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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 hr/d and access to running wheels 23 hr/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

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Supplementary Materials

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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.

Keywords

Activity-based anorexia; anorexia nervosa; cognitive flexibility; attentional set-shifting; reversal learning; food restriction; prefrontal cortex; female rats

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 (greater than 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 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 ($21 \pm 2^\circ\text{C}$) maintained on a 12:12 light-dark cycle (lights on at 800). Rats were housed individually in solid-bottom polycarbonate cages (tub dimensions: 16" x 20" x 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 one hour per day immediately prior to the dark cycle (running wheels locked) and were then given free access to the running wheel for the next 23 hours. 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 (0.1g) 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 minimize the limitation of food restriction given the ABA model; however, this also failed to motivate rats sufficiently and also is theoretically problematic. Last, we attempted food restriction based on published work of others who have used ASST, and we found this method to be consistently effective. The rats were motivated to eat “real” food for over 100 trials.

On formal ASST testing days, rats performed seven consecutive stages with specific rules designed to assess discrimination, set-shifting and reversal learning abilities (Table S1, in Supplement). In the first two stages the rats have to learn to find a hidden reward based on cues in one sensory dimension (e.g., + odor) and to ignore the other (e.g. – digging medium). Thus, rats demonstrate discrimination learning by performing a simple discrimination (SD, where one dimension is held constant, while the other is varied) followed by a compound discrimination (CD, where both dimensions are varied, but the same cues from the SD are used). Rats then perform the first reversal (Rev1), where rewarded and unrewarded cues within the same dimension as the SD/CD are switched. The fourth stage involves an intra-dimensional shift (ID, where a new pair of cues is introduced but the rewarded dimension remains the same, e.g., + odor, – digging medium). ID is followed by the second reversal (Rev2), where the rewarded and unrewarded cues within the same dimension as the ID are switched. The sixth stage entails an extra-dimensional shift

(ED), where a new pair of cues is introduced but the previously irrelevant dimension becomes the new target (i.e. + digging medium, – odor). At the final stage, ED test is followed again by a reversal task (Rev3), in which, as in reversals following SC/CD and ID, the rewarded and unrewarded cues within the same dimension are switched.

2.4. Data Analysis

In a preliminary analysis the possible effect of running itself was tested in both ABA and WPC groups (Fig. S2. in Supplement). Since statistical analysis (RM-ANOVA) showed no significant differences in cognitive function for ASST1A and ASST1B (all p 's $> .05$), baseline values for descriptive and cognitive assessments were calculated as the mean for these two initial test days. Baseline characteristics and test performance for ABA and WPC groups were then compared by repeated measures analysis of variance (RM-ANOVA). Because preliminary analysis showed that the ABA group weighed slightly more than the WPC group at baseline (204 ± 8 versus 192 ± 9 grams, respectively, $p < 0.02$), all further analyses were adjusted for baseline body weight. To assess the effects of ABA on cognitive performance, change from baseline to low weight testing was compared for ABA and WPC groups by repeated measures analysis of covariance (RM-ANCOVA). For any measure showing significant alteration during low weight testing, to test whether this alteration persisted following refeeding, change from baseline to weight recovery testing was also assessed by RM-ANCOVA. All group data are expressed as mean $\pm$ standard deviation (mean $\pm$ SEM in figures). Statistical significance was defined as $p < 0.05$, with Bonferroni adjustment for multiple comparisons. Data were analyzed using SPSS Statistical Software.

3. Results

RM-ANOVA indicated a significant effect of ABA on ASST performance ($F(4,28) = 5.24$, $p = 0.0028$), i.e. total trials to criterion increased from baseline to low weight (Bonferroni-adjusted comparison: $t(28) = 5.32$, $p < 0.001$) and decreased from low weight to weight recovery testing sessions ($t(28) = 5.63$, $p < 0.001$), with no differences between baseline and weight recovery testing ($t(28) = 0.31$, $p > 0.05$). Furthermore, there was a significant interaction effect between discrimination stage and ASST test session ($F(28, 196) = 1.56$, $p = 0.043$) pointing to selective impairment of reversal learning (Bonferroni comparison of baseline and low weight Rev1: $t(196) = 4.98$, $p = 0.001$; for all other discriminations $p > 0.05$). In the WPC group, RM-ANOVA indicated a general decrease in cognitive performance ($F(4,28) = 5.30$, $p = 0.0026$) at low weight (baseline to low weight to recovery: $t(28) = 3.66$, $p = 0.011$ and $t(28) = 4.61$, $p < 0.001$, respectively), but no interaction between test session and discrimination phase ($F(28, 196) = 1.25$, $p = 0.187$) that would indicate specific impairment on any particular discrimination task (Fig. 2).

Further comparison of ASST performance of the ABA and WPC groups focused on three primary cognitive domains: discrimination, set-shifting, and reversal learning at baseline, low weight, and weight recovery testing sessions (Fig. 3). For each testing day, capacity for discrimination was calculated as the combined mean number of trials required for completion of SD and CD; for set-shifting, as the combined mean for ID and ED; and for reversal learning, as the combined mean for Rev1, Rev2 and Rev3.

