Video Article

Using the Activity-based Anorexia Rodent Model to Study the Neurobiological Basis of Anorexia Nervosa

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Abstract

Anorexia nervosa (AN) is a psychiatric illness characterized by excessively restricted caloric intake and abnormally high levels of physical activity. A challenging illness to treat, due to the lack of understanding of the underlying neurobiology, AN has the highest mortality rate among psychiatric illnesses. To address this need, neuroscientists are using an animal model to study how neural circuits may contribute toward vulnerability to AN and may be affected by AN. Activity-based anorexia (ABA) is a bio-behavioral phenomenon described in rodents that models the key symptoms of anorexia nervosa. When rodents with free access to voluntary exercise on a running wheel experience food restriction, they become hyperactive – running more than animals with free access to food. Here, we describe the procedures by which ABA is induced in adolescent female C57BL/6 mice. On postnatal day 36 (P36), the animal is housed with access to voluntary exercise on a running wheel. After 4 days of acclimation to the running wheel, on P40, all food is removed from the cage. For the next 3 days, food is returned to the cage (allowing animals free food access) for 2 hr daily. After the fourth day of food restriction, free access to food is returned and the running wheel is removed from the cage to allow the animals to recover. Continuous multi-day analysis of running wheel activity shows that mice become hyperactive within 24 hr following the onset of food restriction. The mice run even during the limited time during which they have access to food. Additionally, the circadian pattern of wheel running becomes disrupted by the experience of food restriction. We have been able to correlate neurobiological changes with various aspects of the animals' wheel running behavior to implicate particular brain regions and neurochemical changes with resilience and vulnerability to food-restriction induced hyperactivity.

Video Link

The video component of this article can be found at https://www.jove.com/video/52927/

Introduction

Anorexia nervosa (AN) is a psychiatric illness characterized by excessive restriction of food intake, over-exercise, and irrational fears of gaining weight. One of the most deadly psychiatric illnesses[1], AN has no accepted pharmacological treatment to date, and the neurobiological mechanisms and effects of the disease are poorly understood. We are studying an animal model of AN to explore the neurobiological and neurochemical changes associated with hallmark symptoms of the disease.

Activity-based anorexia (ABA) is a bio-behavioral phenomenon described in rodents that models some of the characteristics of AN[2,3]. When rodents with free access to voluntary exercise on a running wheel experience food-restriction, many, but not all, become hyperactive – running more than they ran prior to the onset of food-restriction[3,4]. There have been many suggested explanations for the over-exercise exhibited by ABA animals and AN patients: that it is a form of foraging behavior[5], a mechanism to cope with the stress of food-restriction[6], an attempt to raise body temperature during starvation-induced drop in metabolism[7], or a result of hypoleptinemia[8]. This rodent model reproduces the AN symptoms of body weight loss, hyperactivity, voluntary food restriction by opting to run during limited food access, correlations with anxiety traits[9,10], and vulnerability affected by early life experience[11]. While the rodent model ABA is considered a stress model, this may not accurately reflect AN in human patients, who show increased immune function[12]. Among both rodents and human patients, some individuals show more vulnerability than others. While epidemiological studies strive to elucidate the risk factors for AN, relatively few studies have attempted to understand the neurobiological basis for individual differences in vulnerability to ABA induction in rodents.

It is important to note that the ABA paradigm is widely used, and its use as an animal model of AN has been extensively reviewed[6,13-15]. The contribution of this current work is to outline the specific methods used to induce ABA in adolescent female mice and outline the modifications that were necessary to make to the existing rodent models in order to improve survival in young mice. Additionally, we discuss various techniques that can be coupled with the ABA behavior paradigm in order to study other aspects of the animal model.

