Rodent Model of Activity-Based Anorexia

Olaia Carrera,1 Ángela Fraga,1 Ricardo Pellón,2 and Emilio Gutiérrez1,3

1Unidade Venres Clinicos, Universidad de Santiago de Compostela, Galicia, Spain
2Departamento de Psicología Básica I, Facultad de Psicología, Universidad Nacional de Educación a Distancia, Madrid, Spain
3Departamento de Psicología Clínica y Psicobiología, Universidad de Santiago de Compostela, Galicia, Spain

ABSTRACT

Activity-based anorexia (ABA) consists of a procedure that involves the simultaneous exposure of animals to a restricted feeding schedule, while free access is allowed to an activity wheel. Under these conditions, animals show a progressive increase in wheel running, a reduced efficiency in food intake to compensate for their increased activity, and a severe progression of weight loss. Due to the parallelism with the clinical manifestations of anorexia nervosa including increased activity, reduced food intake and severe weight loss, the ABA procedure has been proposed as the best analog of human anorexia nervosa (AN). Thus, ABA research could both allow a better understanding of the mechanisms underlying AN and generate useful leads for treatment development in AN. Curr. Protoc. Neurosci. 67:9.47.1-9.47.11. © 2014 by John Wiley & Sons, Inc.

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Anorexia nervosa (AN) is a mental disorder characterized by restricted food intake and severe body weight loss (American Psychiatric Association, 1994). Excessive physical activity is also commonly present in patients with an AN diagnosis (Casper, 1998; Hebebrand et al., 2003). All these core symptoms of AN can be reproduced in the rodent model of activity-based anorexia (ABA). It is largely accepted that ABA provides the best analogous model to AN (Gutiérrez, 2013). Under this procedure, rats exposed to food restriction and free access to activity wheels show an increase in activity, subsequent self-starvation, and weight loss that closely parallels the main symptoms of human AN. Additionally, the ABA procedure reproduces other clinical manifestations present in AN, such as the cessation of the estrous cycle in female rats (Watanabe et al., 1992), hypothermia (Paré, 1977), alterations in the circadian sleep-wake cycle (Watanabe et al., 1990), and stomach ulcers (Hall and Beresford, 1989). The ABA model could both allow a better understanding of the mechanisms underlying AN and generate useful clinical leads for its treatment.

Here we describe a protocol to perform the ABA model. The experimental procedure involves food restriction (1 to 1.5 hr per day), but free access to a running wheel (23 to 22.5 hr per day) for the remainder of the day. This treatment corresponds to the experimental group, known as Active, whereas a control group, known as Sedentary, does not have access to the running wheel and is exposed to the identical feeding schedule. Both groups are therefore food restricted, but each group name denotes whether animals have wheel access (Active) or not (Sedentary).

Excessive running and decreased food intake during the food restricted schedule meal time is observed in about three-quarters of the animals exposed to ABA. For these animals body weight sharply decreases, and finally self-starvation and death ensue unless animals
are removed from the experimental conditions. Therefore, in order to minimize animal distress and the number of animals used in experimentation, the protocol should clearly state both the duration of the experiment and the criteria used to withdraw animals from the experiment before the designated duration. Criteria used in ABA research to prevent animals’ death are: (1) minimal food intake (i.e., less than 1 g); (2) minimal body temperature (i.e., less than 33°C), and/or (3) a maximum body-weight loss (i.e., 25% of free-feeding weight). The body-weight loss criterion seems at the same time the most appropriate and easy to be carried out in ABA studies, being additionally conservative in that stomach ulcers have been repeatedly observed in animals losing more than 25% of body-weight (Doerries et al., 1991). With regard to the food intake criteria, the reduction of food intake to less than 1 g per day characterizes the terminal stage that precedes death, and is observed in animals losing well over 30% of their body weights. Food intake of male and female rats still is, on average, higher at 30% weight loss than at the standard 25% weight-loss criterion. At first glance, this seems contradictory to the ABA designation (i.e., activity-based anorexia), because food consumption still increases after meeting the weight-based criterion of 25% weight loss. However, at this stage, food intake of animals in the “Active” group is reduced in comparison to sedentary animals exposed to the same feeding schedule but without running wheel access.