For reversal learning, there was a significant effect for group ($F(1,13) = 15.76, p = .002$) and a significant group by time interaction ($F(1,13) = 7.23, p = 0.019$). Post-hoc testing showed that for the ABA group, there was a significant increase in the number of trials required to complete the reversal learning tests ($p = 0.004$). Following weight restoration, performance on reversal learning did not differ significantly from baseline performance for either the ABA or the WPC group ($F(1,12) = 0.258, p = 0.621$).

On the other hand, baseline performance for the ABA and WPC control groups did not differ significantly for discrimination learning ($F(1,13) = 1.32, p = 0.271$), set-shifting ($F(1,13) = 0.516, p = 0.485$), or reversal learning ($F(1,13) = 0.783, p = 0.392$). For discrimination learning and set-shifting, performance did not change significantly from baseline to low weight testing, and did not show a significant group by time interaction (all p 's > 0.05). Performance did not differ significantly between the two set-shifting tasks (ID versus ED) for either the ABA or WPC groups.

Group comparisons of diet and exercise variables that may alter ASST performance showed that the WPC group weighed less than the ABA group at the start of the weight loss procedures ($p < 0.05$) even though both groups of rats weighed similarly upon arrival until approximately the sixth week in the study (all p 's > 0.05). However, there were no group differences in food intakes ($F(1,14) = 0.685, p = 0.221$) and running distances prior ($F(8,112) = 1.01, p = 0.432$) to weight loss, as well as no differences in the percentage or rate of weight loss ($t(14) = -0.132, p = 0.897$) (Fig. S3 and S4, respectively, in Supplement). There were no correlations between body weights, food intakes, or running distances and ASST performance at any point in the study (all p 's > 0.05), indicating that it is unlikely that these factors contributed to group differences in reversal learning following weight loss procedures.

4. Discussion

The primary finding of this study is that, when tested at low weight, rats exposed to the ABA model demonstrated impaired reversal learning; this effect was no longer observed following weight recovery. ABA is considered the most clinically relevant animal model of AN currently available and is frequently used for studying homeostatic and endocrine regulation in an AN-like state. The results of this study reveals for the first time its potential to also recapitulate a specific component of cortical dysfunction. This study supports the utility of the ABA model for investigations of the neural mechanisms responsible for such deficits.

A major strength of the rodent ASST is its ability to deconstruct complex forms of cognitive/behavioral rigidity associated with different neurocircuits. Rodent and primate electrophysiology, lesion, and pharmacology studies using the ASST have demonstrated that reversal learning selectively involves the OFC, whereas ED set-shifting behavior relies on mPFC (for review, see [15]). Thus, impaired reversal learning in the ABA model found in this study implicates the OFC and related cortico-striatal loop structures [16], in agreement with a microPET study which previously localized ABA-induced metabolic abnormalities to OFC, insular cortex, and ventral striatum [12]. Dysregulation of this ventral (limbic) circuit and a dorsal (cognitive) neurocircuit centered on the dorsolateral PFC was proposed to

underlie different aspects of dysfunctional AN behavior [17]. Neuroimaging studies have also shown abnormal fronto-striato-thalamic activity in patients with AN while performing cognitive flexibility tasks [18, 19].

Reversal learning deficits are indicative of an increase in compulsive or perseverative behaviors. While ABA rats were able to learn stimulus-reward associations after 20% weight loss, they showed greater difficulty in adjusting their behavioral responding to a new rule when the stimulus-reward contingency was swapped. This perseveration may suggest an impairment of extinction learning, which is characterized by the inability to inhibit responding to a previously learned response [6]. Although few clinical studies of AN have examined reversal learning ability separately from set-shifting, one report indicated that low weight adolescent patients performed more poorly on the probabilistic object reversal task, with a lower total score and fewer blocks completed in comparison to healthy controls [20]. Similarly, a recent report showed that low weight women with AN committed more excessive switches and total errors than the healthy controls on a probabilistic reversal learning task, although the effect was below significance level following adjustment for age and IQ differences [21].

We did not observe significant changes in set-shifting behavior at low weight in either ABA or WPC protocols, and there were no differences between the two groups. This stands in apparent contrast with the majority of clinical studies reporting set-shifting deficits in AN [7]. The human literature, while certainly not without its problems, has been much more consistent in highlighting disturbances in set-shifting than in reversal learning. The human literature also suggests that the disturbances in set-shifting are trait-related, and are not reversed simply by weight gain. It is important to note that human research, although limited, has focused on deficits that are not reversed simply by weight gain. Furthermore, the time course of changing cognitive function in AN has so far received little attention. Reversal learning might be relatively prominent early in the course of AN, while deficits in set-shifting may develop more gradually and become more prominent as the illness becomes chronic. It is possible that our design using ABA is more suitable for modeling an early phase of weight loss in the illness.