The mouse ABA model allows exploration strictly of the neurobiology of the disease AN. This is separable from the socio-cultural influences, which, undoubtedly, contribute toward a person's vulnerability. The ABA model can also be used to investigate the effect of recurrent food restriction or other forms of stress in combination with wheel access, so as to capture some aspects of AN relapse[16]. Inhibitory neurotransmitter system function in brain anxiety centers has been studied using electron-microscopic techniques[4,16,17]. Dendritic arborization has been studied

using Neurolucida-assisted tracing and analysis of pyramidal cells in the CA1 field of the hippocampus[18,19] and amygdala[17]. Effects of food restriction and wheel access upon anxiety have been studied using behavioral tests such as the elevated plus maze[10]. The genetic basis of vulnerability has been studied using different inbred strains of mice[9]. Pharmacological manipulations can be tested in an animal model prior to human trials[20-24]. Genetically modified animals and transient knockdown of genes can be used to study how manipulation of particular molecular pathways can affect behavior in the ABA paradigm. The impact of stress during early life upon differential vulnerability to ABA would be another topic that can be addressed through this approach.

Protocol

All procedures described in this protocol are in accordance with the Institutional Animal Care and Use Committee of New York University (Animal Welfare Assurance #A3317-01).
NOTE: This protocol has been optimized for adolescent female C57BL/6 mice. The animals were housed in a facility that maintains RT at 72° ± 2° and room humidity at 50% ± 10%. Room lights turned on from 7 am to 7 pm daily.

1. Preparation of Cages with Running Wheels

1. Set up the computer and USB Interface Hub in a safe area of the animal holding room, away from running water and foot traffic, but close enough to the cage rack to be within the wireless range of the transmitters. Ensure that the computer and USB Interface Hub both receive power from a wall outlet, and the USB Interface Hub connects to the computer via a USB cable. Use a power backup device to power both the computer and the USB Hub.
2. Connect the computer to the USB Interface Hub using the USB cable included with the running wheel equipment.
3. Boot the computer and start the running wheel software by double-clicking on the icon.
4. Install three AAA batteries into the base of each of the running wheels, and confirm that the wheel manager software has recognized the transmitter. List each wheel in the program window under the heading “Wheel Sensors.”
5. Set up the configuration of the data acquisition according to the particular specifications of the experiment.
6. Prepare a cage for each mouse subject with bedding, nestlets, free access to water, and a running wheel. Typically, 8 mice are used per experiment for neuroanatomy studies. More mice may be required for behavioral studies to ensure adequate statistical power.
7. Ensure that the running wheel is able to move freely without touching any of the cage walls, food basket, or cage top. Spin each wheel a few times and confirm that the software is updating the wheel counts for each wheel.

2. Acclimation Phase

1. Place each mouse subject (female C57BL/6 mouse; age P36) individually in a cage with a running wheel.
2. Add a pre-weighed amount of dry food (approximately 100 g) to the food hopper, and place a pre-weighed full container (approximately 50 g) of wet food in the cage.
3. In the program window, begin the wheel activity data acquisition and data storage by selecting the “Start Acquisition” option in the File menu. Choose the directory to which the data will be saved. The software will record revolutions of the wheel continuously until the experiment is stopped manually.
4. Weigh the animal, wet food, and dry food every day at the time that lights are turned off in the room. Refill dry food if the weight falls below 50 g, and replace the wet food container if the food dries out or becomes soiled with bedding. Manually record the wheel count every day at this time as well, in case of loss of the digital data.

3. Beginning Food Restriction

1. Remove all wet and dry food from the cage at noon (or 7 hr before the room lights are scheduled to turn off) on the first day of food restriction.
2. On the same day, at the onset of the dark cycle, record the weight of the animal and the wheel count. Place a pre-weighed amount of dry food (approximately 50g) into the food hopper and a pre-weighed amount of wet food (approximately 5g) into the cage in a weighing boat.
3. Prepare a fresh cage with bedding and nestlets for each animal.
4. After 2 hr, transfer the running wheel to the prepared fresh cage. This cage change ensures that the animal remains food restricted until the next feeding time, in case some food crumbs have fallen or been hoarded in the bedding. In order to reduce the stress of the cage change, add two handfuls (approximately 500 ml) of the soiled bedding from the old cage, and move the animal into the new cage.
5. Record the weight of the remaining wet and dry food to determine the amount of food that was eaten. Record the wheel count at the end of the food access period.