The protocol described here requires only the most basic equipment: a running wheel with mechanical counter of wheel turns attached to a home cage with a sliding door. More sophisticated equipment includes the possibility for automatic recording of wheel turns as well as food and water intake. In this case, software is needed to collect and record data, as well as to activate the brake that blocks the wheel during feeding time. Software can be purchased from the same provider as the rest of the equipment (e.g., VitalView software from Respironics), or can be programmed by the user. MED-PC (Med Associates) is perhaps the most extensively used software nowadays used for running behavioral experiments. MED-PC programs are structured in two main components: the inputs and the outputs, which are nested to work almost in parallel. In the case of ABA, one input is required for the wheel turns and another (optional) for licks at a water-bottle spout, and one output for the brake of the wheel. Programs are constructed to activate the brake for the period of time in which food is being made available to the rat (1 to 1.5 hr a day) and record wheel turns during specified periods of time, normally every minute. Wheel turns can then be aggregated in longer units of time (e.g., every 15 min) in order to give an accurate description of the behavior of the animals during the 23 to 22.5 hr of daily access to the wheel. Once programs are constructed, they need to be compiled (by translating the programs into Delphi, a most modern version of Turbo Pascal) in order to be executable files. Translation and compilation of programs is done in MED-PC IV with the application Trans IV.

NOTE: All procedures in experimentation using live animals should be carried out in accordance with national and international regulations (e.g., European Union Council Directive 2010/63 on the protection of animals used for experimental and other scientific purposes), and the specific protocol must be reviewed and approved by the corresponding ethics committee [e.g., Institutional Animal Care and Use Committee (IACUC)] on the use and care of animals at the institution under whose auspices the research would be conducted.

NOTE: Rats have been the species most often employed in ABA research, but other rodent species have been studied under ABA experimental conditions, such as guinea pigs, hamsters, gerbils (Vincent and Paré, 1976), chipmunks (Vincent et al., 1977), and mice (Epling et al., 1983). Running ABA experiments with mice presents its own peculiarities, as some strains react with decreased running when food restricted (Rikke et al., 2003), and differ in ABA susceptibility (Gelegen et al., 2006, 2007, 2008). Running
an ABA study with mice would require marked changes in the ABA protocol for rats described in this unit (Klenotich and Dulawa, 2012). Thus, the feeding period should be extended up to 6 hr, and both the removal criterion and the pattern of running differ as depicted [e.g., in two recent studies using two different mice strains with marked differences in susceptibility to develop the ABA phenotype such as BALB/cJ (Klenotich et al., 2012) and C57/BL6 mice (Lewis and Brett, 2010)]. Changes to the standard ABA protocol in the sense of extending the daily feeding period are also necessary when testing hyperactive rats such as the Dark Agouti strain (Vidal et al., 2013).

**Materials**

- Male Sprague-Dawley or Wistar rats, 160 to 180 g (minimum of 16)
- Rat chow pellets (Harlan Teklad, cat. no. 2018S, or equivalent)
- Room with control of ambient temperature and relative humidity [ambient temperature should be checked daily and temperature readings should be recorded in the experiment logbook; ambient temperature is a key parameter in ABA research (see Critical Parameters)]
- Polycarbonate cages of the same dimensions depending on the wheel/cage set (48 × 28 × 20 or 28 × 28 × 14–cm) with mesh lids in which food can be placed and a water bottle inserted (all cages are lined with wood shavings)
- Water bottles with rubber stoppers commonly used in animal husbandry
- Identification tags for cages and running wheels
- Top-loading balance
- Wahman-type activity wheels (1.12-m circumference and 10-cm wide running surface of 10-mm wire mesh bounded by clear Plexiglas walls) used to observe running activity
- Computer and spreadsheet software

*NOTE:* We purchase the wheels from a Spanish company, Panlab, that imports them from Allentown (http://www.allentowninc.com). As depicted in Figure 9.47.1, there are different assemblages of wheels with the living chamber. Either the wheel is mounted in the living chamber (polycarbonate cage 48 × 28 × 20–cm), or the wheel is attached to a cage (acrylic 28 × 28 × 14–cm) provided with a sliding door to allow communication between the running wheel and the cage.

**Adaptation period and group assignment phase**

1. Order at least 16 male Sprague-Dawley or Wistar rats (weight range 160 to 180 g upon arrival).
There are some strains of rats that are not appropriate for this research mainly because they are very hyperactive. Almost 100% of ABA research uses Sprague-Dawley or Wistar rats. We obtain animals from the University Animal Resources Center, which, in turn, acquires the rats from Charles River.

Subject characteristics such as gender, age and body weight are relevant for ABA outcome (see Critical Parameters).

2. House the animals in the colony room (four to six animals per cage), and mark them with one or more rings around their tails to identify animals in each cage.

3. Handle the animals briefly, only ~1 min, to weigh the animals on a daily basis for 4 days. Record their body weight and their average food intake each day.

