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Socialization, and its modulation by sex, on the development and recovery of activity-based anorexia in rats☆

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ABSTRACT

The activity-based anorexia (ABA) animal model has been used in the laboratory to study the role of excessive physical activity in the manifestation of anorexia nervosa (AN) in humans. Factors of social context are crucial in human health and the emergence of many psychological disorders, which have also been observed in studies using different mammal species that, like human beings, set their lives in groups. In the present study, the animals' social condition was manipulated to observe the effect of socialization in ABA development, and the possible different influence of the variable sex on the phenomenon. Eighty Wistar Han rats were distributed into four male and four female groups with 10 subjects each, manipulating social conditions (group housing or social isolation) and physical activity (access or not to a running wheel). Throughout the procedure, all groups had food restricted to 1 h/day during the light period. Furthermore, ABA experimental groups with access to the running wheel had two periods of access to the wheel of 2 h each, one before and the other after the food period. In this experiment, socialized rats were less vulnerable to weight loss during the procedure, although there were no differences between the ABA groups. Moreover, social enrichment was shown to be an enabling variable of the animals' recovery after their withdrawal from the procedure, with this effect being more pronounced in females. The results in this study suggest the need to further in the analysis of the role of socialization in the development of ABA.

1. Introduction

Anorexia nervosa (AN) is a psychological disorder characterized by a severe restriction of food intake, intense fear of gaining weight and an alteration in the way of perceiving one's own body [1]. In up to 80% of people affected by the disorder, an excessive physical activity is observed preceding its development, which further increases during its course [2]. This suggests that the emergence of this symptom may be critical in the initiation of the pathology even though it is not directly among its diagnostic criteria [3].

Experimentally, the relationship between diet, weight and activity in AN can be studied through the animal model of activity-based anorexia (ABA), which is carried out by individual isolation of animals, usually rats or mice, in a cage equipped with an activity wheel that they can access 23 h a day and where food intake is limited to 1 h per day [4,5]. In Routtenberg and Kuznesof [6] first studies on this model, it was found that rats that had food restriction and continuous access to the activity wheel progressively performed more exercise despite eating less until death, whereas the group of rats without access to the activity wheel survived as weight loss stabilized.

The ABA model is widely used because of its ability to mimic key symptoms shown in AN, such as weight loss, hyperactivity, and alterations of the estrous cycle in female animals, among others [7]. However, the forced food restriction in the ABA model does not correspond to the somewhat "voluntary" restriction in the AN disorder, which although not imposed by an experimenter, it is dependent on cultural and self-image aspects. Nevertheless, though the ABA model does not replicate the etiology of the human disorder, most animal models of psychopathology also do not. And despite it, animal models still play an important role in the understanding of human disorders, while taking this hindrance into account [8].

The excessive activity that animals develop under the ABA model

☆ Declaration of competing interest
The authors declare that they have no competing interests.

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tends to be concentrated around the time of exposure to food, finding peaks of activity between two to three hours before access to it, showing what is known as food anticipatory activity (FAA) [9]. It has been observed that rats more vulnerable to the ABA model seem to acquire this behavior earlier than the most resistant ones, therefore, FAA can be a predictor of the development of anorexia [10]. Alternatively, Wu et al. [11] found that FAA was not different between a group susceptible to ABA and another more resistant group, while differences were found in postprandial activity (PPA), manifest to a greater extent in the most vulnerable group.

One of the critical factors due to its impact on human health is social stress, being associated with a dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and negatively affecting social behavior [12]. Specifically, it has been found that patients with AN are more susceptible to social isolation and have problems communicating and perceiving their emotions, in addition to the fact that these situations of isolation can be triggers for relapses in the disorder [13]. Moreover, in some cases, this impoverished social functioning appears to be found before the onset of AN symptoms (Gillberg & Råstam, 1992). On the other hand, social skills training in early adolescence can be a protective factor in the development of risk behaviors related to AN [14].

These social influential factors of AN should also be considered throughout rehabilitation and therapy for the disorder. Among the types of therapy that treat these social symptoms is the *Cognitive Remediation and Emotion Skills Training* (CREST) program that focuses on improving the emotional processing of patients and results in the improvement of the social anhedonia and alexithymia found in AN [15]. Moreover, in the treatment of adolescents with AN, family therapy has been established as an important therapeutic strategy. Within family therapy, there are several approaches to the disorder and how the family interacts with it, such as structural family therapy, strategic family therapy, and family-based therapy (FBT), the latter being the most prominent at present [16]. In a study comparing the results of FBT with those of individual therapy, several improvements were observed with respect to the latter, such as lower weight loss at the beginning of treatment and faster weight gain over the months [17].

The impact of social stress on health is common to humans and other social mammals, where it has been observed that adverse social consequences cause pathologies with patterns parallel to those that occur in humans, sharing common mechanisms such as deregulation of the HPA axis or changes in the sympathetic nervous system. These similarities can be useful in the study of social determinants of health thanks to the integration of clinical studies with humans and experimental studies in the laboratory with animals [18].

As previously mentioned, the standard procedure of the ABA model requires isolation of rats. These are social animals that show a preference for social contact over other environmental enrichment factors [19] and even favor social interaction over competition in situations of food deprivation [20]. Moreover, exposing young rodents to different forms of enriched sensory stimulation (i.e., tactile stimulation through handling, the stimulation provided for the mother rat to the puppies, or environments including varied and rich sensory stimulation) has a crucial and long-lasting behavioral and neurobiological impact [21]. Taking this into account, isolation can be an important factor in the promotion of excessive activity. It has been found, for example, that this type of social condition in adolescent rats can cause lasting effects in social behavior and alterations in neuroplasticity and neurochemistry in the animals, such as reduced synaptic density in cortical areas and reduced myelination in the male prefrontal cortex [22]. Additionally, sex differences have been found in terms of the affectation of stress, with a higher incidence and propensity to develop pathologies such as anxiety and depression in female rats than in males [23]. Within the ABA model, early maternal separation results in a more rapid development of the phenomenon [24,25]. However, there are few studies that have analyzed the direct effect of social contact, except for Boakes and Dwyer's [26] Experiment 3. In that study, two groups of rats differentiated by housing in groups of four subjects in their home cages or the isolation of the animals in individual cages were used, and an abbreviated ABA procedure was carried out in which access to the activity wheel was allowed only during the 2 h prior to exposure to food. They found that group-housed rats were less vulnerable to the development of ABA, losing less weight during the procedure than isolated rats. However, the study was carried out only with male rats (see also [27], for comparable results under a different non-ABA procedure), and the remarkable sex differences found in the development of ABA in terms of a greater amount of running in females [28] warrants the comparison of the effects of socialization between rats of both sexes.

The abbreviated ABA procedure, where rats have limited access to both food and the activity wheel, is sufficient for the development of the phenomenon, as has been observed in previous studies. For example, in Dwyer and Boakes [29] access to the activity wheel during the 4 h prior to the intake period was sufficient for the development of ABA, and in Boakes et al. (1997) ABA was observed only with 2 h of FAA. These modifications with respect to the standard procedure have the advantage of the possibility of establishing differential socialization conditions during the time that the rats remain in their home cages, so that the effects on FAA (Boakes et al., 1997) and PPA (Wu et al., 2014) can be studied, in addition to characterizing possible sex differences.

Altogether, our objective in this study was to determine the influence of social conditions (social isolation vs. social enrichment housing the animals in groups) on the development of ABA (food ingestion, weight loss, and hyperactivity) in young male and female rats and establish the differences with the traditional control group without access to the possibility of running. Finally, the present study also analyzed the influence of social enrichment on weight regain after removal of the procedure, with a less restricted food intake period and preventing access to the activity wheel.

Given that rats are social animals for which isolation can have adverse effects on their development [22], it could be expected that social enrichment would be a protective condition against the development of ABA, preventing or attenuating its emergence. Regarding the differential influence of sex on the phenomenon, it is possible that social condition has a greater impact in weight loss and the level of activity on female rats resulting in a higher resistance to ABA, given the higher incidence of pathologies related to a social stress factor among them [23]. Finally, regarding the recovery of weight after withdrawal of the animals from the procedure, an effect would be expected in the same direction as that found in the development of ABA, predicting that social enrichment would facilitate weight gain.

2. Method

2.1. Subjects

Eighty Wistar Han rats, 40 males and 40 females, purchased from Charles River Laboratories (Lyon, France) were used. At arrival, animals were between 4 and 5 weeks old. They spent 1 week of quarantine and 4 days of acclimatization in the room where the experiment was carried out before its beginning. On the second day of acclimatization, the rats were randomly assigned into groups based on the factors of access to the wheel and social status ($n = 10$ in each group), on a first round 4 groups of males and on a second round 4 groups of females (more details of the

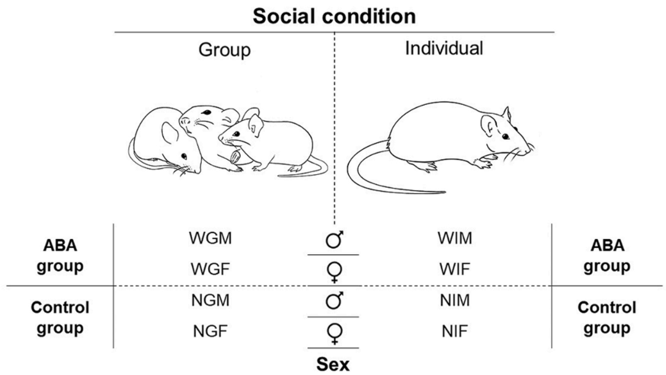


Fig. 1. Conditions of the 8 groups in relation to the 3 independent variables manipulated in the experiment.
Note. ABA: Activity-based anorexia; WGM: Wheel Group Male; WIM: Wheel Individual Male; NGM: No-Wheel Group Male; NIM: No-Wheel Individual Male; WGF: Wheel Group Female; WIF: Wheel Individual Female; NGF: No-Wheel Group Female; NIF: No-Wheel Individual Female.

groups assortment in the Procedure). An attempt was made to keep the rats assigned to the condition of social enrichment in groups of 5 subjects, in accordance to how they were sent by the provider, while the rats in social isolation were individualized. First, the animals were randomly assigned half to the social condition and the other half to the isolated one, and then, again randomly, half of each to the activity condition and the other half to the sedentary one. Fig. 1 shows the details of the conditions of each group. The initial weight of the male rats was between 128 and 190 g ($M = 155.78$, $SD = 13.26$), and the initial weight of the female rats was between 113 and 158 g ($M = 131.\ 29$, $SD = 9.30$). To ensure equality of the groups of males and females with respect to weight at the beginning of the experiment, linear mixed-effects models (LMMs) were built. The LMMs built to analyze the initial weight did not show any significance when the fixed effect of Group was considered regarding the groups of male rats ($\chi^2_{(3)} = 2.23, p = .52$), nor in the groups of female rats ($\chi^2_{(3)} = 3.09, p = .38$). On the other hand, differences were found between males and females in their initial weight ($t = 8.62$, $p = .001$), with males weighing 20.88 ± 2.42 g more than females in a model where initial weight was a function of Sex [Weight ~ Sex + (1 | Subject)] ($\chi^2_{(1)} = 53.53, p = .001$), as can be expected because of sex dimorphism in rats regarding body weight.

