The absence of food restriction prevents the development of activity-based anorexia in rats[1]

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Abstract

The purpose was to study whether re-exposure to an activity wheel with and without food restriction could generate a recurrence of the ABA model in rats. This model consists of exposing a group of rats to an experimental box with access to an activity wheel 23 hours a day, food available one hour a day and water ad libitum. During the 7-day experimental period, the rats gradually increased their wheel activity level, consumed less food, and suffered dramatic body weight loss. When removed from these conditions, they return to their initial body weight and feeding pattern within five days. In this study, two groups of rats (experimental and control) were initially exposed to the typical ABA model procedure. After a recovery period, the control group was re-exposed to this model, while the experimental group was re-exposed to the wheel but with food ad libitum. As a result, we found that control rats suffered from recurrence of the anorexic condition manifested in body weight loss, decreased food consumption, and increased activity. In contrast, the experimental group did not present these same levels, since this group did not increase its activity; their body weight did not decrease, although their food consumption did decrease. It was found that the ABA phenomenon requires the joint availability of the activity wheel and food restriction. These data suggest implications to consider for the study of relapse in human anorexic behaviors.

Key words: Anorexia, re-exposure to the ABA procedure, food restriction, body weight, food consumption, activity wheel, rats.

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Resumen

El objetivo fue estudiar la re-exposición con y sin restricción de comida al modelo de Anorexia Basada en Actividad (ABA) en ratas. Este modelo consiste en exponer a un grupo de ratas a una caja experimental con acceso a una rueda de actividad durante 23 horas al día, comida disponible durante una hora al día y agua *ad libitum*. Durante 7 días del período experimental las ratas aumentan gradualmente su nivel de actividad de la rueda, consumen menos alimento y sufren una dramática pérdida de peso corporal. Cuando se eliminan estas condiciones, las ratas normalmente recuperan su peso corporal inicial y su patrón de alimentación dentro de los cinco días siguientes. En este estudio dos grupos de ratas (experimental y control) inicialmente fueron expuestos al procedimiento del modelo ABA. Después de un período de recuperación, el grupo control se volvió a exponer al modelo ABA, mientras que el grupo experimental fue expuesto a la rueda pero con comida *ad libitum*. Como resultado, las ratas control mostraron nuevamente una pérdida de peso corporal, una disminución del consumo de alimento y una mayor actividad en la rueda. En contraste, el grupo experimental no mostró estos mismos niveles ya que no aumentó su actividad, no disminuyó su peso corporal, aunque sí disminuyó el consumo de alimento. Se confirmó que el fenómeno ABA requiere de la disponibilidad conjunta de la rueda de actividad y de la restricción de comida. Estos datos sugieren implicaciones a considerar para el estudio de la recaída de las conductas anoréxicas en humanos.

Palabras clave: Anorexia, re-exposición al procedimiento ABA, restricción alimentaria, peso corporal, consumo de comida, rueda de actividad, ratas.

The Activity-Based Anorexia model in rats consists in exposing a group of rats to an experimental box with access to an activity wheel 23 hours a day, food available one hour a day, and water *ad libitum*. During an experimental period of several days, rats gradually increase their level of wheel activity, consume less food, and suffer dramatic body weight loss. When they are removed from these conditions, they recover their initial body weight and eating pattern within a few days. Under these conditions, rats show a behavior that is important for scientific research; namely, a progressive increase in the level of activity during the experiment (Gutiérrez, & Pellón, R. (2002a; 2002b).

Bolles and de Lorge (1962) were two pioneering researchers in this field, and since then this model has been proven to be very useful because it allows researchers to isolate the influence of complex factors, including culture, in order to identify the basic processes that cause characteristic anorexic behaviors (Martínez and Gómez, 2011). What is intriguing with these behaviors lies in the consequent decrease in body weight given the context of food restriction, in which physical activity is not required and even counterproductive; however, energy expenditure is continuous and so drastic that rats can starve after an average of seven days of the experiment (Paré, *et al.,* 1978). For such reasons, it is usually applied as an ethical criterion to remove all rats from the experiment when they lose 25% of their body weight (Klenotich and Dulawa, 2012). In our study, we also applied a maximum number of seven days to end the experimental phase as a criterion because the decrease in body weight is usually clearly observable at that point.

