Video Article

Assessing Activity-based Anorexia in Mice

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Abstract

Rodents develop activity-based anorexia (ABA) when exposed to a restricted feeding schedule and allowed free access to a running wheel. These conditions lead to a life-threatening reduction in body weight. However, rodents exposed to only one of these conditions ultimately adapt to re-establish normal body weight. Although increased running coupled with reduction in voluntary food intake appear paradoxical under ABA conditions, ABA behavior is observed across numerous mammalian species.

The ABA paradigm provides an animal model for anorexia nervosa (AN), an eating disorder with severe dysregulation of appetite-behavior. Subjects are singly housed with free access to a running wheel. Each day, the subject is offered food for a limited amount of time. During the course of the experiment, a subject's body weight decreases from high activity and low caloric intake. The duration of the study varies based on how long food is offered daily, the type of food offered, the strain of mouse, if drugs are being tested, and environmental factors.

A lack of effective pharmacological treatments for AN patients, their low quality of life, high cost of treatment, and their high mortality rate indicate the urgency to further research AN. We provide a basic outline for performing ABA experiments with mice, offering a method to investigate AN-like behavior in order to develop novel therapies. This protocol is optimized for use in Balb/cJ mice, but can easily be manipulated for other strains, providing great flexibility in working with different questions, especially related to genetic factors of ABA.

Video Link

The video component of this article can be found at https://www.jove.com/video/57395/

Introduction

Since 1953, rodents have been reported to show a paradoxical hyperactivity on running wheels when they are given free access to the wheels while undergoing voluntary hypophagia when food availability is restricted[1]. Conversely, rodents do not rapidly drop in body weight when offered food on a schedule without running wheels or when housed with running wheels and offered food *ad libitum*[1,2,3]. The ABA model reliably results in dramatic declines in body weight, hypophagia, hypothermia, loss of estrus, and increased stimulation of the HPA axis[4]. Ultimately, ABA results in death, unless the subject is removed from the paradigm[5]. The ABA paradigm provides researchers with an animal model of AN, a complex eating disorder which features severe dysregulation of appetite-behavior, affecting approximately 1 in 100 females and a smaller percentage of males[6]. Patients suffering from AN frequently exhibit hyperactivity, consisting of extreme amounts of exercise, and/or general restlessness[7,8]. With a mortality rate of approximately 10%, AN has the highest mortality rate among all psychiatric disorders[9]. Current treatment for AN is limited to cognitive therapies, as there are no approved pharmacological treatments for those suffering from AN[10,11].

AN has typically been regarded as a disorder affecting primarily females. As a diagnosis that is 10 times more likely in females than males, female subjects are traditionally the focus in AN[12]. However, special consideration should be taken in excluding males from studies. While the diagnosis of AN remains lower in males, muscle dysmorphia (MD) is a condition that has many similarities to AN, in that body image is distorted and diet is often disordered. There is support for the notion that MD and AN may be classified in a similar manner[13,14,15]. This may suggest that some cases of MD represent the "male version" of AN. In the context of animal models, some reports have suggested that males are more susceptible than females to the ABA paradigm. For example, a recent study showed a higher mortality rate and decreased food intake compared to females in C57Bl/6 mice[16]. One predictor of susceptibility to ABA is spontaneous physical activity (SPA). Rats with higher or lower SPA are more likely to lose weight in the ABA paradigm, with male rats showing a stronger effect than females[17]. Conversely, female rodents have been observed to exercise more than males during the restriction phase of ABA[18]. Additionally, studies with Balb/cJ mice have shown the opposite effect of C57Bl/6 mice, where female mice have a higher mortality rate and decreased food intake compared to males (**Figure 1**)[6]. With varying results between the sexes in the ABA paradigm and increasing awareness of males with disordered eating patterns, both male and female subjects should be tested.

Aside from sex differences in the ABA paradigm, age and strain must be considered when choosing subjects. Adolescent mice may more accurately model AN, since AN typically emerges in adolescence, as observed with rats and mice[19,20,21,22]. Strains that are more active than others at a baseline level have a higher rate of susceptibility and severity of ABA[23]. Strains known to have higher levels of anxiety, such as

DBA/2, have increased wheel running activity, which would indicate a faster dropout rate in the ABA paradigm[24]. Depending on the experimental design, the strain of choice can be tailored to maximize the duration of ABA.