The testing room had a mean temperature of 21 ± 2 ºC, a 60% relative humidity, and a light-dark cycle of 12 h (lights on from 8:00 a. m. to 8:00 p.m.). The experiment was carried out during the light period. The animals were always handled by the base of the tail and only for the time necessary to move them from one box to another or to the weighting scale, avoiding stress. Furthermore, a longer handling was refrained from, because of its protective effect on weight loss [30]. All rats, regardless of the experimental condition, had free access to water throughout the procedure. 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).

2.2. Apparatus

The animals in the social isolation condition were housed in individual home cages of transparent Plexiglas (19 × 24.5 × 40 cm^3) and the animals in the social enrichment condition were housed in larger home cages of the same material (19 × 35 × 55 cm^3).

During the two periods of activity (FAA and PPA), the rats assigned to the ABA groups were housed in transparent plexiglass cages with a rectangular base prism (19 × 20.5 × 32.5 cm^3). These cages were equipped with an activity wheel of 34 cm in diameter and 9 cm thick, in addition to a 10 cm diameter entrance hole to the wheel with a manually sliding metal door that prevented or gave access to the wheel. The brakes of the activity wheel were controlled by a MED-PC program for Windows installed in a computer located in the same testing room, as well as the measurement of the number of wheel turns in 1-minute intervals.

During the feeding periods, the ABA groups ate in the cages equipped with a wheel (but without access to it), while the control groups in the social enrichment condition (NG: No-Wheel Group) were transferred to individual cages (18 × 32.5 × 20.5 cm^3) and the control groups in the isolation condition (NI: No-Wheel Individual) ate in their home cages. All cages had a metal grid with a hole 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.

To measure body weight and the amount of food ingested daily by the animals, a precision scale (Precisa 1000C-3000C) was used.

2.3. Procedure

The experiment was divided into two rounds, applying the procedure first to the 40 male rats and then to the 40 female rats. Both rounds finished at Day 16 (cf. [4]). The division into rounds was due to the limitation in the number of activity wheels, having 10 wheels in total. This fact also affected the establishment of two turns of access to the wheel within each round.

The experimental procedure began in Session 0, when the food was withdrawn from all the animals. The order of access to the activity wheel was counterbalanced in the groups assigned to the ABA condition to avoid any effect related to the time of day, dividing the procedure into two turns. As can be seen in Fig. 2, half of the rats underwent the procedure in the first turn (9:00 to 14:00) and the other half in the second turn (14:00 to 19:00).

Each experimental session consisted of three parts. In the first part, the animals belonging to the activity groups (WI – Wheel Individual - and WG – Wheel Group -) were individually placed in the cages equipped with an activity wheel. The activity period lasted 2 h, releasing the brakes at the beginning and activating them at the end. In the second

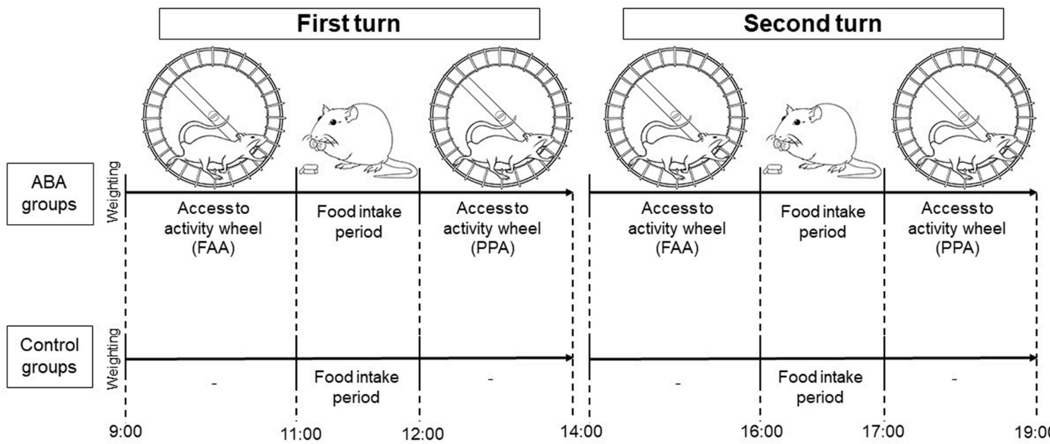


Fig. 2. Timeline of the procedure followed during the experiment.
Note. ABA: activity-based anorexia; FAA: food anticipatory activity; PPA: postprandial activity.

part, the feeding period lasted 1 h during which the subjects of the WI and WG groups ate individually in the wheel cages, while the animals in the NI groups ate in their home cages and those in the NG groups were transferred to individual cages to be able to measure the food intake of each subject. Each animal was given an approximate amount of food of 25 g, which was controlled individually in jars for each subject. At the end of the feeding period, the remaining food was collected for later measurement and the animals of the NG groups were returned to their home cages. In the third part, the second period of activity began for the WI and WG groups with a duration of 2 h. At the end of the experimental session, the rats in these groups were returned to their corresponding home cages.

The weight of each animal was recorded daily before the beginning of experimental sessions, at 8:30 a.m., starting from the three days prior to Session 0 until the end of the procedure. The weight recorded in Session 0 was used as baseline for the weight changes of the animals throughout the procedure and as a criterion for recovery after withdrawal. The weight of the food was recorded before and after the feeding period to calculate the intake of each animal. In addition, the number of wheel turns was recorded for the corresponding groups (WG and WI).

A criterion to withdraw an animal from the procedure was established if its weight was equal to or less than 75% of its initial weight in Session 0 for one day. The body weight and food intake of the withdrawn rats were recorded until they reached or exceeded their starting weight in Session 0 to observe the recovery process. Survival to the procedure was achieved when weight stability was obtained during four consecutive days (weight equal to, or greater than, the weight four days before) (as in [29]).

The recovery phase began on the day of withdrawal. The animals, whichever group they belonged to, did not have access to the activity wheel and the ingestion period was extended to 6 h, 3 h during the first turn of the procedure and 3 h in the second turn. All the withdrawn animals ate individually to enable the measurement of food intake. The rats from the WG group were placed in individual cages during the two ingestion periods and were returned to their home cages at the end. The first period of ingestion took place between 11:00 a.m. and 2:00 p.m. and the second period between 4:00 p.m. and 7:00 p.m.

Wheel turns were counted every minute in two periods of 120 min, each corresponding to the FAA and PPA periods respectively.

2.4. Data analyses

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. Due to the different number of sessions analyzed for the animals caused by removal from the procedure when they reached the withdrawal criterion, linear mixed-effects modeling (LMM) was used for the analysis of the data corresponding to body weight loss, food intake and, regarding to the activity developed, FAA, PPA and total running. LMMs offer a more flexible approach than the typical ANOVA, handling missing data and unbalanced designs, as each measure represents one of many within a subject, so the removal of an observation has a much smaller impact in the mixed model than in an ANOVA [31]. Data analyses were carried out comparing the conditions manipulated in this experiment, Socialization (social/isolated) and Running Wheel (yes/no). In the analysis of activity, the effect of Group (socialized ABA/isolated ABA) was considered. Comparison of sex was only added in the analysis of the percentage of body weight, due to the use of a relative measure of change in body weight in respect to the initial weight. A likelihood-ratio test was used in each analysis to compare the models that best fitted the data with reduced models that lacked the particular effect tested (null model). Every LMM was done using the packages lme4 [32] and lmertest [33] on R (RCore Team, 2012; RStudio Team, 2020). In the survival analysis, the Kaplan-Meier method was applied, using the logarithmic rank test (Mantel-Cox), comparing the eight groups with each other.

3. Results

First of all, to rule out a possible effect of the time of day in the measures taken in the procedure, analyses of the change in body weight, food intake and total activity from all rats were done with LMMs comparing the animals that underwent the procedure in the first turn (9:00 to 14:00) and in the second turn (14:00 to 19:00). In body weight, the best fitted model considered Time of day (first turn/second turn) as a fixed effect ($\chi^2_{(1)} = 916.60$, $p = .001$) and Subjects and Days as random effects. Differences between the two turns were not significant ($t = -1.57$, $p = .12$). Regarding food intake, a likelihood-ratio test showed that [Intake ~ Time of day + (1 | Subject) + (1 | Day)] was the best

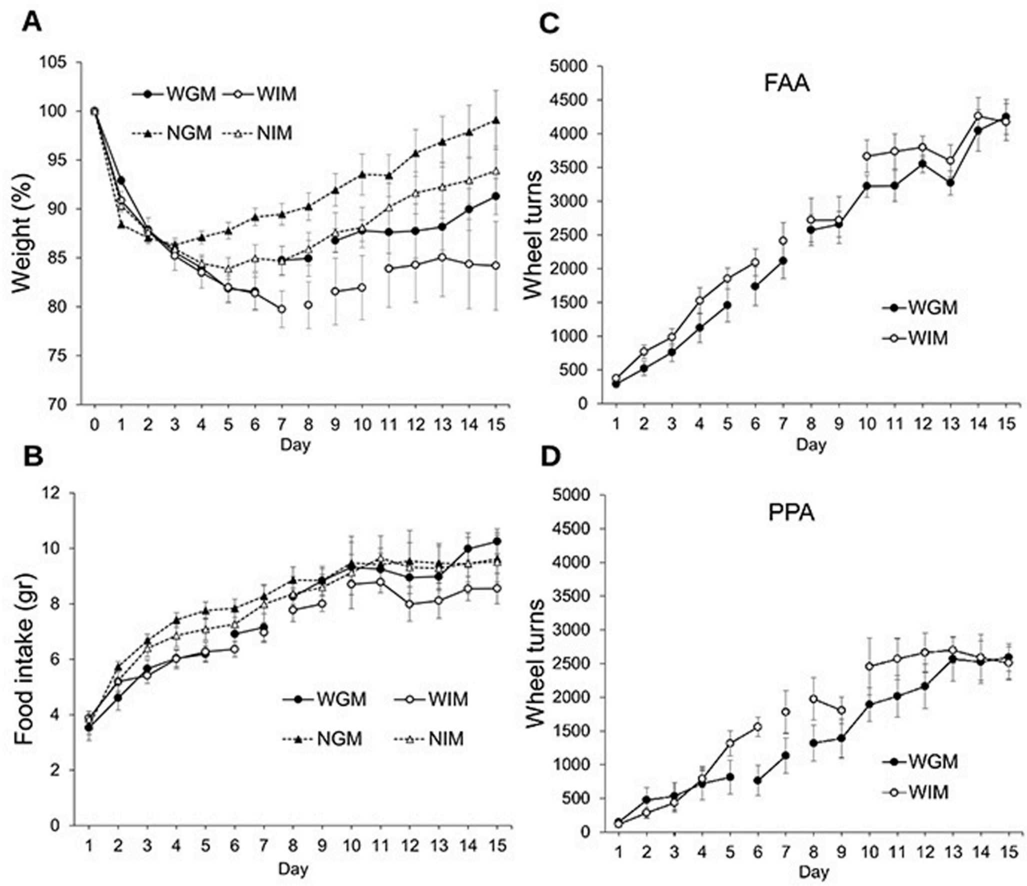


Fig. 3. Measures of percentage of weight, intake, anticipatory activity (FAA) and postprandial activity (PPA) of the 4 groups of male rats.

