

## ARTICLE

# Fear and food: Anxiety-like behavior and the susceptibility to weight loss in an activity-based anorexia rat model

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## Abstract

Anorexia nervosa (AN) is a severe psychiatric disorder characterized by energy restriction, low body weight, a fear of gaining weight, and often excessive physical activity. Anxiety disorders appear to constitute a major risk factor for developing AN and are the most frequent comorbidity. Here, the influence of anxiety-like behavior prior to food restriction on increased physical activity, leading to greater susceptibility to weight loss, was tested in rats. Furthermore, the possible anxiolytic effect of starvation itself was analyzed. A chronic starvation model activity-based anorexia (ABA) was applied to mimic physiological and behavioral characteristics of AN. During the induction of starvation and acute starvation, food intake was reduced by 70% and the rats lost 25% of their body weight, which was kept stable to imitate chronic starvation. Anxiety-like behavior was quantified before and after chronic starvation using the elevated plus maze, based on rodents' aversion to open spaces. Anxiety-related behavior before food restriction was associated with increased running-wheel activity during habituation and during the induction of starvation, and predicted faster weight loss in ABA rats. Additionally, food-restricted animals showed less anxiety-like behavior after chronic starvation. Animals showing more anxiety-like behavior appear to be more susceptible to weight loss, partially mediated by increased physical activity. Anxiety-related behavior was associated with increased physical activity, which in turn was associated with more rapid weight loss. Our data let us assume that food restriction has an anxiolytic effect. These findings demonstrate the importance of considering anxiety disorders in patients with AN.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Anxiety disorders appear to constitute a major risk factor for developing anorexia nervosa (AN) and are the most frequent comorbidity. Food restriction seems to have an anxiolytic effect in rodents and anxiety-like behavior was shown to be related with increased physical activity.

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### WHAT QUESTION DID THIS STUDY ADDRESS?

The influence of anxiety-like behavior prior to food restriction on increased physical activity, leading to greater susceptibility to weight loss in the activity-based anorexia (ABA) rat model was tested. Furthermore, the possible anxiolytic effect of starvation itself was analyzed.

### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The present animal study was able to show that anxiety-like behavior prior to food starvation predicts weight loss in the ABA rats. Anxiety-related behavior was associated with increased physical activity, which in turn was associated with more rapid weight loss. Our data let us assume that food restriction has an anxiolytic effect at least in rats.

### HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

With regard to clinical consequences, our findings underline the relevance of considering anxiety disorders in patients with AN more intensively. Moreover, they could suggest the importance of using exercise as a therapeutic approach, because physical activity might be a means to regulate emotions, such as anxiety. Although excessive activity is counterproductive, controlled and moderate activity under therapeutic supervision could ease internal pressure, increase recovery rates, and prevent the development of osteoporosis.<sup>1,2</sup> Nevertheless, it should be considered that the present results are of observational nature and do not offer insight into mechanistic processes.

## INTRODUCTION

Anorexia nervosa (AN) is a severe psychiatric disorder characterized by restricted energy intake, reduced body weight, body image disturbance, and a fear of gaining weight, primarily affecting adolescent girls and young women.<sup>3,4</sup> Anxiety disorders are the most frequent comorbidities of AN, and anxiety disorders (especially generalized anxiety disorder, social phobia, and obsessive-compulsive disorder) prior to the onset of AN appear to constitute a major risk factor for the subsequent diagnosis of AN.<sup>5-9</sup> However, clear pathological mechanisms underlying this association remain unknown. Approximately 80% of patients with AN had at least one diagnosis of an anxiety disorder in their lifetime and, in 75% of the comorbid cases, it predated the eating disorder.<sup>7,10-12</sup> This observation and recently observed genetic correlations between AN and other psychiatric disorders (including anxiety disorders) indicate a sharing of genes across phenotypes and pleiotropy.<sup>13-16</sup>

Excessive physical activity can be observed in up to 80% of patients with AN.<sup>4</sup> In an AN animal model, increased anxiety-like behavior in mice was associated with higher levels of running-wheel activity,<sup>17</sup> constituting a potential risk factor for more rapid weight loss.