The ABA paradox is not unique to mice. Other mammals including rats, hamsters, gerbils, pigs, chipmunks, and guinea pigs have demonstrated this phenomenon[6]. The conservation of the ABA phenomenon across mammalian species suggests that the ABA paradigm can provide a translational tool to investigate the mechanisms underlying anorexia-like behavior in humans. Mice in particular are well-suited for the study of mechanisms underlying ABA. Mice can be densely housed and generation time is relatively short. Mice have a fully sequenced genome, and numerous inbred, outbred, and special strains, such as congenics, are available. A vast number of genetically manipulated lines have been generated, making them ideal for studies assessing genetic influences on disorders such as AN. Depending on the question at hand, researchers may manipulate complex neural circuitry and/or gene expression to assess behavior in the ABA paradigm, potentially answering questions about genetic influence that are not possible when studying humans.

A limited number of animal models of AN currently exist. Stress models induce hypophagia in rodents using tail pinching, novelty-induced hypophagia, cold swimming, and brain stimulation. By inducing stress, changes in the HPA-axis decrease appetite resulting in reduced body weight[25]. However, the HPA-axis is also potently stimulated by ABA, which also incorporates additional features of AN such as hyperactivity. Another model to consider in studying AN is the chronic food restriction model. By limiting food to a range from 40 to 60% of *ad libitum,* one can mimic the physiological response to malnourishment[26]. Though this method is effective for studying the effects of inadequate feeding, it does not reproduce a core issue of AN, which is voluntary food restriction. In the ABA paradigm, animals are deprived of food access for part of the day, but also voluntarily reduce food intake if a wheel is also present. Genetic models have also been used to examine the etiology of AN. Researchers have found neurochemical and genetic factors implicated in AN, such as the gene BDNF and neurotransmitters dopamine and serotonin[27]. The use of genetic models is crucial to understand the neural mechanisms behind AN. However, genome wide association studies for AN have not yet yielded significant hits, and no rare variants in AN have been identified. Future studies should combine a genetic approach with the ABA model to increase understanding of AN-related phenotypes.

Developing animal models for entire psychiatric disorders is virtually impossible due to the complexity and heterogeneity of human disorders. However, by modeling specific, well-defined components of a psychiatric disorder, unique insights into the underlying neurobiology or pathophysiology may be obtained. Such biological insights may then be used to identify novel treatments. The rodent ABA paradigm therefore provides a preclinical tool for studying the mechanisms underlying AN-like behavior that cannot ethically be studied in humans, such as the effects of genetic manipulations, perturbations to neural circuits, and the consequences of certain environmental factors.

Protocol

All methods described here have been approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, San Diego.

1. Mice

1. Choose the appropriate mouse strain for the study.
2. Purchase mice from a supplier or amplify a line to obtain appropriate experimental numbers.
3. Group-house purchased mice in the animal facility for at least 1 week prior to beginning the study to allow for an ample acclimation period.
4. Begin the pre-experimental acclimation phase when mice are 8 weeks old.

2. Housing

1. Choose cages that are large enough to contain an un-obstructed running wheel, food receptacle, and a water bottle.
2. Regulate room temperature and humidity to complement the hypothesis being tested.
   NOTE: Higher temperatures will reduce the development of ABA[28].

3. Running Wheels

1. Choose wheels with wireless communication to avoid potential distractions and entanglements within the cage, see the **Table of Materials** for an example.

4. Water Bottles

1. Select a water bottle that will not compete for space with the running wheel or food receptacle in the home cage.
2. If providing drug treatment in the drinking water, duct tape the bottle to create a light-sensitive environment.

5. Food

1. Select a low-fat chow appropriate for the hypothesis being tested (*e.g.*, Envigo's Rodent Diet 8604).
   NOTE: Diets high in fat and sugar can reduce the development of ABA[29].
2. Use a small glass jar to provide chow, approximately 65 cm in diameter x 50 cm in height.
   NOTE: An automated feeder may be used instead of a glass jar, which may reduce stress by limiting investigator interference. This may be most advantageous if performing ABA studies with drug manipulations.