Note. The mean (± SEM) of the 4 groups of male rats is shown: WGM (Wheel Group Males), represented by a solid circle; WIM (Wheel Individual Males), represented by an empty circle; NGM (Non-Wheel Group Males), represented by a solid triangle; and NIM (Non-Wheel Individual Males), represented by an empty triangle. Discontinuation of a line indicates when a withdrawal from the procedure occurred as noted in the table attached to this figure (number next to the day of withdrawal denotes the number of subjects withdrawn that day). Four measurements were collected. **(A)** Mean (± SEM) of the percentage of weight with respect to Day 0 of the WGM and NGM groups up to the end of the procedure (Day 15). **(B)** Mean (± SEM) of the intake measured in grams. **(C)** Mean (± SEM) of the number of turns on the activity wheel during the 2 h period of FAA (food anticipatory activity) for the WGM and WIM groups before their intake period. **(D)** Mean (± SEM) of the number of turns during the 2-h PPA (postprandial activity) period for the WGM and WIM groups after the intake period.

Group	Withdrawal Days
WGM	Day 5(2) and 7(1)
WIM	Day 6(2), 7(2) and 9(1)

model to fit the data ($\chi^2_{(1)} = 1347.66$, $p = .001$), although there were no differences in food intake between the first and second turn ($t = -0.17$, $p = .87$). In the ABA groups, total activity was a function of the Time of day ($\chi^2_{(1)} = 671.24$, $p = .001$) [Total activity ~ Time of day + (1 | Subject) + (1 | Day)]. There was not a significant difference between the animals in the first and second turn ($t = -0.21$, $p = .83$).

3.1. Main comparisons in male rats

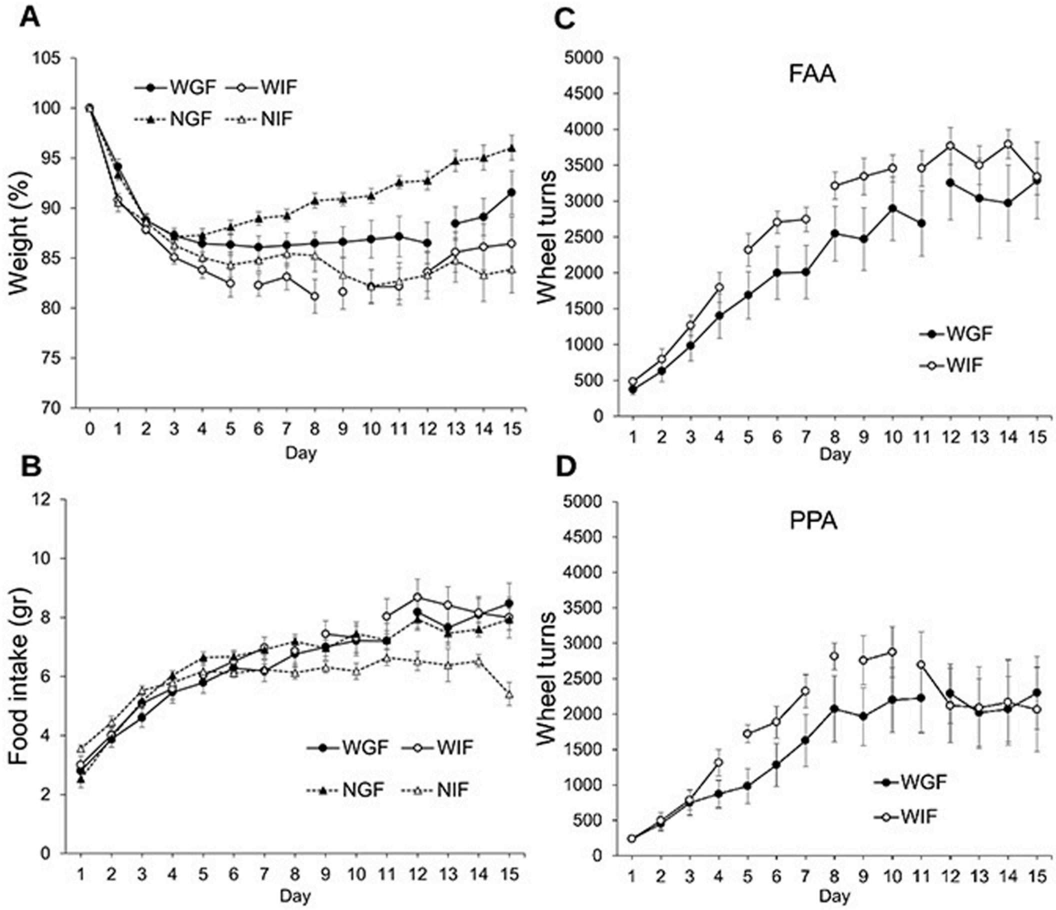
All LMMs were analyzed within the 15 days of the procedure, including the rats withdrawn before the end of the experiment that reached the removal criterion (less than 75% of its initial weight in Day 0 for one day). Days of withdrawal and number of male rats withdrawn from the procedure are reported in Fig. 3.

3.1.1. Body weight in male rats

LMMs were conducted with the conditions Running wheel (yes/no) and Socialization (social/isolated) as fixed effects when modeling the change in percentage of body weight in the groups of male rats. Subjects and Days were considered as random effects. Regarding the Running wheel condition, a likelihood-ratio test was done comparing the constructed models, where the best fitted model was the one including the Running wheel as fixed effect and Subjects and Days as random effects [Weight ~ Wheel + (1 | Subject) + (1 | Day)]. Body weight was a function of the Running wheel condition ($\chi^2_{(1)} = 394.33$, $p = .001$). Loss in weight was 3.85 ± 1.38 greater in the male ABA groups than in the control groups ($t = -2.78$, $p = .009$). In the Socialization condition, the best fitted model considered Socialization as a fixed effect, and Subjects and Days as random effects [Weight ~ Social + (1 | Subject) + (1 | Day)] ($\chi^2_{(1)} = 394.36$, $p = .001$) when compared to the null model. Rats in the socialized groups had 3.5 ± 1.4 more body weight than the isolated ones ($t = 2.49$, $p = .017$). The progression of weight for all groups throughout the procedure can be observed in Fig. 3A. There was no interaction between the conditions Running wheel x Socialization ($t = -1.06$, $p = .29$).

3.1.2. Food intake in male rats

Data from food intake in male rats during the procedure were modeled using LMMs with the same two conditions as before: Running wheel and Socialization. The progression of food intake throughout the procedure can be seen in Fig. 3B. The model that provided a better fit for the data in the Running wheel condition was the one considering access



Group	Withdrawal Days
WGF	Day 12(1)
WIF	Day 5(1), 8(1), 9(1) and 11(1)

Fig. 4. Percentage of weight, intake, anticipatory activity (FAA) and postprandial activity (PPA) measurements for the 4 groups of female rats.
Note. The mean (± SEM) of the 4 groups of female rats is shown: WGF (Wheel Group Female), represented by a solid circle; WIF (Wheel Individual Female), represented by an empty circle; NGF (Non-Wheel Group Female), represented by a solid triangle; and NIF (Non-Wheel Individual Female), represented by an empty triangle. Discontinuation of a line indicates when a withdrawal from the procedure occurred as noted in the table attached to this figure (number next to the day of withdrawal denotes the number of subjects withdrawn that day). Four measurements were collected. **(A)** Mean (± SEM) of the percentage of weight with respect to Day 0 for WGF and NGF up to the end of the procedure (Day 15). **(B)** Mean (± SEM) of the intake measured in grams. **(C)** Mean (± SEM) of the number of turns on the activity wheel during the 2-hour FAA (food anticipatory activity) period of WGF and WIF. **(D)** Mean (± SEM) of the number of turns during the 2-hour period of PPA (postprandial activity) of WGF and WIF after the intake period.

to a Running wheel as a fixed effect, and Subjects and Days as random effects [Intake ∼ Wheel + (1 | Subject) + (1 | Day)] ($\chi^2_{(1)} = 616.08$, $p = .001$). Differences between the ABA and control groups were not significant ($t = -1.79$, $p = .08$). As in the first condition, the best fitted model to account for the data in the Socialization condition was [Intake ∼ Social + (1 | Subject) + (1 | Day)]. Food intake was a function of Socialization ($\chi^2_{(1)} = 619.52$, $p = .001$). There was not a significant difference in food intake between the socialized groups and the isolated ones ($t = 1.44$, $p = .16$).

3.1.3. *Development and distribution of activity in male rats*

LMMs were performed regarding FAA, PPA, and total activity between the ABA groups with wheel access. Figs. 1C and 1D show the development of FAA and PPA respectively. Regarding the FAA analysis, a likelihood-ratio test was done comparing the constructed models. In the best fitted model, FAA was a function of Group, and Subjects and Days were accounted as random effects [FAA ∼ Group + (1 | Subject) + (1 | Day)] ($\chi^2_{(1)} = 461.64$, $p = .001$). There was not a significant difference between WGM and WIM ($t = 0.78$, $p = .44$). In the PPA modeling, the model that better fitted the obtained data account Group as a fixed effect, and the random effects of Subjects and Days. A significant difference in PPA between the ABA groups was not found ($t = 0.63$, $p = .54$). Lastly, the best fitted model to account for the result in total activity in the male ABA groups was [Total activity ∼ Group + (1 | Subject) + (1 | Day)] ($\chi^2_{(1)} = 415.83$, $p = .001$). There were no significant differences between WGM and WIM in total activity ($t = 0.71$, $p = .48$).

