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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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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 can 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 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.

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Keywords

Anorexia Nervosa; Activity-Based Anorexia; ABA; individual susceptibility; SPA; behavioral models; spontaneous physical activity; physical activity; inter-individual variability; predictive models; support vector machines

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 ranges 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 weight loss and if not stopped, eventual death [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 a 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 inherent higher drive for movement [33]. In both rodents and humans, spontaneous physical activity (SPA) is 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 home-cage 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 Figure 1: (1) SPA measurement, (2) RW acclimation and (3) ABA. The methods used to complete these stages are as follows:

2.2.1. SPA measurement—SPA was measured by infrared activity sensors placed around an acrylic cage (17.0" × 17.0", 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 inch above cage floor). Stereotypic behavior is reported as movement within a 3.25 × 3.25 inch 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 acclimation effects [34]. As these data were not available for female rats, we measured SPA for four consecutive 24 h periods (n = 32, Supplementary Figure 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 Figure 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 hours. As SPA on day 4 was measured for a full 24 hours, 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 inches, overall wheel dimension: $19.81 \times 20.45 \times 6.57$ 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 one hour daily starting 4 hours within the lights-on period (11 AM – 12 PM) for a maximum of 7 days. Food intake and body weight was measured daily. Body weight was measured between 1-2 hours into the light cycle. During ABA, animals were monitored daily by investigators and animal technicians for signs of distress (i.e., dehydration, hypoactivity, lack of food intake). However, maximal duration of the experiment was achieved before any distress signs were judged to require removal from the study.

2.3. Models for prediction of ABA susceptibility

The goal of this study was to test whether it was possible to predict ABA susceptibility from a set of baseline characteristics for male and female rats (Figure 1): (a) *history of body weight* (body weight measured at SPA recording, start and end of the RW acclimation period), (b) *Food intake during SPA* (corrected for spillage), (c) *ad-libitum fed RW distance*, (d) *SPA* (time spent ambulating and rearing) and (e) *time spent in stereotypic behavior*. All these parameters, except SPA and stereotypic behavior, have been suggested to predict severity of weight loss during ABA in other studies [25-27].

We defined ABA susceptibility as body weight at the end of ABA (measured as percent of initial body weight) below a given threshold. We used different values for this threshold (65%, 70%, 75%, 80% and 85%). For this analysis, we excluded one male rat for which the food hopper during SPA was broken and thus it was not possible to obtain an accurate food measurement.

We chose Support Vector Machine (SVM) algorithms as the prediction engine. SVM are supervised learning algorithms used for two-class classification problems [38-40]. Briefly, in a two-class classification problem, there is a group of samples (i.e. rats) belonging to

either one of two classes (i.e. ABA susceptible or resistant) and set of features (individual characteristics such as body weight, SPA, food intake and RW activity) for each sample. A SVM algorithm will attempt to find a line (for sets of two features), plane (for sets of three features) or hyper-plane (for sets of four features or more) that can separate the samples according to their categories. This is usually referred to as the training of the SVM. Once a SVM is trained, new samples (i.e., new rats) will be assigned to either category according to features that determine their location relative to the hyper-plane. While SVM can only perform linear classification, the use of kernels (mathematical functions that map a feature space into a euclidean space) allow their use in non-linear classification problems [38].

In this paper we tested different models (combinations of individual features) to predict ABA susceptibility at different thresholds. In the next sections we outline the main ideas implemented in our analysis. A more detailed description of the computational methods used can be found in Supplementary Materials and in [38-40].

2.3.1. Handling imbalanced data—Across different thresholds for ABA susceptibility, there are unequal numbers of susceptible and resistant rats (Table 1). Using imbalanced data for SVM training decreases their performance [41, 42]. Several approaches have been proposed to correct this problem in the analysis stage. Although collecting additional data is the gold standard for handling imbalanced data, we reasoned that using analytical methods for dealing with imbalanced data was preferable to subjecting more animals to ABA. There are two popular methods to deal with imbalanced data during SVM training. One method is to use classification weights during SVM training and allow for correction of the classification errors given the size of different classes [43]. A second popular alternative is to use algorithms to impute synthetic data into the minority class. The SMOTE algorithm is a popular re-sampling procedure that creates synthetic data by interpolation data within the minority class to decrease the imbalance between data sets [44]. Preliminary analyses indicated that using a combination of the SMOTE algorithm and classification weights provided a clear advantage over use the use of weights or a standard SVM, which is consistent with previous studies [45].

2.3.2. Evaluation of SVM performance—To validate models for prediction of ABA susceptibility, it is necessary to test them against data not used in their training. To this end, we used the technique of leave-one-out cross-validation (LOCV). In this statistical technique, a SVM model is trained with all but one of subjects of the data set, which is used for testing. Thus, the performance of a model was calculated as its ability to correctly classify each of the rats included in the training data, but doing so with an SVM trained using data from all other animals. It should be clear that although synthetic data (generated with the SMOTE algorithm) was used during SVM training, their performance was evaluated using real-world data *only*. Using cross-validation during SVM can help prevent over-fitting of the data during SVM tuning.

The performance of a particular model was measured using the geometric mean (G_{MEAN}) based on predictions of the model against all real data using false positive and negative (FP and FN) and true positive and negative (TP and TN). G_{MEAN} is calculated as $\sqrt{x}$, where $x = \text{TP}/(\text{TP} + \text{FN})$ and $y = \text{TN}/(\text{TN} + \text{FP})$. G_{MEAN} has a maximum value of 1, which indicates perfect classification in

both classes (ABA susceptible and resistant). We report SVM performance as percent, such that 100% is perfect classification and 0% indicates miss-classification of all rats. Importantly, a performance of 50% would not be different from random class-assignment. Unlike accuracy (percent of correctly classified subjects), the geometric mean accounts for misclassification of the minority class, and thus is a more robust and accurate indicator of classification performance [42]. This is an important consideration, as our dataset is not balanced for ABA susceptible and resistant rats at each ABA susceptibility threshold.