Data have been documented showing that increased activity is related to food intake. For example, in a study carried out by Carrera et al., (2014) they contrasted food intake between two groups of rats: one that had access to an activity wheel and another that did not. While both groups (active vs sedentary) had a food restriction regime imposed for 23 hours a day, the active group ingested less food than the sedentary rats. Pérez-Padilla, et al., (2010) have shown that even irregular feeding periods during the light period did not prevent the generation of activity-based anorexia. These authors concluded that fixed and variable periods of food availability produced typical anticipatory peaks of activity-based anorexia in rats. However, Kanarek and Collier (1983) had shown evidence that the timing of food availability could influence the development of activity-based anorexia. These authors divided the standard one-hour food access period into two 30-minute or four 15-minute periods per day and rats consumed more food and did not develop behaviors characteristic of activity-based anorexia. These authors concluded that the phenomenon did not occur because the animals did not adapt to the restriction of the feeding schedule. This evidence would demonstrate that periods of food availability plays an important role in developing activity-based anorexia. In addition to modeling the characteristics of human anorexic behaviors in rats, studies have shown that rats are able to regain their body weight and eating pattern after removal from experimental conditions within an average of five days (Boakes, et al., 1999; Dixon, et al., 2003; Gómez and Martínez, 2013).

Hampstead, *et al.* (2003) investigated whether rats could adapt to repeated exposure for five cycles of food restriction plus the activity wheel. The expected adaptation was a decrease in weight loss, an increase in food consumption, and a decrease in excessive activity. Hampstead, *et al.* (2003) used 20 female rats, the experimental (active) group went through the standard anorexia procedure with one hour a day to access food and 23 hours with availability of an activity wheel for seven days; while the control group (sedentary) remained without activity wheel and only with food restriction, repeating this cycle five times. All rats had a recovery phase between each cycle and at the end of the experiment. Their results showed that: (a) rats in both groups increased food consumption throughout the five cycles; (b) those of the active group consumed more food than those of the sedentary group from the third cycle, although in the first and second cycles they consumed less; (c) sedentary rats reduced weight loss further throughout the five cycles; (d) the active rats increased the number of runs with the passage of the cycles until reaching 75% of their weight; and, e) the group of active rats showed an anticipatory behavior to the food increasing the turns before the time to eat. These authors concluded that their results support the hypothesis of Dwyer and Boakes (1997), which was that subjects could adapt to food restriction if there was no interference from the activity wheel. This meant that the activity wheel would have no effect on the behavior of the rats, if they were adapted to food restriction, prior to accessing the wheel. But in the experimental setting of Hampstead, *et al.* (2003), this hypothesis was only corroborated by the results of the group of sedentary rats.

Therefore, it was necessary to explain why the group of active and food-restricted rats did not adapt to the re-exposures, since this was the main objective of the experiment. Another relevant hypothesis to help understand the generation of anorexia in rats is that physical activity acquires a reinforcing value in conditions of food restriction. Pierce, *et al.* (1986, Experiment 1) analyzed the variables associated with anorexia in an operant conditioning experiment. As is well-known, the operant approach holds that the probability of a behavior being repeated increases if, as a consequence, that behavior receive a reinforcement, or an aversive stimulus is eliminated; for example, it is likely that a hungry rat will press a lever repeatedly if this behavior is followed by food (Skinner, 1938). Although

Pierce *et al.* (1986, Experiment 1) allowed a group of food-deprived rats access to an activity wheel, those animals pressed the lever more often than their counterparts that had *ad libitum* access to food. The authors interpreted these data as evidence of an increase in the effectiveness of exercise as reinforcement under conditions of food deprivation. Later investigations have supported this hypothesis and have even complemented it with analyzes of a physiological nature. For example, Pierce and Epling (1994) suggested that exercise may generate some neurochemicals that rats would perceive as pleasurable, and that this would explain the observed reinforcing function, and Kanarek, et al. (2009), on the other hand, concluded that the activity of running activates rewarding dopaminergic pathways.

