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Research article

Establishment of a chronic activity-based anorexia rat model

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HIGHLIGHTS

- Anorexia nervosa is a psychiatric disorder with a typically chronic course.
- The new activity-based anorexia rat modelis characterised by a fixed feeding regime and chronic low weight holding phase.
- This model enables the study of chronic starvation with initial hyperactivity and ensuring complete amenorrhoea.
- A 25% starvation level, early adolescent age and three weeks of starvation were demonstrated to be the best parameters.

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ABSTRACT

Background: Anorexia nervosa (AN) is often a chronic eating disorder characterised by body image disturbance and low body weight often associated with starvation-induced amenorrhoea and excessive exercise. Activity-based anorexia (ABA) is an animal model representing many somatic aspects of this psychiatric illness. We systematically manipulated the extent and length of starvation and animal age to find the optimal parameters to study chronic starvation.
New methods: Wistar rats had 24 h/day running wheel access and received 40% of their baseline food intake until a 20% or 25% weight reduction was reached (acute starvation). This body weight was then maintained for two weeks (chronic starvation). The rats of different ages of 4 or 8 weeks were used to represent early and late adolescent animals, respectively. The complete absence of a menstrual cycle was defined as the primary outcome parameter.
Results: Acute starvation caused a disruption of the oestrous cycle in 58% of the animals. During chronic starvation, a complete loss of the oestrous cycle could be found. Furthermore, 4-week-old rats exhibited higher levels of hyperactivity and amenorrhoea than 8-week-old animals. A 20% starvation level led to 90% loss of cycle, while a 25% starvation level triggered complete loss.
Comparison with existing methods: Most current ABA models focus on acute starvation, while most patients are chronically ill.
Conclusions: The optimal parameters to achieve complete amenorrhoea included early adolescence, chronic starvation and 25% weight loss. The new ABA model allows studying the effects of chronic AN on underlying behavioural, hormonal and brain pathobiology.

Abbreviations: ABA, activity-based anorexia; AN, Anorexia nervosa; GFAP, glial fibrillary acidic protein; NPY, neuropeptide Y; RWA, running wheel activity.

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1. Introduction

The third most common chronic disease in adolescence is anorexia nervosa (AN) (Gonzalez et al., 2007). Amenorrhoea is observed nearly ubiquitously in patients with AN, as it is seen as a reproductive precaution that prevents additional energy demands due to pregnancy in an already malnourished state (Herpertz-Dahlmann, 2015). The treatment success in patients with AN is limited (Espie and Eisler, 2015). At an average of 18 months after treatment, nearly 60% achieved a normal body weight; however, less than 60% of patients developed a normal cycle, and the risk for relapses was between 31 and 41% (Berends et al., 2016; Carter et al., 2004). After 7–10 years, Steinhausen reported that approximately half of AN patients recovered completely, 30% enhanced their condition and 20% of the patients stayed chronically ill (Steinhausen, 2009). Furthermore, the burden for patients, caregivers and costs for society are high (Schmidt et al., 2016). Therefore, research of the aetiology, sustaining factors and therapeutic options is urgently needed.

Activity-based anorexia (ABA) is the most commonly used animal model for studying AN. In 1967, Routtenberg and Kuznesof developed the original anorexia animal model using a restricted feeding schedule and access to running wheels (Routtenberg and Kuznesof, 1967). In 1953, Hall et al. demonstrated that rats were paradoxically hyperactive when food access was restricted, despite furthering their starvation by increasing energy consumption (Hall et al., 1953). Later, this model was called the ABA model (for a review see: Méquinion et al., 2015b). ABA mimics some of the core features of AN, including increased running activity, oestrous cycle disturbances and altered hormone production (Belmonte et al., 2016; Lee and Kinzig, 2017; Paré, 1977; Paulukat et al., 2016; Watanabe et al., 1992). The developing hyperactivity in ABA models can potentially be explained evolutionarily with a food seeking behaviour. This increased activity seems to be partially due to a lack of leptin, as it was shown that hyperactivity was significantly lessened when the rats were substituted with leptin (Exner et al., 2000). Hypoleptinemia is a known symptom of AN (Seitz et al., 2016). The ABA paradigm is well-established for analysing the neurobiological consequences after short-term starvation (Carrera et al., 2014). However, protocols for longer-term starvation that simulate chronic illness are scarce.