4. Monitoring Animal Health during Food Restriction

1. Every day, at the onset of the dark cycle, record the weight of the animal and the wheel count. Place a pre-weighed amount of dry and wet food to the cage.
2. If an animal’s body weight falls below 75% of their initial body weight before food restriction, remove them from the experiment.
   NOTE: Other indications of excessive starvation include a hunched posture and inability to move around the cage. The animal may be cold to the touch and fail to eat during the 2 hr of food access.
3. Prepare a fresh cage with bedding and nestlets for each animal.
4. After 2 hr, transfer the running wheel to the prepared fresh cage. Add two handfuls (approximately 500 ml) of the soiled bedding from the old cage, and move the animal into the new cage.
5. Record the weight of the remaining wet and dry food to determine the amount of food that was eaten. Record the wheel count at the end of the food access period.

5. Ending the Experiment

1. After three days of food restriction, end the ABA experiment. Euthanize the animal for collection of brain tissue, or allow the animals to recover before undergoing additional behavioral testing.
2. Click the “End Acquisition” option under the File Menu in the program window.
3. Remove the running wheels from the cages, and remove the batteries from the wheel base.
4. If allowing the animals to recover, return a pre-weighed amount of dry food to the food hopper and allow the animals *ad libitum* access to food during recovery.

6. Data Analysis

1. Save all wheel data for the experiment in a .wls file in the directory chosen at the start of the experiment.
2. Export data to a spreadsheet by selecting the “Export” option in the File Menu. Select the desired .wls files in the “Source Data File” option. Select the start and end date and time, and select each wheel sensor for export in the Wheel Sensors list.

Representative Results

In order to study the effect of ABA in a similar population to human anorexia nervosa, these experiments have been performed in female adolescent mice. Thus, wheel acclimation begins soon after the onset of puberty in mice, on day P36. The acclimation phase is conducted from P36-P40, and food restriction occurs from P40-P43.

Adolescent mice are continuing to grow, and their body weight continues to increase as they approach full adulthood. During wheel acclimation, the mice generally lose a small amount of weight or plateau in weight. After the beginning of food restriction, body weight of ABA animals sharply decreases (**Figure 1**). The body weight of animals in the ABA group can be compared to control (CON) animals that did not have access to a running wheel and did not experience food restriction.

The wheel activity of each animal can be analyzed in various ways: (1) The daily (24-hr) wheel activity of the ABA animals can be plotted, showing that the animals begin to run excessively after the onset of food restriction (**Figure 2**). (2) Each animal’s wheel activity can be examined at a finer scale using the analysis software, showing the circadian pattern of wheel activity (**Figure 3**). (3) The wheel activity during the 2 hr of food access indicates voluntary food restriction, since the animals are choosing to run instead of eat. (4) After food restriction begins, some animals show an increase in activity in the period of time just prior to the time of feeding. This daily increase in locomotor activity prior to the presentation of food is called “food anticipatory activity” (**Figure 4**). (5) The speed with which animals run can be compared, as both the distance and the dwell-time on the wheel are monitored continuously. Change in these parameters may reflect the learning phase of running on the wheel.

Animals show individual variability in their wheel activity, eating behavior, and weight loss. While this individual variability often makes it difficult to obtain statistically significant group mean differences, it opens an avenue of analysis by correlation. For example, the change in body weight in ABA mice correlates with their daily change in wheel running – that is, animals that showed more wheel activity also lost more weight[16].
In the same study, it was also shown that the GABAergic innervation of hippocampus CA1 pyramidal cells was increased in the animals that showed decreased hyperactivity in a second experience of ABA. In a study using ABA rats, it was found that the expression of GABA receptors containing the α4 subunit correlates with decreased hyperactivity, or resilience to ABA[25].