6. Pre-experimental Acclimation Phase

1. Set up the experimental area by choosing a cage rack close to the laptop and wheel hubs. This will limit issues with data transmission.
2. Turn on the laptop and wireless hubs.
3. Open the wheel management software on the laptop.
    1. Look at the software to ensure both hubs are active.
    2. Open "tools" and select "delete wheels." Repeat this step 5 times.
    3. Close the software, then re-open it.
4. Place 3 AAA batteries into the battery pack of a wheel base.
    1. Connect the battery pack wires to the hardware of the wheel base. This will be disconnected prior to use. Close the battery pack into the wheel base.
5. Check the software to ensure the wheel base is listed with the ID "1" under the appropriate wireless hub. If the base is not listed as ID "1", unhook the battery pack, and start over at step 6.2.
6. Change the wheel ID from "1" to the mouse ID under the name column.
7. Adhere the running wheel's base to the bottom of the cage with duct tape to ensure stability. Do not allow any tape to stick out, as mice will chew on exposed tape.
8. Choose a running wheel disc without a corroded magnetic piece.
9. Place the wheel on the base and rotate the wheel to verify clearance from the cage walls and grating.
10. Spin the disc a specific number of rotations to ensure the wheel running software is properly counting rotations.
11. Prevent pieces of chow from being lost in the bedding or getting wet by duct-taping the underside of the food jar to the cage floor, away from the running wheel and the water bottle.
    NOTE: Wet chow increases food intake compared to dry chow[30].
12. Place 5 pieces of chow into the jar.
    NOTE: The chow during the pre-experimental phase is available *ad libitum* and does not need to be weighed. Exposure to running wheels during the acclimation period may intensify ABA[21,31].
13. Place a full water bottle in the cage and provide water *ad libitum*.
14. Repeat until all cages have a wirelessly connected running wheel, food, and water.
15. Individually place mice into their appropriate cages.
16. In the wheel managing software, go to "file" and click "start acquisition."
17. Watch the software update to ensure wheel rotations are being counted and the system is working well.
18. Acclimate mice to the experimental housing conditions for 2 total days.
19. Assess mice during this phase to ensure they are healthy enough to handle the ABA experiment.
20. Check cages for any flooding from water bottles each day. If bottles have flooded, replace the old cage with a new cage and repeat the necessary steps to adhere wheels and food to the cage without interference.
21. On day 2, check cages for food levels. If food is low, refill with 2 pieces of chow. Do this without disturbing the mice.
    NOTE: If during the pre-experimental acclimation phase any mouse is exhibiting hypophagia or is underweight, remove the mouse from the study.

7. Experimental Baseline Phase - Day 1

1. Select "end", and then "start acquisition" in the wheel management software.
2. Measure out 9 g chow in a small plastic beaker on a scale with 0.00 g sensitivity. Record the exact weight.
3. Gently remove and weigh the mouse in a large plastic beaker on a scale with 0.0 g sensitivity. Record weight.
    NOTE: It is crucial to limit the stress of mice while handling, therefore handle them patiently.
4. Dispose of all acclimation food.
5. Search bedding for any food that may have been removed from the jar and dispose of that as well.
6. Leave the water bottle in place, but ensure it is adequately full.
7. Clean soiled running wheels with 70% isopropanol and paper towels.
8. If cleaning was necessary, after the isopropanol dries, return the mouse to its cage and replace the cage on the rack.
9. Repeat these steps until every mouse is weighed and 9 g of food is measured out and placed in cages.

8. Experimental Baseline Phase - Days 2 - 7

1. Return to the animal facility at the same time every day. Do not vary in taking measurements by over or under 10 min.
2. Measure out 9 g chow in a small plastic beaker on a scale with 0.00 g sensitivity. Record the exact weight.
3. Gently remove and weigh the mouse in a large plastic beaker on a scale with 0.0 g sensitivity. Record weight.
4. Pull the food jar out of the cage and dump all the food into a small beaker on the 0.00 g scale.
    1. If feces and bedding are in the food jar with the food, pick out all of the bedding and feces. Use a small strainer to rub powdered food into the weighing beaker. Remove any feces before doing this with tweezers (if necessary).
    NOTE: Bedding will not break up into smaller pieces to fit through the strainer.
    2. Search for any food remnants in the cage and add them to the beaker to be weighed. Record the amount of food remaining.
    NOTE: If the bedding and cage must be searched, it is best to do so while the mouse is in the weigh beaker or transfer cage to reduce stress.
    3. Dispose of all food.
5. Wipe jar clean and place back into the cage. Replace tape if necessary.

6. Place the previously measured 9 g of new food into the jar.
7. Clean soiled running wheels with 70% isopropanol, then dry the wheels. Do this one cage at a time and do not accidentally interchange wheels.
8. End and restart acquisition in wheel running program every other day.
9. Repeat these steps until the start of the restriction phase.