2.3.3. SVM tuning—Preliminary analyses suggested the Gaussian kernel achieved high SVM performance and it was selected for further analysis. However, the performance of the SVM for a particular model depends on a number of additional numeric parameters, which are problem-specific. This means they depend on the combination of kernel and feature space used. In our case, these numeric parameters include two parameters specific to the Gaussian kernel, (See Supplementary Methods), the SVM weights for each class and the amount of over and under-sampling of the SMOTE algorithm. Identifying the combination of parameters that will result in the highest performance of an SVM for a given problem is usually described as tuning the SVM. We used a genetic algorithm (GA) to tune the SVM for each model tested for each ABA threshold. The GA is a heuristic search algorithm that mimics the process of natural evolution [46]. Supplementary Figure 2 shows an example of the progression of a SVM during tuning with the GA algorithm. Additional details of the implementation of GA can be found in Supplementary Materials.

2.4. Software

All statistical analyses, SVM modeling and graphs were done using the open source statistical software R [47]. We extensively used the packages ggplot2 0.9.3 [48] and plyr 1.8 [49]. The SVM implementation used was from the library LIBSVM [43] through the package e1071 1.6.3 [50]. The GA algorithm used was from the package GA [51]. The SMOTE algorithm used was from the implementation of the package DMwR [52].

2.5. Statistical Analyses

For all experiments, comparisons between three or more groups were done using ANOVA. Pairwise comparisons between groups were performed using t-test with a Benjamini-Hochbergh correction for multiple comparisons [53] when appropriate. One-group comparisons were done with a t-test. The Ansari test was used to compare group variances. Clustering of SVM by performance across ABA thresholds was done with a complete hierarchical clustering algorithm based on distance matrix of euclidean distance between performance vectors across ABA thresholds. Rate of body weight loss during ABA was analyzed using a linear mixed model with random effects for rat and time. For this analysis we report estimates of slopes with standard error. Effects of food intake and RW activity on final body weight were done with an ANCOVA using food intake and RW as covariates. Comparison of distributions was done with the Kolmogorov-Smirnov test. For all analyses, a p-value of less than 0.05 was considered indicative of statistically significant differences. We reported calculated p-values unless they were below 0.001.

3. Results

3.1. ABA body weight loss in male and females

Male and female Sprague-Dawley rats went through ABA for a maximum of 7 days. ABA caused a significant decrease in body weight in males and females compared to the pre-ABA body weight (Figure 2). We used linear mixed models to compare the rate of body weight loss between male and females. A linear mixed model analysis of the time-course of body weight loss (Figure 2A) with random effects for rat and time showed no significant effect of gender ($\beta = 0.016 \pm 0.02$, $t_{39} = 0.82$, $P = 0.43$), a significant effect of time ($\beta = -0.03 \pm 0.03$, $t_{240} = -10.23$, $P < 0.001$) and a significant interaction between gender and time ($\beta = -0.009 \pm 0.003$, $t_{240} = -2.35$, $P = 0.01$). The effect size of this interaction was small but prompted us to explore differences between male and female body weight loss. Comparison of individual body weight at the end of the ABA (Figure 2B) showed larger average body weight loss in males compared to females ($t_{39} = 4.09$, $P < 0.001$), without difference in variance between groups (Ansari test, $AB = 152$, $P = 0.782$). We found no significant differences in cumulative food intake normalized by body weight (Figure 2C, $t_{39} = 0.04$, $P = 0.96$) or its variance (Ansari test, $AB = 147$, $P = 0.45$). We also found no significant differences in average RW distance between male and females (Figure 2D, $t_{39} = 0.91$, $P = 0.36$), but there was a trend for significant differences in variance between groups (Ansari test, $AB = 194$, $P = 0.08$). These results suggest that ABA causes progressive body weight loss in male and females with similar individual variability, but the average and progression of body weight loss caused by ABA appears stronger in males compared to females.

Next, we explored the influence of RW activity and food intake on the rate of body weight loss during ABA. An analysis of time course of food intake and RW distance during ABA showed that while the increase in RW activity is linear over time, the increase in food intake is described by a hyperbole that stabilizes starting at the fourth day (Supplementary Figure 3 and Supplementary Table 1). This suggests that food intake reaches its plateau level during ABA while RW does not. Next, we tested the influence of daily RW distance and food intake as co-variables in a linear mixed model with random effects for rat and day of ABA. The effect of food intake was statistically significant ($\beta = -0.0041 \pm 0.005$, $t_{238} = -7.69$, $P < 0.001$), but the effect of RW distance was not ($\beta = 0$, $t_{238} = -1.29$, $P = 0.19$). Inclusion of these terms eliminated the significant interaction between gender and time ($\beta = -0.06 \pm 0.03$, $t_{240} = -1.72$, $P = 0.08$). Using a power transformation of food intake that provided a linear relationship with time did not change the results (Data not shown), suggesting that food intake, but not RW distance, is a major influence on rate of body weight loss in both genders.

