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Early-life social context affects vulnerability to activity-based anorexia in rats

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Abstract

Rationale

Early-life social adversity is more than often found in patients diagnosed with anorexia nervosa (AN), and it is strongly related to its onset during adolescence. In rats, social adversity can be studied through the chronic social instability model, but its impact has not yet been observed in the development of activity-based anorexia (ABA).

Objectives

The present study aims to analyze the effect of poor social conditions in adolescence in the later development of ABA in rats, an animal model of AN.

Methods

Sixty Wistar Han rats underwent two experimental phases: socialization and the ABA procedure. In the socialization phase, housing conditions were manipulated, and subjects were exposed to either social stability, social instability, or isolation for 20 days. In the ABA developmental phase, all rats had restricted access to food for 1 h/day during the light period and either had access to a running wheel for two 2 h periods or not.

Results

Data showed that socialization prevented fatal weight loss compared to isolation during the ABA procedure. Furthermore, rats exposed to social stress through housing instability were resilient to diet restriction compared to socially stable and more so than isolated rats. Weight loss developed similarly between groups that had access to a running wheel, although adaptation to the procedure happened earlier in the socially stable rats compared to the instable or isolated ones. Additionally, food intake was significantly lower in the isolated subjects compared to the socialized ones.

Conclusions

In general, these results show that socialization, whether stressful or stable, can have a protective effect against ABA development, although it does not prevent it. Social factors need to be considered when approaching the emergence, development and recovery of AN.

Introduction

The social context and interactions that social animals experience shape their development and health through their life. As such, social connection provides humans and other animals with means of survival and adaptability to the environment, being highly associated as a protective factor and impacting our longevity. On the other hand, poor social conditions such as low socioeconomic status or loneliness, substantially increase mortality risks at levels comparable to those associated with smoking, sedentary living or alcoholism (Holt-Lunstad 2018).

The impact of social context is also evident in psychological disorders such as anorexia nervosa (AN). AN is an eating disorder characterized by an intense fear of gaining weight, and the misperception of the physical self-image, in which self-evaluation is excessively influenced by weight and shape (American Psychiatric Association, 2013). Social adversity in the form of family disfunction, neglect or abuse during childhood is strongly associated with the onset of the disorder (Molendijk et al. 2017; Kovács-Tóth et al. 2022). Patients with AN often exhibit deterioration in their social life and interpersonal relationships, and loneliness is often associated with relapses (Meneguzzo et al. 2020). Moreover, impaired social skills, such as deteriorated emotion processing and interpretation, and a tendency to negatively interpret social situations, are usually found preceding the onset of AN and during the course of the disorder (Inoue et al. 2021). Overall, it is clear that social risk factors are influential in the development of AN, while also indicating the likely presence of other predisposing factors (Bozzola et al. 2024).

Activity-based anorexia is a widely used animal model to study the interaction between weight, food intake and excess of activity reproducing key symptoms of AN, such as severe body weight loss, alteration of the estrous cycle, and hyperactivity (de Paz et al. 2020; Gutierrez 2013). A standard ABA procedure typically involves giving individual access to a running wheel (RW) during 21–23 h a day, and 1-1.5 h of limited access to food. Under this procedure, Routtenberg and Kuznesof (1967) first described a so-called “self-starvation” phenomenon and excessiveness of wheel running, accompanied with a severe weight loss that ultimately led to death. Later, Epling et al. (1983) proposed this phenomenon as a model of AN. The main feature of this animal model is the development of excessive physical activity, which parallels a common symptom in AN, since over 80% of AN patients report a significant increase in physical exercise during the disorder (Casper et al. 2020). The temporal structure of the wheel running activity usually consists of peaks of running between two to three hours before food access, which is known as food-anticipatory activity (FAA), and smaller peaks after food intake, known as post-prandial activity (PPA) (Carrera et al. 2014; de Paz, Vidal and Pellón 2020).

Social interaction is not only beneficial, but essential for the rat’s well-being, as these rodents are highly social animals. Ensuring social interaction during housing is a standard requirement under the EU directive on the protection of animals used for scientific purposes (Directive 2010/63). A substantial body of research has documented the benefits of social housing. For example, Chandler et al. (2022) found that group-housed rats consumed significantly less alcohol compared with isolated ones. Moreover, pair-housing prevented anxiety-like behavior induced by social defeat (Nakayasu and Ishii 2008), and long-term social housing prevented the decline of cognitive flexibility in aged rats (Dankert et al. 2025).

If social interaction is essential for rats, its occurrence during adolescence is even more critical, a developmental stage characterized by heightened sensitivity to the social environment. Rough-and-tumble play, the predominant form of social interaction in rats during this period, is critical for the development of normal social behavior and health (Pellis and Pellis 2007). Therefore, altering social interaction during adolescence, such as through deprivation or impairment, can have an impact on rats' health and normal behavior.

There are several animal models that replicate chronic social stress, including social isolation and housing instability, among others. Social isolation consists of housing animals individually. This paradigm of social stress induces depressive-like behavior shown in an open-field and splash test and an excess of locomotor activity in the animals (Amini-Khoei et al. 2024), while also inducing a significant increase in the self-administration of drugs (Noschang et al. 2021) and alcohol abuse (Lesscher et al. 2015). Meanwhile, social instability comprises the unpredictable alternation of periods of individual housing and housing with unfamiliar partners. The duration of these periods can differ between studies from 1 h to 24 h, but also the number of animals during the group housing period, such as pair housing (Mccormick et al. 2008) or five animals per cage (Herzog et al. 2009), or even including crowding as a condition (Brown and Grunberg 1995). This form of social stress can reduce social reward motivation (Leonetti et al. 2025), decrease weight gain, and induce anxiety-like behavior in social interactions and in the elevated-plus maze test, as well as increased aggressiveness (Baranyi et al. 2005) and depressive-like behavior (Mathews et al. 2008).

Socialization is also a seemingly influencing factor in the development of ABA. Early maternal separation, for example, can accelerate the onset of the phenomenon and its key symptoms (Hancock and Grant 2009; Farinetti et al. 2020). Moreover, the characteristics of social housing during the ABA procedure can also affect it, as isolation induces a more pronounced weight loss and an increase in WR activity, when compared to group housing (Boakes and Dwyer 1997), while also slowing down weight recovery after the procedure (Martínez-Herrada et al. 2023). The effects of group housing also appear to be sexually dimorphic, as the benefit of social enrichment play a more pronounced role in the development and recovery of ABA in female rats (Martínez-Herrada et al. 2023).