9. Experimental Restriction Phase - Day 1

1. End and start acquisition in the wheel management software.
2. Weigh mice, old, and new food per baseline protocol.
3. **Do not clean wheels now.** This may distract mice during their limited feeding period.
4. Allow access to new food, 9 g of chow, for **6 h**. Record the time food is offered.
   NOTE: The time during the light cycle that food is available will affect the development of ABA. Since mice normally sleep during the light cycle, giving access to food during this time exacerbates the development of ABA, since mice do not usually consume the majority of their daily food at this time. Conversely, mice given food access during the dark cycle will develop ABA more slowly[32]. Adjusting duration of food access will result in longer or shorter survival times (**Figure 2**). Food anticipatory activity (FAA) is hyperactivity, which occurs immediately before food is offered[32,33,34]. FAA can affect the development of ABA, as it has been shown that the denial of wheel running access during FAA ameliorates ABA behavior[32].
   1. Remove food exactly 6 h later for each mouse and measure the quantity remaining. Record the time food is removed.
5. Clean wheels now, if necessary, and attempt to do so without getting behind on pulling food from subsequent cages.
6. Weigh food and calculate the difference in food to determine the amount of food eaten per mouse.
7. Calculate the dropout weight for each mouse.
   NOTE: Dropout weight is when a mouse must be removed from the experiment when it reaches 75% of its final measurement of baseline body weight. For example, if a mouse weighs 20 g at the beginning of the restriction phase, when it reaches 15 g it must be removed from the experiment.
8. Keep a copy of the dropout weights on hand in the animal facility.
   NOTE: Experiments evaluating recovery from ABA may be attempted; food is either provided *ad libitum,* or wheels are locked to study the process by which mice return to a healthy body weight[34].

10. Experimental Restriction Phase - Days 2 to 14

1. Repeat all measurement steps in the Restriction Day 1 protocol. Do not restart wheel acquisition.
2. If a mouse reaches drop weight (75% of its baseline body weight), remove the wheel and food jar from the cage and provide ample food on the bottom of the cage, then proceed with the other mice. Once all cages have been attended to, euthanize all mice that have reached drop weight.
3. Leave the wheel on for each mouse for the duration of the experiment.
4. Check each mouse daily for any injuries. If necessary, clip damaged toenails, treat sores, *etc.* Ask animal care personnel for advice if unsure about how to proceed with an injury.

11. Ending the Study

1. If the study has lasted 14 days, weigh all the mice on day 15. Restriction day 1 weight is considered a baseline weight.
   NOTE: The length of the study can be modified to address the hypothesis being tested. Longer duration food access periods may be used to extend study length.
2. Euthanize mice as necessary.
3. Take dirty equipment to appropriate rooms.
4. End wheel count acquisition on day 15 to provide 10 full days of data.
5. Turn off wireless wheel hubs.
6. Extract data.
7. Analyze data.

Representative Results

Brain-derived neurotrophic factor (BDNF), a protein that contributes to the regulation of feeding as well as weight maintenance, is reduced in the serum of patients with AN[35]. This experiment assessed the effects of scheduled feeding, running wheel access, or both on BDNF expression within the hippocampus (HPC), ventral tegmental area (VTA), nucleus accumbens (NAc), and medial prefrontal cortex (mPFC). Expression of neuronal cell adhesion molecule 1 (NCAM1) was also assessed to explore the specificity of effects on BDNF within the mesocorticolimbic pathway.

Mice were individually housed within a 12:12 light-dark cycle in a climate controlled room in an animal care facility. Cages were equipped with wireless running wheels which relayed data every 30 s to a laptop. Standard chow was offered in a jar approximately 2" in diameter with walls approximately 1.5" high during both baseline and restriction periods.

Four experimental groups were assigned pseudo-randomly: *ad libitum* (Ad Lib), scheduled feeding (STV), free wheel running and food access (RUN), or free wheel running with food restriction (ABA) (**Figure 3**). Group assignment was determined by initial body weight to form four weight equivalent groups. Mice were individually housed and fed *ad libitum* with constant access to a running wheel for 2 days during the acclimation period, and 7 days during the baseline period.

