

## Effect of auditory isolation on activity-based anorexia

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The objective was to study male and female rats exposed to Activity-Based Anorexia (ABA; simultaneous exposition to food restriction and free access to an activity wheel) under two conditions of sound attenuation, by means of different arrangements of wheels (standard versus isolation) in the laboratory room. Regardless of the sound attenuation condition, all but one male and one female with access to wheels had to be removed from the experiment, but extended ABA endurance was found in rats in the sound attenuation condition. Furthermore, significantly lower levels of running were observed in both males and females under the sound attenuation arrangement in the isolation condition. The results suggest that external stimulation plays a role in the activity displayed by rats exposed to ABA, and that the reduction of external stimulation diminishes running but does not protect rats from developing ABA.

*Efecto del aislamiento auditivo en la anorexia basada en la actividad.* El objetivo fue el estudio de ratas macho y hembra expuestas al procedimiento experimental de Anorexia basada en la Actividad (ABA, exposición simultánea a restricción de alimento y acceso libre a una rueda de actividad) bajo dos condiciones de atenuación acústica por medio de un arreglo experimental diferente de las ruedas de actividad (estándar versus aislamiento) en el laboratorio. Independientemente de la condición de la atenuación del sonido, todos los animales con acceso a las ruedas de actividad menos una rata macho y otra hembra tuvieron que ser retirados del experimento, mientras que la condición de atenuación del sonido aumentó la resistencia a ABA. Además, los niveles de actividad de machos y hembras fueron significativamente inferiores en la condición de atenuación del sonido en la condición de aislamiento. Los resultados sugieren que la estimulación externa desempeña un papel en la actividad desplegada por las ratas expuestas a ABA, y que aunque la reducción de la estimulación externa disminuye la actividad no protege a los animales del desarrollo de ABA.

Routtenberg and Kuznesof (1967) first described the self-starvation process subsequent to the simultaneous exposition of rats to limited food availability and unlimited running-wheel access resulting in extreme weight loss leading to death of most of the animals. This experimental procedure and the behavioral outcome was later denominated as Activity-Based Anorexia by Epling, Pierce, & Stefan (1983). Individual arrangement of the animals is the standard in ABA studies where animals previously group housed in the colony room are transferred to wheels and cages that are usually distributed in a row on shelves in the same room. Although this experimental rearrangement allows some visual, hearing and smelling contact among animals, physical contact is prevented by individual confinement to the cage and attached wheel. Social isolation inherent to this relocation of animals seems to be an important factor in ABA studies, as group housing alleviates ABA development in comparison to the typical individual arrangement in ABA studies (Boakes & Dwyer, 1997; Paré, Vincent, & Natelson, 1985). However, the adverse effect

of individual arrangement on ABA diminished when ambient temperature (AT) is raised to the thermoneutral zone (Gutiérrez, Baysari, Carrera, Whitford, & Boakes, 2006), indicating that group housing facilitates the regulation of body temperature as rats housed in groups had a tendency to cluster, at least during periods of low activity (Boakes & Dwyer, 1997).

Recent research has shown that having access to a warming plate (Hillebrand, Rijke, de Brakkee, Kas, & Adan, 2005), or raising AT to the thermoneutral zone (Gutiérrez et al., 2006) or above it (Morrow et al., 1997) prevented the development of ABA in rats, and reversed ABA once running had become excessive and the rats had already lost a considerable percentage (20%) of initial body weight (Cerrato, Carrera, Vázquez, Echevarría, & Gutiérrez, 2011; Gutiérrez, Cerrato, Carrera, & Vázquez, 2008; Gutiérrez et al., 2009). However, over the course of a series of published (Gutiérrez et al., 2008, 2009; Cerrato et al., 2011) and unpublished ABA studies (Cerrato, 2007), wheel running seems to be less intense in rats when wheels and cages are isolated in a wooden incubator, which provides a relatively isolated condition, in comparison with activity reported in ABA literature with the conventional setup of wheels in a row, as reviewed by Gutiérrez, Vázquez, & Boakes (2002). One probable factor affecting this apparently diminished activity in the animals sheltered in the incubators could be the attenuation of sound by the wooden walls of the incubator, as Campbell & Sheffield (1953) have shown that

activity in food-restricted male rats diminished under controlled external stimulation (noise and light). In this study rats were isolated in a cabinet and individually placed in a sound-proof room to make “doubly sure that auditory stimulation outside the cabinet would not affect Ss” (p. 320). However, activity was measured under different external stimulation conditions comparing only two 10-minute daily periods. Campbell & Sheffield (1953) reasoned that the activity of starved animals might be associated with a lowered response thresholds to external stimulation. However, surgically deafened animals (Hall, Smith, Schnitzer, & Hanford, 1953) submitted to food restriction show the same increase as intact ones (Hall & Hanford, 1954) although both groups remained in the dark for most part of the day.