Finally, the temporal distribution of wheel running in the ABA groups of male rats was analyzed (graphic representations are shown in

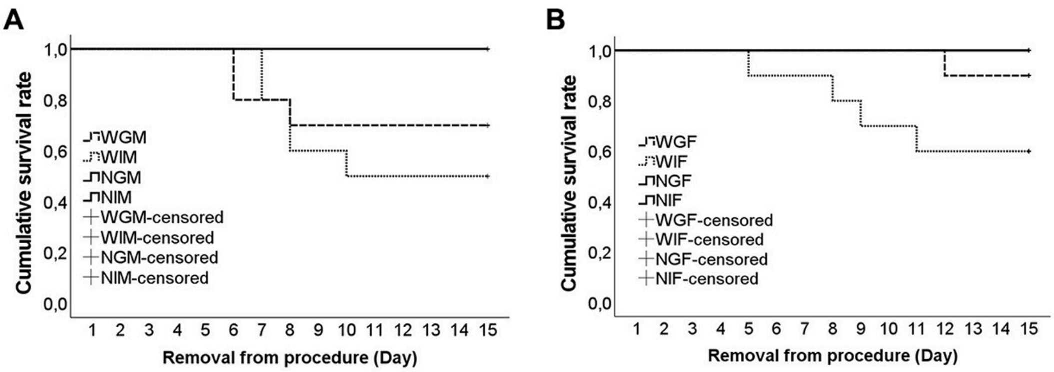


Fig. 5. Survival functions of the eight groups of rats.
Note. Survival functions with respect to the day on which the experimental subjects reached the withdrawal criterion. The censored cases indicated by a cross on Day 15 represent those rats that did not reach the event, that is, reaching the withdrawal criterion after losing 25% of their initial weight. **(A)** The survival rate of the four male groups is shown (WGM - Wheel Group Male; WIM - Wheel Individual Male; NGM - No-Wheel Group Male; NIM - No-Wheel Individual Male) throughout the procedure. WGM is indicated with a broken line and WIM with a dotted line. The cumulative survival of the control groups remains at their survival rate at a value of 1 throughout the procedure, as none of the rats in these groups fulfilled the withdrawal criterion, which is reflected in overlapped horizontal lines (a gray line for NIM and a black line for NGM) from Day 1 to Day 15. **(B)** The survival rate of the four female groups is displayed (WGF – Wheel Group Female; WIF – Wheel Individual Female; NGF - No-Wheel Group Female; NIF - No-Wheel Individual Female). WGF is indicated with a broken line and WIF with a dotted line. As in the case of the male groups, the cumulative survival of NGF and NIF remains at a value of 1, as none of the rats in these groups were removed from the procedure, which is reflected in overlapped horizontal lines (a gray line for NIF and a black line for NGF) from Day 1 to Day 15.

Appendices 1 and 2), comparing the data collected on the first two days and on the last two days of each animal, in order to observe how the activity progressed in relation to the meal period throughout the procedure. In the case of the WGM and WIM groups, a slight increase in activity was observed during the first two days of PPA. On the other hand, and on the last days, apart from the increase in activity with respect to the first days, an anticipation to the meal in the FAA could be observed with an increase in running during the last minutes. Although this anticipation was less in the case of WIM, in which the distribution of the activity seemed to be more stable, without seeing any increase throughout the interval. On the other hand, an acceleration of the PPA was observed, starting with low peaks of activity that progressively increased throughout this phase.

3.2. *Main comparisons in female rats*

All data from the 15 days of procedure were included in the built LMMs, as well as the rats removed before the end of the experiment that reached the criterion for withdrawal (less than 75% of its initial weight in Day 0 for one day). Days of withdrawal and number of female rats withdrawn from the procedure are reported in Fig. 4.

3.2.1. *Body weight in female rats*

For female rats, LMMs (Running wheel × Socialization) were performed in relation to the change in weight percentage during the procedure. The conditions Running wheel (yes/no) and Socialization (social/isolated) were considered as fixed effects when modeling body weight in the groups of female rats. Subjects and Days were considered as random effects. Data are depicted in Fig. 4A. In the Running wheel condition, a likelihood-ratio test showed [Weight ~ Wheel + (1 | Subject) + (1 | Day)] as the best fitted model for the data ($\chi^2_{(1)} = 516.05$, $p =$ .001). However, there were no significant differences in body weight between the ABA and control groups ($t = -1.85$, $p = .07$). Regarding the Socialization condition, in the best fitted model changes in body weight were a function of socialization. Subjects and Days were accounted as random effects [Weight ~ Social + (1 | Subject) + (1 | Day)] ($\chi^2_{(1)} =$ 516.27, $p = .001$). Body weight was 4.34 ± 1.06 greater in the socialized groups than in the isolated ones (t = 4.09, p = .001). There was no interaction between the conditions Running wheel x Social ($t = -0.92$, $p = .36$).

3.2.2. *Food intake in female rats*

Regarding the intake measure of the groups of female rats, models were built to analyze the data in the two conditions: Running wheel and Socialization. Progression of food intake throughout the procedure is represented in Fig. 4B. In the Running wheel condition, the best fitted model was [Intake ~ Wheel + (1 | Subject) + (1 | Day)]. Food intake was a function of the condition ($\chi^2_{(1)}$ = 709.52, p = .001). There were no significant differences between the ABA groups and control groups (t = 0.25, p = .8). Regarding the Socialization condition, the model that provided a better fit for the data in the performed likelihood-ratio test accounted food intake as a function of Socialization ($\chi^2_{(1)} = 708.88$, $p =$ .001), with the random effects of Subjects and Days [Intake ~ Social + (1 | Subject) + (1 | Day)]. No differences were found between the socialized groups and the isolated ones ($t = 0.78$, $p = .44$).

3.2.3. *Development and distribution of activity in female rats*

LMMs were built regarding FAA, PPA and total activity comparing the groups with access to wheel during the 15 days of the procedure. Figs. 2C and 2D show the progression of FAA and PPA, respectively. Regarding the FAA analysis, the best fitted model to account for the development of FAA was [FAA ~ Group + (1 | Subject) + (1 | Day)]. FAA was a function of the fixed effect Group ($\chi^2_{(1)} = 319.33$, $p = .001$). There were no significant differences between WGF and WIF ($t = 1.31$, $p = .20$). A likelihood-ratio test was done comparing the models built to analyze PPA. The model that provided a better fit for the data was [PPA ~ Group + (1 | Subject) + (1 | Day)] ($\chi^2_{(1)}$ = 184.38, p = .001). No differences were found in PPA between WGF and WIF ($t = 1.24$, $p = .23$).

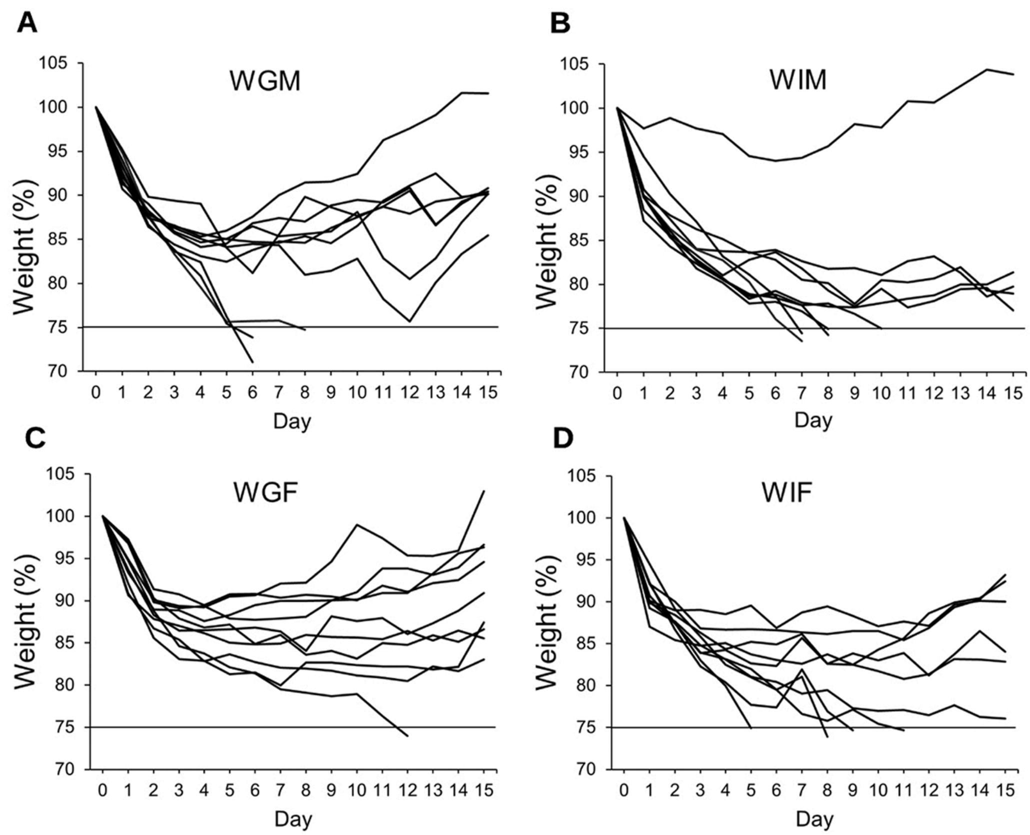


Fig. 6. Individual weight percentage measurements of the 4 ABA (activity-based anorexia) groups, males and females.
Note. The development of the weight of each animal is shown in percentage with respect to Day 0 in the 4-wheel groups (WGM - Wheel Group Male; WIM – Wheel Individual Male; WGF – Wheel Group Female; WIF - Wheel Individual Female) during the entire procedure, from Day 0 to Day 15, when the procedure ended. A horizontal line at the height of 75% reflects the withdrawal criterion. **(A)** In the WGM group, three animals reached the withdrawal criterion: two on Day 6 and one on Day 8. **(B)** In the WIM group, 5 animals reached the withdrawal criterion: two on Day 7, two on Day 8 and one on Day 10. **(C)** In the WGF group, only one animal reached the withdrawal criterion on Day 12. **(D)** In the WIF group, four animals reached the withdrawal criterion on Days 5, 8, 9 and 11. In Annexes 5, 6, 7 and 8, the data with identification of each individual rat are represented.

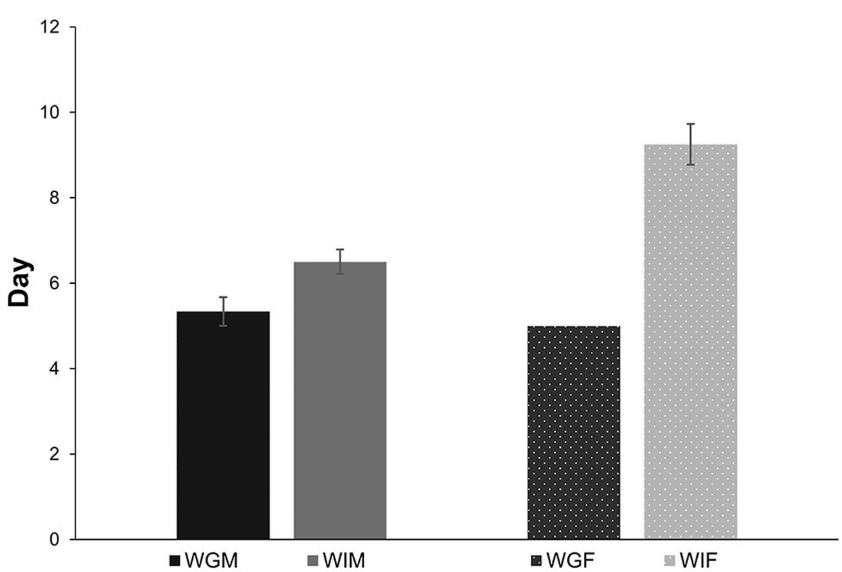


Fig. 7. Mean days of the recovery phase of the animals withdrawn from the 4 ABA (activity-based anorexia) groups.
Note. The mean (± SEM) of days of recovery after withdrawal from the ABA procedure in 3 experimental groups (WGM – Wheel Group Male; WIM - Wheel Individual Male; WIF - Wheel Individual Female) is shown. In WGF (Wheel Group Female), there was only one rat exposed to recovery, so only the value for this subject is shown. The bars for male rats are shown in solid colors, while the bars for female rats are shown with a dotted background.