Due to the role that food plays in this model, the objective of this study was to determine if re-exposure to the activity wheel with and without food restriction could generate the ABA model in rats that had previously shown the development of the ABA phenomenon. We first replicated the typical ABA experimental model. In the first phase, as expected, the rats developed ABA phenomenon. Then we waited for a recovery period of 16 days, we estimate it would be enough for the rats to stabilize their food consumption pattern. After this interval, all the rats were re-exposed to the activity wheel, but under two distinct experimental conditions: a) with food restriction; or, b) with free access to food. The main goal was to reproduce the phenomenon ABA using re-exposure to the activity wheel as a predictive variable due to prior experience to the experimental conditions. Our hypothesis was that re-exposure to the wheel would have a diminishing effect on food consumption and body weight in both groups of rats, and activity on the wheel would be higher for the food-restricted group, but these effects would be greater in the food restriction group.

Method

Subjects. Sixteen 60-day-old male Wistar rats were obtained from the animal-breeding laboratory at the Institute of Neurosciences. Prior to the experiment, they were kept in collective-size household boxes (4 rats per box) with sawdust bedding and *ad libitum* access to food and water. Ambient temperature was maintained between 20 and 25°C and a 12-h light-dark cycle was imposed with changes at 8:00 and 20:00. The experimental procedure and handling of the animals were approved by the Ethics Committee of the Neurosciences Institute of the University of Guadalajara (approval code: # ET112015-200).

Apparatus and Materials. Individual, transparent methacrylate boxes measuring 21 x 45 x 24 cm with a metal grid and wood sawdust were used. Food was placed in a feeding trough and a water dispenser was attached to the box. The feed used was a commercial brand with Purina Rodent Laboratory Chow (3% fat, 23% protein, 7% ash, 1% calcium, 6% fiber, 49% of E. L. N, 6% phosphorus and 12% humidity). The activity wheels were placed in the lateral area of the boxes, with access controlled by a manual device. An automatic counter and *Lafayette Instrument's Activity Wheel Monitor* software, programmed to store data daily every 30 minutes during the 23 hours that the activity wheel was available, recorded the number of laps run. Two types of electronic scales were used: a high-precision one for food (KERN 440-33 Max 200 g), and another for body weight (AND GX-6000, Max 6100 g).

Procedure. The first five days constituted the baseline period, during which all rats received food and water ad libitum while housed in individual home-boxes. Body weight and food and water intake were recorded daily. The first experimental phase (A) began on day 6, when each rat was placed in a box-room with access to an activity wheel, but food was restricted completely for 23-h. During the one hour of food availability, access to the wheel was blocked. Water was available at all times. The rats were held under

these conditions for the seven days of this phase. This part of the procedure lasts seven days because it is the average period in which the animals lose 25% of their body weight. With more days under these circumstances, subjects can die, as reported by Paré, *et al.* (1978). If subjects reach the 25% weight loss criterion before seven days, they were removed from the experiment. After a recovery period of 16 days, in which rats were placed in their individual box with free access to food and without the activity wheel available the re-exposure phase (B) began. It also lasted seven days, but in this phase the experimental group (24-hour group) had access to both the activity wheel and food for 24 hours daily. In contrast, the control group (1-hour group) was subjected to the procedure described above, with only one hour per day of access to food, but 23 hours to the activity wheel. After that phase, the rats were given a period of five days for final recovery. In all phases, when applicable, 50 g of food and 100 ml of water were provided daily; but during the food restriction periods for the experimental group, food was available for only one hour (10:00-11:00 a.m.).

Data analyses. Data were analyzed with a two-factor mixed design ANOVA (Condition: 24h y 1h) x (Session, the number of sessions depended on the phase) using Statistical Package for Social Science (SPSS) software. Analysis of the pair-wise comparisons was performed using the same program, with the minimum level of statistical significance set at p <.05. The dependent variables were the amount of food consumed (grams), body weight (grams), number of laps on the activity wheel, and the amount of water ingested (milliliters). The sphericity assumption was measured by a Mauchly's test, but because the data did not fulfill this assumption, Greenhouse-Geisser correction was applied to the degrees of freedom, although those were reported with their original values.