To the best of our knowledge, only one study has addressed the effect of sound isolation of wheel activity under experimental conditions to those faced by rats exposed to ABA. In an attempt to disentangle the self-starvation effect described by Routtenbergh & Kuznesof (1967), Spatz & Jones (1971) tested the buffering effect of noise reduction reported by Campbell & Sheffield (1953) under the experimental conditions of food deprivation and wheel access used by Routtenbergh & Kuznesof (1967). Spatz & Jones (1971) compared two groups of food-restricted (1h/day) male rats with 22.5 h/day of wheel access in cages with an attached wheels either in a conventional arrangement named “communal” (“wheels together in a table” p. 315), or in an individually isolated disposition where wheels were placed in an isolation box (approx. 70×70×50 cm) “made from 1-inch-thick Celotex and lined with fibre-glass insulation” (p. 315). The results revealed that only the communal group self-starved (food intake  $\leq$  1g/day) and died whereas all the isolated rats reached the recovery criterion. Furthermore, whereas in the Campbell and Sheffield study external auditory stimulation was totally absent in the isolation condition, Spatz & Jones (1971) studied both conditions in the same room though there is no data on the sound attenuation achieved by the isolation boxes. Furthermore, in the two previously mentioned studies only male rats were employed and up to date no study has compared males and female rats under both arrangements.

Thus, the aim of this study was to assess the influence of wheel arrangement in laboratory conditions (isolated or standard), and the impact of external stimulation (light and noise). However, both Campbell & Sheffield (1953) and Spatz & Jones (1971) used male rats heavier than those regularly used in ABA studies (310-410 g and 317-319 g respectively), and these subject characteristics have been reported to influence ABA outcome as older (Paré, 1975) and heavier (Boakes & Dwyer, 1997) rats exhibited lower levels of running activity and weight loss in comparison to their younger and lightweight counterparts. Moreover, since ABA performance is influenced by gender (Boakes, Mills, & Single, 1999) and preliminary evidence indicates that females are differently influenced by isolation (Gutiérrez et al., 2008; Cerrato et al., 2011), this study involved young adult rats of both genders.

## Method

### *Subjects*

A total of 64 Sprague-Dawley 40-day old rats, 32 females (weight range 182-243 g) and 32 males (weight range 205-242 g), obtained from the University Animal Resources Centre were used in the study. Animals were grouped housed (4 rats per cage) and

were handled and weighed daily for four days in the colony room with food and water ad libitum on a 12-hr light–dark cycle, with lights on from 08:00 to 20:00 h, and an ambient temperature set at 21 °C. After the fourth-day colony room adaptation period, both males and females were grouped by weight before being assigned to one of four conditions with eight animals in each: Active Isolated (ACT-IS); Active Standard (ACT-ST); Sedentary Isolated (SED-IS); Sedentary Standard (SED-ST), and then transferred to the laboratory where the AT was set at 21 °C. Animals were weighed daily at the start of the feeding period of each experimental day. The ethics committee on the use and care of animals of Santiago de Compostela University approved all described procedures. The experiment was carried out in accordance with the European Communities Council Directive of 24 November 1986 and D.L. of 27 January 1992 no. 116 (86/609/EEC) on the protection of animals used for experimental and other scientific purposes.

### *Apparatus*

The laboratory contained 16 Wahman-type activity wheels (1.12-m circumference, 10-cm-wide running surface of 10-mm wire mesh bounded by clear Plexiglas walls). These were attached to acrylic cages (28 × 28 × 14 cm). A sufficient number of same size (28 × 28 × 14 cm) acrylic cages without attached wheels were employed for Sedentary animals. Sixteen wooden incubators (60 × 60 × 60 cm) with polycarbonate roofs, provided with a 150W heat wave lamp connected to a thermostat and a probe positioned at the level of the animal, which allowed individual control of ambient temperature for each animal were available for the Isolated condition. Sufficient rooms with control of ambient temperature were not available to test all animals at the same time, so all the female animals were run first and then the study was repeated with males.