The aim of this study was to compare the effects of social enrichment and chronic social stress, namely social isolation and social instability, during adolescence in the subsequent development of ABA in early adulthood in female rats. To this end, each social condition was compared with its corresponding control group without access to a wheel to run.

Given the impact of chronic social stress, greater vulnerability to the ABA procedure could be expected in the groups subjected to social isolation and social instability. This vulnerability could be reflected in a more rapid weight loss and increased wheel running. In contrast, social enrichment may act as a potential protective factor against the development of ABA (Boakes and Dwyer 1997; Martínez-Herrada et al. 2023).

Method

Subjects

Sixty female Wistar Han rats were purchased in three batches of 20 each from Charles River Laboratories (Sulzfeld, Germany; Saint-Germain-Nuelles, France). The experimental procedure was conducted separately for each batch due to limitations in laboratory space and the availability of activity wheels. On the day of arrival, rats were 4 weeks old (PND 28-34). Acclimatization to the laboratory facilities lasted three days, during which all 60 rats were housed in groups of five in Plexiglas cages (19 × 35 × 55 cm^3). The room used throughout the experiment had a mean temperature of 21 ± 2 ºC, at 60% relative humidity. The procedure was carried out during the light period of a 12 h light-dark cycle (gradual light turn-on at 8:00 a.m. and turn-off at 8:00 p.m., simulating dawn and dusk respectively). During the procedure, bottles of tap water were always available in the living cages, regardless of the experimental condition of the rats tested. All international and national guidelines applicable to the care and use of animals under experimentation were followed (European Council Directive 2010/63; Spanish Royal Decree 53/2013) and the procedures were carried out in accordance with institutional ethical standards, being approved by the Committee of Research Ethics of UNED and by the Community of Madrid (PROEX 075/19).

Apparatus

Two different cage sizes were used in this experiment, depending on the social housing condition. For social isolation and overcrowding, type III cages (typically for individual housing) made of transparent Plexiglas and a measure of 19 × 24.5 × 40 cm^3 were utilized. The cages used for stable socialization, type IV cages (typically for group housing), measured 19 × 35 × 55 cm^3 and were made of the same material.

During the five hours of the abbreviated ABA procedure, rats assigned to the activity groups were housed in individual transparent Plexiglas cages with a rectangular base prism (19 × 20.5 × 32.5 cm^3). These cages were attached to an activity wheel of 34 cm in diameter and 9 cm thick, in addition to a 10 cm diameter entrance hole to the wheel. The activity wheel was equipped with brakes controlled by a MED-PC program for Windows. Number of wheel turns in 1-minute intervals was measured with the same program. Sedentary groups were housed in individual cages during this time period.

All cages included wood chip bedding covering the floor and a metal grid on top with a concave space to place the food (Maintenance Diet for rats and mice 1324 10 mm pellets, Altromin Spezialfutter GmbH & Co. KG, Lage, Germany) and a water bottle. Body weight was measured daily at 9:30 a.m. using a precision scale (Precisa 1000C-3000C).

Procedures

As mentioned before, the experimental procedure was done separately in three batches. The social conditions were randomly assigned to each batch of 20 rats, and the experimental procedures were conducted sequentially. The first batch was assigned to the social instability (SI) condition, followed by the social stability (SS) condition, and finally social isolation (IS) condition. In addition to limitations in space and equipment availability, this procedure was implemented to prevent potential effects of emotional contagion between rats from different housing conditions during the social phase, given that rats can experience stress vicariously by observing other distressed conspecifics (Keysers and Gazzola 2023; Eraslan et al. 2024).

The experimental procedure consisted in two distinct phases. In the first phase (socialization), rats were randomly divided into three groups of 20 rats each with different housing conditions: SI, SS and IS. In the second phase (ABA development), these three groups were divided into two each, resulting in a total of six groups of 10 rats, randomly assigned to an ABA or control group (Figure 1).

Socialization phase

Rats were differently housed depending on the social condition assigned to its group for 20 days.

In the *social instability (SI)* set-up, rats were exposed to two daily alternating housing conditions, isolation and overcrowding, based on the procedure done by Herzog et al. (2009), originally adapted from a protocol described by Haller et al. (1999). The duration of the two conditions was assessed randomly for each day in periods of time between 1 to 23 h. In the isolation period, all rats were individually housed in the type III cages, whereas, in the overcrowding period, subjects were housed in groups of five in the same type of cage as in the isolation period, changing cage mates each day.

In the *stable socialization (SS)* condition, rats were housed in clusters of five in the type IV cages. Cage mates were not changed.

In the *isolation (IS)* condition, rats were individually housed in the type III cages.

ABA development phase

After the socialization phase, rats were randomly assigned to ABA (access to a RW) or control groups (without access to a RW). Food was withdrawn for all the animals in Session 0. The housing conditions mentioned in the first phase (socialization) were maintained throughout the days that lasted the ABA development phase, except for the 20 rats that were exposed to the social instability condition, due to the impossibility of maintaining the same randomly alternating housing conditions and running the ABA procedure at the same time.

In the ABA development phase, daily sessions lasted five hours (10:00 to 15:00). Just before the start of the session, all rats were weighed. Then, rats in the ABA groups were individually placed inside the cages equipped with an activity wheel. Access to the activity wheel was given for two periods of 2 h each, before and after a period of food intake of 1 h. Meanwhile, rats in the control groups were placed

individually in type III cages without a RW for the duration of the session and fed at the same time as the ABA groups. Each animal was given approximately 24 gr of food, and the leftover pellets after the 1-h period of food intake were weighed to assess the total grams of food consumed by each rat in that hour. At the end of the 5-h experimental session, rats were returned to their respective home cages for the reminder of the day (see Figure 2).

To ensure the animals' safety, a rat was withdrawn from the procedure if its weight was equal to or less than 75% of its initial weight at Session 0 for one day. On the other hand, a weight stability criterion was established, which was defined by body weight at a given day equal or greater than the weight measured four days before. When all rats reached either withdrawal or weight stability, the experimental procedure for ABA development ended (Carrera et al. 2014).