To test the contribution of food intake and RW distance to total body weight loss caused by ABA we used an ANCOVA with total food intake and RW distance over ABA as covariates. This analysis indicated no significant effect of intake ($F_{[1,36]} = 2.31$, $P = 0.13$), but a significant effect of RW distance ($F_{[1,36]} = 30.108$, $P < 0.001$) and a significant effect of gender ($F_{[1,36]} = 19.96$, $P < 0.001$). We also examined whether SPA was related to RW activity during ABA. An ANCOVA for RW distance using gender as experimental variable

and SPA as a covariate failed to find a significant effect of SPA ($F_{[1,36]} = 2.25$, $P = 0.14$) and gender ($F_{[1,36]} = 0.97$, $P = 0.34$)

These analyses suggest ABA causes larger body weight loss in males compared to female rats, with similar variability within each gender. Use of linear mixed models suggest that differences in rate of body weight loss between male and female rats are explained better by variation in individual food intake rather than running wheel activity. However, it appears the greater body weight loss in males compared to females is explained by lower total RW activity in females compared to males. Overall, our results also suggest that differences in food intake explain the *rate* of weight loss whereas RW activity influences the *amount* of body weight loss.

3.2. Prediction of ABA susceptibility

Using Support Vector Machine (SVM) algorithms as predictor engine to test different models (combination of individual characteristics of animals pre-ABA), we determined whether it was possible to predict ABA susceptibility in a set of combined male and female rats. The rationale for combining male and female rats was to examine whether it was possible to determine a set of predictors with equal validity in both genders. To do this, we classified rats as either susceptible or resistant to ABA depending on whether their percent of initial body weight was less or more than a given threshold. We used different threshold values, from 65% to 85% in 5% increments (Table 1). This resulted in an unbalanced data set for ABA susceptibility with a different proportion of susceptible and resistant rats at each threshold (Table 1). As performance of SVM is affected by imbalanced data, we used a combination of the SMOTE algorithm and internal SVM weights [45] (See Methods for a detailed description of statistical methods) to improve SVM performance. To determine the kernel parameters, SVM weights and numerical parameters of the SMOTE algorithm we used a Genetic Algorithm approach (See Methods and Supplementary Information for details).

First, we compared the performance of a model including all behavioral features plus gender information against a model that did not include gender information. When both predictive models were implemented with a SVM, their performance ranged between 73.2 and 95.4%, which was significantly higher than chance for both models and across all thresholds (Figure 3, one sample t-test against 50% performance, $P < 0.01$ for all groups). A two-way ANOVA showed no significant difference between models ($F_{[1,90]} = 0.029$, $P = 0.86$), a significant difference in performance across thresholds ($F_{[4,90]} = 28.01$, $P < 0.001$) and a significant interaction between models and thresholds ($F_{[4,90]} = 3.28$, $P < 0.015$). This indicates that inclusion of gender as a predictor does not improve the performance of a model that included all behavioral features. Thus, we excluded gender as a predictive factor from further analyses.

Next, we examined the performance of all combinations of behavioral features to predict ABA susceptibility, which resulted in a total of 25 models. Figure 4 shows the average performance of 19-20 independent runs of SVM tuning for each model across all ABA thresholds. A two-way ANOVA showed a significant difference in performance between models ($F_{[26,2554]} = 17.04$, $P < 0.001$), across ABA thresholds ($F_{[4,2554]} = 1086.24$, $P <$

0.001) and a significant interaction between models and thresholds ($F_{[104,2554]} = 12.31$, $P < 0.001$). Pairwise comparisons of differences in average performance between ABA thresholds indicated higher performance at the extreme ABA thresholds (Supplementary Figure 4). Next, we clustered these models by their performance to examine if there was a particular combination of behavioral features that achieved the highest performance (Figure 4). The model with the lowest performance included only SPA and stereotypic behavior. Two models with different combinations of body weight history, ambulatory SPA, RW distance during acclimation and food intake during SPA achieved the highest average performance (Figures 4 and 5). We compared the performance of these two models against each other using a two-way ANOVA. This analysis indicated no significant difference in performance between models ($F_{[2,190]} = 0.354$, $P = 0.55$), a significant difference across ABA thresholds ($F_{[4,190]} = 45.50$, $P < 0.001$) and no significant interaction between models and thresholds ($F_{[8,190]} = 1.11$, $P = 0.351$). The best model included ambulatory SPA, RW distance, food intake and body weight history, while the second to best model included ambulatory SPA, RW distance and body weight history. These two models were not different from each other in terms of average performance across thresholds ($t_{46} = 0.42$, $P = 0.67$), but pairwise analysis indicated their performance was statistically higher compared to all other models (Figure 5). Overall, these analyses indicate that a profile of food intake, SPA, RW and body weight history can predict ABA susceptibility in male and female rats.

The simpler top-ranked model (SPA, RW activity and body weight history) had an average performance of 86.4% across ABA susceptibility thresholds (Figure 5). The model including only body weight history had a performance of 81.7% and the model including body weight history and RW activity had a performance of 80.5%, but this difference was not statistically significant ($P > 0.05$ for pairwise comparison). A comparison of the best model (SPA, RW activity and body weight history) against nested models showed that SPA and RW activity increase the performance of prediction by 5.9% and 3.5% respectively (Figure 5). Overall, these data suggest that ambulatory SPA, RW activity and body weight history can predict ABA susceptibility with an average performance of 86.8% across ABA susceptibility thresholds (range of 81.3 – 97.1%) and that a combination of SPA, RW activity and body weight history achieves a better performance compared to pairwise combination of features.

3.3. Effect of ambulatory SPA on ABA susceptibility

To evaluate the effect of SPA on ABA susceptibility, we explored the effect of artificially varying ambulatory SPA while maintaining all other individual characteristics constant for all rats included in our original dataset. Next, we used the top two performing models (Figures 4-5) to examine how altering SPA levels affected the probability of being susceptible to ABA separately for males and females (Figure 6). For this analysis, we focused on a single ABA susceptibility threshold of 75% of the starting body weight. This analysis showed that either very high or very low levels of SPA increased the probability of ABA susceptibility (Figure 6). The distributions of probabilities were different between male and females for both models (KS test, $P < 0.05$ for both models). Our data suggest that SPA can affect the probability of ABA susceptibility in male and females, but the effects of higher SPA levels on susceptibility to ABA were larger in males compared to females.