The activity-based anorexia (ABA) animal model combines limited food access with running-wheel access, leading to body weight loss and increased physical activity.<sup>18</sup> Along with a behavioral test (elevated plus maze) to quantify

anxiety-like behavior, our experimental setting seems ideally suited to analyze the interaction among anxiety-like behavior, physical activity, and starvation in a controlled experimental setting. The Elevated Plus Maze (EPM) has been widely used in the assessment of anxiety-related behavior in rodents.<sup>19,20</sup> A previous study found an association between anxiety-like behavior and running-wheel activity (RWA) as well as reduced anxiety-like behavior after short-term food restriction in female mice using the EPM.<sup>17</sup>

We used a chronic version of the ABA model and the EPM to quantify the influence of anxiety-like behavior on weight loss and running activity in female adolescent rats. In the present study, we sought to determine whether animals showing more anxiety-like behavior are more prone to weight loss and whether this is dependent on the physical activity levels. Moreover, we aimed to analyze whether starvation indeed has an anxiolytic effect in the animals. Therefore, we chose a longitudinal study design and extended the classical ABA model by experimental groups with and without food restriction combined with and without running-wheel access, respectively.

## MATERIALS AND METHODS

Forty-nine adolescent 4-week-old female Wistar rats (RjHan:WI; Janvier, Hannover, Germany) arrived in the laboratory with an average body weight of 106.22 g  $\pm$  1.84 g.

All animals were single-housed in type IV, 1820 cm<sup>2</sup> cages (Polysulfone, Tecniplast) with sawdust bedding, cotton wool pads as nesting material, and a running-wheel. The rats were treated under standardized conditions with a 12 h day/night cycle (lights on at 7 a.m.). The room was constantly regulated to maintain a temperature of 22°C and 55% humidity. The rats were randomly split into four groups: (1) controls (ad libitum food, no running-wheel,  $n = 12$ ); (2) controls with running-wheel (CRW, ad libitum food, running-wheel,  $n = 12$ ); (3) limited food (LF, starvation, no running-wheel,  $n = 12$ ), and (4) ABA (starvation, running-wheel,  $n = 13$ ). Note, that groups 3 and 4 differ in the absence and presence of the running-wheel but the amount and the composition of food were identical. The sample size was calculated a priori using a two-sided Satterthwaite  $t$ -test leading to a power of at least 80% at a significance level of  $\alpha = 0.05$  (SAS Institute, Cary, NC, USA).

RWA, body weight and food intake were recorded daily at 12 a.m. The daily RWA was measured with a tachometer (Sigma BC 5.12). The animal trial was approved by the Governmental Animal Care and Use Committee (Approval no.: 84-02-04-2017.A036). All experiments were performed in accordance with the German legislation governing animal studies, following the Guide for the Care and Use of Laboratory Animals (NIH, 8th edition, 2011) and the 2010/63/EU Directive on the Protection of Animals Used for Scientific Purposes (Official Journal of the European Union, 2010).

### Activity-based anorexia model

The ABA model is a well-established and currently the most frequently used rodent model to induce physiological and behavioral characteristics of AN.<sup>18</sup> A modified concept of the ABA model was used here.<sup>21</sup> Instead of reducing the feeding time, as conducted in the initial model by Routtenberg and Kuzneshof,<sup>22</sup> a reduced amount of food equivalent to 30% of the ad libitum food intake in the habituation period was implemented in LF and ABA groups. Food-restricted rats received food daily at 12 a.m. The target weight was set at 75% of the baseline body weight. Unlimited water was available for all groups during the entire experiment. The ABA group and the CRW group had unlimited access to a running-wheel, whereas the running-wheel was blocked in the cages of control and LF rats.

The experiment was divided into four phases. In the habituation period, all groups had ad libitum access to food. After 10 days, the induction of the starvation started followed by the acute starvation and the food intake was reduced to 30% in the LF and ABA groups. The maintenance period began once each individual rat had reached its target weight ( $-25\%$  of its baseline body weight), imitating

chronic starvation. In this phase, the amount of food was adjusted daily by increasing or decreasing the quantity of food pellets by 0.5–1.5 g depending on individual body weight, in order to keep the target weight stable. A reduced mortality rate has been shown using this protocol,<sup>21</sup> which allows an observation of chronic starvation that is often seen in patients with AN.<sup>23,24</sup> It also facilitates the analysis of influencing factors without body weight as a confounder.

### Elevated plus maze

Behavioral testing was carried out twice: at the end of the habituation period (day 10, EPM1) and at the end of the chronic starvation period (day 30, EPM2). It was conducted in the morning before feeding to potentially further increase the effect of starvation. The animals arrived in the experimental room 30 min before testing to adapt to the environment. All tests were performed under red light. After every test, the animals were transferred back to their home cages and the maze was disinfected with Antifect N liquid (propanol 35% and ethanol 23.5%).

