Chapter 25

The Activity-Based Anorexia Mouse Model

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Abstract

Animals housed with running wheels and subjected to daily food restriction show paradoxical reductions in food intake and increases in running wheel activity. This phenomenon, known as activity-based anorexia (ABA), leads to marked reductions in body weight that can ultimately lead to death. Recently, ABA has been proposed as a model of anorexia nervosa (AN). AN affects about 8 per 100,000 females and has the highest mortality rate among all psychiatric illnesses. Given the reductions in quality of life, high mortality rate, and the lack of pharmacological treatments for AN, a better understanding of the mechanisms underlying AN-like behavior is greatly needed. This chapter provides basic guidelines for conducting ABA experiments using mice. The ABA mouse model provides an important tool for investigating the neurobiological underpinnings of AN-like behavior and identifying novel treatments.

Key words: Activity-based anorexia, Hyperactivity, Anorexia nervosa, Animal model, Mice, Food restriction

1. Introduction

1.1. Activity-Based Anorexia

In 1953, Hall and Hanford observed that rats housed with running wheels and subjected to restricted food access for 1 h a day had significant decreases in body weight and food intake, and a paradoxical increase in running wheel activity (1). Conversely, rats given running wheels and food ad libitum, or food restricted rats housed without running wheels, were able to maintain a normal body weight (1–3). This model of "self-starvation," later coined the activity-based anorexia (ABA) model, consistently produces rapid decreases in body weight and food intake, hyperactivity, hypothermia, loss of estrus, increases in HPA axis activity, and leads to stomach ulceration and eventually death (2–5). The ABA phenomenon has been observed in many other species besides the rat, such as the hamster, gerbil, guinea pig, chipmunk, pig, and mouse, indicating that ABA behavior is highly conserved across mammalian species (6–9).

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The mechanisms that underlie ABA behavior are generally unknown. Nevertheless, several theories have been proposed to explain this paradoxical behavior. During ABA, animals present with a significant drop in body temperature, a symptom also observed in patients with anorexia nervosa (AN). Lambert suggests that hyperactivity develops to counteract the drop in body temperature that arises when animals fail to adjust to food restriction (1993) (10). ABA has also been suggested to result from autoaddiction to endogenous opiods. This theory posits that dysregulation of the opiod system renders hyperactivity and self-starvation behaviors addictive (11). Another intriguing explanation of ABA behavior comes from the "adapted to flee famine" hypothesis which suggests that hyperactivity and denial of starvation reflect an adaptive mechanism that facilitates migration in response to famine (12). Although each theory presents intriguing arguments to explain ABA behavior, none may fully explain the phenomenon. These theories may not prove to be mutually exclusive, and these processes may work in concert in the development of ABA.

1.2. Anorexia Nervosa

Anorexia nervosa is an eating disorder that affects approximately 0.5–1.0% of females during their lifetime and affects about one tenth of as many males (13). The lifetime mortality rate for AN is approximately 10%, which represents the highest mortality rate of all psychiatric illnesses (14). AN often onsets around mid-adolescence and is characterized by an refusal to maintain a healthy weight, strong pursuit of thinness despite being underweight, fear of weight gain, preoccupation with food and body shape, and inappropriate assessment of body size. Patients usually have disruption of their menstrual cycle, or amenorrhea, and signs of hypometabolism (13). Moreover, patients often exhibit hyperactivity, which can manifest as extreme exercise or as a general restlessness (15, 16). Patients also have hypercortisolism and increases in corticotrophin-releasing hormone (CRH) in their cerebral spinal fluid (CSF), indicating an overactive hypothalamic-pituitary-adrenal (HPA) response during illness (17, 18). AN is highly comorbid with anxiety disorders. Patients often have one or more anxiety disorders in their lifetime, most commonly obsessive-compulsive disorder or social phobia. Onset of anxiety disorders usually precedes the onset of AN (19). Overall, patients often present with signs of perfectionism, distractibility, obsessionality, anxiety, and compulsivity, which are usually present before AN diagnosis and worsen with illness (13, 19).