4. Discussion

The key contribution of this paper is the demonstration that a profile of SPA, RW activity and body weight history can predict ABA susceptibility in both male and female rats (Figures 3-5). We found that inclusion of gender did not significantly improve the performance of a predictive model that included all individual characteristics considered in this study (Figure 3). This result supports the idea that the basic pathophysiology of AN is similar between males and females [8, 54].

Our analysis of RW, food intake and body weight change during ABA suggest that ABA causes progressive body weight loss in males and females with similar individual variability in both genders, but with larger effects in males compared to females (Figure 2). Our results also suggest that differences in food intake explain the *rate* of weight loss whereas RW activity influences the *amount* of body weight loss. The exact nature of the relationship between food intake and RW activity in ABA is not clear. Increasing duration of feeding time in ABA decreases severity of weight loss [55], and pre-exposing the animals to restricted feeding prevents weight loss when exposed to a running wheel [56]. These data suggest that artificially restricting intake in rats triggers an ingestive response, which is then blocked by RW wheel access to further drive negative energy balance. ABA is not self-starvation, and in our protocol, we prevented RW access while food was available. Our analysis of time course (Supplementary Figure 3 and Supplementary Table 1) suggest that food intake has a hyperbolic relationship with time and equilibrates starting the fourth day of ABA for males and females, while RW distance increases linearly over time. This relationship might explain why food intake is more important for the rate of weight loss. Food intake will have a larger impact on weight loss earlier during ABA but eventually it will reach a plateau, while RW distance will continue to increase throughout ABA (thereby highlighting individual differences in RW distance). Therefore, RW distance which could have a larger impact on total body weight once food intake has reached its plateau level.

We used SVM to analyze different combinations of individual predictors (models) for their ability to predict ABA susceptibility (Figures 4-5). The approach we used to create predictive models for ABA susceptibility used analytical methods to deal with imbalanced data for ABA susceptibility during training and tuning of the SVM models (See Methods), but the performance of the SVM models was tested only against non-synthetic data. It is not our intention to state that analytical methods to deal with imbalanced data are superior to collecting more data. Collecting more data is the gold standard and analytical methods are useful in situations in which collecting data is not possible or justifiable. As our analysis was able to replicate previous findings (i.e., importance of running wheel activity as a predictor of ABA susceptibility, see Discussion below) we felt we had little justification to run additional animals in the ABA protocol, especially considering the hardship this behavioral intervention is for the animals.

An interesting conclusion is that, in our dataset, gender does not appear to be a significant predictor for ABA susceptibility when including all other individual features (Figure 3). Prior data are inconclusive regarding whether male or females are more susceptible to ABA [19, 21-23], but ABA does cause body weight loss and hyperactivity in both genders,

suggesting that focusing on common aspects of AN pathophysiology across genders could improve development of new therapies.

The average performance of all predictive models was higher for the extreme cutoffs (Supplementary Figure 4), which might be a consequence of the training dataset being biased against the majority class, despite using the SMOTE algorithms and SVM weights to balance the datasets. The best predictive model was a combination of RW activity during *ad-libitum* feeding, SPA and body weight history (Figures 4 and 5). Previous studies of ABA susceptibility showed that RW activity is a strong predictor of body weight loss during ABA in female rats [18, 25, 26]. Our results add support to the hypothesis that RW activity during *ad-libitum* feeding has predictive value for body weight changes during ABA in female rats and extend this result to male rats (Figure 4 and 5). Human studies suggest that pre-morbid BW correlates with the severity of body weight loss during AN [57] and linear analysis of ABA susceptibility suggest it can contribute to accuracy of prediction of ABA weight loss [18, 25, 26]. Overall, our results align with previous data suggesting that body weight and RW activity during *ad-libitum* food intake contribute to susceptibility to weight loss in ABA.

To our knowledge, this is the first study to show that SPA is relevant for prediction of ABA susceptibility (Figures 4-5) whereas stereotypic activity is not. That a model of RW activity and body weight history showed lower performance than body weight alone, but a combination of SPA, RW activity and body weight history achieved higher performance than either pairwise combination alone suggest complementary abilities of these individual features for prediction of ABA susceptibility. This highlights the necessity of feature selection in the prediction of complex traits, as it is not possible to predict in advance the effect of different combinations of predictors in prediction performance [58].

A previous study in female rats showed that locomotor activity (measured by telemetry) during RW exposure was a predictor of body weight loss during ABA [25]. We measured SPA in the absence of a RW. While this might seem like a technical nuance, this is a key methodological difference, as RW exposure might alter home cage activity [59, 60]. Furthermore, measurement of locomotor activity by telemetry is less sensitive than measurement by beam breaks [61]. Finally, the system we used to measure SPA in this study can identify rearing, locomotor activity and stereotypic behavior separately (See Methods). This higher precision turned out to be important for accurate prediction of ABA susceptibility, as it allowed analysis of different components of physical activity (e.g. rearing and ambulatory vs. stereotypic behavior).

A goal of this paper was to analyze the influence of SPA as a predictor of susceptibility to ABA. Our data suggest a predictive but non-linear relationship between SPA and ABA susceptibility in both male and females, but with higher influence of SPA in males compared to females (Figure 6). Whether the shape of the relationship between SPA and ABA susceptibility is general or specific to the group of animals tested needs to be validated against independent data, but the data seems to clearly support our finding that SPA has predictive power in ABA susceptibility.

