure to ABA will enhance preference for the wheel-associated side of the CPP box in all mice. Together, this work provides some evidence that a subset of mice exhibit excessive running in the activity-based anorexia model because reward circuits have changed in such a way that they prefer running over eating. The neural basis of this effect will be the focus of future research.
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Figures

Figure 1. First condition in pilot study: no wallpaper_red floor

Figure 2. Second condition in pilot study: wallpaper_no red floor
Figure 3. Third condition in pilot study: stripes_no red insert

Figure 4. Fourth condition in pilot study: stripes_red insert
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Figure 5. Fifth condition in pilot study: red insert_both sides

Figure 6. Sixth condition in pilot study: white insert_both sides
Figure 7. Time spent on each side of the conditioned place preference box during the pilot study.

Figure 8. Time spent on each side of the conditioned place preference box during preconditioning.
Figure 9. Percentage of time ABA and FR groups spent on each side of the conditioned place preference box during postconditioning 2.

Figure 10. Increases in time spent on the food- and wheel-associated sides of the CPP box after exposure to the activity-based anorexia model. The CPP score was determined by subtracting
time spent on the corresponding side during postconditioning 2 – time spent on that side during postconditioning 1. Only time spent on the non-preferred side was included in this analysis.

**Figure 11.** Percent baseline body weight during each day of food restriction.

**Figure 12.** Percent baseline body weight on last day in the ABA model.
**Figure 13.** Average food intake during baseline and food restricted days across groups.

**Figure 14.** Running wheel activity of ABA mice during the first and second halves of the light cycle. Wheel counts before (baseline) and during food restriction (ABA days) are shown.
Figure 15. Running wheel activity of ABA mice during the first and second halves of the dark cycle. Wheel counts before (baseline) and during food restriction (ABA days) are shown.

Figure 16. Modifications of the CPP box for Experiment 2.
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**Figure 17.** Time spent on each side of the conditioned place preference box during preconditioning.

**Figure 18.** Time spent on food- and wheel-associated sides during post-conditioning 1.
**Figure 19.** Time that HC, FR, and ABA groups spent on food- and wheel-associated sides during post-conditioning 2.

**Figure 20.** Percentage of time spent on the food- and wheel-associated sides of the CPP box during postconditioning 2. There appears to be two separate behaviors that emerged in the ABA group.
**Figure 21.** Percent baseline body weight during each day of food restriction

**Figure 22.** Percent baseline body weight on last day in the ABA model. Weight on last day in model for the ABA group was significantly lower than for the FR group.
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**Figure 23.** Average food intake during baseline and days of food restriction.

**Figure 24.** Running wheel activity of ABA mice during the first and second halves of the light cycle. Wheel counts before (baseline) and during food restriction (ABA days) are shown.
Figure 25. Running wheel activity of ABA mice during the first and second halves of the dark cycle. Wheel counts before (baseline) and during food restriction (ABA days) are shown.

Figure 26. Percent baseline body weight during baseline, food restriction and after recovery.
**Figure 27.** Food intake during baseline, food restriction, and recovery.

**Figure 28.** Time spent on food- and wheel-associated sides during post-conditioning 3 by mice in each condition.