The EPM was used to quantify the influence of food restriction and RWA on anxiety-like behavior. It consists of four arms arranged in a plus-shape, two of which were open and two of which were enclosed by side walls that were 20 cm high. The arms were 50 cm long and were connected by an open center and positioned on 50 cm high table legs. Although rodents are prey animals which prefer to stay in the covered arms of the maze, they are also curious to explore new territories.<sup>19,20</sup> It is generally assumed that rats display higher levels of anxiety-related behavior when they spend more time in the closed arms of the apparatus.<sup>19</sup>

At the beginning, the animals were positioned at the center of the maze. The experiment was recorded for 10 min with a video camera (Basler, acA1300-60gmNIR) and analyzed using ANY-Maze Video Tracking System (Stoelting Co.). Each rat was tracked at the center of its body. The following parameters were recorded for further analysis: the average time the animal spent in the closed arms, the average number of entries in the closed arms, the average time the animal moved (time mobile), and the average number of entries in both the closed and open arms. Furthermore, two ratios were calculated to correct for general movement activity: the average time the animals spent in the closed arms divided by the total time the animals were active (time in closed arms/time mobile), and the average number of entries into the closed arms corrected for the average number of total entries in the open and closed arms (entries in closed arms/total entries). To evaluate differences between the two time points (before [EPM1] and after chronic starvation [EPM2]) delta ( $\Delta$ ) values were determined.

## Statistical analysis

Body weight and RWA data were separated in different phases: habituation (days 1–10), induction of starvation (days 11–13), acute starvation (days 14–17), and chronic starvation (days 18–30). The induction of starvation was defined as the first days of food restriction (days 11–13) according to Wable et al. (2015),<sup>17</sup> when RWA significantly increases. The acute starvation period (days 14–17) was marked by body weight loss and declining activity levels, whereas chronic starvation (days 18–30) was characterized by a stable body weight and decreased activity levels. Alterations in body weight during the different phases were evaluated in a one-way analysis of variance (ANOVA) with Bonferroni post hoc test. Group differences between control versus LF and CRW versus ABA were of particular interest in this study design. The days needed to reach the target weight were recorded for each rat to investigate its individual susceptibility to weight loss. Group differences for the days until the target weight was reached between LF and ABA were calculated with a two-sided Student's *t*-test. Group comparisons (CRW vs. ABA) for RWA were calculated separately for the different phases using two-sided Student's *t*-tests.

Differences in the EPM parameters were calculated by a repeated measure  $2 \times 2$  ANOVA.

A two-tailed Pearson correlation was calculated for anxiety-like behavior before starvation (EPM1) and days needed to reach the target weight to explore the hypothesis that anxiety-related behavior could predict body weight loss. To test whether increased RWA and/or increased anxiety-like behavior more strongly predict increased body weight loss, a multiple regression analysis was performed. One-tailed Pearson correlations were used to explore the hypothesis that anxiety-like behavior predicts physical activity in the animals, as suggested before.<sup>17</sup>

Effect sizes are displayed as Cohen's *d* for Student's *t*-tests,  $\eta^2$  for ANOVAs, and  $r^2$  for Pearson correlations. Statistical analyses were performed with SPSS Statistics 25 (IBM, Chicago, IL, USA) and the significance level for all comparisons was set at 5%.

## RESULTS

### Body weight, days until target weight, and running-wheel activity

As expected, during the entire starvation period, there were statistically significant differences in body weight between the control and food-restricted groups (induction

of starvation:  $F = 9.380$ ,  $\eta^2 = 0.385$ ,  $p < 0.0001$ , control vs. LF:  $p = 0.148$ , CRW vs. ABA:  $p = 0.016$ ; acute starvation:  $F = 43.238$ ,  $\eta^2 = 0.742$ ,  $p < 0.0001$ , control vs. LF:  $p < 0.0001$ , CRW vs. ABA:  $p < 0.0001$ ; chronic starvation:  $F = 104.684$ ,  $\eta^2 = 0.875$ ,  $p < 0.0001$ , control vs. LF:  $p < 0.0001$ , and CRW vs. ABA:  $p < 0.0001$ ; Figure 1a).