Lastly, in the analysis of total activity, the best fitted model to account for the data considered the variable Group as a fixed effect, and Subjects and Days as random effects [Total activity ∼ Group + (1 | Subject) + (1 | Day) ($\chi^2_{(1)} = 276.93$, $p = .001$). There were no significant differences between the ABA groups ($t = 1.33$, $p = .20$).

Finally, as in the case of males, the temporal distribution of wheel running was analyzed across the ABA groups of female rats (Appendices 3 and 4). Similar to males, in the first days, an increase in activity was observed during the first moments after food intake, although in the case of females these activity peaks were more pronounced than in males. Furthermore, during the last days of the procedure, activity peaks were observed prior to the intake period in anticipation of food, but more marked than in the ABA groups of male rats. Moreover, the acceleration of the PPA was also observed, which increased exponentially throughout this period.

3.3. *Comparison by sex of body weight percentage*

Although there were differences in initial absolute body weight between male and female rats, the use of a relative measure (percentage of weight in relation to body weight measured in Day 0) allowed to build LMMs comparing the eight groups in this experiment regarding body weight in the three conditions manipulated: access to a running wheel, socialization, and sex. In the analysis of Sex, a likelihood-ratio test showed percentage of body weight as a function of Sex, with the random effects of Subjects and Days [Weight ~ Sex + (1 | Subject) + (1 | Day)] ($\chi^2_{(1)} = 916.76$, $p = .001$). However, there were no significant differences between male and female groups in the change of body weight during the procedure ($t = -0.02$, $p = .98$). Regarding the Running wheel condition, the model that better fitted the data of the eight groups included access to the running wheel as a fixed effect, and Subjects and Days as random effects [Weight ~ Wheel + (1 | Subject) + (1 | Day)] ($\chi^2_{(1)} = 917.07$, $p = .001$). There were significant differences between the ABA groups and the control groups, regardless of sex, where body weight was 3.1 ± 0.91 less in the groups with access to the running wheel than in the groups that did not have it ($t = -3.39$, $p = .001$). In the Socialization condition, the best fitted model was [Weight ~ Social + (1 | Subject) + (1 | Day)]. Body weight was a function of socialization ($\chi^2_{(1)} = 916.43$, $p = .001$). Weight in the socialized groups was 3.9 ± 0.87 greater than in the isolated ones ($t = 4.46$, $p = .001$), regardless of sex. There was no statistically significant interaction found between the three conditions (Running wheel × Socialization × Sex) ($t = -0.19$, $p = .85$).

3.4. Survival rates

Considering that the early withdrawal of animals hindered a more complete statistical analysis of the development of ABA throughout the different days that the procedure lasted for each subject in each group, a survival analysis was performed with the Kaplan-Meier method to compare the survival rate in terms of the experimental condition and the sex of the subjects. The result can be observed in Fig. 5. In the pairwise comparison, significant differences were found between WIM and NGM [χ^2 (7,80) = 6.38, $p = .012$] and WIM and NIM [χ^2 (7,80) = 6.38, $p = .012$], and, on the other hand, between WIF and NGF [χ^2 (7,80) = 4.76, $p = .029$] and WIF and NIF [χ^2 (7,80) = 4.76, $p = .029$]. Regarding the variable sex, survival differences were only found between WGF and WIM [χ^2 (7,80) = 4.14, $p = .042$]. In general, it can be observed in Fig. 5 that the group-housed female rats (WGF) were more resistant than the rest of the groups in developing ABA, as not many differences between the other groups were found.

3.5. Observation of individual weight evolution

Fig. 6 shows the development of the percentage change in weight of each individual rat subjected to the ABA procedure throughout the days, representing a group of animals in each panel. In general, in the 4 groups a stabilization of weight can be seen from Day 3–4, except for the vulnerable rats, which continued to lose weight until reaching the criterion and being then withdrawn from the procedure. In the experimental animals of the social enrichment condition, stabilization can be seen at higher ranges of weight than those of the animals in the isolation condition. These differences seem to be less in male rats. The difference in the withdrawal day in terms of sex and social status is noteworthy, with only one rat in the WGF group having reached the criterion towards the end of the procedure, in contrast to the 4 rats that had to be withdrawn in the WIF group from Day 5. This difference is hardly visible in male rats, in which 3 rats reached the criterion in the WGM group, 2 of them on Day 6, being the first to be withdrawn from the procedure, followed the next day by 2 animals from the WIM group.

3.6. Recovery from the procedure

Regarding the recovery phase, Fig. 7 shows the mean number of days it took for the rats withdrawn from the procedure in each group to regain 100% of their weight on Day 0. The comparison is limited by the different number of subjects who reached the withdrawal criterion in each group, especially in the case of WGF, in which only one animal reached the criterion.

In the ABA groups of male rats, there were 3 animals in the WGM group that underwent the recovery phase ($M = 5.33$, $SD = 0.58$) and 5 animals in the WIM group ($M = 6.2$, $SD = 0.83$). Looking at Fig. 7, it can be seen that there is a tendency to a slightly faster recovery in the socialized groups in comparison to the isolated ones. This effect seems to be greater in the female groups, where the one rat in the WGF group regained 100% of its weight on Day 0 in almost half the time that the 4 rats in the WIF group ($M = 9.25$, $SD = 0.96$) needed.

4. Discussion

Social stress is a critical factor in the deterioration of health and in a high mortality in humans, and is related to the appearance of various pathologies, such as heart diseases, diabetes, and respiratory infections [18]. Specifically, in AN, an impoverished social functioning has been observed prior to the onset of the disorder (Gillberg & Råstam, 1992) and as a triggering factor in relapses [13]. The incidence of social stress on health is also found in rats, where adverse social experiences in adolescence lead to behaviors related to anxiety, depression, and substance abuse [23]. Therefore, the present study sought to analyze the effect of socialization on the development of ABA using an abbreviated version of the model, with access to a running wheel during the 2 h before and the 2 h after food intake, which was restricted to 1 hour per day. The experiment included control groups with the same time of access to food, but without the possibility of exercising on the running wheel, common to the standard ABA model [4], and young male and female rats in order to observe the possible differences according to sex. Sexual dimorphism has been found in previous studies related to susceptibility to social stress and the development of pathologies, more prominently in females [23], and a greater amount of activity and suppression of intake in adolescent females than in males of the same age in an ABA model manipulating social stress due to maternal separation [24].

Regarding the groups of male rats, the data show that access to a running wheel and social condition affect the change in percentage of body weight, with a greater weight loss in the ABA groups and in the isolated ones. Although isolated rats were, in general, more prone to weight loss, there was no difference in weight between the socialized and isolated ABA groups, which does not seem to be in line with the data in Experiment 3 of Boakes and Dwyer [26] (see also [27]). Furthermore, the random effect of Days had more influence in food intake than the manipulated conditions, as it increased equally during the days on the procedure for all the male groups regardless of access to a running wheel or socialization. These results point to adaptation to food delivery in the diurnal cycle, and to a lack of relation between the effect of socialization in ABA development and a greater food intake. This lines up with data in past experiments where food consumption was not the key aspect to weight loss [34,35]. Nor was an influence of social condition on the amount of activity developed in the ABA groups found. In both ABA groups, total wheel running increased progressively throughout the procedure.

Regarding the groups of female rats, access to the running wheel did not affect the change in body weight during the procedure, but socialized rats were less vulnerable to weight loss than the isolated ones. This vulnerability to weight loss is also supported by the difference in the days of the first withdrawal between the WIF4 rat (Day 5) and the WGF3 rat (Day 12), being in the latter case the only rat in the female social group that was withdrawn during the entire procedure. No differences were observed in food intake, which points out to the same case as in the male groups, where consumption of food had no role in ABA development, rather than excess of exercise. Regarding the total activity developed in the ABA groups, no influence of social condition was found. Activity progressively increased over the days, FAA increasing more rapidly than PPA.

In relation to sex differences regarding the influence of social condition on vulnerability to the development of ABA, there was no difference in the change of percentage of body weight between the male and female groups in this experiment. However, access to a running wheel seemed to be more determinant in weight loss in the male rats than in the female ones. This greater vulnerability seen in male rats to an abbreviated ABA procedure is consistent with the data in Hancock and Grant [36], where male rats lost significantly more body weight and reached less the adaptation criterion to the procedure compared to the female ones. These results were latter replicated by Farinetti et al. [37].

Regarding the recovery of weight after withdrawal from the procedure in the ABA groups, data could not be statistically analyzed properly due to the small number of subjects in this phase, although a qualitative overview of the results seems to show that socialization versus individualization facilitated weight gain. This is coherent with other studies on the benefits of socialization after a period of stress, such as Biggio et al. [38], who found that male rats housed in groups for 4 weeks recovered from structural and molecular damage in the hippocampus after a situation of social isolation. Furthermore, in humans, benefits have been found in group therapy with respect to the treatment of AN, for example, in terms of cognitive remediation therapy (CRT), where the group format was advantageous in improving cognitive flexibility and self-esteem of the patients, while social communication was reinforced [39]. In addition, in adolescence, the importance of family therapies focused on the social support of the patient stands out, in which weight gain and an improvement in mood are observed [40], as well as less internalization and loss of weight at the beginning of therapy [17]. On the other hand, the data found in the present study point towards a sex difference in recovery, with the facilitating effect of socialization being greater in females than in males, which will correspond with its greater impact in the previous development of ABA. All in all, it is hoped that a larger sample of subjects in future experiments would help to verify the tendency to an easier recovery due to socialization that was shown here.

In general, the present study shows that social enrichment attenuate weight loss in a food restriction procedure, although it does not prevent it, and it seems to have a facilitating effect in the recovery of ABA. It is possible that the effect would have been greater if a standard ABA procedure had been used, but this standard procedure makes it difficult to individually measure activity of each animal if they had remained in a group for the 22–23 h that they should have had access to the activity wheel. Additionally, group housing may need to be established for a longer period of time for the benefits of social enrichment to have a significant effect on the development of ABA. Nevertheless, in the present study we observed, in a non-systematic way, the presence of play and affiliative relationships between group-housed animals compared to the isolation of individualized rats, confirming the existence of social ties that enrich the environment in which animals live and develop. Furthermore, social contact might have an impact on the elevation of body temperature, which it is known to attenuate the deleterious effect of activity anorexia [41,42].