SPA is a process regulated by multiple neuropeptide systems and over multiple brain regions [34, 62]; thus while it is unlikely that a single neuronal pathway or activity of a specific neuropeptide could explain the variance in SPA and its role in the pathophysiology of AN, the orexin neuropeptides have a large influence on SPA levels [34, 63, 64]. Current evidence suggests that in AN there is higher activity of the orexin peptides. Lower energy availability increases the activity of the orexin neurons while higher nutrient availability decreases their activity [65-70]. Expression of food anticipatory activity in a restricted feeding schedule requires orexin signaling [71, 72] and peripheral injection of an orexin receptor 1 antagonist has been shown to decrease weight loss and food anticipatory activity in ABA in female rats [31], suggesting that orexin receptor activity is necessary for the physical activity needed to acquire food during ABA. Yet others report that ABA increases [31] or does not affect orexin mRNA expression [73] and both higher and lower plasma levels of orexin-A and higher orexin mRNA expression in AN patients has been reported [74-76]. Currently, it is unclear what accounts for the inconsistency between these reports. Overall, we interpret these data to suggest that in some rats, higher basal orexin activity results in higher SPA, leading to higher RW activity during ABA and larger weight loss. Since lower SPA levels can also predict high ABA susceptibility, there are likely different behavioral patterns that predict susceptibility to weight loss in the ABA model. Whether these different predictive patterns indicate different brain network states is a subject for future study.

5. Conclusions

The data presented in this paper suggest that a common set of behavioral and anthropomorphic features can predict ABA susceptibility in male and females, supporting the hypothesis that the basic pathophysiology of AN is similar between males and females. Our data suggest SPA can be a predictor for ABA susceptibility in male and female rats, but there is not a linear relationship between individual SPA levels and weight loss during ABA, and SPA has larger effects in ABA susceptibility in males compared to females. The data from this paper support the hypothesis that pre-morbid activity levels could predict ABA susceptibility. Finally, these data suggest that future prospective human studies for susceptibility to AN should consider both the merit of measuring general activity as well as volitional exercise.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

REFERENCES

- [1]. Davis C, Katzman DK, Kirsh C. Compulsive physical activity in adolescents with anorexia nervosa: a psychobehavioral spiral of pathology. *J Nerv Ment Dis.* 1999; 187:336–42. [PubMed: 10379720]
- [2]. Davis C, Kennedy SH, Ravelski E, Dionne M. The role of physical activity in the development and maintenance of eating disorders. *Psychol Med.* 1994; 24:957–67. [PubMed: 7892363]
- [3]. Beumont PJ, Arthur B, Russell JD, Touyz SW. Excessive physical activity in dieting disorder patients: proposals for a supervised exercise program. *Int J Eat Disord.* 1994; 15:21–36. [PubMed: 8124324]

- [4]. Holtkamp K, Hebebrand J, Herpertz-Dahlmann B. The contribution of anxiety and food restriction on physical activity levels in acute anorexia nervosa. *Int J Eat Disord.* 2004; 36:163–71. [PubMed: 15282686]
- [5]. Kaye WH, Fudge JL, Paulus M. New insights into symptoms and neurocircuit function of anorexia nervosa. *Nat Rev Neurosci.* 2009; 10:573–84. [PubMed: 19603056]
- [6]. Fichter MM, Quadflieg N, Hedlund S. Twelve-year course and outcome predictors of anorexia nervosa. *Int J Eat Disord.* 2006; 39:87–100. [PubMed: 16231345]
- [7]. Muise AM, Stein DG, Arbess G. Eating disorders in adolescent boys: a review of the adolescent and young adult literature. *J Adolesc Health.* 2003; 33:427–35. [PubMed: 14642705]
- [8]. Woodside DB, Garfinkel PE, Lin E, Goering P, Kaplan AS, Goldbloom DS, et al. Comparisons of men with full or partial eating disorders, men without eating disorders, and women with eating disorders in the community. *Am J Psychiatry.* 2001; 158:570–4. [PubMed: 11282690]
- [9]. Preti A, Girolamo G, Vilagut G, Alonso J, Graaf R, Bruffaerts R, et al. The epidemiology of eating disorders in six European countries: results of the ESEMEd-WMH project. *Journal of psychiatric research.* 2009; 43:1125–32. [PubMed: 19427647]
- [10]. Raevuori A, Hoek HW, Susser E, Kaprio J, Rissanen A, Keski-Rahkonen A. Epidemiology of anorexia nervosa in men: a nationwide study of Finnish twins. *PLoS One.* 2009; 4:e4402. [PubMed: 19204790]
- [11]. Keski-Rahkonen A, Hoek HW, Susser ES, Linna MS, Sihvola E, Raevuori A, et al. Epidemiology and course of anorexia nervosa in the community. *Am J Psychiatry.* 2007; 164:1259–65. [PubMed: 17671290]
- [12]. Hudson JI, Hiripi E, Pope HG Jr, Kessler RC. The prevalence and correlates of eating disorders in the National Comorbidity Survey Replication. *Biol Psychiatry.* 2007; 61:348–58. [PubMed: 16815322]
- [13]. Wade TD, Bergin JL, Tiggemann M, Bulik CM, Fairburn CG. Prevalence and long-term course of lifetime eating disorders in an adult Australian twin cohort. *Aust N Z J Psychiatry.* 2006; 40:121–8. [PubMed: 16476129]
- [14]. Routtenberg A, Kuznesof AW. Self-starvation of rats living in activity wheels on a restricted feeding schedule. *J Comp Physiol Psychol.* 1967; 64:414–21. [PubMed: 6082873]
- [15]. Beneke WM, Schulte SE, vander Tuig JG. An analysis of excessive running in the development of activity anorexia. *Physiol Behav.* 1995; 58:451–7. [PubMed: 8587951]
- [16]. Casper RC, Sullivan EL, Tecott L. Relevance of animal models to human eating disorders and obesity. *Psychopharmacology (Berl).* 2008; 199:313–29. [PubMed: 18317734]
- [17]. Gutierrez E. A rat in the labyrinth of anorexia nervosa: contributions of the activity-based anorexia rodent model to the understanding of anorexia nervosa. *Int J Eat Disord.* 2013; 46:289–301. [PubMed: 23354987]
- [18]. Gelegen C, Pjetri E, Campbell IC, Collier DA, Oppelaar H, Kas MJ. Chromosomal mapping of excessive physical activity in mice in response to a restricted feeding schedule. *Eur Neuropsychopharmacol.* 2010; 20:317–26. [PubMed: 19896807]
- [19]. Hancock SD, Grant VL. Sexually dimorphic effects of postnatal treatment on the development of activity-based anorexia in adolescent and adult rats. *Dev Psychobiol.* 2009; 51:679–95. [PubMed: 19757457]
- [20]. Dixon DP, Ackert AM, Eckel LA. Development of, and recovery from, activity-based anorexia in female rats. *Physiol Behav.* 2003; 80:273–9. [PubMed: 14637226]
- [21]. Boakes RA, Mills KJ, Single JP. Sex differences in the relationship between activity and weight loss in the rat. *Behav Neurosci.* 1999; 113:1080–9. [PubMed: 10571490]
- [22]. Carrera O, Gutierrez E, Boakes RA. Early handling reduces vulnerability of rats to activity-based anorexia. *Dev Psychobiol.* 2006; 48:520–7. [PubMed: 17016834]
- [23]. Doerries LE, Stanley EZ, Aravich PF. Activity-based anorexia: relationship to gender and activity-stress ulcers. *Physiol Behav.* 1991; 50:945–9. [PubMed: 1805285]
- [24]. Gelegen C, Collier DA, Campbell IC, Oppelaar H, van den Heuvel J, Adan RA, et al. Difference in susceptibility to activity-based anorexia in two inbred strains of mice. *Eur Neuropsychopharmacol.* 2007; 17:199–205. [PubMed: 16735105]