Statistical analysis

The variation in the percentage of body weight during the procedure was calculated in relation to the body weight registered for each rat at the beginning of Session 0. Linear mixed-effect models (LMMs) were used to analyze data related to the change of body weight, food intake and physical activity developed (FAA and PPA) during the ABA procedure. Data analyses were carried out comparing the following conditions: socialization (isolation/instability/stability), access to a RW (yes/no) and Days, accounting for the individual differences of the subjects as a random effect in each Day of the procedure. LMMs models were fitted using restricted maximum likelihood (REML). A likelihood-ratio test was used in each analysis to compare the models that best fitted the data with constructed null models that only included random intercepts and slopes for subjects (e.g., Weight ~ 1 + Day + (1+ Day|Subject)). Satterthwaite's approximation was used to evaluate significance of the fixed effects in the fitted models. All LMMs were fitted in R (RCore Team, 2012; RStudio Team, 2020 using the lme4 (Bates et al. 2015) and lmertest (Kuznetsova et al. 2017) packages. Least-squares means for pairwise comparison between conditions was done with the lsmeans package (Lenth 2016). In the survival analysis, the Kaplan-Meier method was applied, using the logarithmic rank test (Mantel-Cox), comparing each social condition in the ABA groups and control groups, separately. This analysis was carried out using IBM SPSS Statistics (Version 27).

Results

To ensure that all animals started the ABA development procedure with no significant differences in body weight, a simple linear regression model [Weight ~ Group + (1 | Subject)] was done. There were no significant differences in the rats' body weight among the groups before the start of the procedure.

Percentage of body weight loss

Percentage of body weight during the ABA procedure was modelled using LMMs with the conditions RW (yes/no) and Socialization (IS/SI/SS) as fixed effects.

Firstly, there was a two-way interaction found between the fixed effect RW and the effect of Day [$F(1, 54.76) = 33.42$, $p < .001$]. On the one hand, the comparison between the ABA groups showed no effect of the social condition on body weight loss. On the other hand, comparing the control groups [Weight ~ +1 + Day + Social + Social*Day + (1 + Day | Subject)] ($\chi^2_{(2)} = 23.23$, $p < .001$), SI rats gained significantly more weight during the procedure than SS rats ($t = 3.66$, $p < .001$) and to IS conditions ($t = 2.53$, $p = .04$).

Secondly, there was also a two-way interaction between Socialization and the effect of Day [$F(2, 54.56) = 3.87$, $p = .027$]. A comparison between the ABA and control group was done for each social condition.

In the SI condition (Figure 3A), there was a significant interaction between the effect of RW and Day [$F(1, 18) = 50.90$, $p < .001$]. In the best fitted model, body weight was a function of this interaction, accounting for random intercept and slopes for each Subject within each Day [Weight ~ +1 + Day + Wheel + Wheel*Day + (1 + Day | Subject)] ($\chi^2_{(2)} = 27.09$, $p < .001$). In this model, the group without access to a RW (SIC), gained more weight during the procedure than the group with access (SIA) ($t = 7.13$, $p < .001$).

In the SS condition (Figure 3B), there was a two-way interaction between RW and Days [$F(1, 20.24) = 41.20$, $p < .001$]. The best fitted model had body weight as a function of the two-way interaction, with the random effect of Subjects [Weight ~ +1 + Day + Wheel + Wheel*Day + (1 + Day | Subject)] ($\chi^2_{(2)} = 23.95$, $p < .001$). SSC, the control group, gained more weight throughout the procedure than the experimental ABA group SSA ($t = 6.42$, $p < .001$).

Regarding the IS condition (Figure 3C), only the fixed effect of Days had a significant effect on the changes in body weight in the isolated rats [$F(1, 15.96) = 18.35$, $p < .001$]. Body weight changes did not differ significantly between the ABA and control groups.

Food intake

Food intake during the ABA procedure was modelled using LMMs with the conditions RW (yes/no) and Socialization (isolation/instability/stability) as fixed effects.

A three-way interaction was found, where the fixed effects RW and Socialization, modulated by Days, accounted for the changes in food intake during the ABA procedure [$F(2, 56.85) = 4.9$, $p = .01$].

Firstly, there was a two-way interaction between the fixed effect RW and the effect of Day [$F(1, 48.68) = 27.09$, $p < .001$]. On the one hand, the comparison between the ABA groups showed no effect of the social condition in body weight loss. On the other hand, comparing the control groups [Intake ~ +1 + Day + Social + Social*Day + (1 + Day | Subject)] ($\chi^2_{(2)} = 29.76$, $p < .001$), food intake was significantly higher in SIC animals than in ISC animals ($t = 2.54$, $p = .04$).

Secondly, there was also a two-way interaction between Socialization and the effect of Day [$F(2, 60.98) = 7.27$, $p < .001$]. Comparison between the ABA and control group was done for each social condition.

In the SI groups (Figure 4A), there was a significant interaction between RW and Days [$F(1, 361.24) = 19.7$, $p < .001$]. In the best fitted model, food intake was a function of this interaction, accounting for random intercept and slopes for each Subject within each Day [Intake ~ +1 + Day + Wheel + Wheel*Day + (1 + Day | Subject)] ($\chi^2_{(2)} = 21$, $p < .001$). A significant increase in food intake was found in the SIC group compared with the SIA group ($t = -4.44$, $p < .001$).

In the SS condition (Figure 4B), there was only a significant effect from the RW condition [$F(1, 18) = 7.14$, $p = .015$]. Best fitted model [Intake ~ +1 + Day + (1 + Day | Subject)] ($\chi^2_{(1)} = 6.07$, $p = .014$). SSC rats had a higher food intake than SSA rats ($t = 2.57$, $p = .019$).

In the IS condition (Figure 4C), there was a significant interaction between RW and Days [$F(1, 16.60) = 20.09$, $p < .001$]. In the best fitted model, food intake was a function of this interaction, accounting for random intercept and slopes for each Subject within each Day [Intake ~ +1 + Day + Wheel + Wheel*Day + (1 + Day | Subject)] ($\chi^2_{(1)} = 17.82$, $p < .001$). ISC animals showed significantly higher food intake than ISA animals ($t = -4.48$, $p < .001$).

Development and distribution of activity in the running wheel

Physical activity during the ABA procedure was measured before (FAA) and after (PPA) food intake. In both cases, social condition did not affect the amount of RW activity (Figure 5), but there was an effect of Days in FAA [$F(1, 27.92) = 289.58$, $p < .001$] and in PPA [$F(1, 26.77) = 156.90$, $p < .001$].