During the scheduled food access period of up to 12 days, both STV and ABA groups had food access for only 6 h per day (09:00 - 15:00) and were monitored and removed if they met their calculated drop weight of 25% of their starting body weight. Daily adjustments were made to limit food access for the STV group, to ensure that the STV group had a similar dropout rate as the ABA group, whose members normally lose body weight more rapidly. Each RUN mouse was yoked to an ABA mouse for removal from the study, and each Ad Lib mouse was yoked to an STV mouse. Yoking ensured that all mice were removed from the study at similar time points. This design allowed for comparison of gene expression between groups of mice that experienced various experimental conditions for the same duration. The number of days to removal was interpreted as a measure of survival. Another possibility is to include an additional RUN group which is not restricted to eat as little as the ABA group, but is provided with an excess of food during the 6 h period. Furthermore, 5 experimental groups can be used, including both forms of the RUN group. The choice of groups depends upon the goals of the study.

After removal from the ABA paradigm, mice were decapitated for rapid brain extraction. Tissue from the mPFC, HPC, NAc, and VTA was removed using a brain matrix and tissue punch and immediately snap frozen using dry ice. Samples were kept in a -80 °C freezer before RNA extraction.

Statistical analysis of the data was performed using analysis of variance (ANOVA) tests. For baseline data, ANOVA is applied to each dependent variable (body weight, food intake, and wheel running). *Post hoc* analyses of variance or interactions were performed. Bonferroni adjustments were made when *post hoc* analyses of variance were applied.

To analyze data from restriction, general linear models (PROC GLIMMIX; SAS v9.2; code available from corresponding author upon request) are used to assess each variable. *Post hoc* analyses are used to resolve interactions using the false discovery rate method. Survival analysis was performed using the Kaplan-Meier test with Logrank (Mantel-Cox) and Peto-Peto-Wilcoxon *post hoc* tests. Significance was set at p <0.05.

Wheel running did not affect body weight during baseline, while food consumption increased when running wheels were available on baseline days 2 - 7. Prior to scheduled feeding, activity on the running wheels was not different between the RUN and ABA groups.

Since Ad Lib and RUN mice were yoked to STV and ABA mice, respectively, there was no difference in survival between experimental groups during scheduled feeding (**Figure 4a**). Without yoking, survival of ABA mice is dramatically reduced compared to RUN and STV groups[35]. However, body weight was reduced on days 1 - 5 in both groups exposed to scheduled feeding (STV and ABA groups) (**Figure 4b**). Scheduled feeding also reduced food intake on days 1 - 5 in both groups (**Figure 4c**), but had no effect on wheel running activity (**Figure 4d**). In this study, hyperactivity of the ABA group was brief (days 1 - 3) and nonsignificant compared to the RUN group, perhaps due to the rapidly declining health of mice in the ABA condition.

Wheel running significantly increased BDNF expression within the VTA (**Figure 5a**) while food restriction increased NCAM1 mRNA expression in the VTA (**Figure 5b**), but did not alter BDNF mRNA expression. There were no main effects of wheel or food access, or interactions on BDNF or NCAM1 mRNA expression within the NAc (**Figure 6**). In the mPFC, scheduled feeding decreased BDNF mRNA expression, but did not affect NCAM1 mRNA expression (**Figure 7**). Wheel access did not alter gene expression, and no interactions of food restriction and wheel access were found. Similarly, in the HPC, there were no interactions of food restriction and wheel access on BDNF or NCAM1 mRNA expression, although food restriction increased NCAM1 mRNA expression (**Figure 8**).

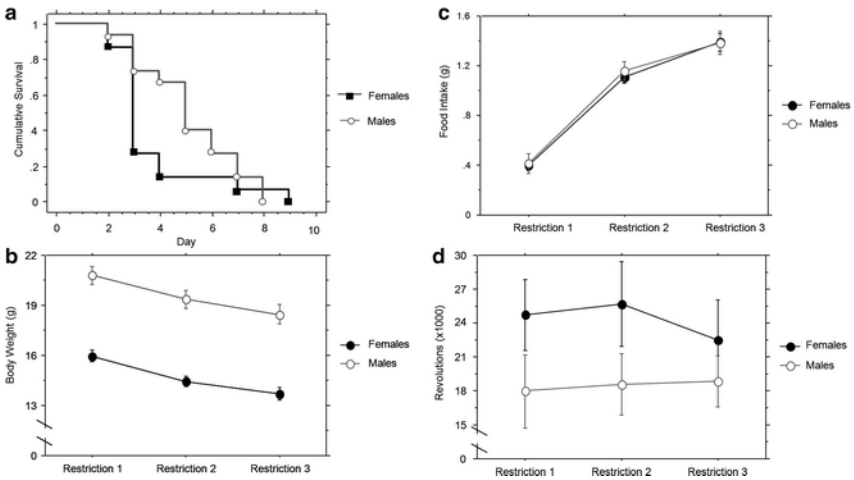

Figure 1: Sex Differences in the ABA Paradigm. (**a**) Cumulative survival between male and female mice over time. (**b**) Body weight between male and female mice over restriction days (**c**) Food intake between male and female mice over restriction days. (**d**) Wheel running between male and female mice over restriction days. This figure has been modified from the original publication[6]. Please click here to view a larger version of this figure.