Results

Body weight. The upper graph in Figure 1 shows the body weight in (g) and percentage of lost weight of the rats in the lower graph for the experimental (24-hour food) and control groups (1-hour food) during the experimental sessions (phase A and B) and recovery periods. At the beginning of the experiment (baseline), the rats had an average age of 60 days and a mean body weight of 275 g. As it was expected, there was no main effect of Condition nor was there a significant interaction between Condition and Sessions of the phase A. Both groups decreased their body weight progressively with no significant differences through the sessions. There was a main effect of Session [F (1, 66) = 203.001, p < .001], which indicates that the sequence of the sessions affected the decrease in body weight.

The percentage of lost weight basically replicated the body weight curves so that in Phase A both groups of subjects were around 75% of lost weight in the last session. To be specific, all subjects in the experimental group met the criteria and in the control group only 3 subjects did not meet the criteria in session 7. In the first recovery period, both groups increased their body weight over the 16 days, so an effect of Session did exist [F (15, 210) = 378.881; p < .001], though there was no effect of Condition or for the interaction of these two factors. These results were expected because both groups were held under the same conditions in these phases.

In phase B, also in the last session, the subjects in the experimental group reduced their body weight to approximately 80% relative to the body weight of the last session of the first recovery (none met the criteria of 75% because the calculation was taken from the baseline). The statistical analysis of this phase B showed a main effect of Condition [F (1, 14) = 6.648; p < .05], Session [F (6, 84) = 25.149; p < .001], and an interaction between condition and session [F (6, 84) = 77.923; p < .001]. The 24-hour experimental group had greater body weight than the 1-hour control group and this difference reach the level of significance on the fourth day of this phase. Finally, regarding the last recovery period, the analysis

of body weight showed no main effect of Condition, but a main effect of Session [F (4, 56) = 80.028; p <.001], and the interaction Condition x Session was significant [F (4, 56) = 31.476; p <.001]. This indicates that the final period of recovery did affect in a different way to both groups. The body weight of the experimental group was stabilized in this period, and the body weight of the control group implied a progressive and significant increase between each day.