Reach of body weight stability and survival rates

Regarding vulnerability to the ABA procedure, only four rats were withdrawn due to extreme body weight loss, all of which were exposed to the IS condition. Two rats were withdrawn from the ISA group in days 13 and 17, and two rats in the ISC group in days 15 and 19.

Among rats that reached weight stability, SIA animals did so between days 10 and 21 ($M = 14.8$, $SD = 0.96$), whereas SIC animals reached weight stability between days 7 and 13 ($M = 10$, $SD = 0.6$). In the SS condition, SSA rats reached weight stability between day 7 and 16 ($M = 11.8$, $SD = 0.84$) and SSC rats between day 6 and 11 ($M = 7.6$, $SD = 0.56$). Lastly, in the IS condition, ISA rats reached weight stability between day 9 and 21 ($M = 13.9$, $SD = 1.47$) and ISC rats between day 8 and 12 ($M = 10$, $SD = 0.62$).

A survival analysis was then performed with the Kaplan-Meier method to compare the survival rate to the procedure considering the day of weight stability, as defined previously in the Method section as body weight at a given day equal or greater than the weight measured four days before. Survival analyses were done comparing social conditions between the ABA groups and control groups separately. Firstly, between the ABA groups (Figure 6A), pairwise comparisons revealed differences between the socially enriched group (SSA) and the groups exposed to social stress, SIA [χ^2 (2,30) = 4.86, $p = .027$] and ISA [χ^2 (2,30) = 3.92, $p = .048$], with no differences between SIA and ISA groups. In a similar manner, between the control groups (Figure 6B), SSC reached weight stability significantly before SIC [χ^2 (2,30) = 5.94, p =

.015] and ISC [χ^2 (2,30) = 9.1, p = .003] groups. In general, social enrichment facilitated weight stability during the ABA procedure compared to the groups exposed to social stress, regardless of access to a RW.

Discussion

The social context of gregarious animals can have a great impact in their development, especially during early life, which can have a major role in affecting the animal's health and behavior, with long lasting effects that can be seen later in adulthood. In humans, perceived loneliness or lack of social connection is a predisposing factor related to mental and physical health (Hawkley and Cacioppo 2010), and especially during adolescence social disfunction represents a risk factor in the development of psychological disorders (Lamblin et al. 2017). In rats, social stress affects how the animal interacts with conspecifics, such as acute aggression (Meng et al. 2010) or reduced social interaction (Hodges et al. 2017), with related increased stress levels to those interactions (Asgari et al. 2021). Moreover, social stress can also promote addiction (e.g., alcohol consumption) (Marcolin et al. 2020) and alter reward sensitivity (Herlehy et al., 2022).

The aim of the present study was to compare the effects of adolescent social stress, provoked by social instability or social isolation, with those of social enrichment, on the subsequent development of ABA in female rats. For this purpose, an abbreviated ABA model was used, allowing social housing throughout the procedure, unlike the standard ABA paradigm (Carrera et al. 2014).

Firstly, regarding the changes in body weight during the ABA procedure, the results showed that it was mostly influenced by the social housing condition when comparing the "sedentary" or control groups, in stark contrast with the similar changes in body weight that happened in the three groups that had access to a RW. It is, then surprising, to see that the group that was exposed to social instability in adolescence (SIC) was less affected by food restriction than rats that had a stable grouping since the start of the whole experiment (SSC). Typically, during the first sessions of the ABA procedure, rats experience a decline in body weight as they consume less food than expected while adapting to the food-restriction schedule. As shown in Fig. 3A, the level of body weight in the SIC group is mostly the same from the start to the end of the procedure, and unusually higher than the other groups. This fact could point to an adaptation to food restriction in the social phase, as rats under chronic social instability show a lower food intake when compared to socially grouped ones (Herzog et al. 2009). Prior experience with food restriction is shown to lessen its impact (Lett et al. 2001; Boakes and Dwyer 1997), but does not prevent it (Ratnovsky and Neuman 2011). Although it prevented body weight loss, this only happened in the control group but did not have such an effect on ABA, as the SIA group had a similar body weight loss and food intake as the other ABA groups (SSA and ISA), and a severe weight loss compared to its control group (SIC). Moreover, the lessened impact of food restriction in the SIC contrasted with the SSC one could also be explained by the fact that rats that undergo early life social stress seem to be resilient when exposed to similar challenges later in life, as long as they are no longer exposed to stress later on (Buwalda et al. 2013). These findings suggest that rats that experienced low

food intake under the SIC condition during adolescence appeared more resilient to subsequent food restriction, with the added protective factor of social stability during the ABA procedure. While SIC rats could benefit from this resilience, the control rats under no social stress (SSC) had no challenges during their adolescence, resulting in a greater impact of food restriction later on.

As for social isolation (IS), its impact on body weight loss was so pronounced that access to a running wheel (RW) appeared to exert little additional effect, as both isolated groups exhibited a similar pattern of body weight loss. The impact of isolation is further confirmed in the higher vulnerability to ABA development, as four isolated rats (from both ISA and ISC) had to be withdrawn from the procedure to prevent fatal weight loss, but none from both of the social groups. In comparison, social enrichment facilitated survivability, as the SS groups reached weight stability sooner than both groups under social stress models (SI and IS). This is in line with a previous study comparing survival rates between socially housed and isolated rats during the abbreviated ABA procedure (Martínez-Herrada et al. 2023).

The effect of social isolation observed in the present study should be taken into consideration when interpreting finding from the standard ABA model, as all rats that undergo this procedure remain isolated throughout the experiment (Carrera et al. 2014). Although this is an inherent feature of the model, its impact on ABA development should not be overlooked. Rats subjected to the ABA procedure experience not only food restriction, but also social deprivation, and both factors may contribute to the overall development of ABA.

A second relevant finding concerns food intake. There was a significant difference regarding chronic social stress between instability and isolation, whereas SI rats had a greater intake than the IS ones, pointing to the aforementioned resistance to food restriction induced by the instability condition. In each social condition, food intake was affected by whether or not the rat had access to a RW, increasing in sedentary rats, which differs from other studies regarding the ABA model (Pérez-Padilla et al. 2010; de Paz et al. 2019). It is possible that while RW has no effect on food intake, it can interact with the fact that chronic social stress can affect food motivation and reward seeking behavior (Herlehy et al. 2022; Herzog et al. 2009). The potential influence of social factors on food motivation during ABA development warrants further investigation.