The results of the present study suggest the need to further in the analysis of the role of socialization in the development of ABA. Future research is needed regarding positive or negative ways of socialization, in peer groups created from birth, and the effect of the reversion of social conditions at different stages of development, among others, to deepen our knowledge of this phenomenon in all its different variables and how it affects the development and recovery of AN in hopes that better prevention and therapy is given to the patients that need it.

Author's contribution

AMH, ADP and RP reviewed the literature and contributed to defining the theoretical frame and the design of the experiment. AMH and ADP carried out the experiment in the animal laboratory facilities. AMH and ADP conducted statistical analyses of the data. AMH, ADP and RP discussed the interpretation of the results obtained. AMH prepared a first draft of the manuscript, and tables and figures. RP and ADP revised and amended all the material. All authors have read and approved the final manuscript.

Ethical statement

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).

Data availability

Data will be made available on request.

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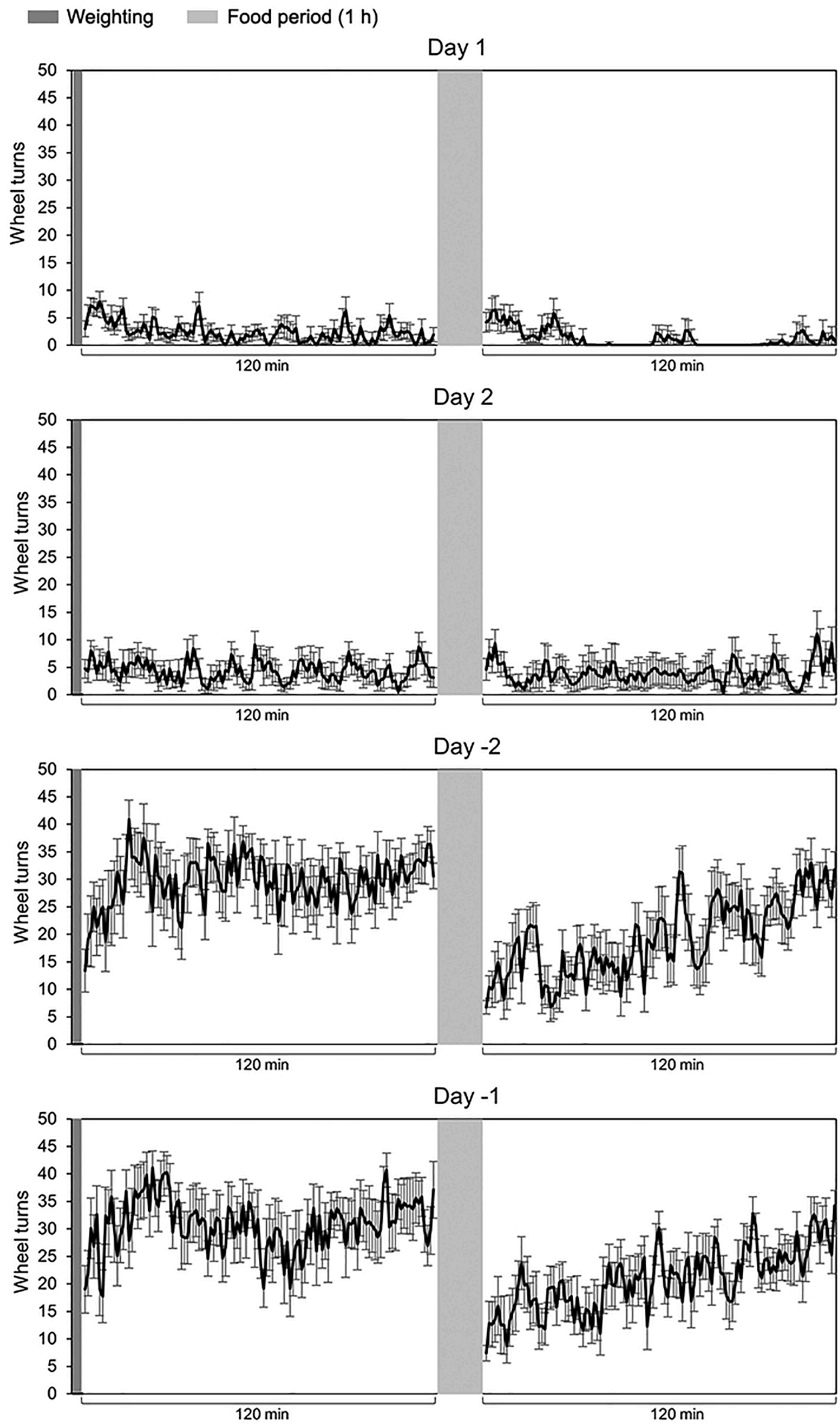
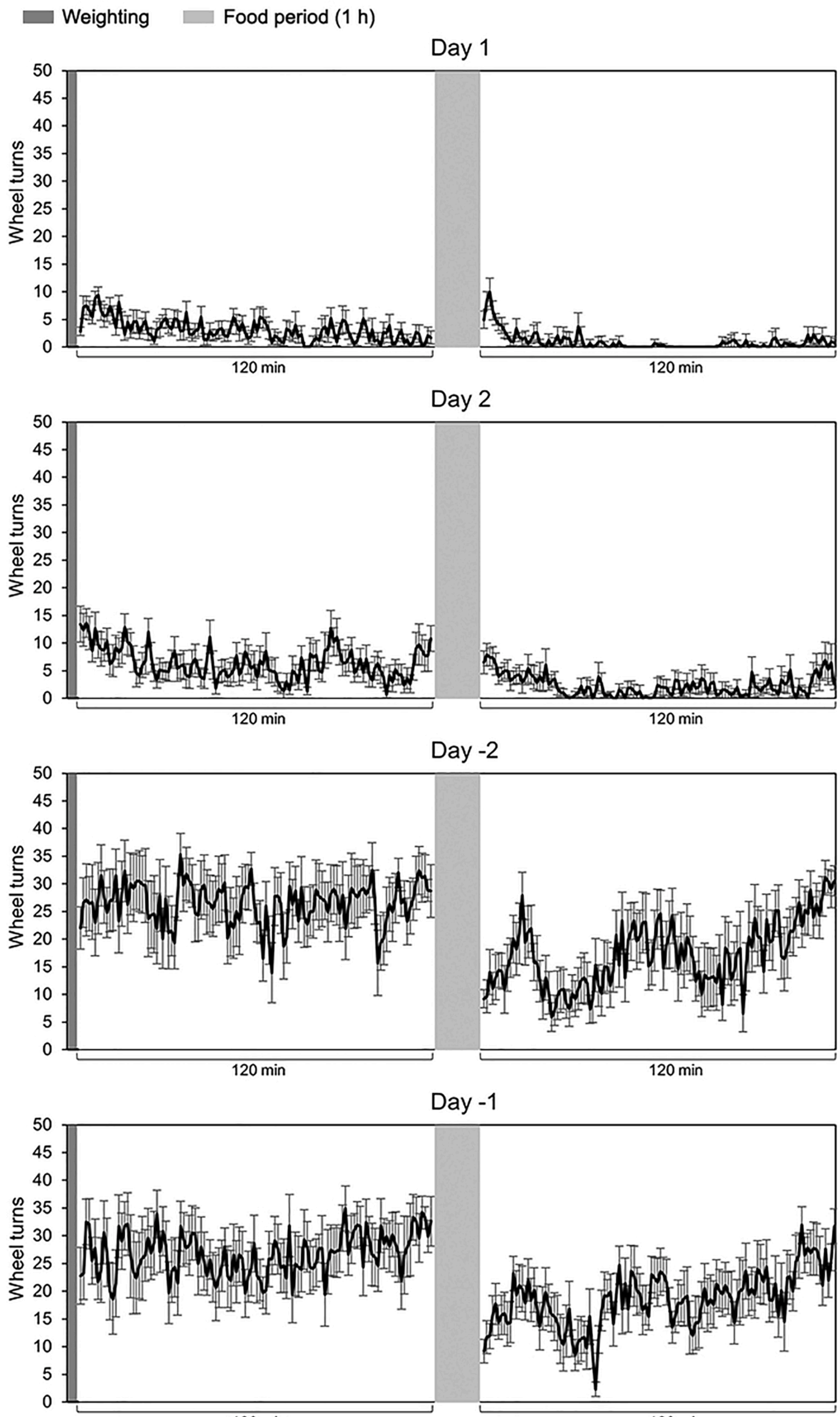
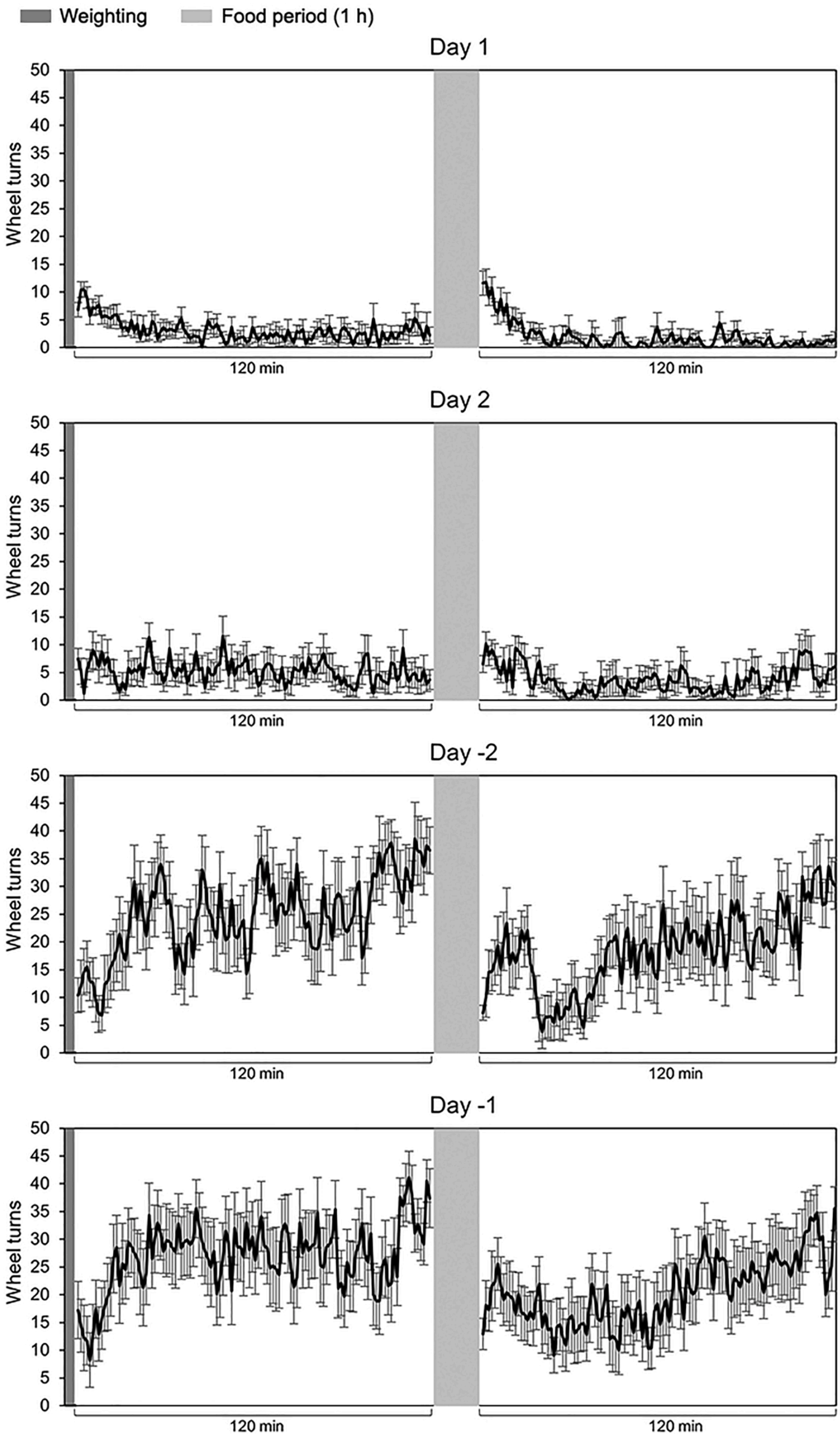
Appendix 1. Running distribution within days of the WGM group (Wheel Group Male)