ABA animals needed significantly fewer days to reach the target weight (75% of baseline body weight) compared to the LF group (5.8 vs. 11.1 days;  $t = 5.73$ , Cohen's  $d = 0.22$ ,  $p < 0.0001$ ), which is most likely the result of a higher caloric requirement due to physical exercise (see black rectangles and dots in Figure 1a).

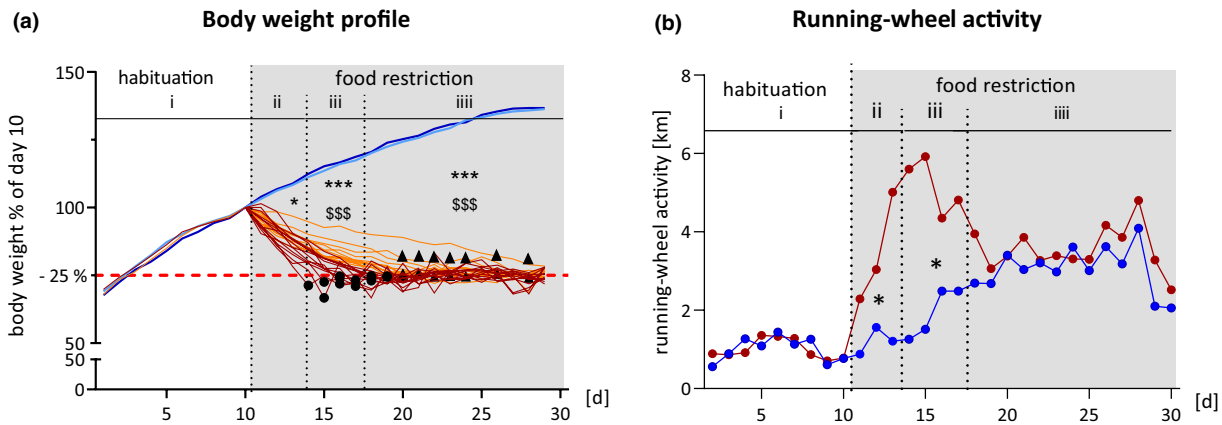
RWA was significantly higher in ABA rats compared to CRW rats during the induction of starvation ( $t = -2.649$ , Cohen's  $d = 1.07$ ,  $p = 0.014$ ) and the acute starvation period ( $t = -2.504$ , Cohen's  $d = 1.07$ ,  $p = 0.02$ , Figure 1b). During the chronic starvation, there was no difference in RWA ( $t = -0.218$ , Cohen's  $d = 0.08$ ,  $p = 0.832$ ).

### Elevated plus maze

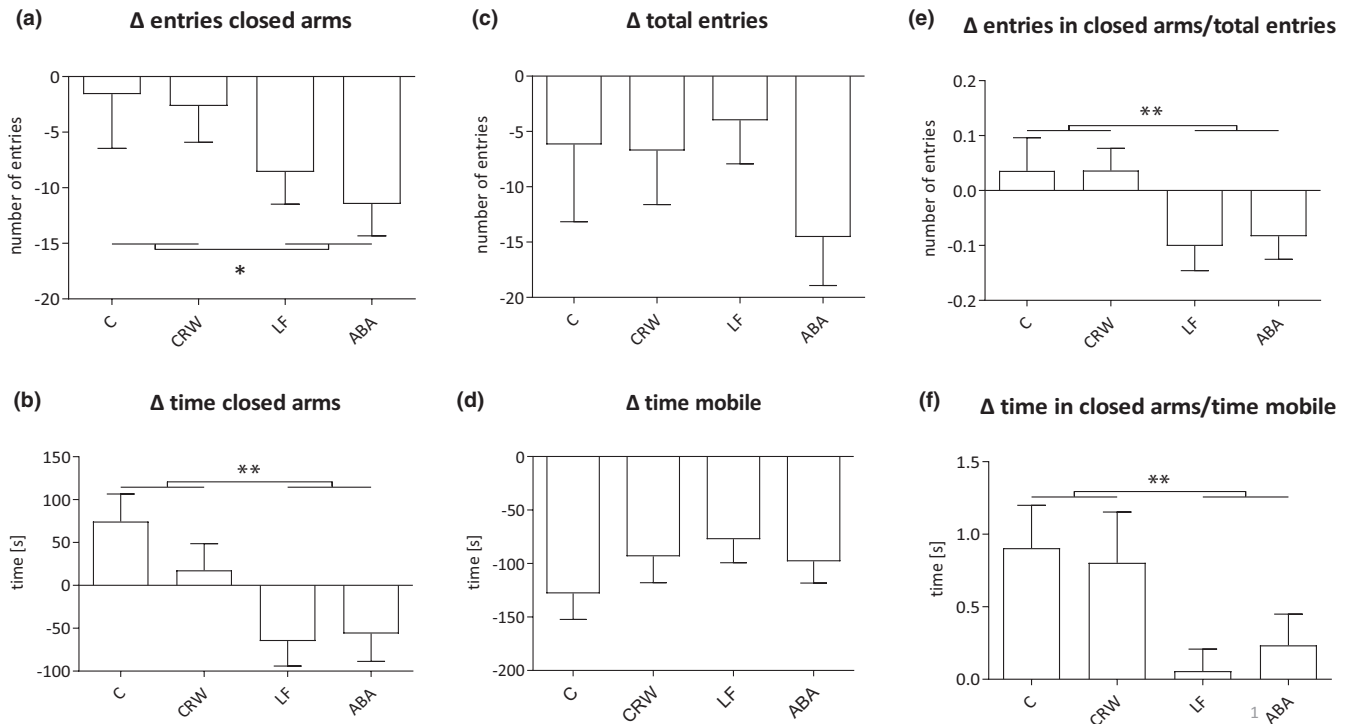
#### Food-restricted rats show less anxiety-like behavior