In regard to the development of activity, the three ABA groups showed a similar rate of running in the RW, with no difference between the social conditions. It seems that the shorter duration of access to a RW, compared with the standard ABA model, may have limited the development of excessive activity. As shown in Fig. 5, wheel-running activity appeared to reach a plateau during the final days of the procedure, reducing the likelihood of detecting differences between groups. A similar finding was reported by Martínez-Herrada et al. (2023).

As limitations of this study, it needs to be pointed out the impossibility to keep the social instability condition during the ABA development procedure in the manner that it was structured. The change to group stability during ABA for the SI groups may have masked or lowered the impact of social stress on these groups, but it also had the unexpected effect of resilience shown in the SIC group, which presents

a possibility for future studies in this matter. Furthermore, regarding vulnerability to the procedure in the isolated groups, it is possible that this effect could be explained by the lack of body heat compared to the socially grouped rats, as isolated rats cannot huddle to maintain body temperature as grouped rats can. Body weight loss results in a decreased body temperature that can accelerate ABA development, as shown for example in Gutierrez et al. (2008).

In spite of limitations, this is the first study to adapt the social instability model to study its influence on ABA, furthering our understanding of the determining health factor that is the social context of gregarious animals. In turn, the translational value is readily apparent, as adverse social conditions are usually found in the childhood of later AN patients (Kovács-Tóth et al. 2022). Moreover, although accidental, this study showed stable socialized housing as a surprising protective effect that prevented the expected deleterious effects of the social instability model during ABA. This could point towards the possibility of resocialization as an effective treatment to lessen the vulnerability to the disorder that early-life social stress can induce. Nevertheless, more research is needed in this regard.

Conclusions

The experiment reported here shows that social housing can exert complex effects on the development of ABA, influencing food motivation in ways that are not typically observed in standard ABA procedures. The findings also highlight social isolation as a relevant altering component of the model, with an impact comparable to that of RW access or food restriction.

Overall, the social context appears to be a key factor in the initiation, progression, and recovery of AN. As such, it should be carefully considered in future research, as well as in the design of preventive and therapeutic interventions for the disorder.

Declarations

Conflict of Interest

The authors declare that they have no conflicts of interest relevant to the content of this manuscript.

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Author Contribution

A.D.P. conceived the research project and research question. A.D.P., A.M.H., and R.P. contributed to the study design, literature review, interpretation of the findings, and critical revision of the manuscript. A.M.H. and A.D.P. conducted the experimental work and performed the statistical analyses. A.M.H. drafted the manuscript. All authors reviewed and approved the final submitted version of the manuscript.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Clinical trial number

Not applicable.

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Figures

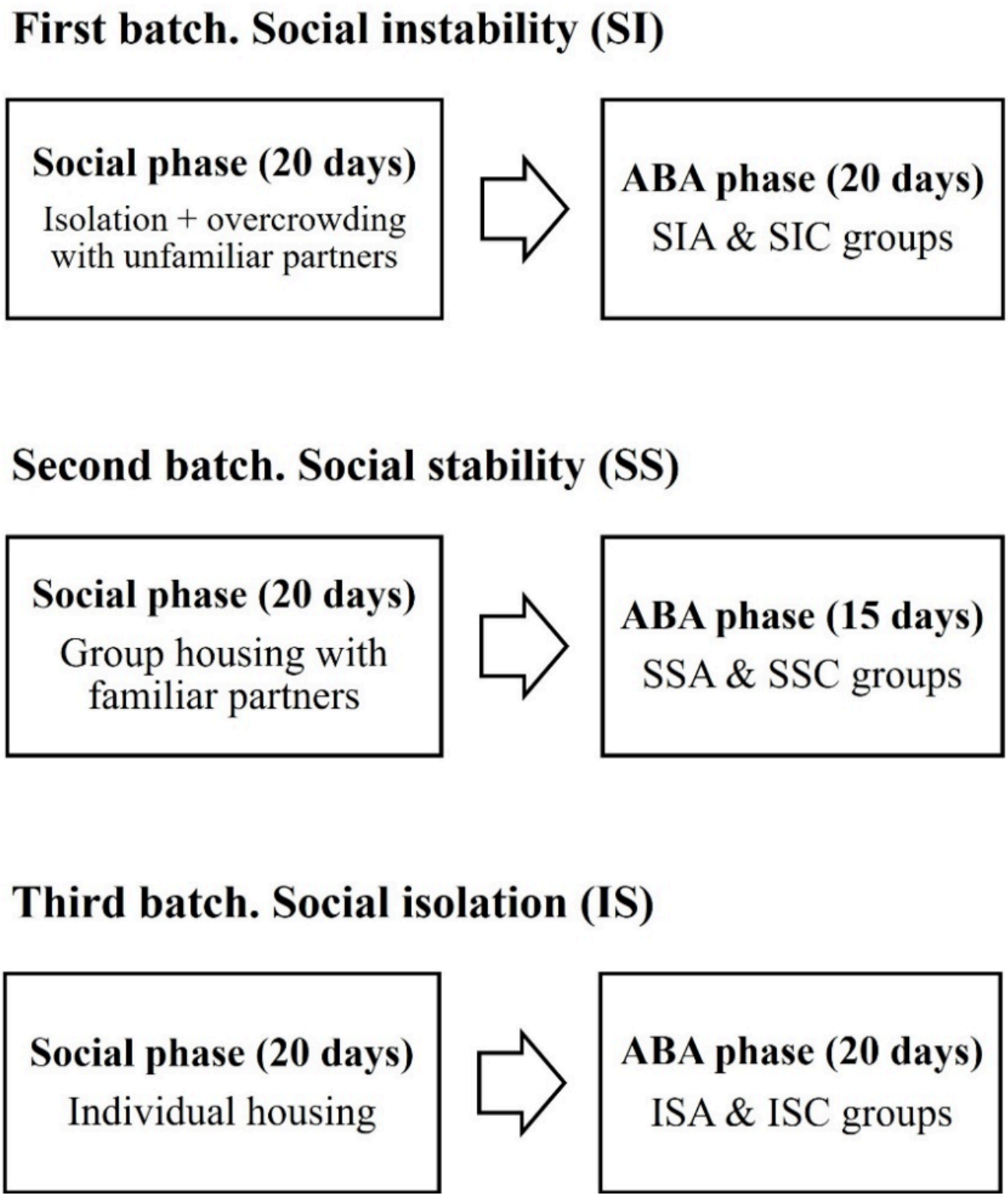


Figure 1

The figure shows the order of experimental procedures for the three batches. The duration of the ABA phase procedure was not fixed and was determined by the time of withdrawal of all the rats or the achievement of weight stability within each batch. ABA: activity-based anorexia; SIA: social instability ABA; SIC: social instability control; SSA: social stability ABA; SSC: social stability control; ISA: social isolation ABA; ISC: social isolation control