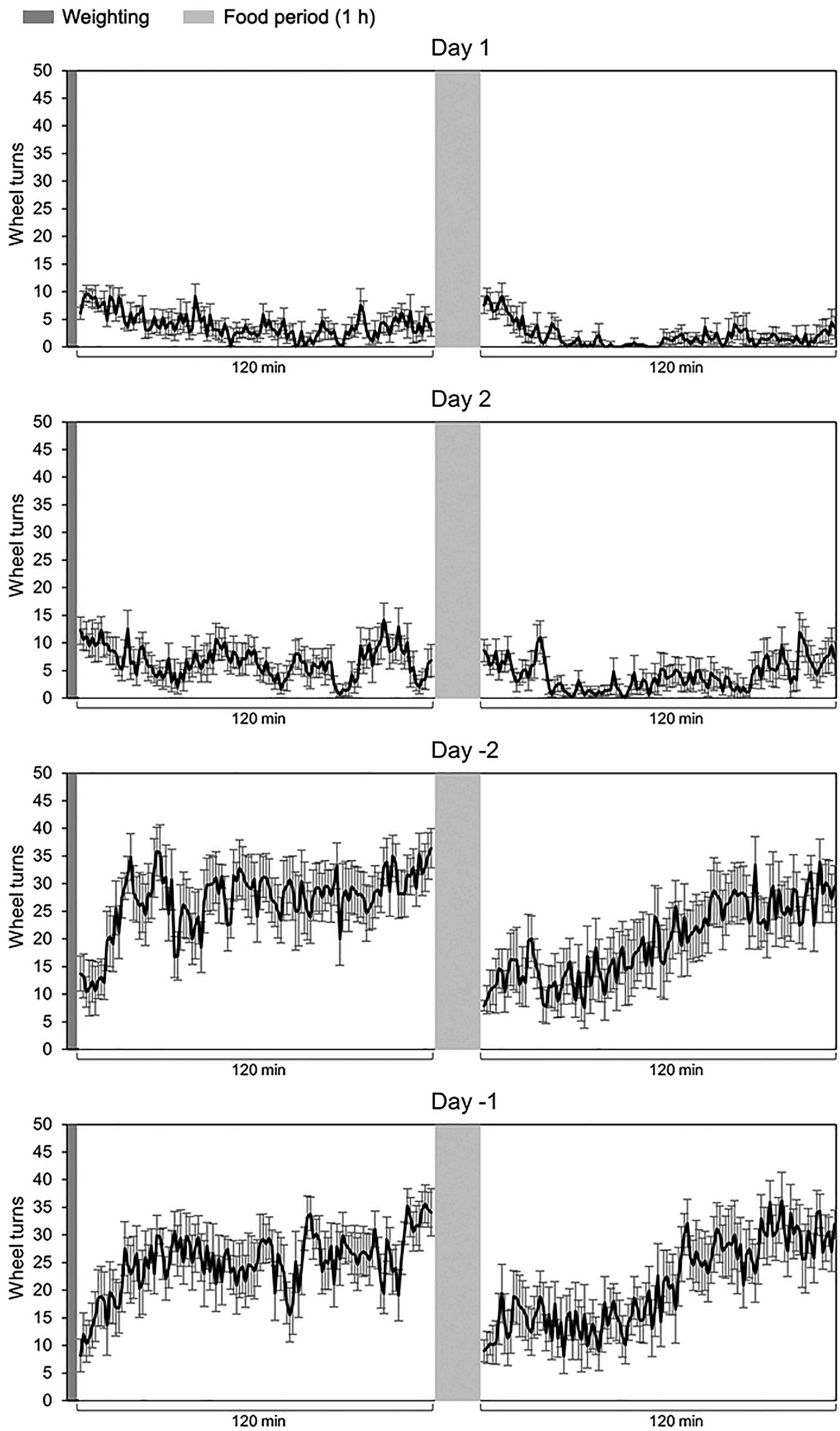
Appendix 2. Running distribution within days of the WIM group (Wheel Individual Male)



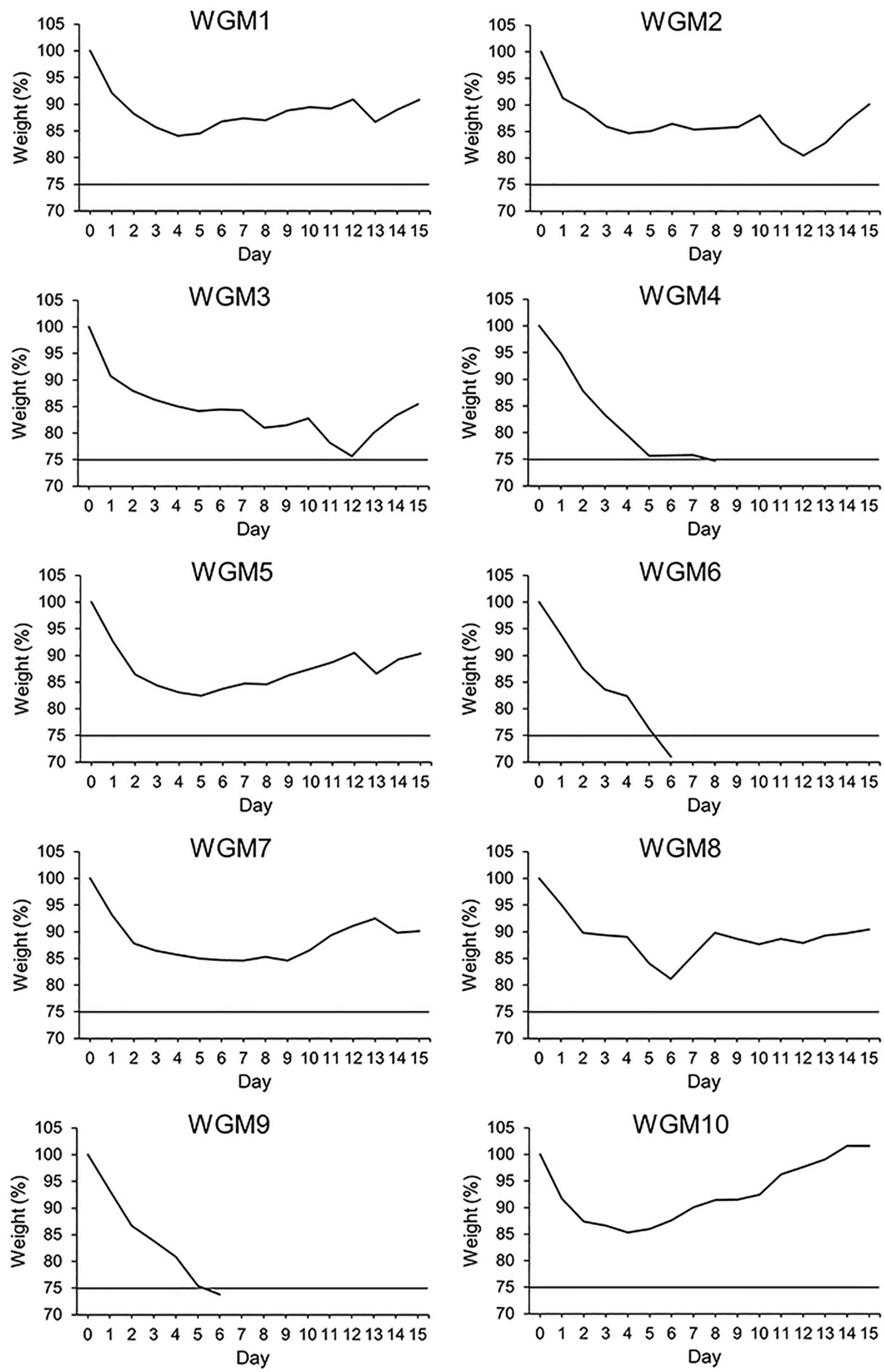
Appendix 3. Running distribution within days of the WGF group (Wheel Group Female)