Using a repeated measure  $2 \times 2$  ANOVA, a significant difference in the average number of entries in the closed arms was found comparing the two measurement time points before (EPM1) and after chronic starvation (EPM2;  $F = 11.577$ ,  $\eta^2 = 0.208$ ,  $p = 0.001$ ). Both food reduced groups showed significantly less anxiety-like behavior after chronic starvation compared with controls ( $F = 4.924$ ,  $\eta^2 = 0.101$ ,  $p = 0.032$ ; Figure 2a,b). Similarly, all food restricted rats spent significantly less time in the closed arms after chronic starvation, also indicating less anxiety-related behavior ( $F = 11.517$ ,  $\eta^2 = 0.207$ ,  $p = 0.001$ ; Figure 2c,d). No differences were seen due to the running-wheel access. Thus, food restriction but not running-wheel access led to a reduction of anxiety-like behavior in the rats after chronic starvation. The change over time in the control groups probably represents a repetition effect as the rats generally move less at the second measure time point and preferably spent time in the closed arms. To exclude general movement as a confounder, the total numbers of entries in all arms and the total time the animals were mobile during the behavioral test were analyzed. For both parameters there was a statistically representative main effect for the time in all groups (total number of entries:  $F = 9.210$ ,  $\eta^2 = 0.093$ ,  $p = 0.004$ ; Figure 2c; total time mobile:  $F = 75.387$ ,  $\eta^2 = 0.560$ ,  $p < .0001$ ; Figure 2d). This finding could be explained by a repetition effect in the behavioral test, because all animals were generally

— CRW  
 — C  
 — LF (▲ day target weight reached)  
 — ABA (● day target weight reached)



**FIGURE 1** Body weight and running-wheel activity profiles. (a) Average body weight in % of day 10. Individual body weight profiles are depicted for LF and ABA rats during the food restriction period. Black dots and rectangles represent the day target weight was reached in ABA and LF rats, respectively. (b) Mean RWA normalized to the average RWA of the habituation period: (i) habituation, (ii) induction of starvation, (iii) acute starvation, and (iv) chronic starvation. Differences between four groups were calculated with one-way ANOVA and Bonferroni correction and between two groups with two-sided Student’s *t*-test. \* Represents differences between control and LF, \$ represents differences between CRW and ABA. ABA, activity-based anorexia; C, control; CRW, control+running-wheel; LF, limited food; RWA, running-wheel activity. \*/\$ =  $p \leq 0.05$ , \*\*\*/\$\$\$ =  $p \leq 0.001$