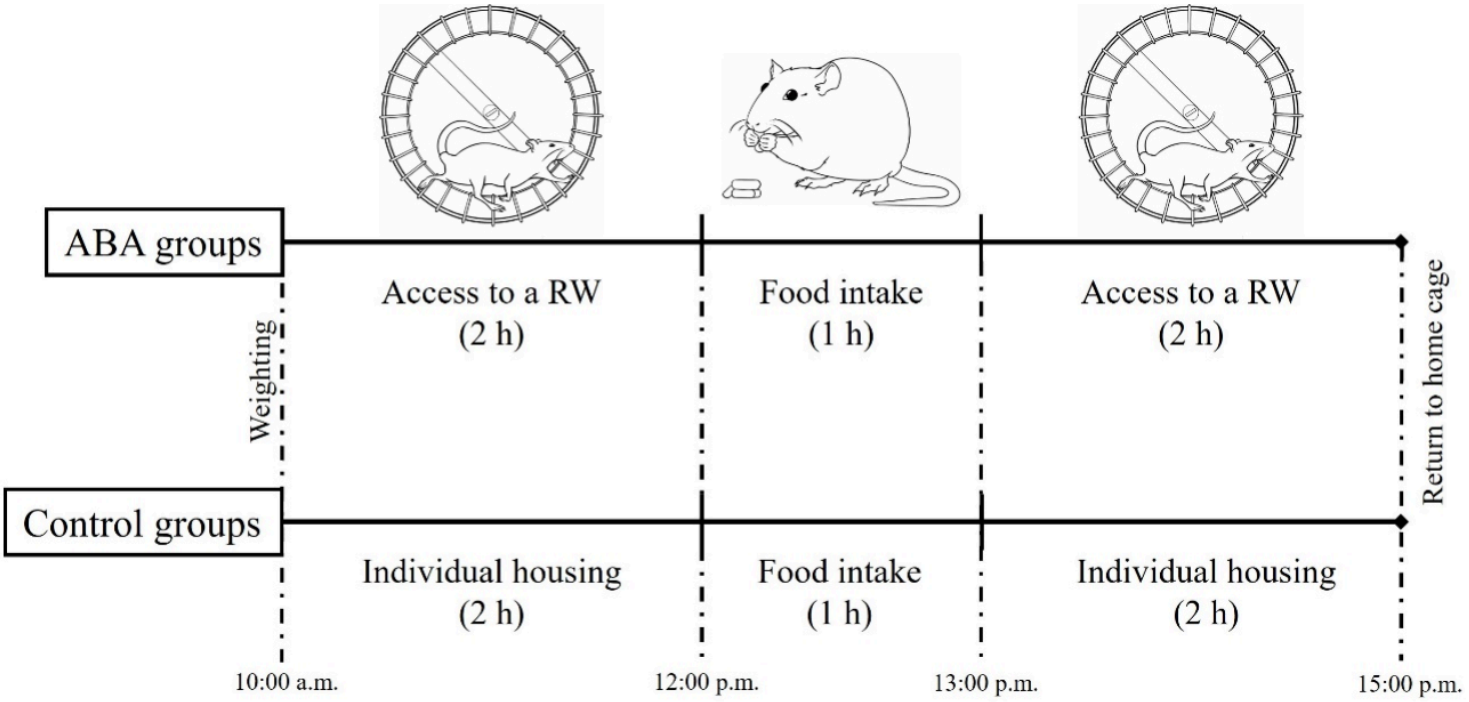


Figure 2

Timeline of the abbreviated activity-based anorexia procedure. ABA: activity-based anorexia; RW: running wheel