AN is often a chronic illness with a high rate of relapse (13, 20). About 30–50% of patients relapse within a year of weight-restoration (21, 22). Currently, treatment of AN remains highly limited. Patients show variable improvement following various psychological interventions including cognitive-behavioral therapy,

interpersonal therapy, behavioral programs, and family-based therapy (13, 23–26). There are currently no approved pharmacological treatments for AN, although studies examining the effects of the selective serotonin reuptake inhibitor (SSRI) fluoxetine (27, 28) have produced conflicting results. However, recent studies assessing the potential utility of the atypical antipsychotic olanzapine have been promising (29). Considering the high mortality rate associated with AN, studies aimed at identifying potential treatments and the neurobiology underlying this severe disorder are critically needed.

1.3. Utility of an ABA Mouse Model

Since the sequencing of the mouse genome and the widespread availability of numerous inbred strains, the use of mouse models in all facets of basic research has become commonplace. Mice are easily bred, handled, and housed, and their genome can be manipulated to develop knockout or transgenic mice which allow for the study of single genes and their roles in normal or disease processes. The experimental conditions required to assess ABA (discussed further below) require carefully selected experimental and control groups and specialized equipment. The ABA model is a useful tool for studying aspects of AN-like behavior.

1.4. Validity of Animal Modeling When Employing ABA

Developing and using animal models of psychiatric disorders is inherently difficult due to the complex nature of these illnesses. Although a mouse model that recapitulates all of the symptoms a disorder is intuitively appealing, modeling an entire syndrome is practically impossible and also unnecessary for the model to be useful (30). Modeling specific aspects of a disorder can provide insight into the pathophysiology of the disorder and indentify potential treatments. The ABA model has been proposed to provide a model for several aspects of AN, including hyperactivity, self-starvation, weight loss, amenorrhea, hypothermia, and increased HPA axis activity.

Animal models should exhibit predictive validity for the disorder they are intended to model to justify their initial use. That is, the animal model should make accurate predictions about the human phenomenon of interest. Specifically, variables that influence the disorder should influence the dependent variable in a similar fashion. For example, pharmacological treatments that are effective in treating the disorder should also modify the expression of dependent measures (30). Thus, any potential treatments for AN identified in the clinic should also reduce ABA, and vice versa. The ABA model exhibits predictive validity for some aspects of AN in that adolescent mice and rats are more vulnerable to ABA than older rodents ((31–34); unpublished results). Furthermore, female rats and mice are more vulnerable to ABA than male rodents ((35); Fig. 1). Thus, the ABA model can be used as a preclinical tool for studying AN-like behavior.