   It is important to have an appropriate balance for weighing small quantities of food (±0.1 g accuracy). It is useful to have two balances, one for weighing animals and water bottles (range 0 to 600 g), and a more sensitive one (range 0 to 100 g) for weighing food consumption.

   Daily food intake can be monitored by weighing food when delivered and weighing what remains 24 hr later. Body weight and food intake should be weighed at the same time every day.

4. During these 4 days, provide the rats with unlimited access to food and water.

5. On the fourth day, assign animals to two weight-matched groups (Active and Sedentary conditions) and transfer them to the laboratory room, where ambient temperature should be controlled (21 ± 1°C is the standard ambient temperature; see Troubleshooting) with a 12:12 hr light/dark schedule.

   Animals are then switched from group to individual housing.

6. House rats allocated to the Active condition in the cages attached to the running wheels, and tag the cage.

   From this point onward rats are individually housed, so it is not necessary to mark the rat, only the cage.

7. House the rats allocated to the sedentary condition in the acrylic cages and tag each cage.

Pre-exposure to the wheel phase (days −4 to −1) (Optional, see Troubleshooting)

8. Give the animals assigned to the active group 2 hr of access to the running wheels for each of these days (from 09:30 to 11:30).

   During this phase animals have unrestricted access to food and water.

9. Record wheel turns, body weight, and food intake for each day.

Activity-based anorexia phase (days 1 to 14)

10. The main procedure starts with the removal of food at 11:00. At the same time, open the doors to the wheels for the Active rats. This day is known as day 0.

11. From day 1 onward, weigh animals just before the feeding period, between 09:10 and 09:30.

12. Give the rats access to food (the same food as always) from 09:30 to 11:00. During the feeding time, put several food pellets (20 to 25 g) on the feeder section of the cage lid.

13. Close the doors to the wheels (or activate their brakes) during this feeding period.
14. Once the feeding period has finished (11:00), monitor daily food intake by weighing food at the start and at the end of each feeding period (including food crumbs from the cage floor).

15. Open the doors to the wheel for Active animals.

16. Give the animals access to the water throughout the whole experiment.

17. For ethical reasons, any animal weighing 75% (or less) than their original start weight for two consecutive days has to be removed from the experimental setting (impeding any possibility of access to the wheel) and be given free access to the food. Recovery criterion is defined as body weight on any particular day, day \( n \), greater than the weight of the animal 4 days before, day \( n - 4 \) (Weight day \( n \) weight day \( n - 4 \)).

Active rats hardly survive in a laboratory environment when they are food-restricted to a single meal and have unrestricted access to an activity wheel. The removal criterion prevents animals from unnecessary discomfort. In comparison, Sedentary rats usually successfully adjust to food restriction and tolerate the 14-day experiment (but should be removed if their bodyweight loss reaches the 25% with respect to day 0).

18. The experiment ends on day 14, after the feeding period. Remaining Active animals that have not met either the removal or the recovery criterion are nevertheless removed from the experimental conditions and housed in the colony room as they were during the adaptation period (housed in groups and with ad libitum food access).

COMMENTARY

Background Information

Antecedents of ABA can be traced to observations of increased physical activity in semistarved laboratory rats since the early 20th century (see Boakes, 2007; Gutiérrez, 2013). However, it was the research by Routtenberg and Kuznesof (1967) describing self-starvation of rats living in activity wheels that formed the basis of the experimental work by Epling and Pierce, who coined the term ABA. Research by these authors showed that rats exercised harder when hungry and that the exercise seemed to override the effects of food deprivation, since the rat’s lever pressing for food pellets diminished following exercise (Epling et al., 1981, 1983; Pierce et al., 1986; Epling and Pierce, 1988).

There is another experimental procedure related with ABA known as “semistarvation-induced hyperactivity” (SIH) (Broocks et al., 1990), which focuses on the role of food restriction on running activity. SIH rats with continuous access to wheels display a high rate of wheel running while they are kept at a very low weight (25% to 30% baseline). However, instead of having a time-restricted period of access to food as in the ABA procedure, SIH rats receive a constant percentage of their previous ad libitum food consumption without any time restriction.

There are different hypotheses explaining excessive wheel running behavior in semistarved rats. Excessive wheel running has been interpreted in terms of foraging behavior triggered by insufficient energy replenishment caused by food restriction. Alternatively, increased locomotor activity in food-restricted rats may also be understood as a consequence of the reinforcing properties of running via the activation of dopamine and opioid limbic reward circuitry (Epling and Pierce, 1991). Furthermore, hyperactivity could also be related to low leptin levels associated with weight loss (Exner et al., 2000), as well as functioning as a surrogate thermoregulatory behavior triggered by rat’s hypothermia (Gutiérrez et al., 2002).