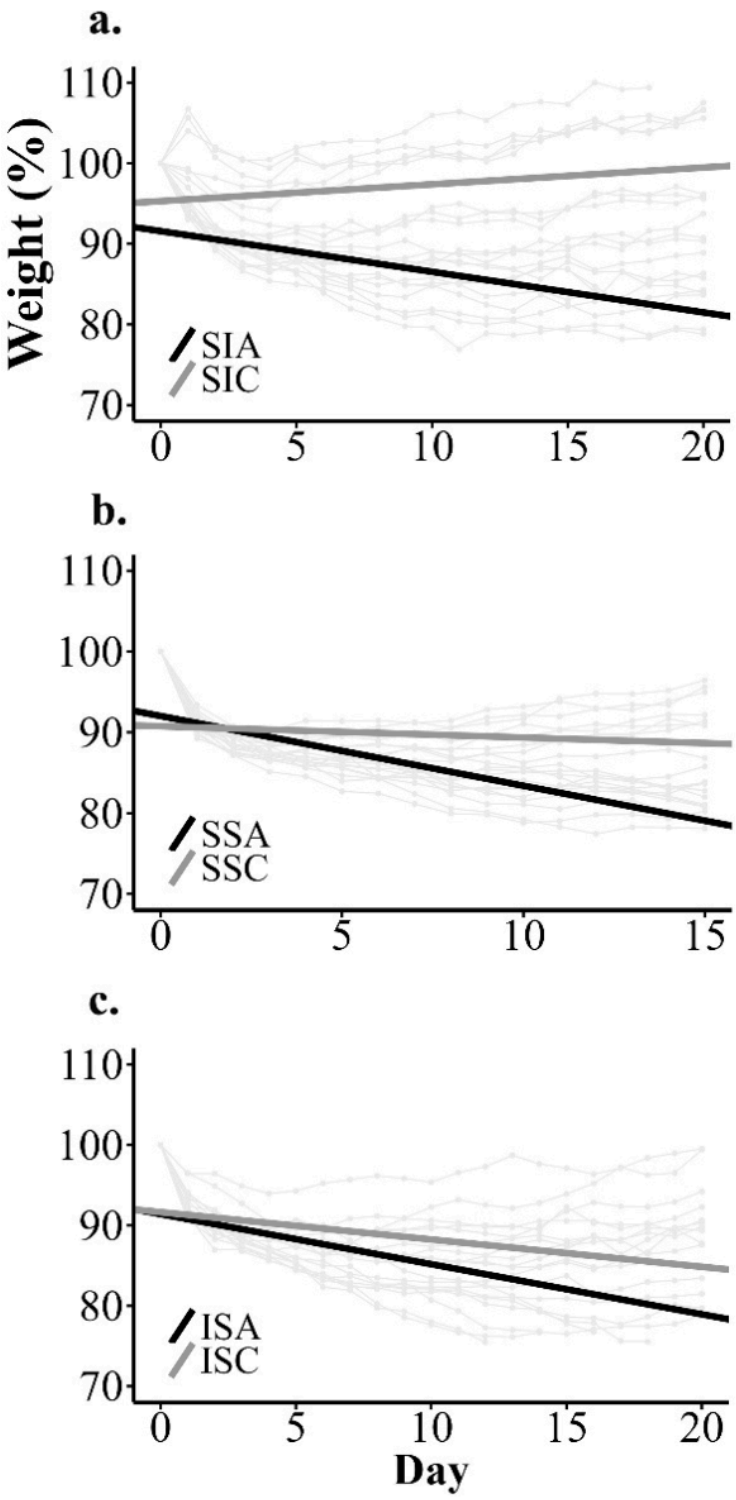


Figure 3

Body weight changes during the ABA procedure. Linear regression of percentage of body weight during the procedure. Comparisons of each social condition between the corresponding ABA (activity-based anorexia) and control group. ABA groups are represented by a solid black line and control groups by a solid gray line. Individual data are represented by light gray lines with solid round dots. A. Social instability condition: SIA (social instability ABA) vs. SIC (social instability control). B. Social stability

condition: SSA (social stability ABA) vs. SSC (social stability control). C. Social isolation condition: ISA (social isolation ABA) vs. ISC (social isolation control)

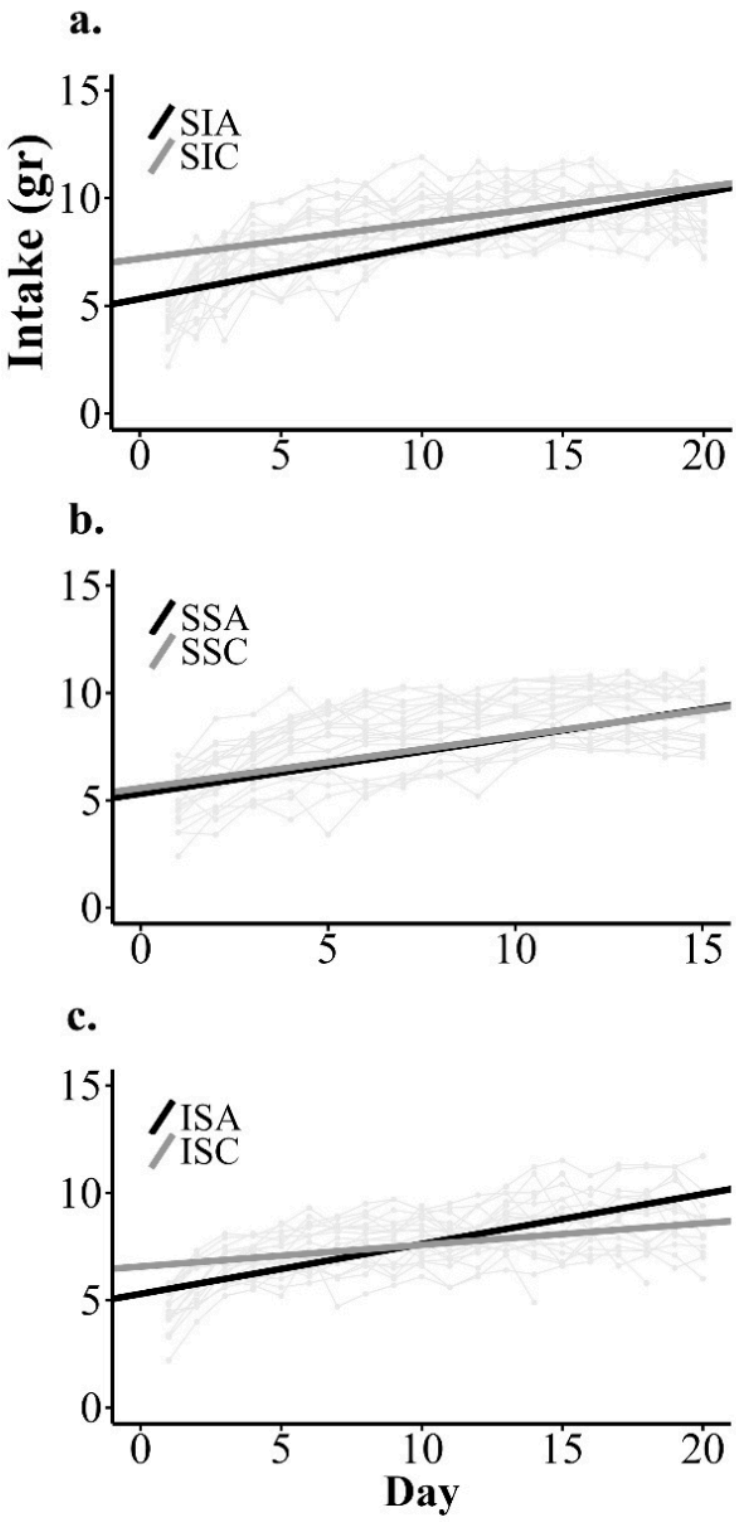


Figure 4

Food intake changes during the ABA procedure. Linear regression of food intake measured in grams during the procedure. Comparisons of each social condition between the corresponding ABA (activity-

based anorexia) and control group. ABA groups are represented by a solid black line and control groups by a solid gray line. Individual data are represented by light gray lines with solid round dots. A. Social instability condition: SIA (social instability ABA) vs. SIC (social instability control). B. Social stability condition: SSA (social stability ABA) vs. SSC (social stability control). C. Social isolation condition: ISA (social isolation ABA) vs. ISC (social isolation control)