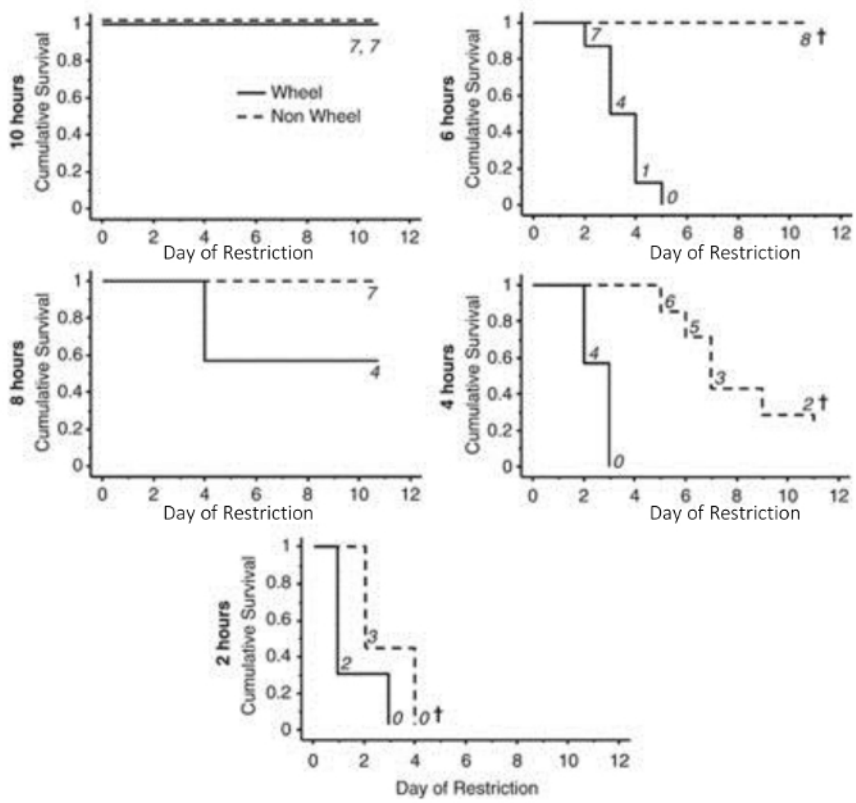

Figure 2: Effects of Feeding Times on ABA Duration. This figure shows how many days mice will remain in restriction depending on how long food is available. This is shown for mice with and without running wheels. This figure has been modified from the original publication[35]. Please click here to view a larger version of this figure.

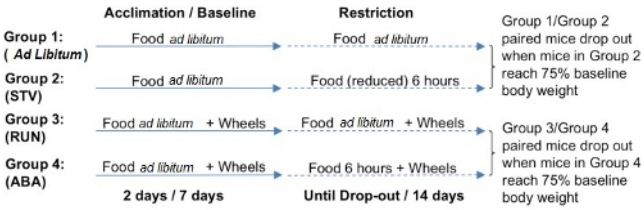

Figure 3: ABA Paradigm Experimental Design. This image shows the set up for ABA experiments. This figure has been modified from the original publication[36]. Please click here to view a larger version of this figure.