**FIGURE 2** Entries and time in closed arms, total time mobile and total entries in the EPM. Differences of mean number of entries in the closed arms (a), mean time in the closed arms (b), total entries (c), and total time mobile (d) between EPM1 and EPM2. Ratio of mean entries in the closed arms and total entries (e) and mean time in the closed arm and total time mobile (f). Differences between groups were calculated with repeated measure ANOVA. The *p* values represent the interaction effect of time\*food restriction. ABA, activity-based anorexia; C, control; CRW, control+running-wheel; EPM1/2, elevated plus maze at day 10/day 30; LF, limited food. \* $p \leq 0.05$ , \*\* $p \leq 0.01$



less active during the second measurement time point. Correcting the number of entries in the closed arms for the number of total entries in all arms and correcting the time spent in the closed arms for the total time the rats were mobile led to similar effects as the uncorrected comparisons ( $F = 7.333$ ,  $\eta^2 = 0.143$ ,  $p = 0.01$ ; Figure 2e;  $F = 7.533$ ,  $\eta^2 = 0.146$ ,  $p = 0.009$ ; Figure 2f). Hence, we found a significant reduction of anxiety-like behavior following food restriction (EPM2) that cannot be explained by increased general movement. See Table S1 for raw data of the EPM.

### Anxiety-like behavior predicts weight loss

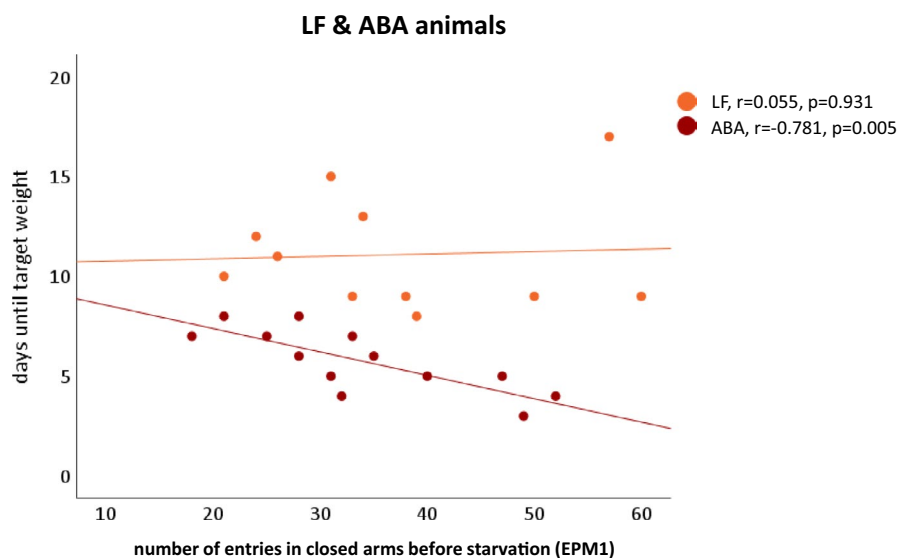
An explorative correlation analysis showed a strong relationship between anxiety-like behavior in ABA animals before starvation (EPM1) and fewer days until they reached the respective target weight ( $r^2 = 0.609$ ,  $p = 0.005$ ; Figure 3). No such association was found in LF animals ( $r^2 = 0.003$ ,  $p = 0.931$ ; Figure 3), proposing a dissociation between anxiety-related behavior and weight loss caused by the animals' running-wheel access. Anxiety-like behavior before starvation (EPM1) and increased RWA during the induction of starvation significantly contribute to the explanation of body weight loss ( $F = 15.699$ ,  $p = 0.001$ ). Seventy-one percent of the variance of faster body weight loss could be explained by these variables (see Table S2).

