

Research report

Rapid development of semistarvation-induced hyperactivity in Dark Agouti rats. Excessive wheel running and effect of 3,4-methylenedioxymethamphetamine (MDMA) [☆]

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ABSTRACT

Clinical studies have found that patients with anorexia develop high activity levels. These data suggest a possible implication of activity in the aetiology of anorexia and are in line with findings obtained in animals during experimental procedures to model interactions between activity and weight loss. Activity-based anorexia (ABA) and semistarvation-induced hyperactivity (SIH) develop when laboratory rats have food access restricted to a single period in the day and are given free access to an activity wheel. This experiment sought to show the effect on weight loss of the excessive activity normally seen in Dark Agouti rats and of hyperactivity induced by 3,4-methylenedioxymethamphetamine (MDMA). To this end, 32 female rats of the Dark Agouti strain were selected and divided into four groups in accordance with a 2 × 2 factorial design, in which one factor was treatment (saline or MDMA) and the other was access or lack of access to an activity wheel. Animals with wheel running access displayed a marked increase in running combined with accelerated weight loss. Although pharmacological treatment resulted in no observable effect on weight loss, rats treated with 12.5 mg/kg MDMA generally registered more wheel running than did those treated with saline. Analysis of data on the temporal distribution of wheel running revealed an alteration in circadian activity patterns as a consequence of MDMA. These results, by showing a general high level of wheel running in Dark Agouti rats, once again emphasise the close relationship between activity and weight loss in the development of SIH and related phenomena such as ABA.

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Introduction

Increases in activity and mortality in rats deprived of food were early documented at different laboratories (Bolles & De Lorge, 1962; Finger, 1951; Hall & Handford, 1954; Reid & Finger, 1955) but it was not until Routtenberg and Kuznesof (1967; see also Routtenberg, 1968) that it was observed that the increased activity and mortality was also accompanied by a reduced food intake during the periods of restricted feeding. This self-starvation phenomenon was later named activity-based anorexia (ABA) by Epling, Pierce, and Stefan (1983) attending to the “levelling off” in food consumption of rats displaying intense running activity, well below the food ingestion of sedentary rats under the same food

restriction conditions (see Boakes, 2007; Gutiérrez & Pellón, 2002 for reviews). Some authors, however, have found that the exposure to wheel running plus food restriction do not affect food consumption, and named this phenomenon semistarvation-induced hyperactivity (SIH) (Broocks, Liu, & Pirke, 1990).

ABA and SIH are experimentally provoked by restricting access to food to a single period during the day and permitting free access to a wheel running during the rest of the time. To demonstrate that these effects are not exclusively due to the feeding schedule a control group is used, which is exposed to the same feeding conditions but it is not given access to the wheel running. After a number of days in which both groups lose weight, the group without access to the wheel running soon stabilises at around 80–85% of its free-feeding weight, whereas weight loss continues in the experimental group (for demonstrations of this phenomenon at our laboratory, see Cano, Gutiérrez, & Pellón, 2006; Gutiérrez & Pellón, 2008; Pérez-Padilla, Magalhães, & Pellón, 2010).

It seems as if physical activity plays a crucial role in the development of ABA/SIH, with this being also true for the anorexia observed in the human population (for review see Hebebrand et al., 2003). Food restriction contributes to increase physical activity

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levels in human anorexic patients (Holtkamp, Heberbrand, & Herpertz-Dahlman, 2004), a relationship that is also present in the animal model of ABA/SIH. For this reason, the animal model has been useful for testing potential treatments in anorexia nervosa (Gutiérrez, Cerrato, Carrera, & Vázquez, 2008; Hillebrand, van Elburg, Kas, van Engeland, & Adan, 2005).

One possible experimental manipulation to bring about changes in activity is the use of psychoactive drugs which have effects on such activity. Hillebrand et al. (2005) found that administration of the antipsychotic olanzapine reduced the development of ABA in rats, with one of the mechanisms implicated being a reduction in wheel-running activity. Similarly, Nergårdh et al. (2007) observed that treatment with neuropeptide Y can increase wheel-running activity and decrease food intake, thus favouring the development of anorexia; this is in sharp contrast to what happens when it is administered to rats enjoying unrestricted food access and leads to increased consumption.