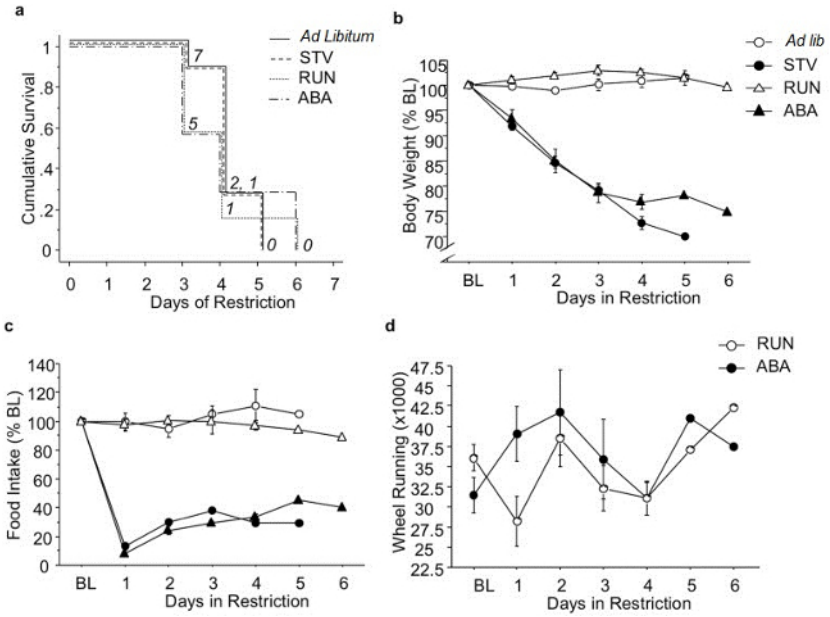

Figure 4: Effects of the ABA Paradigm. (**a**) Survival, (**b**) daily body weight, (**c**) food consumed, and (**d**) wheel running during food restriction. Numbers italicized depict number of mice remaining in the ABA paradigm. Results are expressed as mean ± SEM. This figure has been modified from the original publication[36]. Please click here to view a larger version of this figure.

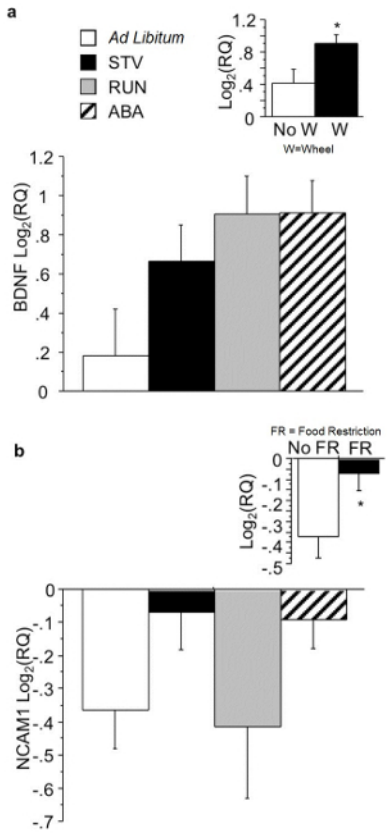

Figure 5: Effects of ABA on Gene Expression in the VTA. (**a**) BDNF, (**b**) NCAM1 expression in the VTA of mice exposed to ABA conditions. Insets indicate the mean BDNF and NCAM1 expression during restriction for the independent measure depicted. Results shown as log2(RQ) ± SEM p <0.05. This figure has been modified from the original publication[36]. Please click here to view a larger version of this figure.

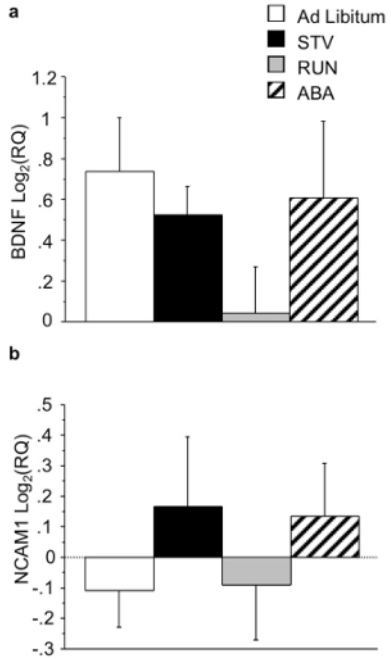

Figure 6: Effects of ABA on Gene Expression in the Nucelus Accumbens. (**a**) BDNF, (**b**) NCAM1 expression in the NAc of mice exposed to ABA conditions. Results shown as log2(RQ) ± SEM p <0.05. This figure has been modified from the original publication[36]. Please click here to view a larger version of this figure.