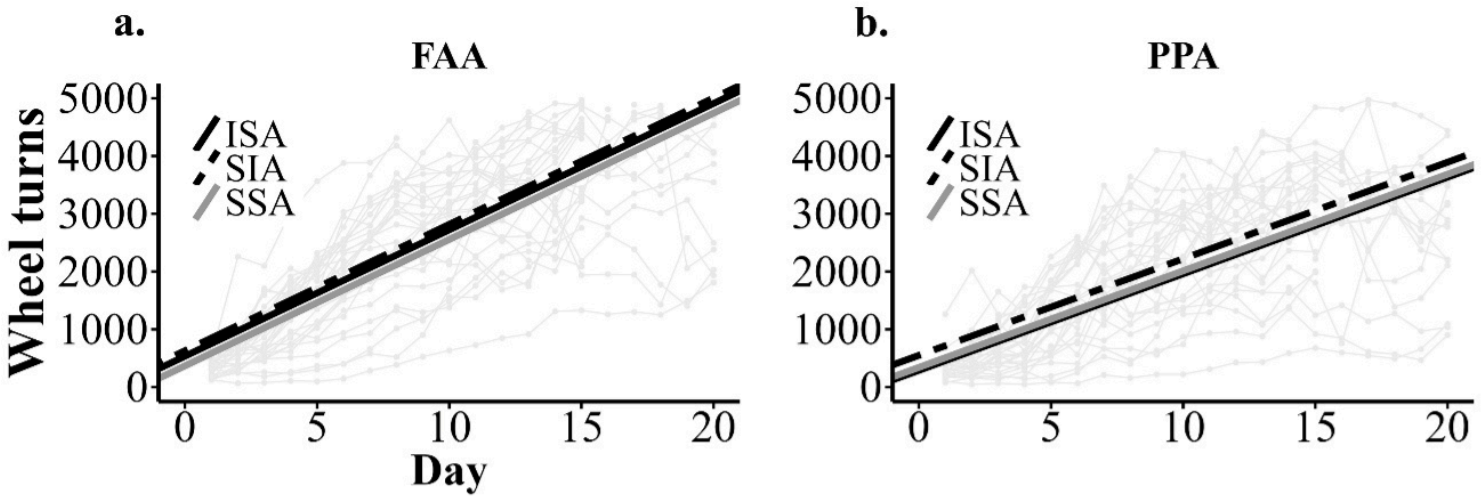


Figure 5

Wheel running changes during the ABA procedure. Linear regression of number of wheel turns during the procedure. Comparison between the three ABA (activity-based anorexia) groups. SIA (social instability ABA) is represented by a two-dash discontinued black line. ISA (social isolation ABA) is represented by a solid black line. SSA (social stability ABA) is represented by a solid gray line. A. FAA (food-anticipatory activity) B. PPA (post-prandial activity)

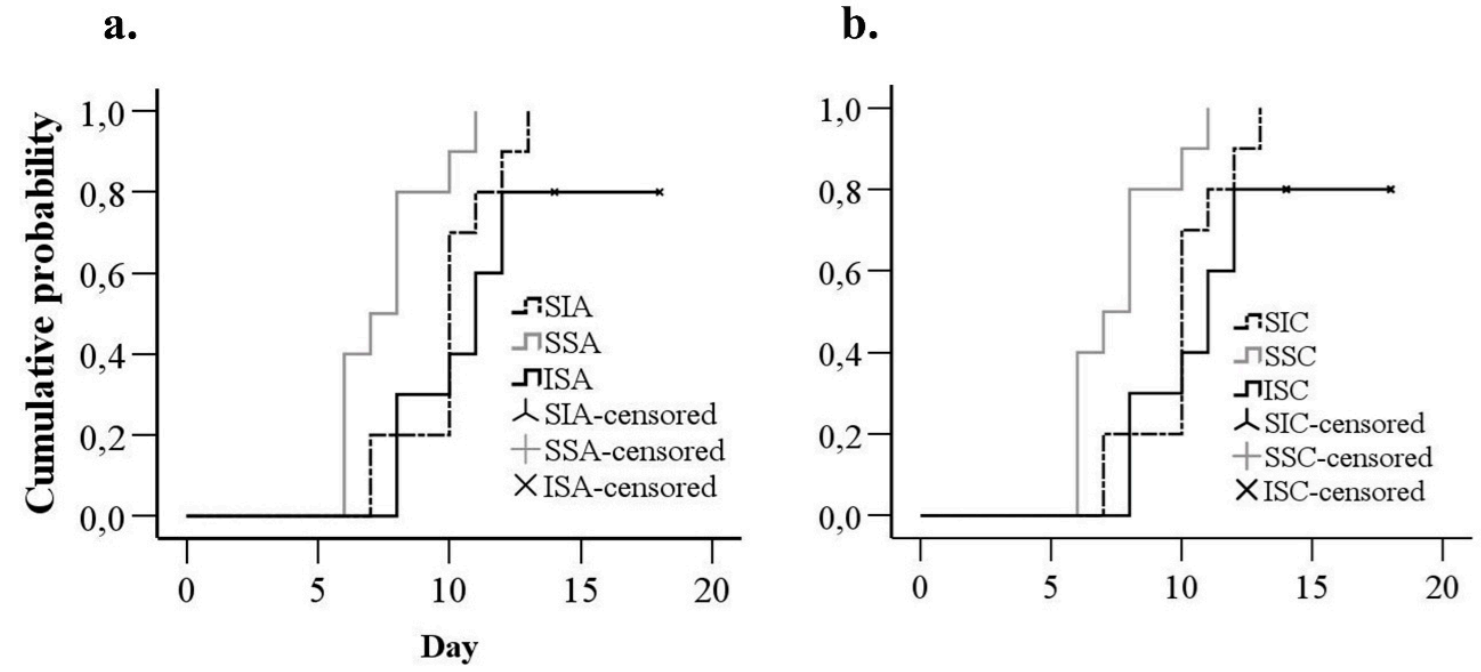


Figure 6

Survival functions after the ABA procedure. Minus one survival rate plots. Survival functions with respect to the day of reach of stability as in reach of body weight at a given day equal or greater than the weight measured four days before. Crossed events indicate withdrawal from the procedure as in reaching the withdrawal criterion after losing 25% of their initial weight. A. Comparison of the three ABA (activity-based anorexia) groups. B. Comparison of the three control groups