Critical Parameters

ABA is an experimental procedure easily reproducible in the laboratory. However, research has shown that certain subject characteristics (e.g., age, body weight) and other experimental parameters (e.g., feeding schedule, ambient temperature) can either protect, alleviate, or aggravate the outcome in animals exposed to ABA.

Age and body weight of rats

Age of the rats is inversely related to survival time under food restriction and free access to a running wheel. Older (Paré, 1975)
and heavier (Boakes and Dwyer, 1997) animals show less running activity and weight loss than their lighter counterparts. Therefore, age effect on ABA is related to the rat’s body weight and activity level. Rats of the same age, but lower body weight, show greater vulnerability to ABA (Boakes and Dwyer, 1997).

**Gender of rats**

Gender of the rats is another factor modulating energy balance when exercise and caloric restriction are involved. Running activity tends to be higher for females than for males under food restriction (Paré, 1975; Watanabe et al., 1990), but also under ad lib feeding conditions (Carrera et al., 2011). In spite of these higher levels of activity, females are better equipped than males to compensate for the cost of running and require a couple of days longer to reach the 25% body weight loss removal criterion in ABA studies (Cerrato et al., 2012).

**Prior experiences to ABA**

In addition, the animal’s previous experience is important for ABA development. Early manipulations of mother-pup interaction, such as early weaning (Glavin and Paré, 1985), can aggravate ABA outcome. In contrast, other early experiences like handling (Carrera et al., 2006) and growing in an enriched environment (Paré and Vincent, 1989) mitigate ABA consequences. Research has shown contradictory results for early maternal separation, both accelerating (Hancock and Grant, 2009) and moderating (Carrera et al., 2009) ABA. Also, more proximal experiences to ABA, such as receiving shocks, cold and enforced running have been found to minimize the effects of running on weight loss (Paré, 1986).

**Feeding schedule**

The feeding schedule influences the development of ABA. The restricted feeding schedule during the light phase of the light-dark cycle employed in the majority of ABA research departs from the natural dark pattern of eating in rats and this is a limitation to process a large amount of food in a relative short period of time (Pérez-Padilla et al., 2010). Therefore, ABA is better tolerated by animals fed during the dark phase (Paré, 1975; Boakes and Dwyer, 1997), or even the light phase if rats have been previously adapted to the restricted feeding regime (Pérez-Padilla et al., 2010). Moreover, with longer feeding periods, animal’s food intake is less affected and body weight is better preserved (Routtenberg and Kuznesof, 1967), a result also reported when the duration of the feeding period is distributed in multiple rather than in a single meal (Lambert and Peacock, 1989). The caloric dietary composition of food also influences ABA outcome, that is, high-fat diet diminishes ABA effect (Brown et al., 2008).

**Ambient temperature**

Ambient temperature (AT) has been overlooked in ABA research (Gutiérrez et al., 2002), mainly due to the fact that the choice of AT (typically between 20°C and 22°C) is often set to the convenience of the laboratory staff. However, several studies have directly addressed the critical role of AT in the development and maintenance of ABA. In 2005, Hillebrand et al. showed that having access to a heat source reduced hyperactivity and body weight loss in ABA rats. Raising AT to 28° to 33°C prevented hyperactivity and body weight loss under ABA conditions (Gutierrez et al., 2006). More interesting, once animals have developed excessive running and have overcome a 20% of body weight loss—and the probability of recovery is null unless experimental conditions are suspended—elevating AT can reverse hyperactivity, resulting in body weight recovery (Gutiérrez et al., 2008; 2009; Cerrato et al., 2012). In line with these results, physical activity in AN patients has been found to be more elevated in the cold semester in comparison with the warm semester (Carrera et al., 2012). Therefore, ABA studies on AT can give clinicians some clues to address the treatment of hyperactive behavior in AN.

**Troubleshooting**

**When rats do not exhibit hyperactivity**

Hyperactivity is one of the most important variables in ABA research, but due to the spontaneous variability in activity, ~20% to 30% of rats are not interested in running (Mon- don et al., 1985), and when exposed to ABA they do not show the typical pattern of progressive increase of running, which results in a preserved meal efficiency and a nonsignificant lose in body weight. Apart from the subject’s characteristics that we mentioned before, which influence physical activity (such as the age and weight of the rats), there are complementary methods to ensure that rats will exhibit high levels of running. One such procedure has been included as an alternative in the Basic Protocol: the pre-exposure phase. The presence of wheel exposure prior to food deprivation produces higher running rates than
when rats have not become familiar with the wheels (Boakes and Dwyer, 1997). Another way to ensure hyperactivity during the ABA procedure is to make a selection of active animals according to the running wheel activity displayed by the animals prior to being exposed to ABA (Paré, 1975). This second alternative allows the experimenter to disqualify rats exhibiting low levels of activity from the study.