### *Procedure*

Active rats were housed individually in acrylic cages with attached running wheels and Sedentary animals were housed in acrylic cages of the same size as Active animals. The experiment was run in two separate rooms of similar dimensions with an air-conditioned system for the control of ambient temperature. In the standard arrangement (ST) condition all the running wheels for Active animals and the cages for Sedentary animals were arranged in a row on shelves mounted at the same distance from the floor (1 m). Each set of wheel attached cages for the active animals and cages in the case of sedentary animals were 30 cm apart and separated by a thin cardboard panel (70 × 50 × .5 cm) that prevented animals from seeing each other although these panels did not prevent animals from hearing and smelling each other. In the Isolation (IS) arrangement condition wheels and cages were placed inside wooden incubators (60 × 60 × 60 cm) with polycarbonate roofs. Before ABA, the Active animals were given access to the running wheels for 2 h per day for a three-day period while sedentary rats remained in their cages.

ABA procedure began on Day 0 with the removal of food at 14:00h for both for Active and Sedentary groups. At the same time the doors of the running wheels were opened for the Active rats. For the next two weeks all rats were fed for 1.5-h daily between 13:00 and 14:30h and the doors of the wheels were closed during this feeding period. Food intake was measured by weighing the

food at the beginning and end of every feeding period. Weight and wheel turns were recorder daily before the feeding period. Animals were removed from the experiment if their weight dropped below 75% of body weight on Day 0 for two consecutive days. Animals were considered to be recovered if body weight on any particular day, Day n, was greater than the weight of the animal four days before, Day n-4. The day on which an individual rat met one of the above mentioned criteria was considered the Last Day for the participation of the rat in the experiment. Sedentary animals were withdrawn according to the outcome achieved by the Active animals they were yoked to. The mean room temperature was 20.7 °C (range 19.1-21.2 °C) and 20.6 °C (range 19.3-21.1 °C) respectively for the rooms where the ST and IS arrangements were run, and the temperature ranged between 20-21 °C inside the incubators.

*Statistical analyses*

Statistical analyses were performed with the SPSS 15.0 package. The four dependant variables were: body weight, food intake, wheel turns, and recovery/removal outcome according to the recovery or removal criteria defined above. Body weight (g), food intake (g), and wheel turns (distance in meters= wheel turns × 1.1m) in both males and females were analyzed using analysis of variance (ANOVA), with Activity (Active/Sedentary) and Arrangement (ST/IS) as independent factors, and with repeated measures over the days until the first rat was removed in order to have an equal number of animals for each group. Univariate ANOVAs were used to assess any group differences on Day 0 and Last Day with the two factor being activity (Active vs. Sedentary) and arrangement (Standard vs. Isolation). Days to removal were examined using non-parametric analysis (Mann-Whitney U-test). An alpha-rate of 0.05 was used for all analyses.

**Results**

Measures of noise in the two wheel arrangements revealed a significantly noisier environment in the ST arrangement of wheels and cages than in the IS, as can be seen form the differences in average peak levels of noise over the last five days (MEAN ± SEM, 66.7 dB ± 1.1 and 58.8 ± 0.7 dB respectively), *p*<.0001. Measures were obtained using the data logger sound level meter model PCE-322A (Tursdale Technical Services Ltd, Durham, UK).

*Females*

The assignment of the animals to the different groups did not yield differences in body weight on Day 0 as revealed by the univariate ANOVA, neither for the Activity (MEAN ± SEM, 202 ± 1.99 g, and 203 ± 2.03 g, respectively for ACT and SED groups), nor the Arrangement conditions (MEAN ± SEM, 204 ± 2.06 g and 200 ± 1.84 g, respectively for the IS and ST conditions), or for the interaction of these factors (all *p*>.05).

Once ABA had started, all ACT animals lost weight and there were differences in days to meet the removal criterion between ST and IS animals. As shown on Table 1, six animals from the ST group reached the removal criterion on Day 6 (median 6 days, range 6-9), and a day later the first rat from the IS group was removed (median 10 days, range 7-12 days), Mann-Whitney U= 2.5, *p*= .001. Furthermore, one rat from the IS group did not reach

the removal criterion as its weight fluctuated without meeting the survival criterion during the two-week period that ABA was maintained active. Thus the number of IS rats for analysis on the Last Day was 7.

