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Role of spontaneous physical activity in prediction of susceptibility to activity based anorexia in male and female rats

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HIGHLIGHTS

- Spontaneous physical activity (SPA) might be a trait for anorexia nervosa.
- Activity-based anorexia (ABA) is an animal model of anorexia nervosa.
- Tested models for prediction of ABA susceptibility in male and female rats
- Best model included running wheel activity, SPA and body weight history.
- SPA affects probability of ABA susceptibility across genders.

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ABSTRACT

Anorexia nervosa (AN) is a chronic eating disorder affecting females and males, defined by body weight loss, higher physical activity levels and restricted food intake. Currently, the commonalities and differences between genders in etiology of AN are not well understood. Animal models of AN, such as activity-based anorexia (ABA), can be helpful in identifying factors determining individual susceptibility to AN. In ABA, rodents are given an access to a running wheel while food restricted, resulting in paradoxical increased physical activity levels and weight loss. Recent studies suggest that different behavioral traits, including voluntary exercise, can predict individual weight loss in ABA. A higher inherent drive for movement may promote development and severity of AN, but this hypothesis remains untested. In rodents and humans, drive for movement is defined as spontaneous physical activity (SPA), which is time spent in low-intensity, non-volitional movements. In this paper, we show that a profile of body weight history and behavioral traits, including SPA, can predict individual weight loss caused by ABA in male and female rats with high accuracy. Analysis of the influence of SPA on ABA susceptibility in males and females rats suggests that either high or low levels of SPA increase the probability of high weight loss in ABA, but with larger effects in males compared to females. These results suggest that the same behavioral profile can identify individuals at-risk of AN for both male and female populations and that SPA has predictive value for susceptibility to AN.

1. Introduction

Anorexia nervosa (AN) is a chronic eating disorder defined by restricted eating, body weight loss, anxiety, and fear of weight gain [1–6]. Epidemiological data show that AN is predominantly a female disease, yet it does occur in males [7,8]. Estimates of life-time prevalence of AN in female range from 0.3% to 2.2% and for males is estimated between 0% and 0.9%, depending on the population studied [9–13]. Research on AN mechanisms in males and females is needed to understand the commonalities and differences between genders and to decide whether gender-specific therapies are necessary.

The most common AN animal model is activity-based anorexia (ABA). In ABA, animals are food restricted while given *ad-libitum* access to a running wheel (RW). In ABA, both male and female rats increase their RW activity, which coupled with limited food intake results in

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weight loss and if not stopped, eventual death due to starvation [14,15]. Thus, ABA models only the physiological consequences of food restriction and hyperactivity that occur during AN and is not equivalent to the human disease [16]. For an in-depth discussion regarding the validity of the ABA protocol as a model of AN, see the excellent review of Gutierrez [17].

Weight loss caused by ABA requires combined RW access and food restriction, as either intervention alone does not cause sustained weight loss [14]. The range of body weight loss in ABA appears to be between 10% and 30% of the body weight before ABA [18,19]. Thus, one could define whether an animal is susceptible to ABA if their final body weight is below a specific threshold percent of their initial body weight, such as 75% [20]. Finally, while exposure to ABA causes body weight loss and increases RW activity in male and female rodents, available data are inconclusive regarding differences in weight loss rate and RW activity between genders [19,21–23].

Considering the severity of health consequences of AN, it would be advantageous to identify at-risk populations before onset of disease. There is large variability between mouse strains in body weight loss during ABA [18,24,25]. Studies in female mice and rats indicate that RW activity during *ad-libitum* feeding predicts body weight loss during ABA [25,26]. In female rats, locomotor activity (measured by telemetry) during RW exposure with *ad-libitum* feeding is a predictor of body weight loss during ABA [25]. These studies support the idea that RW activity can predict ABA susceptibility, consistent with RW as a model of voluntary exercise [27] and its putative role as a cause and symptom of AN.

A higher level of physical activity is a common symptom [28–31], and may be a trait for AN [32,33]. Hyperactivity might be caused by an inherent higher drive for movement [33]. In both rodents and humans, spontaneous physical activity (SPA) describes the drive for movement. SPA includes low-intensity movements, such as fidgeting, time spent standing, and ambulating [34]. In humans, SPA can account for up to 30% of daily energy expenditure [35,36]. In rodents, SPA is operationally defined as time spent moving (ambulating and rearing) in the homecage or an open field after acclimation [37]. In this paper, we hypothesized that SPA levels have predictive power for ABA susceptibility in male and female rats.

We measured individual characteristics of male and female rats (body weight history, food intake during SPA, RW distance during *ad-libitum* feeding, SPA and stereotypic behavior) and tested the performance of different combinations of these individual characteristics (models) to predict ABA susceptibility. To implement these predictive models, we used Support Vector Machine (SVM) algorithms [38–40].