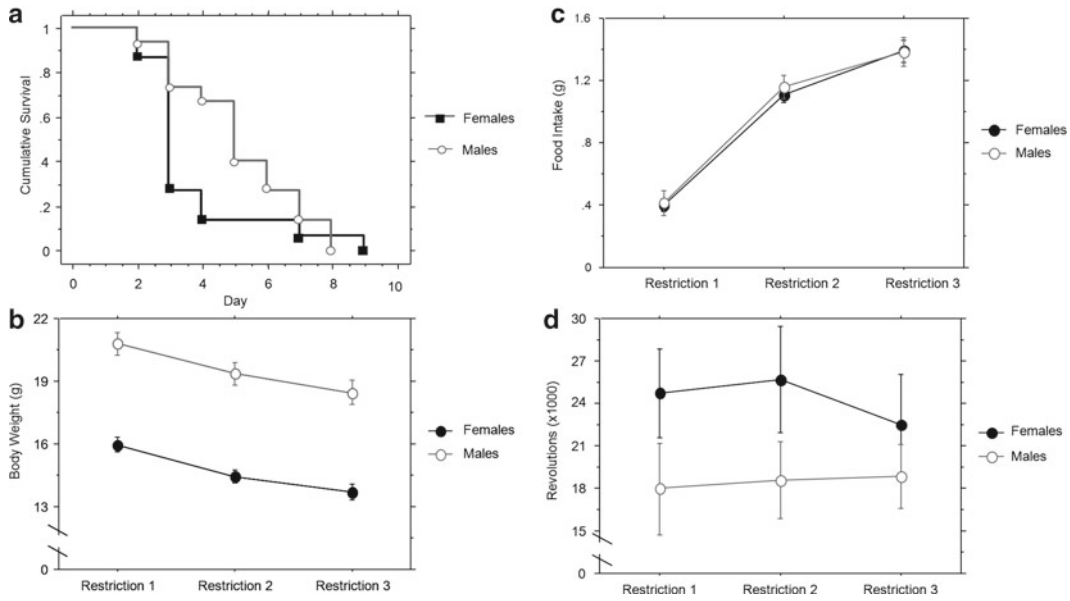

Fig. 1. Sex differences in ABA in Balb/cJ. (**a**) Cumulative survival of male and female Balbc/J mice during ABA ($p = 0.0293$, Breslow test). Survival represents time until mouse was removed from ABA (dropout) due to loss of 25% of baseline body weight. (**b**) Body weight during food restriction before dropout. (**c**) Food intake during restriction before dropout. (**d**) Running wheel activity before dropout. $N = 30$ (15 male, 15 female) Balb/cJ mice.

2. Materials

Setting up the ABA mouse model in the lab involves choosing the most appropriate equipment, mouse strain, route of drug administration (if applicable) and experimental design given the aims of the study and the resources of the lab. To date, very few studies have examined ABA using mice (9, 36–39). Here, we present basic guidelines for assessing ABA in mice and provide some of our own experimental results.

2.1. Selecting Mice

2.1.1. Gender

1. To date, most experiments performed using mice have used females, since AN is about ten times more prevalent in girls.
2. Normally, female rats run significantly more than males and reduce their food intake during estrus (40–42). During ABA, female rats continue to run more than males (34, 35). Female rats also eat more than males during ABA, and therefore, females have been reported to be more resistant to ABA in some studies (35). By contrast, some evidence indicates that females are more at risk than males (43), or that no sex difference exists (34).

3. We recently conducted a study examining the effects of sex on ABA. We used both male and female mice 8–10 weeks of age on a Balb/cJ background. We found that female mice were more vulnerable to ABA, and showed significantly fewer days to lose 25% of initial bodyweight (Fig. 1). Additionally, females lost more weight ($p < 0.0001$) than male mice even though they did not differ in food intake. Although statistically insignificant, female mice also showed increased running wheel activity in comparison to male mice ($p = 0.20$).
4. The use of female mice for ABA experiments may more accurately model the clinical epidemiology of AN, allowing inferences to be drawn more readily from mice to humans. Several mouse strains (discussed further below) are commercially available (Harlan Laboratories, Charles River, The Jackson Laboratory).

2.1.2. Age

1. Younger rats are more vulnerable to the ABA paradigm, exhibiting more rapid weight loss than adult rats (31–34). The smaller size of younger animals may contribute to their increased susceptibility to ABA, as rats with higher initial body weights are less susceptible to ABA behavior (44, 45).
2. Younger rats exhibit more running wheel activity during ABA in comparison to older rats (33, 34).
3. Although younger rats are more susceptible to ABA, they also recover from ABA faster in terms of body weight (34).
4. Recently, we have observed the same phenomenon in mice aged 4–6 weeks (our unpublished findings).
5. Since AN onsets in mid-adolescence, the use of adolescent mice in the ABA model may more accurately model AN. Given the accelerated manner in which younger rodents develop ABA, the experimental design of the ABA paradigm can be adjusted to reduce the rate at which young animals progress (see Note 1).

2.1.3. Strain

1. The large number of strains commercially available (Harlan Laboratories, Charles River, The Jackson Laboratory) for laboratory use vary widely in their vulnerability to ABA. The hypothesis being tested should be considered when selecting a strain to work with.
2. Selecting a strain with high ABA is desirable when testing compounds hypothesized to reduce ABA. Furthermore, using a strain that develops high ABA levels may better model AN in humans than strains that are more resistant to ABA.
3. Choosing a strain with intermediate levels of ABA may be desirable when examining the effects of a manipulation, whether pharmacological or genetic, which might either increase or reduce ABA.