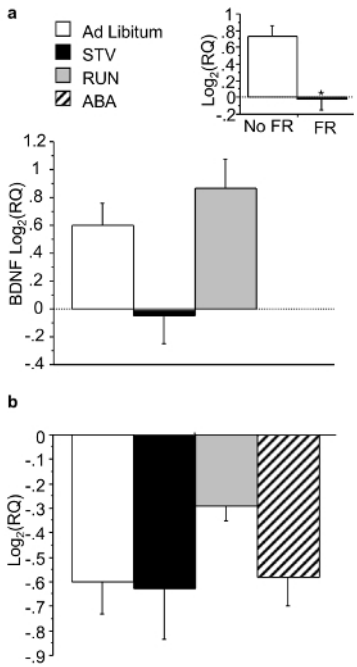

Figure 7: Effects of ABA on Gene Expression in the mPFC. (**a**) BDNF, (**b**) NCAM1 expression in the mPFC of mice exposed to ABA conditions. The inset indicates mean BDNF expression during restriction for the independent measure depicted. Results shown as mean log2(RQ) ± SEM p <0.05. This figure has been modified from the original publication[36]. Please click here to view a larger version of this figure.

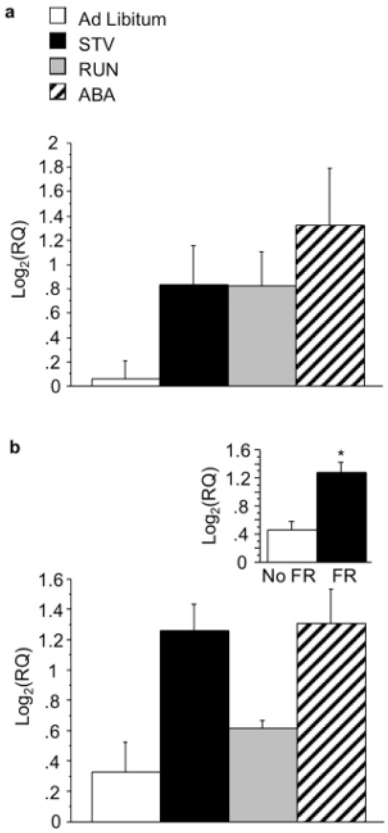

Figure 8: Effects of ABA on Gene Expression in the HPC. (**a**) BDNF, (**b**) NCAM1 expression in the HPC of mice exposed to ABA conditions. Insets indicate mean BDNF and NCAM1 expression during restriction for the independent measure depicted. Results shown as mean log2(RQ) ± SEM p <0.05. This figure has been modified from the original publication[36]. Please click here to view a larger version of this figure.

Discussion

The ABA experiment can be modified by experimenters to test different strains, ages, drugs, and various other variables of AN. Bearing in mind genetic variability, adjustments to the running wheel or food access period would increase or decrease the severity of symptoms and rate of dropout. This can serve to increase or decrease the length of the experiment, depending on the experimental question of interest.

Accurate measurement of food intake is a critical part of the ABA paradigm, but can often be made problematic. Bedding, feces, and urine in the food jar, coupled with food being moved into the cage space provides difficulty in accurately weighing remaining food. Mice also leave small bits of food and powdered food in the jar and throughout the cage. A strainer helps mitigate the problem of sifting through bedding to find food remnants for proper weight recordings, but the method is not infallible. Handling the mice to obtain their weights is another crucial, yet confounding factor in the ABA paradigm. With investigator interference, mice are more vulnerable to stress, which can exacerbate the conditions of ABA.

ABA is a useful method in assessing AN, however this technique does come with limitations. The first limitation to consider is that the ABA model does not mimic all aspects of anorexia. For example, rodents given access to a high fat diet will not develop ABA under typical experimental conditions[30]. However, virtually no animal models exist that recapitulate all aspects of a psychiatric disorder. Thus, ABA could still yield important insights into AN-related behaviors.

Drug treatments can also be implemented in the ABA paradigm. Drug administration via the drinking water is ideal, as it avoids daily injections that can have unwanted effects including local irritation at the injection site and short-term sedation induced by certain drugs. However, it is crucial to adjust the concentration of drug daily to maintain consistent dosing, since daily water intake changes dramatically over the course of ABA, first with increases in drinking and then later with reductions in fluid consumption. Using subcutaneous mini pumps to deliver drug continuously is also possible with the ABA paradigm; however, care must be taken that the mini pumps are small enough to allow mice to get in and out of food jars and run unimpeded on the running wheel. Furthermore, the use of optogenetic fiber cables is theoretically feasible, but would require a modified set-up. Alternatively, manipulation of the activity of specific circuits using designer receptors exclusively activated by designer drugs (DREADD) could be used with the ABA paradigm without the complication of indwelling cords. Thus, a variety of modern neuroscience tools could be incorporated to study the neural mechanisms of ABA behavior.

Disclosures

The authors have nothing to disclose.

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