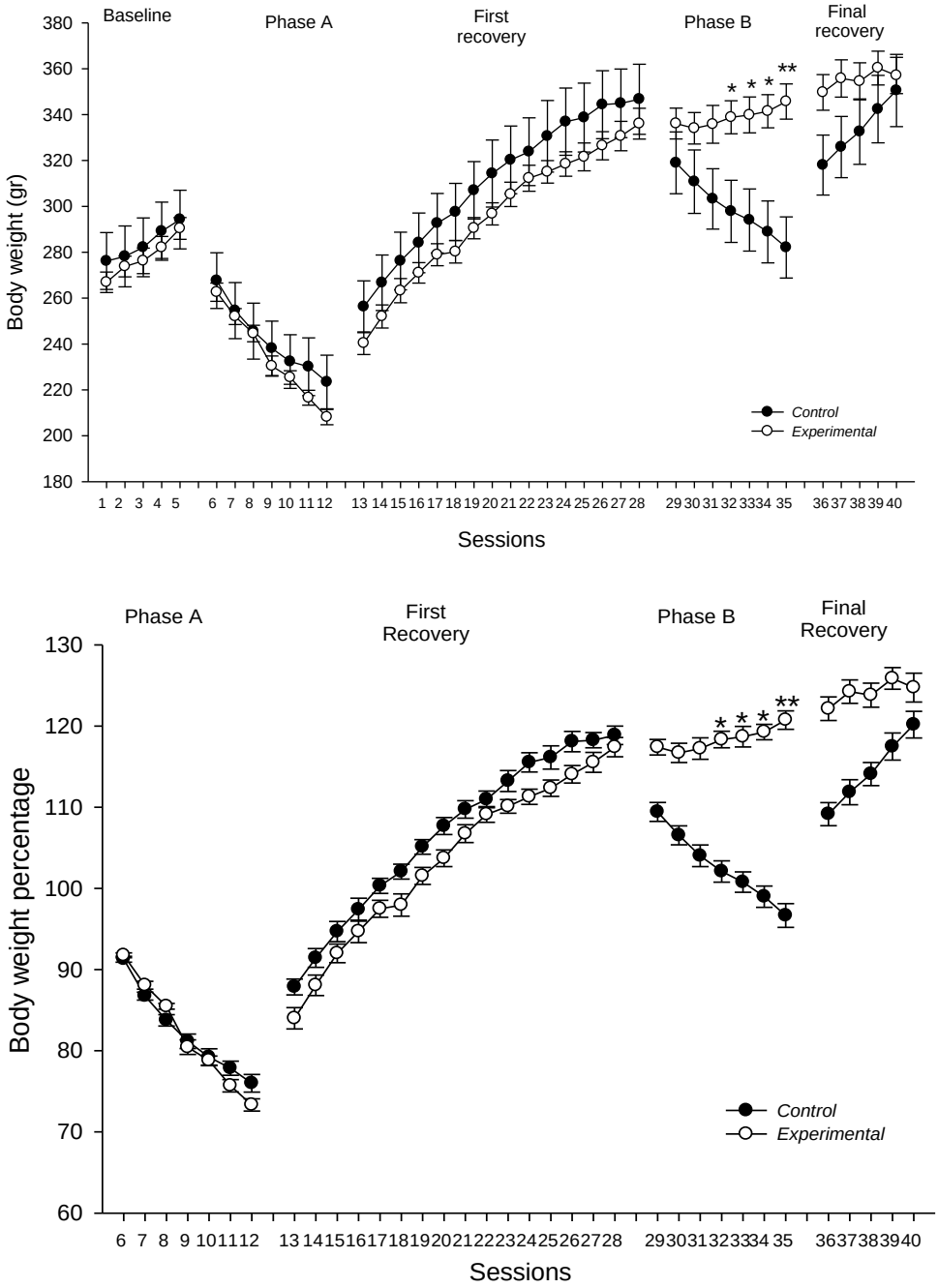

Figure 1. Average body weight in grams (top panel) and percentage of weight loss (lower panel) of the rats in the 24-hrs experimental group (white circles) and 1-hr group control (black circles) during all the experimental and recovery phases. Standard errors are plotted for each mean. Asterisks represent significant between-group differences: one asterisk indicates p < .05; two indicate p < .01.

Food consumption. Figure 2 shows the results of the amount of food consumed by the rats in both groups during the baseline, the experimental sessions and recovery periods. In Phase A both groups had different amounts of food consumption, in the first day of Phase A both groups had a similar media, but in the last day the control group had a higher consumption (M = 12.85, SD = 0.59) than the experimental (M =6.19, SD =1.11). The statistical analysis of Phase A found an effect of Condition [F (1, 11) = 81.02; p < .05], a main effect of Sessions [F (6, 66) = 55.23; p < .05], and a significant interaction between them [F (6, 66) = 19.92; p < .05]. As it was expected the Phase B showed the most radical differences, the control group returned to low consumption (M = 10.37, SD = 1.62, example of the last day) and the experimental group had higher consumption than this group (M = *24*.82, SD = 2.03, example of the last day) but lower compared to their previous consumption in the recovery period (M = *28*.22, SD = 1.50) A main effect of Condition was found in Phase B [F (1, 14) = 392.04; p < .001], a main effect of Sessions [F (6, 66) = 5.39; p < .01], and a significant interaction between them [F (6, 84) = 3.40; p < .05].