4. One may compare strains that vary in ABA levels to investigate potential genetic differences underlying this phenomenon.
5. The C57BL/6J inbred mouse strain is relatively resistant to the developing ABA, and does not exhibit the significant increases in running wheel activity that DBA/2J (9), A/J (37, 38), and Balbc/J mice exhibit. C57BL/6J mice also tend to eat more than DBA/2J mice during ABA (9).
6. Vulnerability to ABA may correlate with anxiety levels in inbred mouse strains, since Balbc/J mice are known to be quite anxious (46), while C57BL6/J mice show low levels of anxiety (9).
7. An anxious strain may be desirable to use for ABA studies, since AN patients often exhibit increased anxiety even before the onset of the disorder (13, 19). Therefore, using a naturally anxious mouse strain in ABA could more closely model the human disorder.
8. Initially comparing several mouse strains for their vulnerability to ABA can be useful to identify an optimal strain for further use.

2.2. Housing and Equipment

2.2.1. Caging

1. During ABA, mice should be housed in cages that will be spacious enough to include access to a running wheel, food containers, and water bottles. Depending on the equipment chosen, ABA can easily be performed in standard facility mouse cages (Thoren Caging, Inc., Tecniplast, Allentown, Inc., Animal Care Systems, Inc., Columbus Instruments) (Fig. 2).
2. Normally, mice are housed in groups. However, when conducting ABA studies, individual housing is necessary for individual running wheel activity and food intake measurements to be recorded. Interestingly, there is conflicting evidence as to

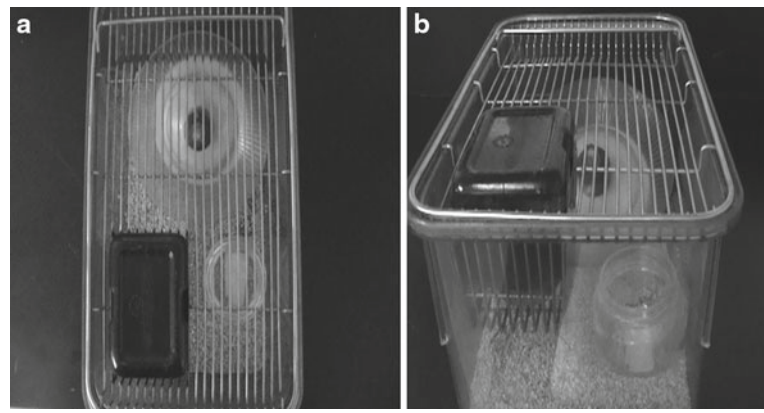

Fig. 2. Equipment setup for ABA in standard mouse housing (**a**) Overhead and side (**b**) view of cage with wheel (Med Associates, Inc.), food jar and water bottle for singly housed mouse.

whether group housing rats reduces ABA behavior (34, 45), or exacerbates it (47).

3. The temperature at which animals are housed can also effect the development of ABA. It has been shown that increasing the ambient temperature (32°C) during ABA lowers running wheel activity, increases body weight and food intake, even after rats had reached a 20% drop in initial body weight (48). In the same respect, rats given access to a warm plate during ABA reduced running wheel activity and body weight loss in rats (49). Moreover, rats subjected to ABA in cooler temperatures (19.4°C) had reduced survival rates in comparison to those housed at warmer temperatures (25°C) (50).
4. It is important to choose the ambient temperature at which mice will be exposed to ABA with respect to desired outcome and maintain a consistent temperature to avoid variable results.