3,4-Methylenedioxyamphetamine (MDMA) (see Green, Mehan, Elliott, O'Shea, & Colado, 2003, for a review) is a derivative of amphetamine that was originally used as an anorexygen and is currently being used as a recreational drug (for a clinical review see Gouzoulis-Mayfrank & Dauman, 2009). Administration of MDMA is reported in the literature as causing hyperthermia under normal laboratory conditions (e.g., Brown & Kiyatkin, 2004) and locomotor hyperactivity in rats (Callaway, Wing, & Geyer, 1990; Rodsiri, Spicer, Green, Marsden, & Fone, 2011). This increase in locomotor activity has been shown 28 days after the end of treatment (Balogh et al., 2004). In addition, the effects on locomotor activity were higher in female than male rats, and these effects were still maintained up to 10 days after the last MDMA administration (Walker et al., 2007). Some studies suggest that an increase in muscular thermogenesis is the cause of this hyperthermic response (Mills, Rusyniak, & Sprague, 2004; Sprague et al., 2005).

The present study used rats of the Dark Agouti (DA) strain. These animals are characterised by maintaining a high degree of intrinsic activity and, in several parameters related with aerobic exercise, such as wheel running, are superior to other strains of rats commonly used in animal research (Barbato et al., 1998). One possible explanation for such hyperactivity may be linked to greater thermoenergetic activity in brown adipose tissue (Larue-Achagiotis, Gubern, Laury, & Louis-Sylvestre, 1994). For this reason, these rats have been used as a genetic model of high activity in exercise-related studies (Koch et al., 2005) and have also been presented as a high-anxiety model in studies of anxiogenic behaviour (Mehan et al., 2002).

Because acute administration of MDMA causes hyperactivity in DA rats (Callaway et al., 1990; Green, Mehan, Elliott, O'Shea, & Colado, 2003; McNamara, Kelly, & Leonard, 1995; Rodsiri et al., 2011; Spanos & Yamamoto, 1989) that can last for 10–28 days (e.g. Balogh et al., 2004; Walker et al., 2007), the present study characterised ABA/SIH in DA rats and ascertain whether the long-term effect of MDMA-induced hyperactivity would facilitate its development. To examine the long-term effects on activity reported in previous literature, drug administration was performed by a single injection of 12.5 mg/kg and the behavioural experiment began 1 week after that administration. As regards the intrinsic nature of the phenomenon of ABA/SIH, this study is also of interest from a theoretical standpoint because it is able to assess the role played by hyperactivity in weight loss. The excessive activity of DA rats should facilitate the development of ABA/SIH if activity and weight loss are related. For these purposes, we used a 2 × 2 factorial design, in which one factor was pharmacological treatment (MDMA or saline) and the other was access or lack access to a wheel running, to study: firstly, the effect of food restriction and wheel running on ABA/SIH development in DA rats, by comparing saline groups with to those without a wheel running; and

secondly, the effect of MDMA on weight loss, food consumption and wheel-running activity. As a result, we expect to find higher activity in rats treated with MDMA compared to untreated rats, and that treated rats will loss weight to a greater degree.

Preliminary laboratory evidence on a separate experiment with a different set of DA rats showed that restricting the availability of food to only 1 h daily caused drastic weight losses, not only among rats with wheel access but also among those without wheel access, so that, despite the fact that this has been the generally used procedure for inducing ABA in other strains of rats (e.g., Wistar in Pérez-Padilla et al. (2010)), the feeding period in our study was extended to 3 h daily for all animals, regardless of the experimental treatment applied.

Method

Subjects

We used 32 female DA/OlaHsd experimentally naïve rats, age 60 days and obtained from Harlan Laboratories Models (Horst, Holland). On arrival the rats were housed in groups of four until they were placed individually at the start of the behavioural procedure. All subjects were monitored daily and maintained on an *ad libitum* food and water regimen.