- [25]. Pjetri E, de Haas R, de Jong S, Gelegen C, Oppelaar H, Verhagen LA, et al. Identifying predictors of activity based anorexia susceptibility in diverse genetic rodent populations. *PLoS One*. 2012; 7:e50453. [PubMed: 23226287]
- [26]. Barbarich-Marsteller NC, Underwood MD, Foltin RW, Myers MM, Walsh BT, Barrett JS, et al. Identifying novel phenotypes of vulnerability and resistance to activity-based anorexia in adolescent female rats. *Int J Eat Disord*. 2013; 46:737–46. [PubMed: 23853140]
- [27]. Garland T Jr, Schutz H, Chappell MA, Keeney BK, Meek TH, Copes LE, et al. The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives. *J Exp Biol*. 2011; 214:206–29. [PubMed: 21177942]
- [28]. Casper RC, Schoeller DA, Kushner R, Hnilicka J, Gold ST. Total daily energy expenditure and activity level in anorexia nervosa. *Am J Clin Nutr*. 1991; 53:1143–50. [PubMed: 1850575]
- [29]. Hebebrand J, Exner C, Hebebrand K, Holtkamp C, Casper RC, Remschmidt H, et al. Hyperactivity in patients with anorexia nervosa and in semistarved rats: evidence for a pivotal role of hypoleptinemia. *Physiol Behav*. 2003; 79:25–37. [PubMed: 12818707]
- [30]. Klein DA, Mayer LE, Schebendach JE, Walsh BT. Physical activity and cortisol in anorexia nervosa. *Psychoneuroendocrinology*. 2007; 32:539–47. [PubMed: 17462830]
- [31]. Scheurink AJ, Boersma GJ, Nergardh R, Sodersten P. Neurobiology of hyperactivity and reward: agreeable restlessness in anorexia nervosa. *Physiol Behav*. 2010; 100:490–5. [PubMed: 20361989]
- [32]. Kron L, Katz JL, Gorzynski G, Weiner H. Hyperactivity in anorexia nervosa: a fundamental clinical feature. *Compr Psychiatry*. 1978; 19:433–40. [PubMed: 679677]
- [33]. Casper RC. The ‘drive for activity’ and “restlessness” in anorexia nervosa: potential pathways. *J Affect Disord*. 2006; 92:99–107. [PubMed: 16448703]
- [34]. Teske JA, Billington CJ, Kotz CM. Neuropeptidergic mediators of spontaneous physical activity and non-exercise activity thermogenesis. *Neuroendocrinology*. 2008; 87:71–90. [PubMed: 17984627]
- [35]. Ravussin E, Lillioja S, Anderson TE, Christin L, Bogardus C. Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. *J Clin Invest*. 1986; 78:1568–78. [PubMed: 3782471]
- [36]. Levine JA, Lanningham-Foster LM, McCrady SK, Krizan AC, Olson LR, Kane PH, et al. Interindividual variation in posture allocation: possible role in human obesity. *Science*. 2005; 307:584–6. [PubMed: 15681386]
- [37]. Kotz CM, Teske JA, Billington CJ. Neuroregulation of nonexercise activity thermogenesis and obesity resistance. *Am J Physiol Regul Integr Comp Physiol*. 2008; 294:R699–710. [PubMed: 18160530]
- [38]. Ben-Hur A, Ong CS, Sonnenburg S, Scholkopf B, Ratsch G. Support vector machines and kernels for computational biology. *PLoS Comput Biol*. 2008; 4:e1000173. [PubMed: 18974822]
- [39]. Noble WS. What is a support vector machine? *Nat Biotechnol*. 2006; 24:1565–7. [PubMed: 17160063]
- [40]. Steinwart, I.; Christmann, A. Support vector machines. 1st ed. Springer; New York: 2008.
- [41]. Japkowicz N, Stephen S. The class imbalance problem: A systematic study. *Intelligent data analysis*. 2002; 6:429–49.
- [42]. Van Hulse, J.; Khoshgoftaar, TM.; Napolitano, A. Experimental perspectives on learning from imbalanced data; Proceedings of the 24th international conference on Machine learning: ACM; 2007; p. 935–42.
- [43]. Chang C-C, Lin C-J. LIBSVM: a library for support vector machines. *ACM Transactions on Intelligent Systems and Technology (TIST)*. 2011; 2:27.
- [44]. Chawla NV, Bowyer KW, Hall LO, Kegelmeyer WP. SMOTE: synthetic minority over-sampling technique. *Journal of Artificial Intelligence Research*. 2002; 16:321–57.
- [45]. Tang Y, Zhang Y-Q, Chawla NV, Krasser S. SVMs modeling for highly imbalanced classification. *Systems, Man, and Cybernetics, Part B: Cybernetics, IEEE Transactions on*. 2009; 39:281–8.