Figure 1 (upper panel) shows the mean body weight (±SEM) measured as percentage of Day 0 body weight for the four groups of female rats over the first days before the first animal was removed in the active condition, and the Last Day's body weight when animals reached the removal criterion. A 2 × 2 × 2 ANOVA over Day 0 and the first six days (body weight in grams) detected no main effects for the Arrangement and Activity factors, both *p*>.05. Significant results were identified for Arrangement × Days interaction, F (6, 168)= 10.090, *p*<.001, indicating that rate of body weight loss was faster for ST animals. Also a significant interaction for the Activity factor was detected, F (6, 168)= 16.165, *p*<.001, indicating that ACT animals lost weight faster than SED animals. Finally, there was a significant result for the Arrangement × Activity interaction over days, (6, 168)= 4.948, *p*<.02, indicating that having access to the wheel differently affected IS and ST animals. In order to further explore this interaction, a repeated measures ANOVA was conducted separately for IS and ST animals. This analysis revealed that access to the wheel had only a significant effect on weight loss rate for ST animals as shown by a significant interaction for the Activity factor over days F (6, 84)= 19.380, *p*<.001 (all other effects for the ST and IS were not significant, *p*>.05). As it can be appreciated on the upper panel of Figure 1, differences in body weight loss rate were evident on Day 6, where body weight for ACT-ST animals was significantly lower than in ACT-IS animals [148.1 ± 5.32 g vs. 169 ± 4.47 g, *t* (14)= 3.21, *p*= .007].

A univariate ANOVA for Last Day revealed that ACT animals lost more weight than SED animals, F (1, 31)= 60. 830, *p*<.001, and that ST animals lost more weight than IS animals, F (1, 31)= 6. 170, *p*<.02. The interaction Arrangement × Activity was not significant, *p*>.05.

Figure 1 (medium panel) shows the daily mean distance (±SEM) run by both ACT-ST and ACT-IS females during days before the first animal was removed as well as the activity for the Last Day. Differences in wheel arrangement had a main effect on activity during these days, F (1, 14)= 4.80, *p*= .046, indicating that overall activity was greater in the ST group. The presence of an interaction effect, F (5, 70)= 8.52, *p*= .001, revealed that the level of activity over days increased at a faster rate for the ACT-ST group than for the ACT-IS group, mainly due to the sudden jump of activity displayed by ST animals on Day 6. A two tailed Student *t* test for Day 6 revealed that the IS animals ran on average (± SEM) almost a third of the distance run by the ST animals [4097.38 ± 1058.35 m

*Table 1*  
Rats on running wheels reaching the removal criterion

		Days to reach the removal criterion									
		5	6	7	8	9	10	11	12	13	14
Female	ACT-IS			1	1		3	1	1		
	ACT-ST		6	1		1					
Male	ACT-IS			2		1	2			1	1
	ACT-ST	2	4	2							

Abbreviations: Active Isolated (ACT-IS); Active Standard (ACT-ST). In both female and male ACT-IS groups n = 7 (see text)

vs.  $11198.5 \pm 1911.59$  m, respectively,  $t(14) = 9.39$ ,  $p = .004$ ]. On the Last Day the ACT-ST animals [ $148.1 \pm 5.32$  g vs.  $169 \pm 4.47$  g,  $t(14) = 3.21$ ,  $p = .007$ ].

With respect to food intake on Day 0, after the three-day period of pre-exposure to the wheel (2h/day), an univariate ANOVA revealed that food intake was greater for ACT than SED animals,  $F(1, 31) = 18.262$ ,  $p < .001$ . Food intake did not differ for the Arrangement condition, and the interaction Arrangement  $\times$  Activity was not significant, all  $p > .05$ . Figure 1 (lower panel) shows the mean daily food intake ( $\pm$ SEM) for female rats over days before the first animal was removed in the active condition, and for the Last Day of participation in the study when animals reached the removal criterion. An ANOVA revealed significant results for the Arrangement factor: main effect  $F(1, 28) = 4.400$ ,  $p = .045$ , interaction  $F(5, 140) = 6.589$ ,  $p < .001$ , indicating that overall food intake and the rate of increase were higher in IS animals. Thus, on Day 6 food consumption observed in ACT-IS animals significantly differed from that of the ACT-ST rats [MEAN  $\pm$  SEM  $7.59 \pm .43$  g vs.  $5.08 \pm .67$  g,  $t(14) = 3.47$ ,  $p = .001$ . Also, a significant main effect (but no interaction) was detected for the Activity factor,  $F(1, 28) = 45.845$ ,  $p < .001$ , indicating that overall food intake was greater

in the SED group. During the six-day period ACT animals ate almost 30% less than their SED counterparts (a 29.7% and a 27.2% respectively for the IS and ST arrangements). The Arrangement  $\times$  Activity interaction was not significant,  $p < .05$ .