The ambient conditions of the room were rigorously controlled and kept at a temperature of 21 °C, 60% relative humidity and a 12-h light–dark cycle (light from 8:00 a.m. to 8:00 p.m.) The mean (±SEM) baseline weight of animals at the commencement of the behavioural procedure was 177.58 (±1.66) g. During the experiment, all animals were weighed daily at the start of the feeding period, with water being freely available to all animals throughout. Animal-use procedures were in accordance with the European Communities Council Directive 86/609/EEC and Spanish Royal Decree 1201/2005.

Apparatus

Temperature was measured using an MC 8700 thermometer fitted with a digital read-out, and an H-RB3 rectal probe (EXACON A/S, Roskilde, Denmark) lubricated with lanolin hand cream.

During the behavioural procedure, experimental animals were housed in individual transparent Plexiglas chambers measuring 21 × 45 × 24 cm. The wheel running, width 9 cm and diameter 34 cm, was positioned at the left-hand side of each chamber. In addition, all running wheels were equipped with a brake mechanism. Eight such chambers were available, being the experiment run on two series that included identical number of animals from each experimental condition.

The control animals were housed in transparent Plexiglas chambers measuring 18 × 32.5 × 20.5 cm.

In both groups, each chamber was provided with a water receptacle, inserted into the right-hand side of the roof and permanently accessible. Along side each water container there was a concave area in which the food was deposited.

The data-programming and -collection equipment (MED-PC for Windows, MED Associates Inc., Georgia, VT, USA) was placed in a separate room. Data on wheel turns and licks to the water receptacle were recorded at 15-min intervals for each subject.

Procedure

After 1 week of acclimatisation, the animals were randomly separated into two groups: one ($n = 16$) was administered an acute dose of MDMA (LIPOMED, Arlesheim, Switzerland) at a concentration of 12.5 mg/kg via the intraperitoneal route; and the other

($n = 16$) was administered a saline solution of the same volume, likewise via the intraperitoneal route. MDMA was dissolved in saline (0.9% NaCl) and given in a volume of 1 mL/kg. Dose is reported in terms of the base.

Data on animals' rectal temperature was recorded pre- and post-treatment for the purpose of obtaining a temperature curve. Prior to administration of the drug, rectal temperature was measured at two points in time (-30 min and -60 min); after injection of the drug, temperature was measured at 30 min and, thereafter, at 1-h intervals until 5 h had elapsed. Each rat was lightly restrained by hand for approximately 20 s, during which time the probe was inserted approximately 2.5 cm into the interior of its rectum and a stable reading was obtained. After this, the animals were placed for a week in home cages awaiting the start of the behavioural procedure.

The experiment started 1 week after the injection of MDMA or saline, with half the subjects in each group (MDMA and saline) being randomly allocated to the experimental (activity) or control (inactivity) condition respectively. Each session lasted 24 h. For all animals, feeding time was restricted to 3 h daily, from 11:00 a.m. to 2:00 p.m., with the brakes of the wheels being activated during this period. All animals had free access to water.

Individual food and water consumption, wheel turns and body weight were measured daily. Each experimental animal (and its respective control) was removed from the procedure on the date on which its body weight fell below 75% of its initial value, a commonly accepted starvation criterion (Dwyer & Boakes, 1997).

Data analysis

To analyse the results in terms of percentage body weight, food and water consumption, a three-factor analysis of variance (ANOVA) was performed, with two between-group factors (MDMA or Saline, and Activity or Inactivity) and one within-group factor (Days). In the case of wheel turns, the results were analysed using a two-factor ANOVA, with one between-group factor (MDMA or Saline) and one within-group factor (Days). All analyses were performed using the SPSS 17.0 statistics software package.

Results

Figure 1 depicts rectal-temperature data by reference to treatment (MDMA or Saline) and the period before and after the time of administration (0 on the x-axis). It was observed that there were no differences in rectal temperature pre-treatment, and that, post-treatment, the rectal temperature of rats treated with MDMA was much higher than that of rats treated with saline serum, with this difference remaining in evidence until 5 h after the time of administration.