2. Methods

2.1. Animals

Male (n = 25) and female (n = 32) Sprague–Dawley rats (SD, Charles River, Kingston, NY, USA) were used in these studies. All rats were approximately 8 weeks old upon arrival at the Minneapolis VA Animal facility. Unless indicated otherwise, animals were housed individually in solid-bottom cages with corn-cobb bedding and chewing substrate (Nylabone, natural flavor, BioServ, Frenchtown, NJ, USA), with a 12-h light/12-h dark photo-cycle (lights on at 0600 h) in a temperature controlled room (21–22 °C). Rodent chow (Harlan Teklad 8604) and water were allowed *ad-libitum*, except when noted. Baseline body weights were recorded after one week acclimation during the SPA recordings and they were 315.41 ± 5.48 g for males and 232.93 ± 4.45 g for females. All studies were approved by the Institutional Animal Care and Use Committee at the Minneapolis VA Health Care System.

2.2. Behavioral experiments

After arrival to the animal facility, animals were housed individually with *ad-libitum* food and water for one week prior to the experiments. The experimental design had three stages as shown in Fig. 1: (1) SPA measurement, (2) RW acclimation and (3) ABA. The methods used to complete these stages are as discussed in the following sections.

2.2.1. SPA measurement

SPA was measured by infrared activity sensors placed around an acrylic cage (17.0 in. × 17.0 in., Med Associates, St. Albans, Vermont, USA). SPA was calculated as the sum of time spent ambulating (detected by two infrared arrays in the x and y-axes at floor level) and vertical movement (detected by a third elevated x array at 3 in. above cage floor). Stereotypic behavior is reported as movement within a 3.25 × 3.25 in. area centered at the center of mass of the animal.

Male SD rats were acclimated for 24 h to these chambers, which is sufficient to eliminate the effects of a novel environment on activity [34]. As these data were not available for female rats, we measured SPA for four consecutive 24 h periods (n = 32, Supplementary Fig. 1). Next, we randomly selected 15 female rats for further studies. For females, SPA used for prediction of ABA susceptibility was measured on the fourth day for several reasons. First, we wanted to avoid a novelty effect in SPA (Supplementary Fig. 1). Secondly, because of equipment problems, SPA on days 2 and 3 was not measured for a full 24 h period, but for between 23 and 24 h. As SPA on day 4 was measured for a full 24 h, we decided it was not possible to average total SPA over days 2–4. Finally, we needed to make the SPA data comparable between male and females (measured over a full 24 h after adaptation to testing conditions). During SPA measurements, food intake (corrected for spillage) and body weight were measured daily.

2.2.2. RW acclimation

After completion of the SPA measurement, all rats returned to their home cages. All animals were 10–12 weeks old at time of RW acclimation.

For acclimation to RW, rats were transferred to individual cages with RW attached (RW diameter, 18 in., width, 5.25 in., overall wheel dimension: 19.81 × 20.45 × 6.57 in., Lafayette Instrument Company, Lafayette, IN, USA) and were given *ad-libitum* access to RW for 7 days. Running was monitored using Activity Wheel Monitoring Software (Lafayette Instrument Company). These cages were of the same dimensions, with the same type of bedding and enrichment as the animals' home cages. Body weight was measured at the beginning and end of this period. Food intake could not be corrected for spillage as small food residues could not be separated from the bedding without disturbing the animals on a daily basis. Therefore, we did not include food intake during RW acclimation as a predictive feature in the analysis of predictive models for ABA susceptibility.

2.2.3. Activity-based anorexia (ABA)

The day after completion of RW acclimation, food access was restricted to 1 h daily starting 4 h within the lights-on period (11 AM–12 PM) for a maximum of 7 days. Food intake and body weight were measured daily. Body weight was measured between 1 and 2 h into the light cycle. During ABA, animals were monitored daily by investigators and animal technicians for signs of distress (*i.e.*, dehydration,

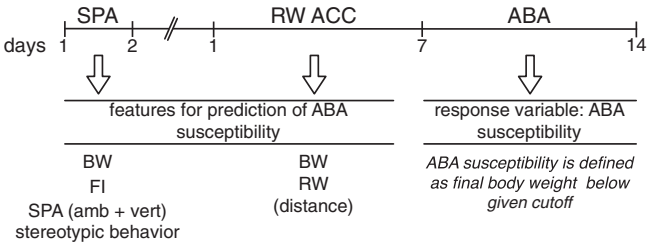

Fig. 1. Diagram of study design. Predictors for ABA susceptibility are SPA, stereotypic activity, food intake (FI) during SPA, body weight (BW) history pre-ABA and RW activity.