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# Impaired reversal learning in an animal model of anorexia nervosa

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ABSTRACT

*Background:* Clinical investigations indicate that anorexia nervosa (AN) is associated with impaired cognitive flexibility. Activity-based anorexia (ABA), a rodent behavioral model of AN, is characterized by compulsive wheel running associated with voluntary food restriction and progressive weight loss. The goal of this study was to test whether ABA is associated with impaired cognitive flexibility.

*Methods:* Female Sprague-Dawley rats were trained to perform the attentional set-shifting test (ASST) to assess cognitive flexibility, including capacity for set-shifting and reversal learning. Rats were assigned to ABA or weight-loss paired control (WPC) conditions. Following baseline testing, the ABA group had access to food for 1 h/d and access to running wheels 23 h/d until 20% weight loss was voluntarily achieved. For the WPC group, running wheels were locked and access to food was restricted to reduce body weight at the same rate as the ABA group. ASST performance was assessed after weight loss, and again following weight recovery.

*Results:* Compared to baseline, the ABA group (but not the WPC group) showed a significant decrement in reversal learning at low weight, with return to baseline performance following weight restoration. The other components of ASST were not affected.

*Conclusions:* Impaired reversal learning, indicative of increased perseverative responding, in the ABA model reveals its potential to recapitulate selective components of cortical dysfunction in AN. This finding supports the utility of the ABA model for investigations of the neural mechanisms underlying such deficits. Reversal learning relies on neural circuits involving the orbitofrontal cortex and thus the results implicate orbitofrontal abnormalities in AN-like state.

## 1. Introduction

A preclinical behavioral syndrome known as “activity-based anorexia” (ABA) is considered the most clinically relevant animal model of anorexia nervosa (AN) currently available. ABA is associated with compulsive-like behaviors in rodents that are analogous to the human disorder, including self-starvation and driven exercise [1], with additional disturbances in neurotransmitter and neuroendocrine systems [2,3]. In the ABA model, when rats are simultaneously given limited access to food and housed with access to running wheels, they voluntarily decrease food intake, dramatically increase running wheel activity, and lose significant amounts of body weight (> 20% free-feeding weight in less than two weeks) [4]. This paradoxical phenomenon is more severe in adolescent female rats in comparison to males; disrupts estrous and circadian sleep-wake cycles; and unless the experimenter intervenes, induces stomach ulcers, exhaustion, malnutrition and eventually death [5].

Clinical studies in AN have documented impaired cognitive flexibility, a behavioral correlate of compulsivity that relies heavily on prefrontal cortex (PFC)-dependent executive function [6]. Cognitive inflexibility in AN is associated with rigid patterns of thought, perfectionism, obsessional ideas, and perseverative actions to control body weight and shape, and has been linked to more severe eating disorder symptoms, increased symptoms of depression and anxiety, and higher rates of mortality [7,8]. In comparison to controls, patients with AN commit more perseverative errors on cognitive testing (e.g., with the Wisconsin Card Sort Test), with medium to large effect sizes [9] implicating neural circuits underlying this type of compulsivity as promising targets for drug development.

To test the feasibility and utility of the ABA paradigm to model cognitive inflexibility, we utilized the standard attentional set-shifting test (ASST, [10]). The ASST assesses discrimination, set-shifting, and reversal learning. Set-shifting involves switching attention between relevant stimulus dimensions or “attentional sets” (e.g., shifting between odors, digging mediums, or textures), whereas reversal learning involves switching attention in response to alternating stimulus-reward

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associations when the relevant stimulus dimension or set is held constant (i.e., shifting between two types of odors, ignoring other sets) [11]. We hypothesized that ABA would result in greater impairments in the ASST compared to a weight loss-paired control group (WPC) proving its utility to model cognitive deficits relevant for AN. Importantly, the ASST is PFC-dependent where lesions of the medial (mPFC) and orbitofrontal (OFC) regions of the PFC induce selective impairments in set-shifting or reversal learning, respectively. Thus, in the ABA model, a more severe impairment in reversal learning, which is indicative of increased perseverative responding, would be consistent with previous studies in ABA rats that have shown metabolic changes in OFC and related neural circuits [12].

## 2. Methods and materials

### 2.1. Animals

Sixteen female Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC), six weeks of age upon arrival and weighing between 150 and 175 g at the beginning of the experiment, were housed in a temperature-controlled room (21 ± 2 °C) maintained on a 12:12 light-dark cycle (lights on at 800). Rats were housed individually in solid-bottom polycarbonate cages (tub dimensions: 16″ × 20″ 8.25″H) with running wheels attached (wheel diameter: 14 in., I.D., run distance: 1.10 m/revolution, Lafayette Instrument Company, Lafayette, IN, USA). Running wheels remained locked except where noted below. When wheels were unlocked, running was a voluntary activity and was monitored using Activity Wheel Monitoring Software (Lafayette Instrument Company). Rats were given ad libitum access to Purina chow (#5001) and tap water, except as noted below. All procedures were approved by the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee.