2.2.2. Running Wheels

1. A running wheel system should be chosen based on how the investigator intends to record activity (i.e., manually, automatically), the system requirements, and how the animals will be housed with wheels.
2. Fortunately, there are several systems that can record 24 h running wheel activity without need for a human observer to manually tally revolutions. Cages with built in running wheels are commercially available, as are free running wheels which can be placed into standard facility caging (TSE Systems, Inc., Med Associates, Inc., Lafayette Instruments, Columbus Instruments, Tecniplast, Harvard Apparatus, IntelliBio).
3. Wheel systems with external hardware or wireless capabilities are ideal so that mice do not get caught up in equipment, chew wires, or have difficulty running in the space provided.
4. It is important to be able to easily clean and reuse wheels to avoid damage and increase the lifetime of the equipment.
5. The ability to lock wheels at any point during the experiment allows the investigator to regulate wheel access during certain periods of the day. Placing a locked wheel in the cage also creates a nonwheel control group that has the same home cage environment as animal with wheels.

2.2.3. Water Access

1. Water should be easily accessible and consistently available to mice during ABA. Mice can become dehydrated quite easily and significantly increase their drinking levels when they have access to a wheel (51).
2. Water intake can be variable during ABA, as rats drank significantly less water prior to food access and significantly more during food access (52). Others have found a general decrease in water intake during ABA (53).

3. Water intake may be a variable of interest and can be manipulated to investigate its effects on the development of ABA. When manipulating water availability during ABA, the investigator should follow guidelines outlined by animal care committees to avoid animal dehydration.
4. Overall, when choosing the caging and wheel apparatus for ABA studies, where and how water will fit into the setup should be considered.
5. We have found that smaller water bottles, which create more room in the cage for a running wheel, are more suited for ABA experiments (Thoren Caging, Inc.) (Fig. 2).

2.2.4. Food Presentation

1. Food can be provided in a small container or jar that the animal can easily fit into and eat within. This setup prevents the spillage of smaller pellets or powder into bedding (see Note 2) (Specialty Bottle, The Jar Store, LLC, SKS Bottle, U.S. Plastics Corp.).
2. The cage may have a specific compartment for food that can be blocked during restriction (Columbus Instruments, IntelliBio).
3. Animals should be acclimated to the method of food presentation before the experiment begins.
4. The type of food given to mice will also be an important consideration. For instance, rats given a sweet, high-fat diet show a reversal of weight loss and an increase in caloric intake during ABA in comparison to standard chow conditions, despite increases in running wheel activity (54).
5. Addition of sucrose, saccharin, or fat to standard chow does not significantly affect the development of ABA (54).
6. Administration of wet chow versus dry standard chow to rats during ABA ameliorated weight loss and increased food intake (52). Rats given wet chow never reached criterion for removal from ABA, whereas all rats given standard chow were removed by day 7.
7. Delivery of standard chow in pelleted or powered form did not affect survival (53) or food intake (47) in ABA.
8. Varying the type of food available during ABA could affect experimental outcome.

3. Methods

3.1. Experimental Design

The ABA model consists of a food restriction period in which animals have access to running wheels. Mice may be exposed to various manipulations including genetic, pharmacological, or

environmental manipulations. The dependent variables food intake, body weight, running wheel activity, and survival are monitored daily and compared between groups. Employing a carefully planned experimental design is key to obtaining results that address the questions of interest to the investigator.

3.1.1. Acclimation

1. Before beginning ABA, mice should be acclimated to the experimental equipment and single housing.
2. Typically, animals are given about 3 days to acclimate to single housing with a running wheel (9, 48, 55, 56).
3. Interestingly, acclimation to running wheels can also exacerbate subsequent ABA (33, 45).

3.1.2. Food Access

1. Mice can survive for several days when receiving 2–4 h of food access a day. The shorter the duration of food access, the more rapidly ABA will develop and advance (47, 48, 55). Therefore, increasing food access duration will allow animals to survive longer, and allow collection of more data points.
2. Typically, most mouse studies to date have used 2–4 h of food access during ABA ((9, 37–39); our unpublished data).
3. The time of day that animals receive food can affect the severity of ABA. Animals given food access in the light cycle develop ABA behavior much more quickly that those with access in the dark cycle (57).
4. Whether food is given at a fixed time or variable intervals does not affect the initiation of ABA, although presentation of food at irregular intervals does speed up its progression (58).
5. There are conflicting results regarding whether preadaptation to food restriction before wheel access reduces ABA behavior (47, 57, 59).
6. Duration and timing of food access should be chosen with the desired length of survival in mind. For example, to test the hypothesis that a particular drug treatment reduces ABA, a shorter food access period may be desired. However, to assess whether a particular genetic mutation worsens ABA, a longer food access period may be ideal.