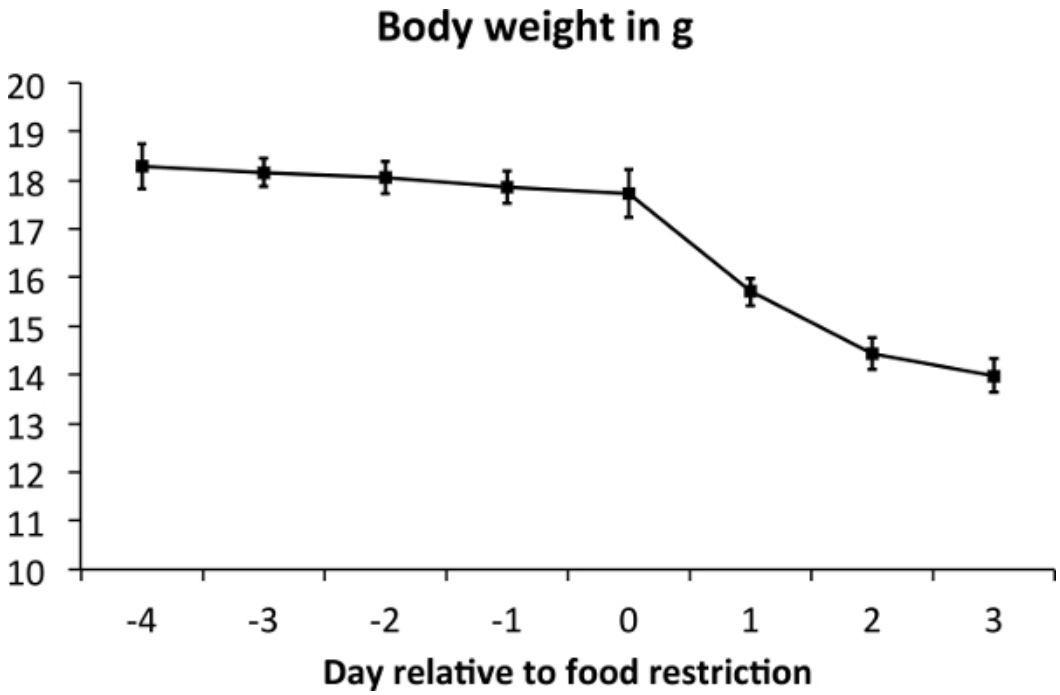

Figure 1. Body weight changes during ABA. Body weight data is shown from one cohort of five adolescent female mice. The mice had running wheel access for the full 7 days of the experiment. The first four days were the acclimation phase, after which food restriction was imposed for an additional three days. Day 0 indicates the beginning of food restriction. Error bars indicate standard error of the mean. Please click here to view a larger version of this figure.

Figure 2. Daily wheel activity before and after the onset of food restriction. Daily (24 hr) wheel activity is shown for one mouse. Day 0 indicates the beginning of food restriction. Total daily wheel activity increases by almost two-fold after the onset of food restriction. Please click here to view a larger version of this figure.