### 2.2. Study design

Following a one-week habituation period, rats were trained to perform the ASST and were tested at baseline, following weight loss, and following weight recovery (Fig. 1). All sessions were conducted during the light phase, and test sessions were video recorded and scored by an experienced observer. Rats were tested in two consecutive groups of eight, the experimental condition (ABA) or the control condition (WPC), using the same methods and materials.

Following the ASST training phase, the first baseline testing session (ASST1A) was completed. To allow for acclimation to wheel running, rats were then given ad libitum access to a running wheel for approximately two weeks to begin to establish stable levels of running [13]. Running wheels were then locked for the next four to five days, after which time animals completed a second baseline testing day (ASST1B). After the baseline ASST testing phase, rats were allowed running wheel access for an additional week and a half to establish baseline running levels prior to ABA. Each animal's free-feeding weight (FFW) was calculated as the average body weight during the last four days of this running phase.

At the start of the weight loss phase, rats assigned to ABA were given free access to food for 1 h/day immediately prior to the dark cycle (running wheels locked) and were then given free access to the running wheel for the next 23 h. This ABA induction phase, which lasted between 5 and 18 days for individual animals (Fig. S1), was terminated when the animal's weight decreased to 80% of its FFW (Fig.1).

For the WPC group, running wheels were locked and food was restricted so as to match the changes in body weight of the ABA animals on a pair-wise basis, i.e. each rat in the WPC group was given daily food rations calculated to replicate the rate of weight loss and the total weight loss of one rat (its pair) in the ABA group (Fig. S1 in Supplement).

In both groups, low weight phase testing (ASST2) was conducted immediately after rats reached the weight loss criterion. After testing at low weight, rats in both groups immediately entered the weight restoration phase with ad libitum access to food and running wheels in locked state. As each animal reached 100% FFW (approximately one week), weight-recovered cognitive testing was performed (ASST3).

### 2.3. ASST testing protocol

ASST training and testing was conducted as described previously [10,14]. Briefly, rats were placed in a testing box (15 in. height, 28 in. length, 18 in. width) with a choice chamber and a waiting room separated by a movable plastic divider. Two identical bowls were placed in opposite corners of the choice chamber, but only one bowl contained a hidden food reward (~0.1 g chow). Rats were trained by successive approximation to dig for the buried rewards. To motivate foraging behavior, rats were limited to 10 g chow per day for four days prior to each ASST session (resulting in an 8–10% reduction of free-feeding body weight). Once reliable digging was established, the rats began exemplar training on two simple discriminations of odor (i.e., almond versus lemon scent) or digging medium (i.e., gravel versus marbles). Training sessions continued for each exemplar until the rat reached six consecutive correct trials.

Using ground chow presented in small (0.1 g) pieces in ASST has been described before in previous reports [14], and it was also successfully piloted in female rats in our lab prior to the experiments reported in the present manuscript. We attempted three pilots to test motivation of rats based on three different methods. First, we tried a more palatable treat (e.g., honey nut cheerios), but we found that the female rats did not remain motivated to dig for cheerios for enough trials to complete the task (the test sometimes requires over 100 trials per session). Second, we tried water deprivation, both in an effort to increase motivation to complete all 7 stages of ASST as well as to

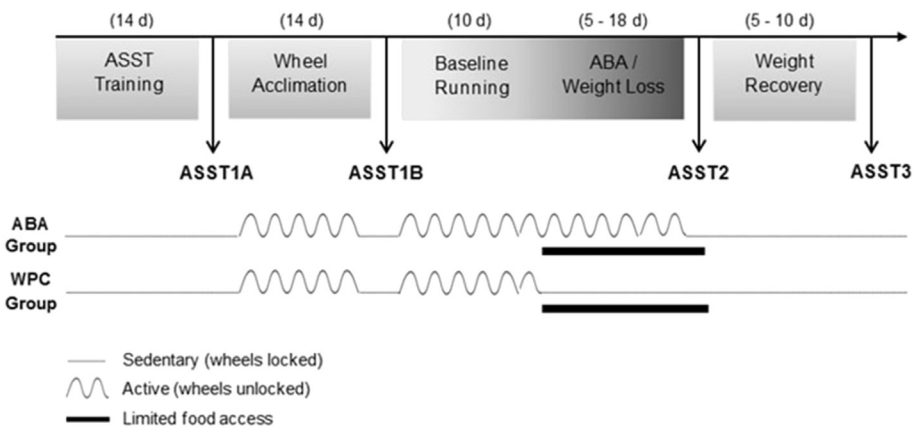


**Fig. 1.** Timeline of behavioral testing, food access, and running wheel activity. Following behavioral training, baseline ASST performance was measured before (ASST1A) and after access to running wheels (ASST1B). Rats were tested again at low weight (ASST2) and then following weight recovery (ASST3).