3.1.3. Running Wheel Access

1. When running wheel activity increases during ABA, there is a significant increase in activity just prior to the feeding period (53, 57, 60) termed food anticipatory activity (FAA) (61).
2. FAA appears to play an important role in development of ABA, as denying access to wheel running during this time ameliorates ABA behavior (57).
3. Wheel access is an important variable to consider during experimental design and can affect the rate at which ABA develops (see Note 3).

3.1.4. Experimental Design: Independent Variables

There are several different independent variables that can be manipulated during an ABA experiment. Food restriction and the presence of a running wheel are both required to produce the ABA phenomenon, and both can be presented in a between-subject or within-subject fashion.

1. A within-subjects design, in which animals first receive food ad libitum and then have restricted access to food, decreases the number of mice and wheels needed and may also increase statistical power.
2. A between-subjects design in which separate groups receive food ad libitum or restricted access reduces the length of the experiment.
3. Other independent variables, such as drug treatments, are difficult to administer in a within-in subjects design and are usually presented in a between-subjects manner.
4. Choosing the appropriate experimental design for the independent variables of interest will depend on each individual variable, practicality, expense, and the animals and equipment available. Although many different experimental designs are possible, an experimental design we have used frequently is presented below as an example (Fig. 3).

3.1.5. Experimental Design: An Example

The present design uses both between- and within-subject factors to increase statistical power, and reduce the number of animals and wheels needed (Fig. 3a). Food access is manipulated in a within-subjects fashion, with animals first receiving food ad libitum, and then receiving food under restricted conditions (2–4 h daily). Therefore, animals serve as their own internal control with respect to the effects of food restriction on food intake, running wheel activity levels, and body weight. Both wheel access and drug treatment are presented in a between-subjects manner. Thus, for each group with running wheels, there is a corresponding group without wheels. Groups without wheels provide a control for any effects of drug treatment on food intake or bodyweight in the absence of running. Drug treatments are often difficult to administer in a within-subjects design due to carry over effects and the often fast progression of ABA. This experimental design is executed through the protocol below (Fig. 3b):

1. Prior to acclimation, animals should receive any necessary drug pretreatment (if applicable) (see Subheading 3.2).
2. Begin acclimation by singly housing all animals. Animals in the wheel access groups should receive wheels at this time (unlocked). Administer food and water ad libitum.
3. After 3 days of single housing and wheel acclimation, obtain daily measurements of the dependent variables (body weight, food intake, running wheel activity) for 5–7 days (baseline

a

	Wheel	No Wheel
Treatment X	Treatment X/Wheel	Treatment X/No Wheel
Control Treatment	Control/Wheel	Control/No Wheel

b

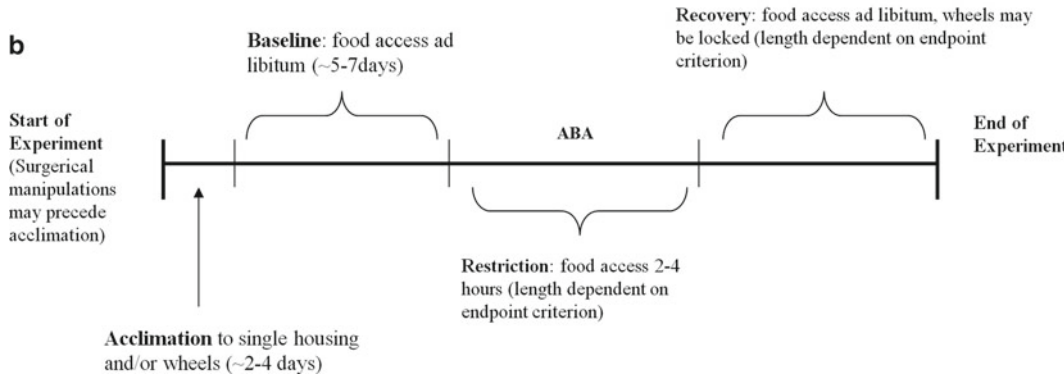

Fig. 3. ABA experimental design and timeline example. (**a**) Experimental design includes animals housed with and without wheels, with or without food restriction, and receiving control or treatment. This strategy employs a within-subjects for food access conditions, and a between subjects design for wheel condition and treatment condition. (**b**) Experimental timeline for this design.