Further studies are required to resolve this discrepancy, both in animal models and human AN. Clinical studies frequently use multidimensional tasks, such as the Wisconsin Card Sort Test, which cannot separate set-shifting failures from reversal failures [19, 22]. Our finding of unimpaired set-shifting on the ASST is consistent with three clinical studies that found no significant deficits in set-shifting in low weight AN using the CANTAB intra-/extra-dimensional (IED) task, which does distinguish set-shifting ability from reversal learning [23–25]. However, these studies using the IED also did not find reversal learning impairments, which may be attributed to the simplicity of the reversal task used. Taken together, this study highlights the need to clarify impairments in cognitive flexibility observed in AN by examining set-shifting and reversal learning separately.

Similar modifications may also be needed for more reliable assessment of set-shifting in the ABA model. A potential limitation of the current study relates to the finding that during baseline testing only six of the eight animals in the ABA group and 7 in the WPC group

showed the expected increase in trials required for completion of the extra-dimensional set-shifting subtest in comparison trials for the less challenging intra-dimensional subtest (Figure 2). Although few rodent studies have examined set-shifting performance in females, it is of note that females have more difficulty forming an attentional set in comparison to males, whereas there appear to be no sex differences in reversal learning [26]. Thus testing female rats may require a separate IED task, as for example the modified ASST paradigm proposed by [27] in which reversals are replaced with multiple ID discriminations to induce more robust attentional set before the ED stage [28].

Several further limitations should be noted regarding this initial study associating ABA with impaired reversal learning. First, the ABA and WPC groups were studied sequentially rather than in parallel and although both groups were similar in age, for unclear reasons the ABA group weighed more than the controls at baseline testing. The sequential design had a major advantage however that it allowed individual control of daily food rations of each WPC rat to match the changes in body weight of one particular ABA rat on a pair-wise basis (Fig. S1 in Supplement). Second, a potential limitation of the current implementation of the ASST testing paradigm is that it required modest food restriction for several preceding days in order to motivate foraging behavior in the baseline and weight recovered stages. Additionally, although animals in both groups were sufficiently motivated to complete all seven stages of the ASST at low weight, we did not assess whether ABA and control rats equally valued food during the weight loss phase.

Previous work has demonstrated that reduced brain levels of serotonin, especially in the OFC, selectively impair reversal learning across species [29]. Different types of 5-HT receptors might be involved and their effect may depend, among other factors, on their distribution patterns within the PFC. 5-HT₇ receptors for example are expressed more heavily in OFC, compared with mPFC [30], and were also implicated in reversal learning [31]. Extrapolating from this evidence, we hypothesize that reversal learning impairments observed during an AN-like state following ABA resulted in reduced serotonergic neurotransmission and OFC dysfunction. It is of note that impaired cognitive flexibility [9] and abnormal serotonergic function [32, 33] are separately well-documented in AN but have not yet been investigated together.

5. Conclusions

The present study demonstrated that the preclinical ABA model of AN is associated with impaired reversal learning during testing at low weight. Follow-up studies are needed to replicate this finding, and to explore possible remediation of this cognitive deficit with serotonergic and other neuropharmacological agents targeting cortical neurocircuitry. Based on the outcome of these studies, the ABA model may play a role in development of new therapeutic interventions to address the cognitive rigidity associated with AN.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Cognitive flexibility is often impaired in individuals suffering from anorexia nervosa (AN).
- This study assessed cognitive changes during activity-based anorexia (ABA), an animal model of AN.
- Reversal learning and set-shifting tasks were assessed using the attentional set-shifting test.
- Results showed selectively impaired reversal learning capacity in the ABA model during weight loss.

Figure 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).

Figure 2.

Effect of ABA on ASST performance. A–B. Average number of trials to meet criterion ($\pm$ SEM) for each discrimination stage of the ASST following baseline, weight loss, and weight recovery phases in the ABA (A) and WPC (B) groups. RM-ANOVA indicated a significant effect of ABA on ASST performance with significant interaction effect between discrimination stage and ASST test session pointing to selective impairment of reversal learning. In the WPC group, RM-ANOVA indicated a general decrease in cognitive performance but no interaction between test session and discrimination phase C–F. Performance in reversal learning and extra-dimensional set-shifting in individual experiments. C–D. Comparison of the performances in each rat on the first reversal learning (Rev 1) and on the preceding compound discrimination (CD) test across study phases in the ABA (C) and CPW groups (D). Note consistent increase in Rev 1 in the ABA group during the low weight phase compared with either the baseline or weight recovered phase. E–F. Comparison of ID and ED performance in each rat across study phases in the ABA (E) and CPW groups (F). Note relatively weak attentional set formed in both groups in even though trial to criterion values were larger in ED compared with ID in Baseline in 7 out of 8 rats in each group.

Figure 3.

Mean scores for each cognitive domain across study phases. At baseline, there were no group differences in discrimination learning, reversal learning, or set-shifting capacity. At low weight, the ABA group needed more trials to complete reversal stages in comparison to the WPC group ($p < 0.05$). Performance returned to baseline levels following weight restoration. There were no other statistically significant differences for any other ASST measures.