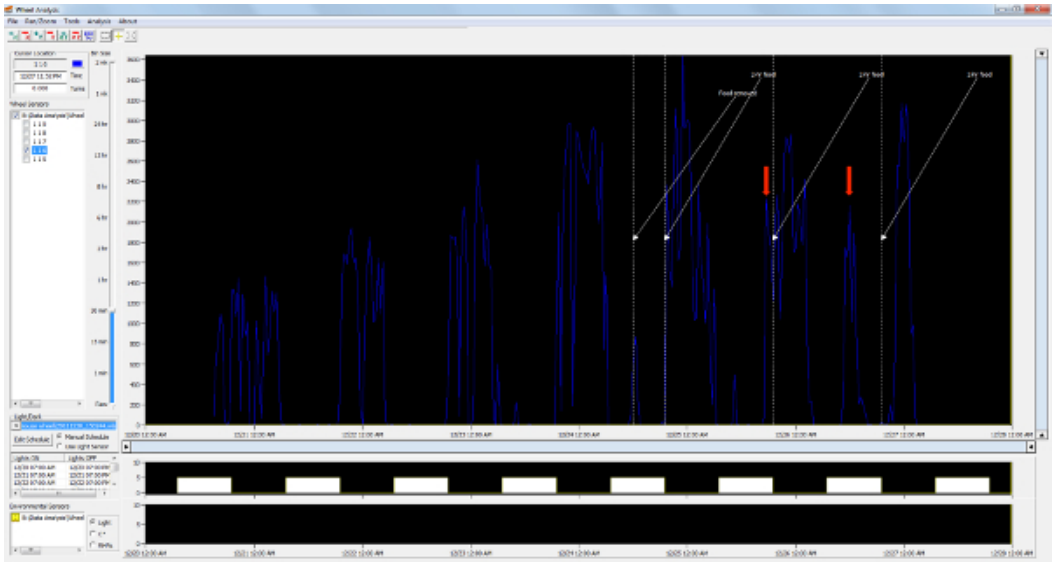

Figure 3. Continuously monitored running wheel activity over the eight day experiment. A screenshot is shown from the Wheel Analysis software. This shows the wheel activity (wheel counts on the vertical axis) of a single mouse over eight days (time on the horizontal axis) of access to a running wheel. Below the activity plot is an overlay indicating the times when lights are on and off in the room. Before food restriction begins, the animal shows minimal activity during the light cycle. The first vertical dashed line indicates the onset of food restriction, the three subsequent lines indicate the 2 hr feeding start each day, and red arrows indicate the emergence of food anticipatory activity during the light phase. Please click here to view a larger version of this figure. Please click here to view a larger version of this figure.

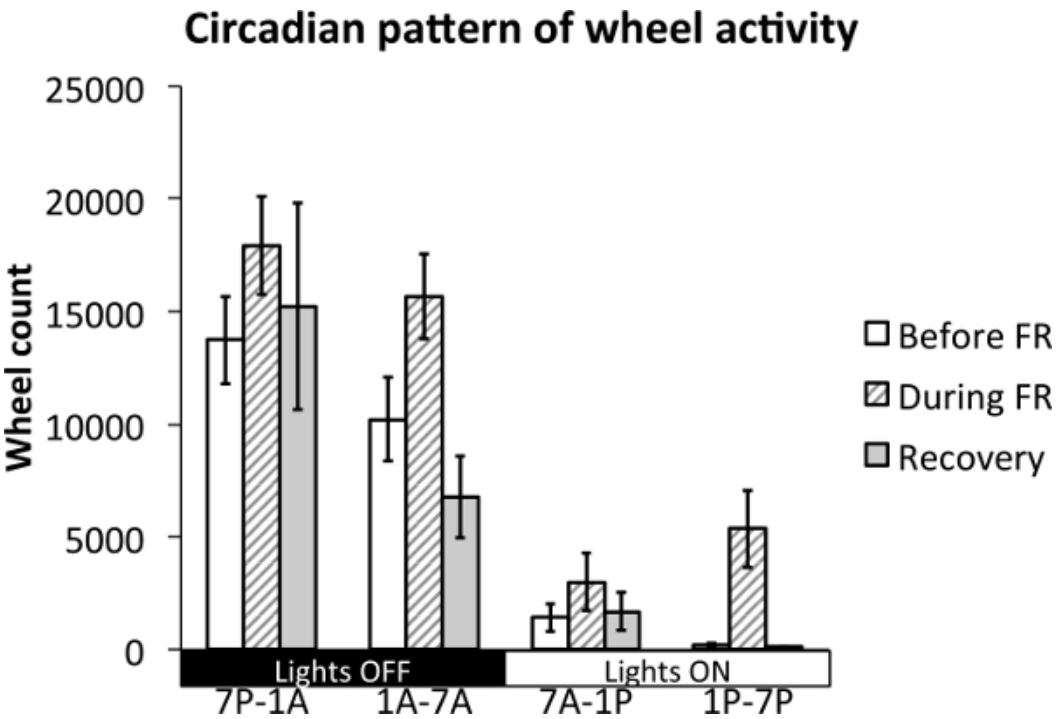

Figure 4. Wheel activity is increased across all hours of the day, but most dramatically in the period prior to food access. Wheel running is shown for four 6 hr sectors of the day. Bars labeled “Before FR” indicate the average number of wheel counts during the latter two days of the acclimation phase. Bars labeled “During FR” indicate the first two days of the food restriction phase. “Recovery” indicates the levels of activity after animals were allowed to recover without a running wheel for at least 6 days. Please click here to view a larger version of this figure.