measurements). It is best to take these measurements all together, and at the time of day the investigator plans to manipulate food access during restriction to acclimate animals to being handled at this time.

4. Following baseline, begin daily food restriction, or the ABA period. Water is still given ad libitum. Continue taking daily measurements of the dependent variables until animals reach end point.
5. Once animals reach the chosen end point (see Subheading 3.1.6), recovery from ABA (see Subheading 3.1.7) can be evaluated.

3.1.6. Determining End Point

As stated above, mice exposed to food restriction and running wheels will increase their activity levels and decrease their food intake to until death occurs. In general, three signs of death are present 48 h prior to stomach ulceration and death (62).

1. There is a large drop in body weight, which levels off and drops dramatically again before death.
2. Food consumption initially increases day to day, but then drops off rapidly before death.

3. Running wheel activity increases very quickly in line with increases in food intake, but drops off dramatically 24 h before death.
4. Defining an end point at which the animals will be removed from ABA is ethically important to reduce suffering and/or examine recovery. Since mice and rats will develop stomach ulcers, and have a low rate of recovery after losing 30% of their initial body weight, most investigators choose to remove animals from ABA when they have lost 25% of their baseline body weight. On the other hand, human AN patients are typically diagnosed when they have lost 15% of their ideal body weight.
5. Regardless of the chosen end point, one should be selected to prevent animals from dying from ABA which is unnecessary and unethical.

3.1.7. Recovery

1. Most investigators define recovery as a return to a stable body weight once unlimited food access is reinstated (3, 57, 63).
2. In addition to weight gain, return of estrus is another sign of recovery (63).
3. Recovery after loss of 25% of initial baseline body weight can be variable, and some mice may not fully recover.
4. Some groups define recovery as a maintenance or increase in body weight during a consecutive 4-day period (3, 52, 53).
5. Recovery from ABA may be based on body weight, or on a predetermined amount of time that must pass before mice are considered "unrecovered."
6. Locking or removing wheels during recovery will aid in increasing the rate of recovery.

3.2. Drug Treatment in ABA

The ABA model can be used to test potential drug treatments for AN. Furthermore, selective drugs can be used to dissect the neural substrates that modulate ABA. Drugs can be delivered to mice subjected to ABA via different routes of administration which have different advantages and disadvantages.

1. Many drugs can be dissolved in the drinking water. The concentration required to deliver a given dose is determined by measuring the bodyweight, and daily water intake of animals. Although this route of administration is noninvasive and delivers drug in a steady manner, drinking rates during baseline and the food restriction period can vary greatly and alter the target dose (see Note 4).
2. The administration of drug by daily injection ensures the accuracy of the dose delivered. However, some drugs have short half lives, and daily injection does not produce steady-state

levels for such drugs. Also, daily injections are invasive, and introduce stress into the experiment. Stress derived from manipulations outside of the ABA paradigm can confound results (see Note 5).

3. Osmotic minipumps allow for precise administration of drug that lasts up to several weeks. Delivery of drug via minipumps is relatively stress free following recovery from implantation. However, implantation of minipumps requires a minimally invasive surgery in which animals must be anesthetized. Therefore, animals will need to fully recover (2–3 days) before beginning experimentation. Certain drugs have poor long-term stability when dissolved, or require vehicles incompatible with pumps, and are better administered by injection or drinking water.