Figure 2 depicts the percentage weight lost by animals under the four conditions, taking subjects' weight at the start of the procedure as the initial value (100%). It was seen that, as from day 5, the animals in the control condition (Inactivity) started maintaining their weight, whereas the experimental subjects (Activity) continued to lose weight gradually. The ANOVA showed effect for Days [$F(7,196) = 610.20, p < 0.001$] and the Activity \times Days interaction [$F(7,196) = 16.51, p < 0.001$]. Nevertheless, neither the Treatment nor its interaction with the remaining factors was found to have any reliable effect. *Post hoc* through Newman-Keuls tests revealed differences between the activity and inactivity groups, both in days 1 and 2 ($p < 0.05$) and in days 5, 6 and 7 ($p < 0.001$).

Figure 3(top) corresponds to the amount of food in grams ingested by subjects in all groups across the days. From this, it was seen that all animals, regardless of their respective condition, tended to increase their food intake over the course of the procedure.

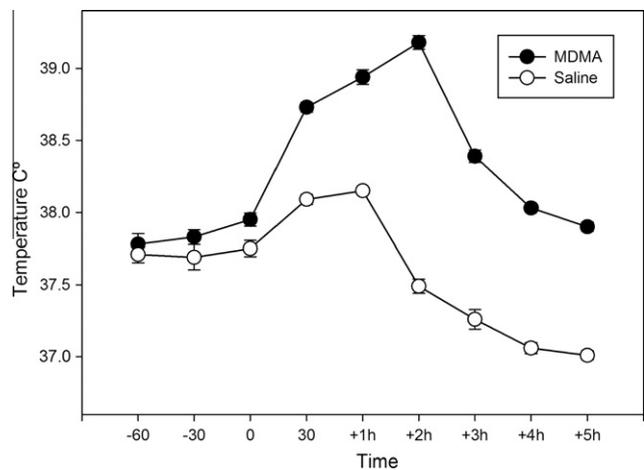


Fig. 1. Mean rectal temperature in $^{\circ}\text{C}$ (\pm SEM) measured for all animals by reference to treatment (MDMA or Saline), across the pre- and post-injection periods (0 on the x-axis).

While the statistical analysis revealed an effect for Days [$F(6,128) = 178.24, p < 0.001$], it showed no effects for either Activity or Treatment.

Figure 3(bottom) shows mean water consumption during the feeding period for all conditions. It was observed that: there was a slight increase in water intake in all groups, which appeared to stabilise after the fourth day; and, similarly, that subjects in the experimental condition maintained their water intake above that of controls. The ANOVA yielded effects for Days [$F(6,128) = 24.20, p < 0.001$] and Activity [$F(1,28) = 4.42, p < 0.05$].

Figure 4 depicts the wheel turns of subjects in the two experimental groups by reference to treatment (MDMA or Saline). It was seen that: there was a progressive increase in wheel turns across the procedure under both conditions (MDMA and Saline); and, similarly, that the group injected with MDMA engaged in more wheel-running activity than did the group injected with saline serum in all cases except the last day, in which the groups drew even. The ANOVA showed effects for Days [$F(6,84) = 42.66, p < 0.001$], and a trend towards statistical significance for Treat-