The protocol described here presumes a standard arrangement of wheels and cages usually distributed on shelves in the same room. This distribution allows animals to hear and smell each other, but not to have visual or physical contact between animals. This standard arrangement allows the noise generated by the turns of the contiguous wheels to have a facilitation effect on running, and any other arrangement resulting in an attenuation of the noise produced during the wheel running of other animals would diminish the rate of wheel running (Fraga et al., 2012).

**Accelerated body weight loss**

If the accelerated body weight loss is affecting the whole group of rats, then check ambient temperature in the laboratory room. Rats are more physically active at lower AT and, subsequently, body weight loss occurs more rapidly. Increasing the AT in the laboratory room a couple of degrees (to 22° to 23°C) is a technique to reduce activity and make body-weight loss more gradual. If a single rat exhibits an unexpected fast decline of its body weight, water availability should be checked. It could happen that the water bottle leaked and the animal was also water deprived.

**Anticipated Results**

As it can be seen in Figure 9.47.2, under ABA conditions (simultaneous food deprivation and access to the wheel) performed at standard AT (21° ± 1°C), Active animals become hyperactive—they can run more than 10 km per day—and they show an increased body weight loss and a reduced food intake in comparison with the Sedentary control group (see Fig. 9.47.3). As can be seen in the upper panel of Figure 9.47.3, almost all Active animals lose weight and reach the removal criterion in 6 to 10 days, while their Sedentary counterparts initially lose weight but then stabilize and can tolerate a 14-day experiment (Gutiérrez et al., 2008; Cerrato et al., 2012). Most of the Sedentary animals often attain the recovery criterion. Regarding food intake (lower panel of Fig. 9.47.3), the sudden change from ad lib to the 22.5/23 hr deprivation schedule severely depress food intake. Henceforth, rats face the one meal condition increasing their daily food consumption steadily from day to day. However, as these changes in food intake happen in the context of a progressive increase of running activity, Active animals are not able to increase their food intake at the same rate as Sedentary animals do and when food intake of Active animals approaches the efficiency shown by Sedentary animals they usually have meet the removal criterion of 25% weight loss.
Figure 9.47.3  (A) Changes over days in body weight relative to weight on day 0 until the first Active rat was removed from the experiment (see Basic Protocol, step 17 for additional details about the 25% removal criterion). (B) food intake over days (daily 1.5 hr feeding period). Active animals (n = 9) had free access to the wheel, whereas Sedentary animals (n = 9) were just food deprived. Figure adapted from Cerrato et al. (2012).

Figure 9.47.4 shows that the typical expected pattern of running is characterized by an increase in wheel turns at the start of the dark period of the day (marked in the figure by horizontal black bars on the abcissae) and in anticipation of the next food occurrence. Food periods are denoted by vertical dash columns. Exposure to the running wheel just 3 or 4 hr before the feeding episode produces a moderate weight loss that in a study was sufficient to reach the ABA criterion (Dwyer and Boakes, 1997), although animals generally do not show the rapid and substantial weight loss that results from the application of the standard procedure.

**Time Considerations**

When an experiment is performed at a standard AT (21° ± 1°C), it is expected that at the end of the first week of ABA exposure (food restriction and wheel access), almost all Active animals will develop hyperactivity and considerable weight loss. The removal criterion is usually reached in 7 to 10 days by most
of the Active animals, whereas Sedentary animals usually attain the recovery criterion by the end of the experiment. In 14 days, most of the animals will reach the removal or recovery criterion. At the end of the experiment, however, some animals might still hover between the removal and recovery criterion but will not fully reach any of them. These animals are defined as intermediate animals.

Taking into account the adaptation period (4 days), the optional pre-exposure to the wheel phase (4 days), and the ABA phase (0 to 14 days), a typical ABA experiment will last 23 days. In order to save time, the pre-exposure to the wheel can be shortened to 2 days, or even eliminated. But bear in mind that the absence of wheel exposure produces lower running rates than when rats have already become familiar with the wheels prior to food deprivation (Boakes and Dwyer, 1997).

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Literature Cited
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