3.3. Statistical Analysis

3.3.1. Analysis of Variance

1. During baseline, group means obtained for the dependent measures body weight, food intake, or running wheel activity can be compared using standard repeated-measures analysis of variance (ANOVA), since these dependent measures will be gathered daily for each animal.
2. Repeated-measures ANOVA can also be applied to data collected during the restriction period, but before animals are removed from the experiment.
3. Standard repeated-measures ANOVA can handle a minimally unbalanced design in which few animals have dropped from the study, but the mixed effects model should be used to analyze data with several missing values (see Subheading 3.3.2).

3.3.2. Mixed Effects Model

1. The mixed effects model allows for a more complete analysis of dependent measures through the end of the experiment when all animals have reached end-point criterion. Animals will reach end point intermittently, thus creating datasets with several missing values.
2. The mixed effects model (or mixed ANOVA model) can compare subjects despite unbalanced datasets and is written as:

$$y = X\beta + Z\gamma + \varepsilon$$

The model has both fixed effects parameters (β), random effects parameters (γ), and an error variable (ε) that all vary as a function of each particular case.

3. This model is a generalization of the standard linear model in which errors are permitted to exhibit correlation and nonconstant variability, which would violate assumptions made in standard ANOVA. For more information on this model, see Cnaan et al. (64).

3.3.3. Survival Analysis

1. Survival analysis is concerned with studying the time between entry into a study and a defined event. For example, survival analysis can compare the time it takes different groups of animals to reach end-point criteria (e.g., loss of 25% of initial body weight).
2. Kaplan-Meier is one type of survival analysis that can be used to assess group differences in time to dropout. Kaplan-Meier is appropriate when time is the only variable of interest, therefore if other covariates exist, the Cox regression may be more suited for analyses.
3. Kaplan-Meier survival analysis generally outputs results of three statistical comparisons, those being the Log rank test, the Breslow or Wilcoxon test, and the Tarone-Ware test, each of which weight the time points in a different manner.
4. The Log rank test compares equality of survival functions by giving each time point equal weight.
5. The Breslow test compares equality of survival functions by weighing time points with consideration of number of cases present at each time point, and is subject to making more type II errors. Figure 1 shows a typical output from this type of analysis.
6. The Tarone-Ware test compares equality of survival functions by weighing all time points by the square root of the number of cases at each time point and is considered a compromise between Log rank and the less conservative Breslow test.

3.4. Summary and Future Scope

ABA can be induced when mice housed with running wheels are subjected to daily food restriction. The subsequent hyperactivity, reduction in food intake, and extreme body weight loss that can lead to death closely mimics the symptoms of AN observed in humans. The ABA mouse models can be used to identify potential treatments for AN and elucidate the neural substrates of this disorder.

4. Notes

1. The severity of ABA behavior can be ameliorated, and the length of the experiment increased, by reducing running wheel access, or increasing the food access period. Conversely, ABA behavior will develop more severely and more quickly if running wheel access is increased and food access is decreased.
2. The measurement of food intake is often complicated by mice defecating and moving bedding into the food jar. Mice also chew food pellets into very small pieces or powder. We have found that using forceps and a small strainer to sift through the contents of the food jar makes removing bedding and fecal matter much easier, and allows for more accurate data collection.

3. In many ABA experiments, investigators lock the running wheels during the food access, preventing animals from running during this period. This may ameliorate the development of ABA, as wheel running will not compete with food intake. This procedure can be useful if the investigator wants to extend the time until end point, or increase the rate of recovery from ABA. Alternatively, allowing wheel access during the food access period allows the animal the choice to either run or eat.
4. Administering drugs via the drinking water offers many advantages, such as avoiding daily injections. Nonetheless, during ABA, water intake levels can fluctuate. Therefore, as water intake varies, the dose of the drug received will also fluctuate. Measuring daily water intake and adjusting the concentration of the drug is needed to maintain the desired dose, but can be labor intensive.
5. Using daily injections to administer a drug of interest can have unexpected effects. Certain drugs and vehicles can cause local irritation at the injection site, resulting in changes in behavior. Some drugs may also cause short-term sedation, which can interfere with both feeding and running behavior. If drug must be injected daily, the time of day of the injection should be carefully considered based on potential sedating or activating effects of the drug.

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