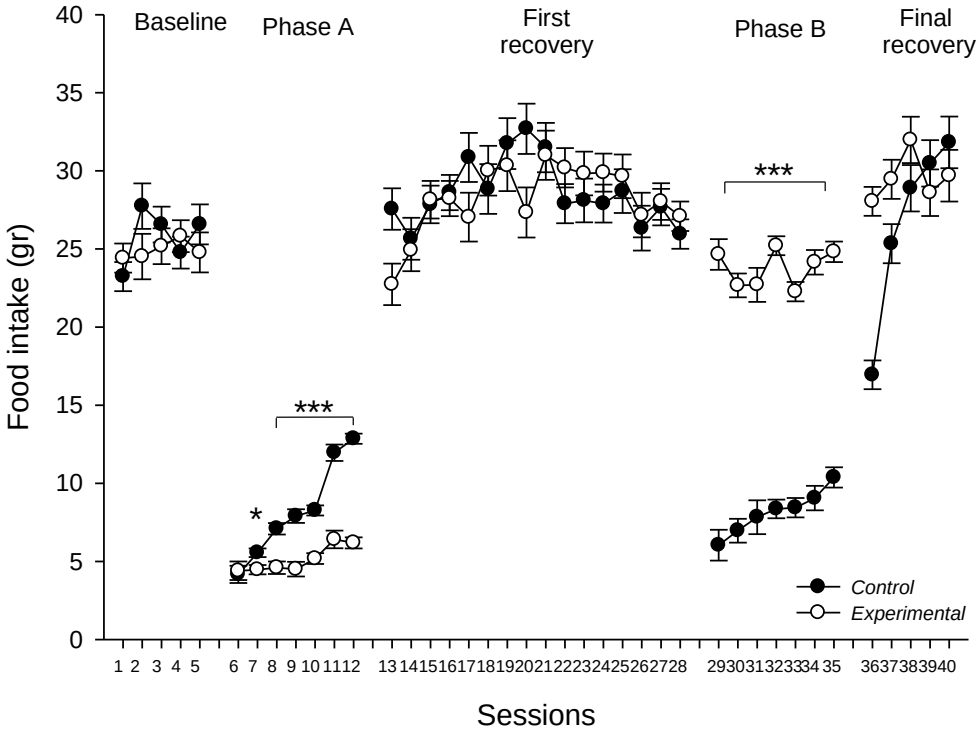

Figure 2. Means of food consumed in grams (± SEM) in each phase of the experiment. The control group (1-hr group) is represented by the black circles; the experimental group (24-hrs group) by the white circles. Asterisks indicate significant differences: two asterisks indicate p <.01; three indicate p < .001.

Activity on the wheel. Figure 3 displays the average number of laps on the activity wheel during the 23-hour sessions in the two experimental phases for both groups. The statistical analysis of Phase A showed that there was no effect of Condition nor an interaction. But there was a main effect of Session [F (6, 66) = 23.53; p <.001], which indicates that the number of laps was significantly different between sessions. Activity increased progressively in both groups with no significant differences between them. Regarding Phase B, analysis showed a main effect of Condition [F (1, 14) = 9.24; p < .01], a main effect of Session [F (6, 84) = 10.98; p < .001], and the interaction Condition x Session was significant [F (6, 84) =

9.97; $p < .001$]. The *post hoc* analysis indicated that the difference between groups was significant on the third day, $p < .01$, since the control group repeated the progressive increase in the number of laps, while the experimental group remained stable.