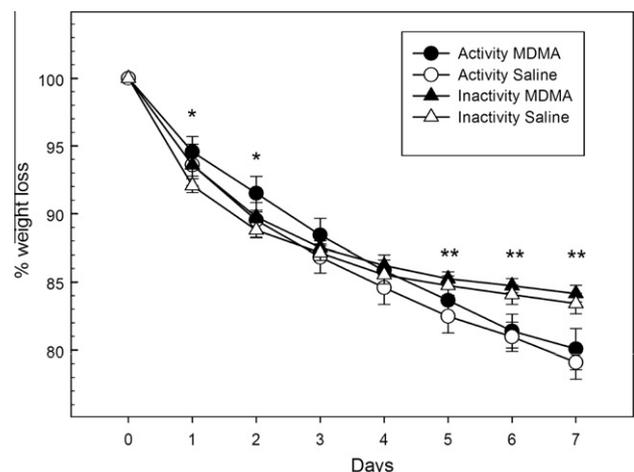


Fig. 2. Mean percentage body weight (\pm SEM) of the four groups of animals in each day across the experiment vis-à-vis their weight at the commencement of the procedure. Circles denote animals with wheel access (Activity), and triangles denote animals without wheel access (Inactivity). Black symbols mark the scores of animals that received the MDMA dose, and white symbols mark those of rats that received the saline injection. * $p < 0.05$, ** $p < 0.001$, for comparisons between activity vs. inactivity.

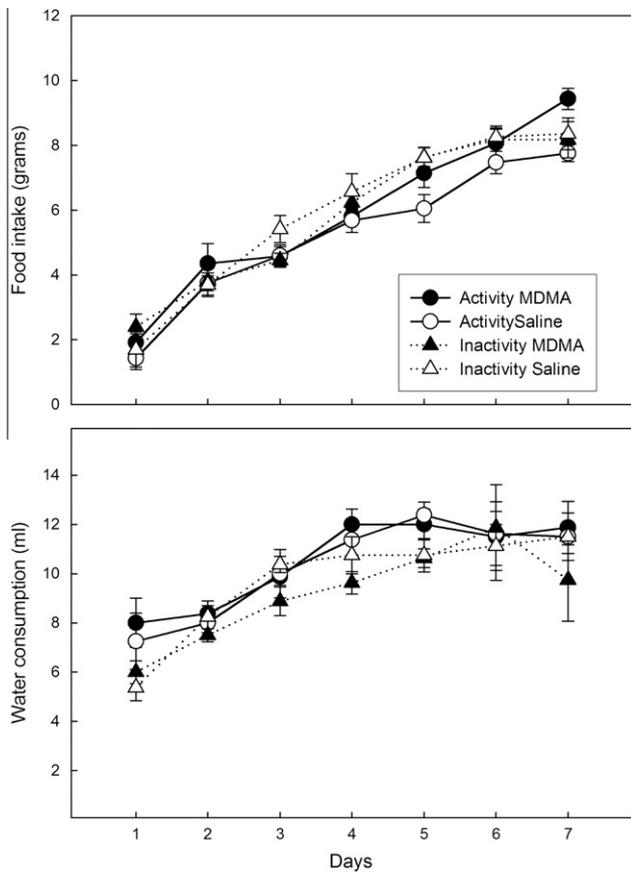


Fig. 3. Shown, at top, are mean grams of food (\pm SEM) and, at bottom, millilitres of water (\pm SEM) consumed during the feeding interval (3 h), both measurements having been taken during the experiment under all four conditions (Activity vs. Inactivity/MDMA vs. Saline) and for each experimental day.

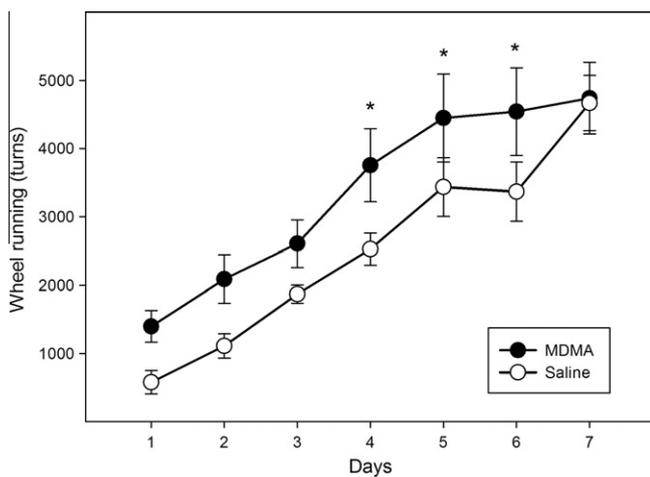


Fig. 4. Mean (\pm SEM) wheel turns registered by groups with wheel access during each day (21 h), across the procedure. Black symbols denote animals injected with a dose of MDMA, and white symbols denote animals that received an injection of saline serum. * $p < 0.05$.

ment [$F(1,14) = 3.97, p = 0.06$] which reflects the general greater activity of MDMA versus saline animals counteracted by their similar final levels of activity.