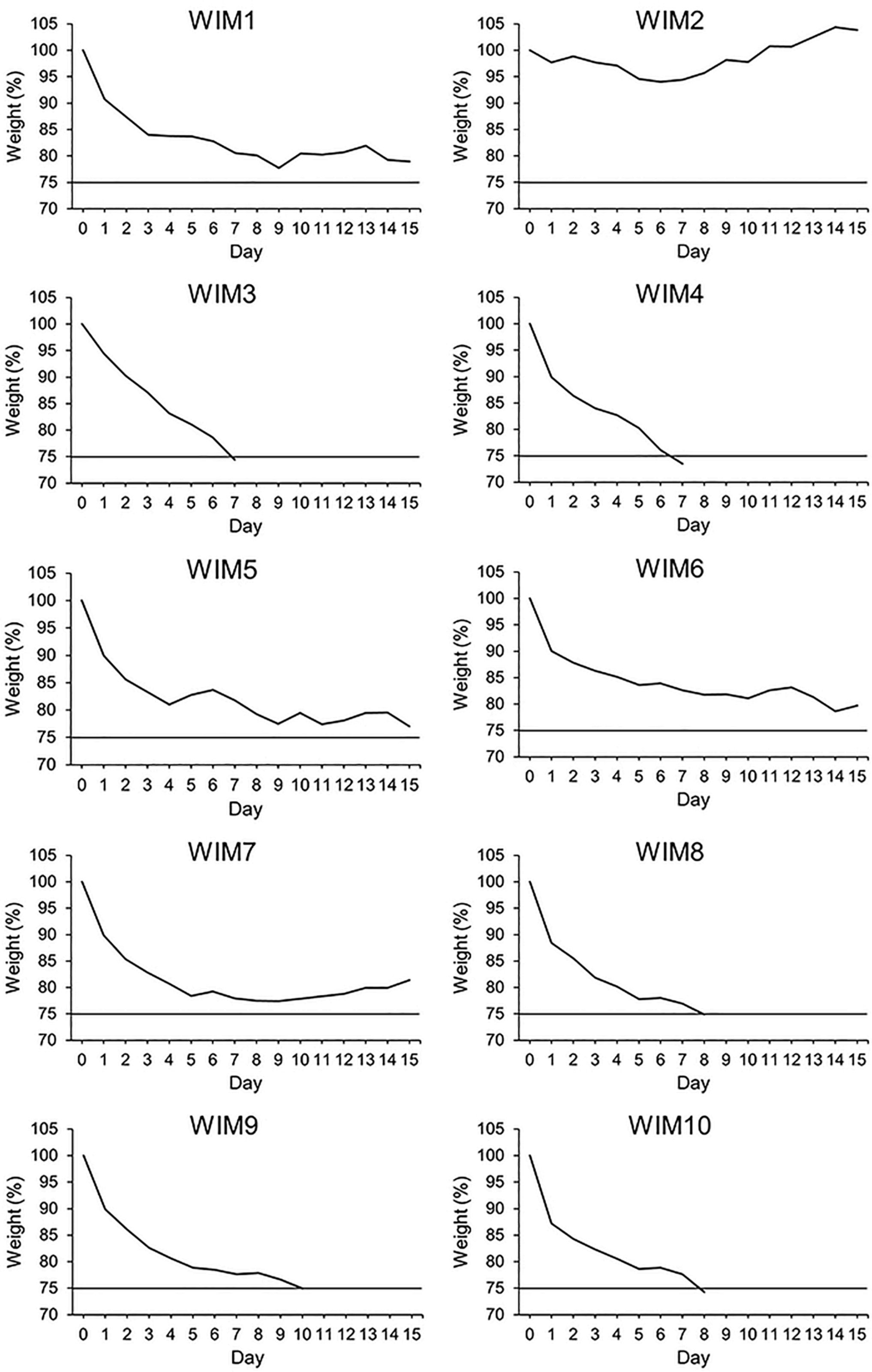
Appendix 4. Running distribution within days of the WIF group (Wheel Individual Female)



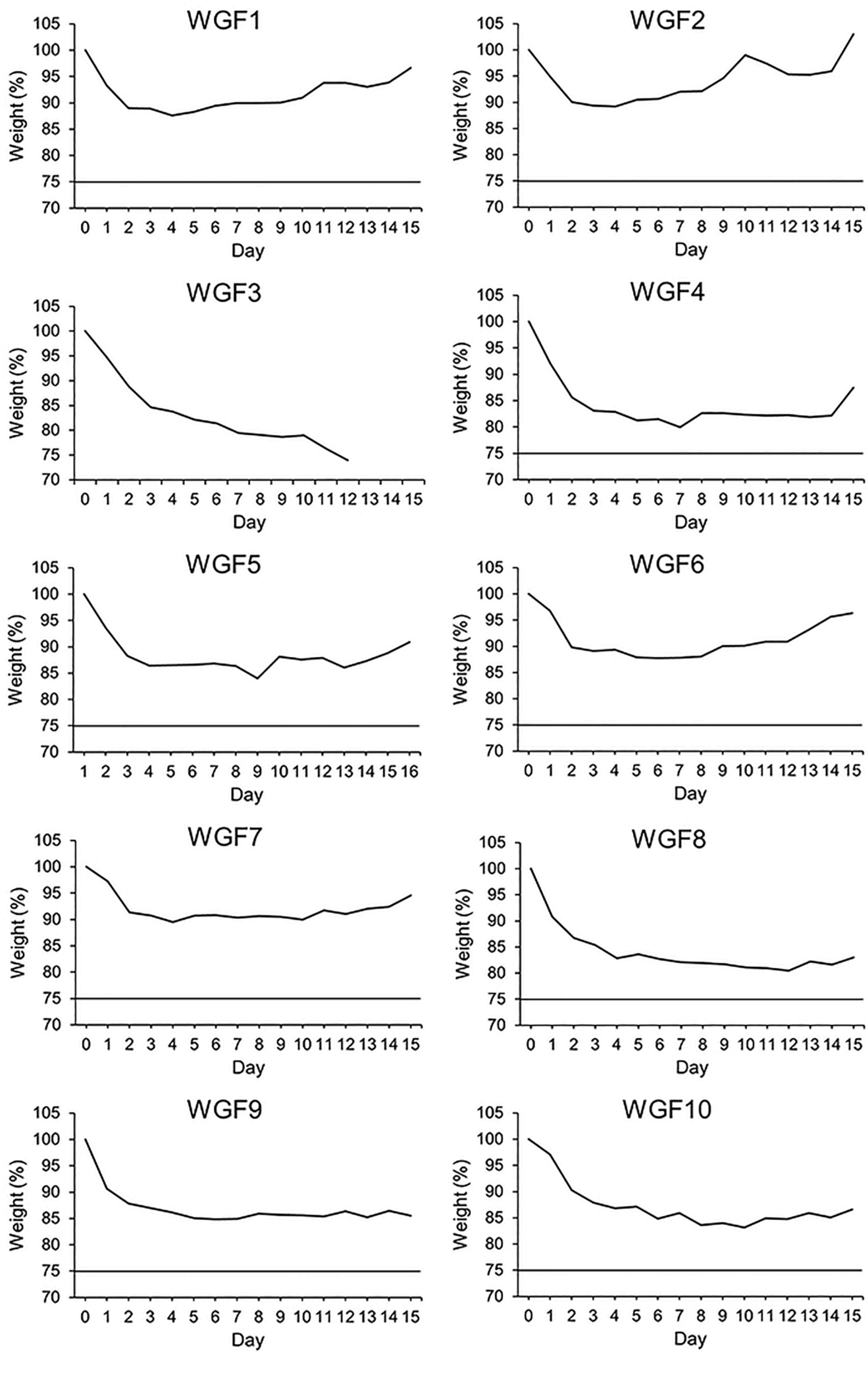
Appendix 5. Development of weight percentage along the days for each rat in the WGM group (Wheel Group Male)



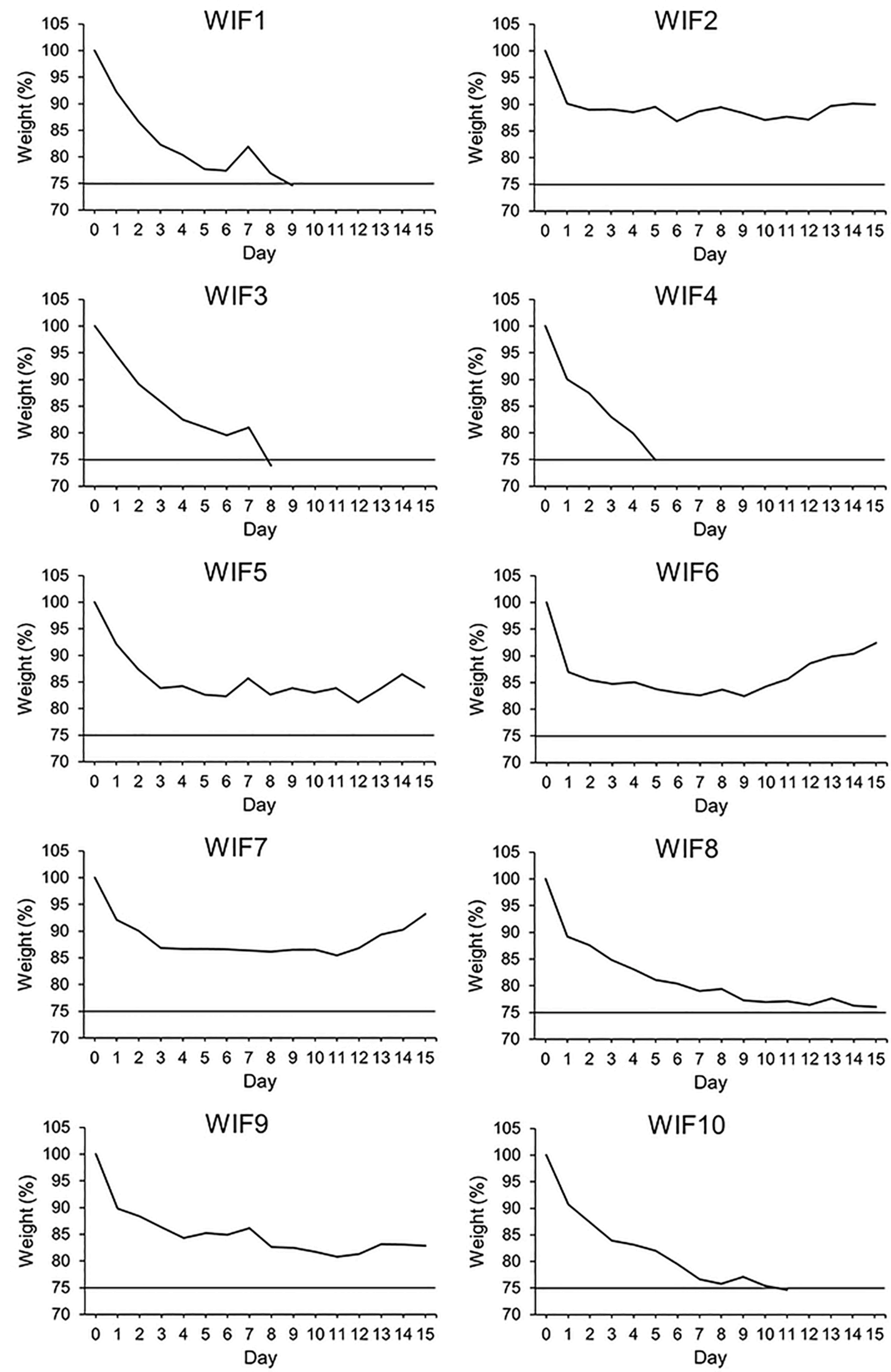
Appendix 6. Development of weight percentage along the days for each rat in the WIM group (Wheel Individual Male)



Appendix 7. Development of weight percentage along the days for each rat in the WGF group (Wheel Group Female)



Appendix 8. Development of weight percentage along the days for each rat in the WIF group (Wheel Individual Female)



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