### Anxiety-like behavior is correlated with physical activity

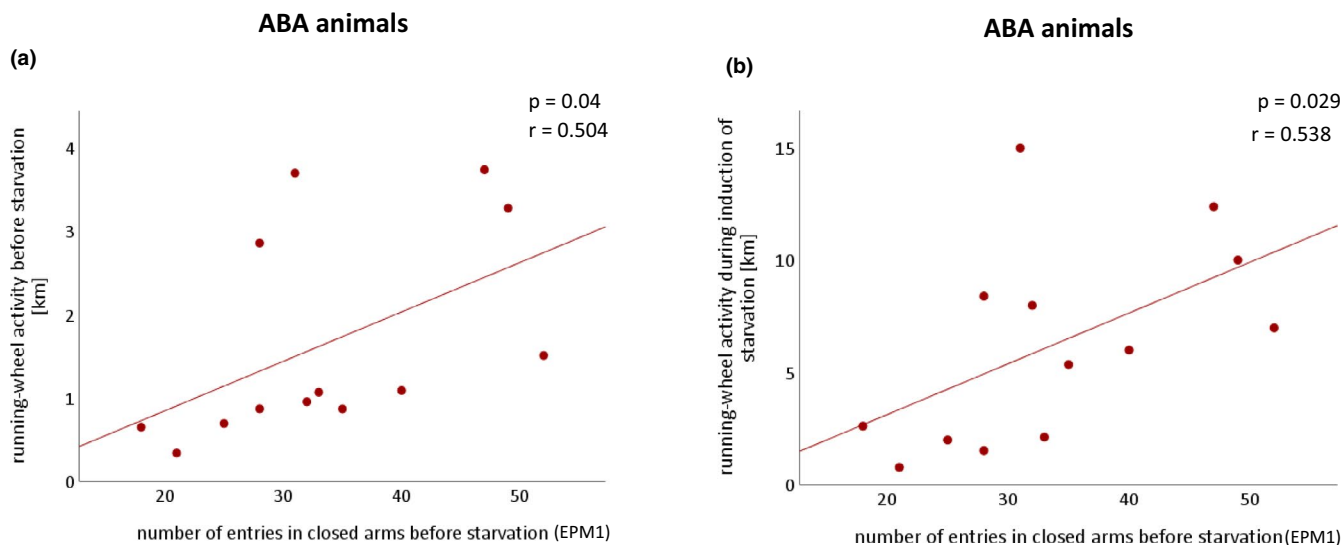
Subsequently, a possible association between anxiety-related behavior and physical activity as shown in an acute starvation mouse model was analyzed.<sup>17</sup> Correlation analyses suggest a similar effect in the present rat ABA model. Animals that show more anxiety-like behavior before starvation (EPM1) showed higher RWA at the same time ( $r^2 = 0.254$ ,  $p = 0.04$ ; Figure 4a). Increased anxiety-related behavior prior to starvation also predicted increasing RWA during the induction of starvation ( $r^2 = 0.290$ ,  $p = 0.029$ ; Figure 4b). However, no such correlation could be shown regarding the acute starvation and chronic starvation (all  $p > 0.05$ , data not shown).

## DISCUSSION

We examined whether high levels of anxiety-like behavior measured by more and longer visits to the closed arms of the elevated plus maze lead to a greater susceptibility to weight loss in the ABA model, potentially via increased voluntary running. Furthermore, we wanted to confirm the potentially anxiolytic effect of starvation. Higher susceptibility to body weight loss could have important implications for a better understanding of the role of previously existing or comorbid anxiety in patients' with AN pathogenesis.



**FIGURE 3** Association between body weight loss and anxiety-like behavior in the EPM. Negative correlation between the days needed until target weight was reached and the mean number of entries in the closed arms before starvation (EPM1) in ABA rats (red). No such correlation in LF rats (orange). One value for LF animals is missing because this animal did not reach the target weight until the end of the protocol. Correlations were calculated by two-tailed Pearson correlation. ABA, activity-based anorexia; EPM1, elevated plus maze at day 10; LF, limited food



**FIGURE 4** Associations between anxiety-like behavior in the EPM and RWA. (a) Positive correlations between the mean number of entries in the closed arms before starvation (EPM1) in ABA rats with the RWA during habituation and (b) during induction of starvation. Correlations were calculated using one-tailed Pearson correlations. ABA, activity-based anorexia; EPM1, elevated plus maze at day 10; RWA, running-wheel activity.

Increased levels of anxiety-like behavior before food starvation appeared to make rats more prone to faster body weight loss in the ABA model. These results could support the clinical hypothesis that high trait anxiety is a subsequent risk factor for the development of AN.<sup>8,9</sup> In the vast majority of cases, the first onset of an anxiety disorder predated the onset of AN.<sup>5,7</sup> Furthermore, findings suggest that anxiety disorders lead to a more severe clinical variant of AN<sup>25,26</sup> and that individuals with a genetic predisposition to pronounced anxiety identified by high scores in anxiety questionnaires (i.e., State Trait Anxiety Index) seem to be more susceptible to AN.<sup>15,27</sup> There is also proof that increased anxiety can complicate recovery from AN and lead to high relapse rates.<sup>28</sup> Our findings in the ABA paradigm thus support the clinical relevance of anxiety in AN. Because of the well-controlled nature, animal studies can help to determine influencing factors of AN even though the ABA model does not mimic all aspects of AN psychopathology.