Figure 5 shows the mean millilitres of water consumed by animals in the two activity groups, recorded for each day over the 21 h during which they enjoyed free running-wheel access. In the initial days, the animals registered a high water intake, which gradually

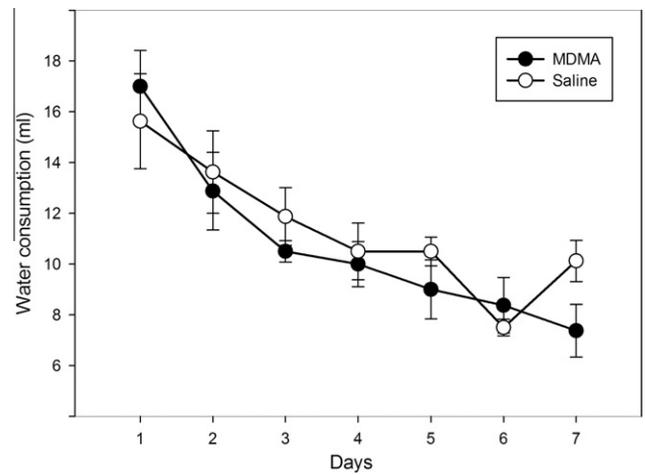


Fig. 5. Mean (\pm SEM) millilitres of water consumed by animals under conditions of activity in each experimental day during times of free wheel running access (21 h).

declined as the experimental procedure progressed, with rats injected with MDMA almost always displaying a slightly lower consumption. While the statistical analysis yielded effect for Days [$F(6,84) = 24.24, p < 0.001$], no reliable effect was obtained with respect to Treatment or Days \times Treatment interaction.

Figure 6 depicts the temporal distribution of the wheel turns given by experimental subjects over the 21 h of activity, with data shown at 15-min intervals. The information shown refers to the last four days of the ABA procedure, with the upper panels corresponding to animals that received a dose of MDMA and the lower panels to those that were injected with saline serum. The black bars on the x-axis indicate the beginning and end of each dark period (20:00–8:00 h), and the striped vertical columns indicate the food presentation period. It was seen that during the nocturnal period both groups registered an activity peak, which fell off from approximately halfway through until the end of the period and in respect of which the maximum wheel-turn values per time interval were recorded, with such values proving somewhat higher in the MDMA than in the saline animals. The activity performed from the end of food presentation until the beginning of the nocturnal period increased over the course of the procedure, reaching levels almost equal to those of nocturnal activity in the last day (particularly in the MDMA group). Lastly, a peak in wheel-running activity is to be observed just before food administration, a peak which continued to rise over the course of the days and during which greater activity was registered by the MDMA-treated rats. Insofar as licks to the water bottle were concerned, there was a high degree of parallelism between their occurrence and activity times (data not shown).

Discussion

Among the DA rats, exposure to the ABA/SIH procedure led to a considerable loss of body weight; this loss was not as pronounced and, indeed, ceased at levels of around 80–85% of their initial weight, among rats that had no access to the activity wheel but had the same restricted feeding schedule. Weight loss among the “activity animals” was very rapid, with the first rat having to be withdrawn from the procedure at day 7, on fulfilling the withdrawal criterion of presenting with a body weight below 75% of its initial value, a rate of decline substantially swifter than that normally seen in strains such as Wistar (e.g., 9 days in Pérez-Padilla et al. (2010)). Due to being extremely active and having low body weight, DA rats developed hyperactivity-induced semistarvation