- [46]. Goldberg DE, Holland JH. Genetic algorithms and machine learning. Machine learning. 1988; 3:95–9.
- [47]. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing; Vienna, Austria: 2013.
- [48]. Wickman H. ggplot2: elegant graphics for data analysis. Springer New York: 2009.
- [49]. Wickman H. The Split-Apply-Combine Strategy for Data Analysis. J Stat Softw. 2011; 40:1–29.
- [50]. Dimitriadou E, Hornik K, Leisch F, Meyer D, Weingessel A, Leisch MF. 2009 The e1071 Package.
- [51]. Scrucca L. GA: a package for genetic algorithms in R. Journal of Statistical Software. 2012
- [52]. Torgo, L. Data mining with R: learning with case studies. Chapman & Hall/CRC; 2010.
- [53]. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological). 1995:289–300.
- [54]. Wooldridge T, Lytle PP. An overview of anorexia nervosa in males. Eat Disord. 2012; 20:368–78. [PubMed: 22985234]
- [55]. Watanabe K, Hara C, Ogawa N. Feeding conditions and estrous cycle of female rats under the activity-stress procedure from aspects of anorexia nervosa. Physiol Behav. 1992; 51:827–32. [PubMed: 1594681]
- [56]. Dwyer DM, Boakes RA. Activity-based anorexia in rats as failure to adapt to a feeding schedule. Behav Neurosci. 1997; 111:195–205. [PubMed: 9109638]
- [57]. Coners H, Remschmidt H, Hebebrand J. The relationship between premorbid body weight, weight loss, and weight at referral in adolescent patients with anorexia nervosa. Int J Eat Disord. 1999; 26:171–8. [PubMed: 10422606]
- [58]. Guyon I, Elisseeff A. An introduction to variable and feature selection. The Journal of Machine Learning Research. 2003; 3:1157–82.
- [59]. Waters RP, Pringle RB, Forster GL, Renner KJ, Malisch JL, Garland T Jr, et al. Selection for increased voluntary wheel-running affects behavior and brain monoamines in mice. Brain Res. 2013; 1508:9–22. [PubMed: 23352668]
- [60]. de Visser L, van den Bos R, Spruijt BM. Automated home cage observations as a tool to measure the effects of wheel running on cage floor locomotion. Behav Brain Res. 2005; 160:382–8. [PubMed: 15863235]
- [61]. Teske JA, Perez-Leighton CE, Billington CJ, Kotz CM. Methodological considerations for measuring spontaneous physical activity in rodents. Am J Physiol Regul Integr Comp Physiol. 2014
- [62]. Kotz C, Nixon J, Butterick T, Perez-Leighton C, Teske J, Billington C. Brain orexin promotes obesity resistance. Ann N Y Acad Sci. 2012; 1264:72–86. [PubMed: 22803681]
- [63]. Kotz CM, Wang C, Teske JA, Thorpe AJ, Novak CM, Kiwaki K, et al. Orexin A mediation of time spent moving in rats: neural mechanisms. Neuroscience. 2006; 142:29–36. [PubMed: 16809007]
- [64]. Teske JA, Levine AS, Kuskowski M, Levine JA, Kotz CM. Elevated hypothalamic orexin signaling, sensitivity to orexin A, and spontaneous physical activity in obesity-resistant rats. Am J Physiol Regul Integr Comp Physiol. 2006; 291:R889–99. [PubMed: 16763079]
- [65]. Karnani MM, Apergis-Schoute J, Adamantidis A, Jensen LT, de Lecea L, et al. Activation of central orexin/hypocretin neurons by dietary amino acids. Neuron. 2011; 72:616–29. [PubMed: 22099463]
- [66]. Burdakov D, Alexopoulos H. Metabolic state signalling through central hypocretin/orexin neurons. J Cell Mol Med. 2005; 9:795–803. [PubMed: 16364191]
- [67]. Burdakov D, Gerasimenko O, Verkhatsky A. Physiological changes in glucose differentially modulate the excitability of hypothalamic melanin-concentrating hormone and orexin neurons in situ. J Neurosci. 2005; 25:2429–33. [PubMed: 15745970]
- [68]. Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, et al. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. Neuron. 2003; 38:701–13. [PubMed: 12797956]