In our experiment, rats displaying more anxiety-like behavior showed increased physical activity during normal feeding conditions. The same animals were additionally more susceptible to the typical increase in running-wheel activity following food reduction. As exercise is associated with increased energy expenditure, this observation could partially explain how anxiety-related behavior leads to rapid weight loss in the ABA model. These results fit well with a previous study showing increasing running levels in anxious mice, also tested in the EPM, under acute food restriction.<sup>17</sup> Mice carrying genes responsible for elevated trait anxiety similarly demonstrated greater hyperactivity when stressed.<sup>29</sup> Hyperactivity following food

restriction in the ABA model is often interpreted as a foraging instinct.<sup>30</sup> The above results allow us to speculate that anxiety traits could be an additional cause. In support of this, there are indications that both anxiety and food restriction can induce hyperactivity in animal models and healthy humans.<sup>31</sup> Apart from the obvious motivation to exercise in order to lose weight, some patients with AN describe physical activity as a means to regulate emotions and desires.<sup>32</sup> Long-distance runners commonly describe a feeling of excitement called the runner's high, which, among others, leads to anxiolysis. It has been suggested that the runner's high results from the stimulation of endorphins via opioid signaling during exercise.<sup>33</sup> In female ABA mice, transiently increasing levels of proopiomelanocortin RNA and peripheral  $\beta$ -endorphin levels have been detected.<sup>34</sup> These findings could partially explain the association between running and reduced anxiety-related behavior observed in the present study.

Adding evidence, we found that anxiety-like behavior and hyperactivity explain a great variance of faster body weight loss. This possibly clarifies how anxiety-like behavior associated with increased RWA leads to faster weight loss in the ABA model. Obviously, greater energy expenditure due to increased physical exercise has been repeatedly shown in patients with AN, with some studies describing a disturbed energy homeostasis.<sup>32</sup> Of notice, hyperactivity in patients with AN has been linked to a worse prognosis.<sup>35</sup>

In our experimental setting, both LF and ABA groups showed a reduction of anxiety-like behavior after chronic starvation regardless of RWA. This corresponds well to similar results studying short-term food reduction in

mice.<sup>17</sup> Independently from AN animal research, an anxiolytic effect of mild to moderate calorie restriction in mice was previously observed.<sup>36,37</sup> This effect also seems to persist during chronic starvation in the present study. We speculate that food-restricted animals are more likely to venture into dangerous areas as a result of their foraging instinct and predominant struggle for survival. However, in preclinical studies, it has been reported that starving animals in the ABA model show GABAergic alterations within the hippocampus that are central to regulating anxiety-related behavior and influence susceptibility to ABA.<sup>38</sup> Moreover, dietary restriction has been suspected to improve anxiety-related behavior via reduced brain levels of serotonin, noradrenalin, and its precursors, which are known to modulate anxiety-like behavior.<sup>39–41</sup> Weight loss in humans can be initially rewarding, goal-driven, and positively reinforced.<sup>42,43</sup> The reduction of anxiety can thus be considered as a decisive factor leading to the maintenance of self-starvation.<sup>44–46</sup> Although the irrational fear of excessive body mass persists or even grows regardless of body weight and weight loss during starvation,<sup>47</sup> some aspects of anxiety (e.g., blushing during public speaking) seem to diminish during acute starvation.<sup>48–50</sup> This may be caused by a shift of attention toward eating and body weight concerns and away from social issues.<sup>51,52</sup> Thus, the anxiolytic effect of starvation seen in the animal model can only partly be confirmed in clinical observations.

## Strengths and limitations

ABA is a well-controlled experimental model that has been widely proved to mimic physiological and behavioral characteristics of AN. We were able to assess the effects of starvation, RWA, and anxiety-like behavior separately using different experimental groups. Furthermore, we examined not only acute but also chronic starvation creating a realistic clinical course of AN. Using an animal study, cognitive components that possibly influence the process of weight loss were excluded. To assess the clinical reality of AN even more accurately, it may be beneficial to define anxiety categorically by using rodent strains bred for both high and low anxiety-like behavior. The association between anxiety-like behavior and physical activity was most prominent during the induction of starvation, whereas this association was not well observable in the acute and chronic starvation period. RWA probably decreases during prolonged food restriction to prevent exhaustion.

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## CONFLICT OF INTEREST

The authors declared no competing interests for this work.

## AUTHOR CONTRIBUTIONS

C.S. wrote the manuscript. B.H.D., C.B., J.S., and S.T. designed the research. C.S., C.V., V.K., A.S., and S.T. performed the research. C.S., J.S., and S.T. analyzed the data. [Correction added on 2 March 2022, after first online publication: The author contributions has been modified in this version.]

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