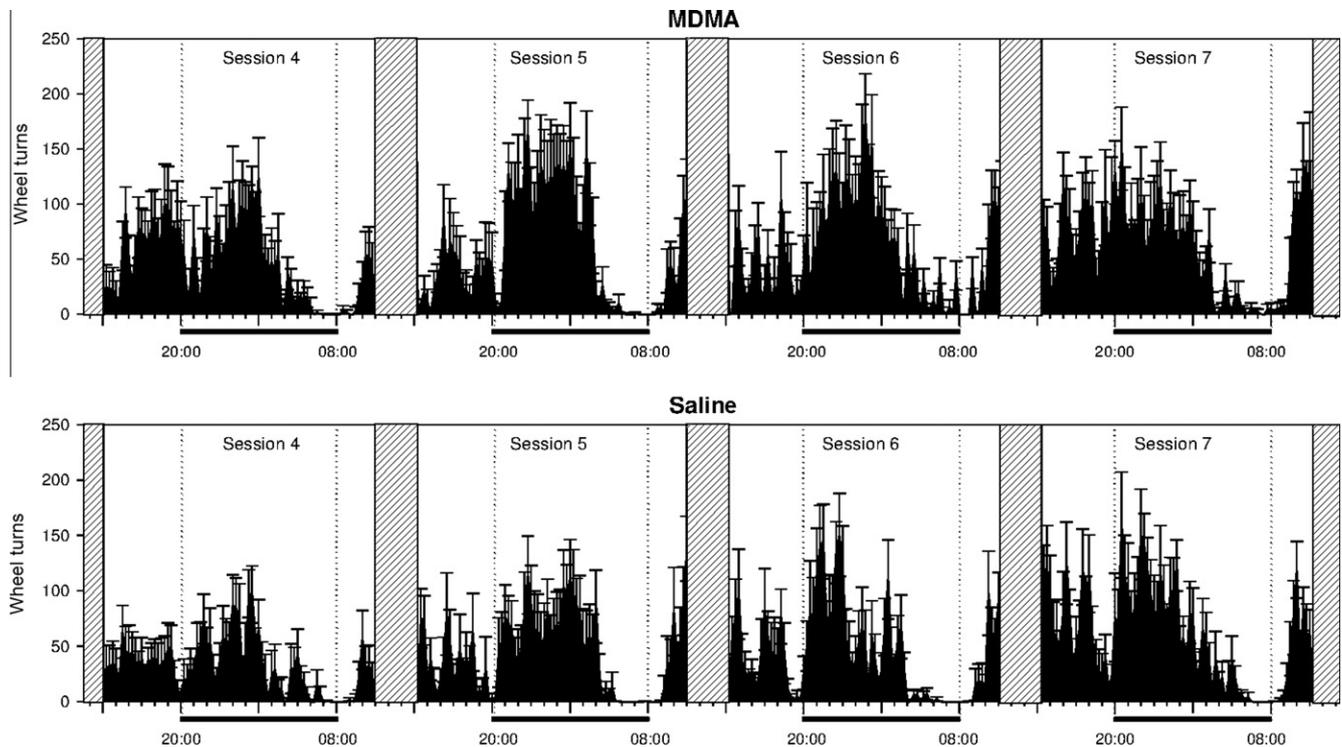


Fig. 6. Temporal distribution of wheel turns of groups of animals exposed to the activity-based anorexia procedure. Each point represents the mean number of turns (\pm SEM) every 15 min, with only the data for the last four days being shown, since these were the days during which the highest activity levels were recorded.

extraordinarily swiftly. The final activity levels displayed by these rats were higher than those normally found among Wistar rats (e.g., the more than 500 wheel turns per hour shown in Fig. 4 versus the 200 turns per hour reported in comparable conditions by Pérez-Padilla et al. (2010)). This is the first time that the development of ABA/SIH in DA rats is analysed, the data show a similar pattern to that found in Wistar rats but with an extremely rapid development and a reach of maximum running in fewer days and to a much higher level.