- [69]. Leininger GM, Opland DM, Jo YH, Faouzi M, Christensen L, Cappellucci LA, et al. Leptin action via neurotensin neurons controls orexin, the mesolimbic dopamine system and energy balance. *Cell Metab.* 2011; 14:313–23. [PubMed: 21907138]
- [70]. Louis GW, Leininger GM, Rhodes CJ, Myers MG Jr. Direct innervation and modulation of orexin neurons by lateral hypothalamic LepRb neurons. *J Neurosci.* 2010; 30:11278–87. [PubMed: 20739548]
- [71]. Kaur S, Thankachan S, Begum S, Blanco-Centurion C, Sakurai T, Yanagisawa M, et al. Entrainment of temperature and activity rhythms to restricted feeding in orexin knock out mice. *Brain Res.* 2008; 1205:47–54. [PubMed: 18343358]
- [72]. Mieda M, Williams SC, Sinton CM, Richardson JA, Sakurai T, Yanagisawa M. Orexin neurons function in an efferent pathway of a food-entrainable circadian oscillator in eliciting food-anticipatory activity and wakefulness. *J Neurosci.* 2004; 24:10493–501. [PubMed: 15548664]
- [73]. de Rijke CE, Hillebrand JJ, Verhagen LA, Roeling TA, Adan RA. Hypothalamic neuropeptide expression following chronic food restriction in sedentary and wheel-running rats. *J Mol Endocrinol.* 2005; 35:381–90. [PubMed: 16216917]
- [74]. Bronsky J, Nedvickova J, Krasnicanova H, Vesela M, Schmidtova J, Koutek J, et al. Changes of orexin A plasma levels in girls with anorexia nervosa during eight weeks of realimentation. *Int J Eat Disord.* 2011; 44:547–52. [PubMed: 21823139]
- [75]. Janas-Kozik M, Stachowicz M, Krupka-Matuszczyk I, Szymaszal J, Krysta K, Janas A, et al. Plasma levels of leptin and orexin A in the restrictive type of anorexia nervosa. *Regul Pept.* 2011; 168:5–9. [PubMed: 21338627]
- [76]. Janas-Kozik M, Stachowicz M, Mazurek U, Zajdel A, Wilczok A, Krupka-Matuszczyk I, et al. Preliminary study of the expression of genes connected with the orexigenic and anorexigenic system using microarray technique in anorexia nervosa. *Neuropsychobiology.* 2008; 57:116–20. [PubMed: 18552512]

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.

Figure 1. Diagram of study design

Figure 2. Effects of ABA in male and female rats

(A) Time-course of body weight (BW) change during ABA in male (M, $n = 25$) and female (F, $n = 15$) rats. (B) Body weight at end of ABA was lower in male compared to female rats. We found no differences in (C) Food intake (FI) normalized by body weight and (D) Total running wheel distance. Line above bars indicate statistical significance (t-test). Open circles in B-D indicate individual animals. Y-axis indicates mean $\pm$ SEM.

Figure 3. Prediction of ABA susceptibility and effect of gender as a predictor

This plot shows the average of 10 independent SVM model using two different sets of predictors. One included body weight ambulatory SPA, stereotypic activity, RW distance during ad-libitum feeding, food intake during SPA, and gender (*open bars*), while another excluded gender (*closed bars*). Statistical analysis found no significant difference between models indicating that gender did not improve performance of predictive models. Letters above bars indicate significant differences between cut-offs. Y-axis indicates mean $\pm$ SEM.

Figure 4. Comparison of models for prediction of ABA susceptibility

We tested all combinations of predictive features (right black/white matrix, $N = 19-20$ independent replications of SVM training) and clustered for their performance to predict SVM susceptibility across all thresholds. M column indicates average performance across all ABA thresholds. Heat matrix indicates average G_{MEAN} for all models and threshold combination. This analysis indicates that a cluster of two models achieved highest performance. These models included different combinations of ambulatory SPA (*spa*), RW

distance during *ad-libitum* feeding (*rw*), food intake during SPA (*food intake*), and body weight history (*bw history*).

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Figure 5. Comparison of average performance of models for prediction of ABA susceptibility
 Barplot shows the average performance of all the models. Models were ranked based on decreasing average performance from left to right. Lower matrix shows absence (white) or presence (black) of individual features in predictive models. Individual features were ambulatory SPA (*spa*), RW distance during *ad-libitum* feeding (*rw*), food intake during SPA (*food intake*), and body weight history (*bw history*). The two top ranked models have significantly higher performance compared to all other models tested ($P < 0.05$, t-test corrected for multiple comparisons).

Figure 6. Effect of SPA in ABA susceptibility

Ambulatory SPA was varied for all rats included in the study in the range 30 – 240 min/24 h while maintaining constant all other individual features. This synthetic data set was evaluated in the three top ranked models (Figure 5) that included (A) ambulatory SPA, body weight history, RW distance during *ad-libitum* feeding and food intake during SPA and (B) ambulatory SPA, body weight history and RW distance during *ad-libitum* feeding. For both models, a KS test indicated significant differences between probability distributions (left plot, $D = 0.41$, $P < 0.01$; right plot, $D = 0.36$, $P < 0.01$).

Table 1
Composition of ABA susceptibility data per body weight loss threshold

ABA threshold (percent initial body weight)	65 %	70 %	75 %	80 %	85 %
Total					
ABA resistant	34	26	16	11	5
ABA susceptible	5	13	23	28	34
Male					
ABA resistant	19	13	7	3	0
ABA susceptible	5	11	17	21	24
Female					
ABA resistant	15	13	9	8	5
ABA susceptible	0	2	6	7	10

ABA susceptible describes animals whose body weight at the end of the ABA period was below the specified threshold. Data corresponds to number of animals per category.