Other models of AN include a dehydration-induced rodent model (DIA), which included a hyperosmotic drink to induce dehydration (Reyes-Haro et al., 2016; Watts and Boyle, 2010). This model is performed for 4–14 days and characterised by a reduction in meal duration and weight loss (Callahan and Rinaman, 1998). However, changes in osmolarity in the blood and brain in this model are very high and can potentially influence research results, such as the difficulty of the brain to be able to distinguish between starvation and dehydration effects. Some groups mimicked AN with a mild food restriction in which 30–40% less food was given compared with the controls (Austad, 2001; Bi et al., 2003; Bruss et al., 2010; Hamrick et al., 2008; Yamamoto et al., 2009). Mild food restriction led to altered gene expression (e.g., increased arcuate NPY expression) and decreased leptin levels. However, these studies only examined short-term starvation lacking the hyperactive component, which is seen in 30–80% of the patients with AN (Herpertz-Dahlmann, 2015). However, ABA is the only model to combine (self-aggravated) food restriction and weight loss with hyperactivity. Most ABA protocols study short-term starvation ranging from 3 to 14 days (Méquinion et al., 2015b). In the original ABA model, the mortality rate was increased after chronic starvation, where the rodents continued to run during the feeding time instead of eating (Exner et al., 2000; Routtenberg and Kuznesof, 1967). Only one ABA study used a longer starvation period with free wheel access (Méquinion et al., 2015a).

The purpose of this study was to establish a modified ABA model that avoids the increased mortality rate of the original model while allowing for the analysis of the effects of chronic starvation, thus best representing the often chronically ill patient population. We modified the model of Mequinon et al. that used fixed amounts of food instead of time windows for feeding. However, instead of determining the fixed amounts of food given by comparing with the average of a control group, we calculated them individually by comparing with the same rat's food consumption during the ten days of acclimatisation. Furthermore, we preset a defined target weight rather than a reduction in quantity to increase control over the starvation process. This allowed us to reduce the variability of body weight and to minimise mortality within the treatment. We started with the acute starvation phase, where the animals received 40% of their previous food consumption until the target weight reduction of 20–25% body weight was achieved. Upon reaching this target weight, the food was adjusted daily to maintain this precise level of reduced body weight for another two weeks to mimic chronic starvation while insuring animal survival.

The second aim of this study was to optimise the new chronic ABA model in finding the optimal duration of starvation, adolescence or pre-adolescence, and level of weight reduction. Amenorrhoea was selected as a primary target parameter to eliminate the oestrous cycle as completely as possible to mimic this important clinical symptom in female AN and to obtain the maximum effect of gonadal suppression and hormonal change. As a secondary parameter, we selected the amount of running wheel activity (RWA) to be the seminal distinctive symptom of the ABA model. To find the best parameters, we tested acute (1 week) vs. chronic (3 weeks) starvation, 4 vs. 8-week-old rodents as well as a 20 and 25% extent of starvation. To minimise the distress of the rats, it was also important to determine the minimal necessary extent of starvation for complete amenorrhoea. We concentrated on female rats because most of the patients with AN are female (Herpertz-Dahlmann, 2015). However, the ABA model has also been shown to work in male rodents, but with gender differences in physical activity (Achamrah et al., 2017).

2. Materials and methods

2.1. Animals and care

Adolescent 4 and 8-week-old female Wistar rats (Charles River, Sulzfeld, Germany) maintained under a 12/12-h light/dark cycle (lights on at 7:00 am) were used in this study. The acute starvation group included one week of starvation, with the animals getting 40% of their average daily food intake during the acclimatisation phase until a 25% body weight loss was reached (Controls_acute_4W: n = 9; ABA_acute_4W25%: n = 9). Furthermore, the chronic ABA groups were subjected to an additional 2 weeks of starvation using different ages of rats, 4 and 8 weeks, and two different extents of starvation, 20 and 25% (Controls_chronic_4W: n = 12; ABA_chronic_4W20%: n = 6; ABA_chronic_4W25%: n = 12; Controls_chronic_8W: n = 12; ABA_chronic_8W20%: n = 6; and ABA_chronic_8W20%: n = 5). A schematic summary of the age, duration and extent of starvation and animal numbers in the different groups is shown in Fig. 1. The room was maintained at a constant temperature (21 ± 1 °C as the standard temperature). The facility was specifically pathogen free according to the FELASA Guidelines and certified according to DIN ISO 9001/2008.

2.2. Prearrangements of cages

Type IV polycarbonate cages (1820 cm^2, Polysulfone, Tecniplast GmbH) were provided with a cover and with an integrated run-