In terms of food consumption, all animals, regardless of experimental condition, were observed to register a comparable, gradual increase in intake across the procedure. In keeping with this it might seem more appropriate to consider the present results as an instance of SIH rather than ABA. The results show the typical pattern found in studies of ABA/SIH (e.g., Gutiérrez & Pellón, 2008; Pérez-Padilla et al., 2010), being this increase in food intake enough to stabilize the weight of the control subjects, something that, in the case of animals with wheel access, failed to prove sufficient to halt weight loss. Even though there were not statistical significance differences, after day 4 activity rats treated with MDMA seemed to had more food intake than activity saline rats (see Fig. 3 upper panel, black versus white circles). The absence of differences between these two groups in terms of weight loss (Fig. 2) despite differences in running (Fig. 4) could be related to small (but perhaps relevant) differences in food consumption. With regard to water consumption during feeding periods, there was a slight increase that tended to stabilise, with this increase being slightly higher among animals having wheel running access. The absence of relevant differences in food intake between the “activity” and “inactivity animals” indicates that the process of adaptation to the food schedule was similar across all groups and, consequently, cannot be accountable for the differential weight loss between animals with and without wheel access (for explanations of ABA as a failure in food-schedule adaptation due to interference from wheel-running activity, see Dwyer & Boakes,

1997; Kanarek & Collier, 1983). For the same reason (i.e., the lack of differences in terms of food intake between “activity” and “inactivity animals”), it is also difficult to attribute the development of SIH to any potential aversion to the taste of the food among the “activity rats” (Lett & Grant, 1996) or to the impact of a satiety signal associated with intense exercise (Pierce, Epling, & Boer, 1986). Excessive wheel-running activity is the most relevant factor in weight loss, though perhaps not for the reasons outlined above, all of which are linked to a decrease in food intake (for alternative explanations see Gutiérrez & Pellón, in preparation; Gutiérrez, Vázquez, & Boakes, 2002). The activity in this study, albeit intensely manifested at the commencement of the dark cycle and in the pre-feeding period, was likewise manifested in the post-feeding period, a factor that may differentiate DA from other rats and cause them to develop SIH more quickly. The activity in these three periods increased over the course of the successive experimental days, so that on the final day of the procedure all rats (treated and untreated with MDMA) showed an almost identical pattern of running.

Since the SIH phenomenon developed so swiftly among the DA rats, there was little room for observing effects of MDMA administration. Animals treated with MDMA and their saline controls displayed a similar loss of body weight, something that occurred under conditions of activity but also when comparing inactivity conditions. Similarly, there were no important differences in food and water intake when MDMA and saline were compared in animals with and without wheel access. With regard to wheel running, MDMA led to an increase in wheel-running activity compared to saline from the beginning to just the very last day of the procedure, where saline-treated rats increased considerably their activity to reach the level of MDMA-treated rats. MDMA rats were running at plateau during the last 3 days, thus MDMA seemed to facilitate the development of maximum running values faster than untreated rats. These higher levels of wheel running, however, did not further facilitate the development of SIH

measured by body-weight loss. Both active groups (treated and untreated with MDMA) run in excess; severe weight losses accompanied these excessive running, a plausible reason by which the extra running resulting from MDMA did not contribute to additional weight losses.

Earlier studies have repeatedly reported an increase in locomotor activity among rats exposed to MDMA (Balogh et al., 2004; Callaway et al., 1990; Roodsiri et al., 2011) and its long-term maintenance (Balogh et al., 2004; Walker et al., 2007), a finding which has been replicated here under the SIH procedure. Animals treated with MDMA registered elevated peaks of anticipatory activity preceding food presentation and a gradual increase in nocturnal activity, resulting in a greater flattening and longer duration of such nocturnal activity over the course of the successive days and, ultimately, in greater homogenisation of activity peaks across the day. The explanation for this fact may lie in the alteration of circadian rhythms, e.g., Ogeil, Rajaratnam, Redman, and Broadbear (2010) observed that acute administration of MDMA altered circadian rhythms and distribution of wheel-running activity.

In brief, the fact that DA rats developed SIH to such a marked degree and so swiftly – doubtlessly due to their excessive activity – prevented MDMA treatment from showing marked effects on the development of the phenomenon, notably on body-weight loss. The data reported here go to re-emphasise the crucial importance of activity in the development of SIH and related phenomena such as ABA.

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