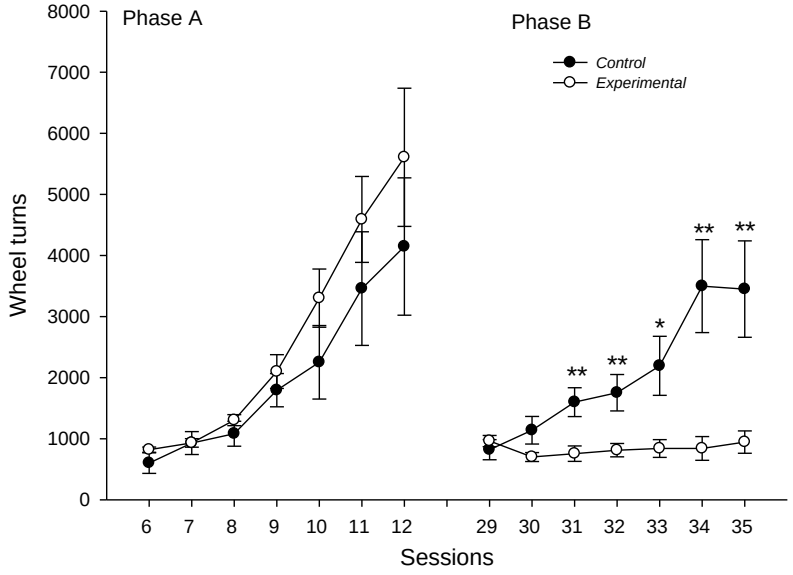

Figure 3. Average number of laps on the activity wheel (± SEM) during the sessions in Phases A and B. The white circles represent the data of the experimental group (24-hrs group) and the black circles those of the control group (1-hr group). One asterisk indicates $p < .05$; two indicate $p < .01$.

Water intake. Water intake during the 23 hours of access to the activity wheel declined in both groups during the sessions of the phase A. There was no effect of Condition nor any interaction between these two factors. There was an effect of Session [F (6, 66) = 4.17; $p < .001$], the decrease of water intake during sessions did not differ significantly between the groups. In contrast, in Phase B the amounts of water intake recorded differed significantly between the two groups, since the control group intake was recorded for 23 hours during each one of the seven sessions, while the experimental group had free access to water for 24 hours. The analysis of Phase B showed an effect of Condition [F (1, 14) = 115.85; $p < .001$]. However, no differences were found between the Sessions and there was no effect for their interaction. These results suggest that water intake by the two groups did not differ significantly between sessions, but remained stable.

Discussion

The research literature using the activity-based animal model of anorexia contains few experimental designs that propose a variable as a predictor of recurrence in anorexia. Therefore, the main objective of this study was to evaluate whether re-exposure to an activity wheel with and without food restriction could generate a recurrence of the ABA model in rats that previously developed the characteristics of the model. To explore this issue, a study protocol was designed to contrast two groups of rats: one was a control group (1-hour group), which we expected would have the highest probability of relapsing into anorexia, because it would be re-exposed to an activity wheel and food restriction; the other

was designated as the experimental group (24-hours group) because the effect of re-exposure to the wheel with no food restriction after a long recovery period would be explored for the first time.

Regarding the results on body weight, we found significant differences between the groups during the final four days of Phase B (the re-exposure phase). These differences could be explained by the variable of food availability. Martínez and Gómez (2011) reported that when rats have simultaneous access to an activity wheel and *ad libitum* access to food, they show a stable and greater weight gain than rats that have food restriction. The subjects in our experimental group (24-hours) had higher body weight in this phase because they had free access to food and showed less activity on the wheel than the 1-hour-control group. The results of this study refer to food consumption, where an unexpected but significant difference was found between the two groups in the first exposure to the experimental program (Phase A). One possible explanation for this finding is that the control group had a slightly higher body weight at baseline coupled with the fact that the lower the body weight, the greater the propensity to develop anorexia (Boakes and Dwyer, 1997). However, the difference between the groups in the re-exposure phase (Phase B) was larger because the experimental group had free access to food, while the control group (1-hour group) did not significantly change its food consumption between the first phase and the re-exposure period.

Considering the control group in experimental phase B, the data could suggest that the dopaminergic and serotonergic systems play an important role in relation to the reinforcing value of eating and running. However, in the experimental group, by contrast, there would be no corresponding reward effect or activation of dopaminergic and serotonergic pathways, so these rats did not trade food for time on the activity wheel. Rather, they kept on eating without running on the wheel to gain that potential reward and so did not gain body weight. In comparison, the rats that experienced *ad libitum* access to food and the activity wheel, and those with restricted food access with no access to the activity wheel, typically do not lose significant amounts of weight and can subsist normally (Brown *et al.*, 2008).