Discussion

The critical aspects of the ABA model are (1) free access to voluntary exercise on a running wheel and (2) food restriction with food access limited to a restricted period of time. Access to a running wheel allows the animal to choose to use the wheel and gives an indication of the effect of food restriction on the motivation of the animal to exercise. Conversely, time-restricted food access (rather than calorie-restriction) allows

the experimenter to measure voluntary food restriction by monitoring the extent to which animals choose to run during the limited hours of food access. In this way, ABA is an excellent model of the self-starvation that occurs in AN.

In order to minimize the noise in the mouse behavior data, it is important to minimize the amount of unpredictable stress that the animals experience. For example, handling of animals should be kept to a minimum, with the animals only being disturbed during weighing, once a day. The experimenter handling the animals should be trained and comfortable with handling the animals. If possible, one person should handle the animals throughout the experiment to avoid additional stress. Scents and perfumes should be avoided. The time of weighing and food delivery should be made to be as regular as possible, to minimize any unpredictability. As a precaution against data loss, it is best to power the computer through a backup power supply in case of a power outage; even a brief interruption of power will cause the computer to restart and data acquisition will cease. Additionally, it is important to monitor the battery life of the wheel transmitters daily. If the battery level becomes weak, the transmitter may intermittently fail to send data to the hub, thus underestimating the activity of the animal.

The mouse protocol described here was modified from the standard protocol that has been used for rats[4]. Adolescent female mice are much more vulnerable to excessive weight loss and death due to starvation. Therefore, the following changes were made in order to improve survival to at least three days of ABA. First, the first day of food restriction was shortened by removing food at noon rather than at 8 pm of the previous day. Further, the time period of food access was increased from 1 hr to 2 hr and the availability of wet food was also added to minimize the effects of dehydration. We found that administering wet food to the mice greatly improved their condition through three days of food restriction. Without the wet food, body weight was falling much faster and animals had to be removed from the food restricted environment. These changes were sufficient to allow the mice to survive through three full days of food restriction and readily recover from ABA.

This protocol for ABA has some important limitations to consider. First, it is necessary to house the mice individually in cages with a running wheel in order to monitor the wheel activity of each mouse independently. This results in social isolation of the animals, a known stressor that may affect the behavior of the animals during ABA as well as some of the neural circuits that are being studied[26]. So far, there is no equipment available that is able to monitor the individual activity of co-housed mice, but this would seem to be a solvable problem using RFID technology and tracking tags tethered to each animal. Another potentially unavoidable consequence of co-housing animals during food restriction is the risk that the animals may become aggressive toward their cage-mates. Changing the animals' cage after each feeding session is another stressor that we had to introduce due to one animal hoarding food under the bedding. We aim to minimize the stress of a new cage by introducing a substantial amount of soiled bedding from the previous cage into the fresh cage.

Other groups using the ABA model have chosen different parameters for their feeding schedule. The choice of feeding time during the dark phase of the light-dark cycle is not standard. We chose to feed the animals at the time that the lights turn off to allow a more natural time for the animals to eat, since the nocturnal mouse is habitually more alert and active during this time. Some groups feed the animals during the lights-on period of the day[13,27]. This may be for the sake of convenience of the experimenter, and it is important to note that the time-period for food allowance during the light phase should be increased to improve survival. It has also be suggested that blocking access to the running wheel during feeding may improve survival, but we feel that this removes the very interesting aspect of behavior that is the decision made by some animals to run rather than eat, further exacerbating the self-starvation aspect of the ABA model, but capturing a hallmark of the human behavior associated with AN.

It is important to note that this protocol has been optimized specifically for adolescent female C57BL/6 mice. If a different mouse strain, sex, or age group is to be used, some parameters of the protocol may require modification. It has also been shown that RT affects the severity of ABA in rodents[28]. While we did not attempt to vary the RT for our studies, increasing the RT is likely to improve survival rates among ABA animals.

The advantage of using an animal model of a human disease, such as AN, is that is possible to study the brain anatomy and physiology and changes induced by access to voluntary exercise and food restriction in a controlled setting. The use of mice in the ABA model allows the use of powerful genetic approaches using transgenic animals and viral infection for gene manipulation. Future studies are aimed at studying the effect of particular genes in the resilience or vulnerability to food restriction-induced hyperactivity and self-starvation.

Disclosures

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