In contrast to the above, Hamsptead, et al. (2003) reported an increase in food consumption in the second exposure phase of the experimental procedure, as their control group (24-hour group) showed lower consumption in the re-exposure phase than in the first recovery period. This finding confirms the hypothesis that re-exposure to the wheel has the effect of reducing intake after restoration of the feeding pattern, even though these rats were maintained on food ad libitum. To corroborate whether this reduction was due to re-exposure to the wheel, this result was contrasted with what was reported by Martínez and Gómez (2011), who showed that before the first exposure to the wheel with free access to food there was an effect reduction in food intake compared to baseline. Hampstead et al. (2003) set out to show that the activity wheel had no effect on rats when they were adapted to food restriction prior to accessing the wheel (see Dwyer and Boakes, 1997). However, the results of the present study and others (e.g., Martínez and Gómez, 2011; Lett et al., 2001) have shown that food intake decreases whenever an activity wheel is accessed. With respect to the variable activity, it is well-known that in experimental contexts, animals are motivated to run by various factors. The first is the search for food, but others include restriction of activity prior to experimentation, and defensive or aggressive behaviors, until other environmental elements in the box room are restricted (Killeen, 2014). In our experiment, the experimental group ran significantly less than the group that was restricted in the final five days of Phase B. This decrease in the number of laps of the wheel is more likely explained by free access to food than by the previous experience with the wheel and the restriction imposed, since Martínez and Gómez'

experiment (2011), in which there was no previous experience, also found a decrease in the level of activity when the wheel was combined with *ad libitum* access to food.

Södersten, *et al.* (2016), meanwhile, found that reduced food intake increases the risk of anorexia nervosa by engaging mesolimbic dopamine neurons that, initially, reward dieting. In contrast, diet restriction-induced exaggerated feedback control over 5-HT synthesis, together with the lower availability of tryptophan (a serotonin precursor), decreases serotonin neurotransmission at postsynaptic sites; thus, leading to hyperactivity, depression and behavioral impulsivity (Haleem, 2012). In our results, dopamine and serotonin may have generated greater hyperactivity and decreased food intake, which could constitute a physiological description of why the ABA model caused a rewarding effect of exercise and decreased food consumption in both groups in Phase A, when the rats only had access to food for 1 hr. These results suggest that ABA rats, and anorexia patients, possibly reject the food reward at the same time as they become addicted to physical activity.

Turning, finally, to the water consumption variable, few studies using the ABA model have included this measurement. In our results, the amount of water ingested differed between the groups on every day of Phase B. When the wheel was available without food restriction, water consumption remained stable, but when a food restriction was imposed with access to the wheel, this measure decreased significantly. These data confirm what Verplank and Hayes (1953) called self-deprivation; that is, the interaction between water intake and food intake, which shows that restrictions on water consumption affect food consumption, and *vice versa*. This effect on water intake probably contributed to the decrease in body weight during the experimental phase. Therefore, drinking behavior could be included among the parameters that define the ABA model.

Conclusions

As a conclusion, results of this research integrating a re-exposure phase with or without food into the ABA model make it possible to obtain a better analogue to a relapse or recurrence, since the measurements of the dependent variables show a similarity to what occurs during first exposure; that is, similar percentages for weight loss and activity on the wheel, together with reduced consumption of food and water. In contrast, re-exposure to the wheel *without* the restriction only generated a reduction effect on food consumption, compared to the food pattern obtained in the first feedback period. As future lines of research regarding the methodological improvement of different procedural aspects, we would include continuing with the experimental phases beyond seven days; keep the animals in the recovery period only until they recover their initial weight and the incorporation of a control group, which did not have access to the activity wheel in any period of the experiment. Finally, some limitations of this study should be mentioned. An important limitation of our study is that feed intake levels can be controlled in such a way that they are equivalent, and therefore, the experimental groups can be considered similar before being exposed to different experimental conditions. We should also mention that although we do not include the manipulation of any neurophysiological variables, we nevertheless point out that the activity-based model of anorexia provides a methodology to explore effects at the neurobiological level. Another limitation is that given that diagnosis of anorexia nervosa is more prevalent among female populations, future studies should test the ABA animal model and recurrence with female rats. It is also possible that other behavioral interpretations may be plausible and could contribute to a more complete understanding of the activity-based anorexia phenomenon. As an example, by appealing to well-known learning mechanisms such as behavioral contrast, resurgence or renewal. These issues will surely be in the future

directions of this field of basic research and applied implications. Indeed, future research in this area could have implications to increase our understanding of anorexia in humans and methods that may be used to prevent it in the long term.

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