Concerning the univariate ANOVA for the Last Day, the Arrangement factor was significant,  $F(1, 31) = 14.738$ ,  $p = .001$ , while the Activity factor only approached statistical significance,  $F(1, 31) = 3.228$ ,  $p = .083$ , and the interaction Arrangement  $\times$  Activity was not significant,  $F(1, 31) = 2.205$ ,  $p > .05$ .

### Males

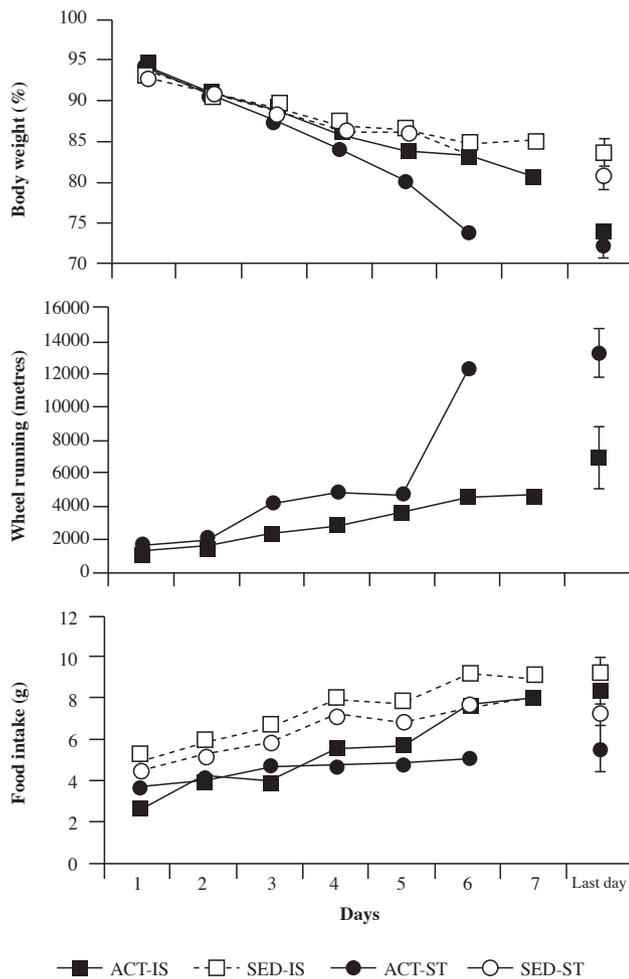
The assignment of the animals to the different groups did not yield differences in body weight on Day 0, as revealed by the univariate ANOVA, neither for the Activity (MEAN  $\pm$  SEM,  $236.1 \pm 3.01$  g and  $239.2 \pm 2.68$  g respectively for ACT and SED groups), nor the Arrangement factor (MEAN  $\pm$  SEM,  $237.9 \pm 2.87$  g and  $237.4 \pm 2.89$  g for the ST and IS groups respectively), or for the interaction of these factors (all  $p > .05$ ).

As was the case with females, one male rat from the IS group did not reach the removal criterion during the two-week period in which ABA was maintained active, whereas all ST rats had to be removed (see Table 1). As for the rats that met the removal criterion, two rats were removed on Day 5 from the ST group (median 6 days, range 5-7 days) while the first two rats reached the removal criterion for the IS group on Day 7 (median 10 days, range 7-14 days). Non-parametric analysis of the data revealed a significant U effect = 2.00,  $p = .001$ .

Figure 2 (upper panel) shows the mean body weight ( $\pm$ SEM) measured as percentage of Day 0 body weight for the four groups of male rats over days before the first animal was removed in the active condition (five days for the ST groups and seven days for the IS groups), and the body weight for the Last Day of participation when animals reached the removal criterion. Similar tendencies observed in female were found in male animals. A  $2 \times 2$  ANOVA over Day 0 and the first five days detected no main effects for the Arrangement and Activity factors, all  $p > .05$ . A significant interaction for the Activity factor was detected,  $F(5, 140) = 12.804$ ,  $p < .001$ , indicating that ACT animals lost weight faster than SED animals. Also, a significant interaction for the Arrangement factor was detected,  $F(5, 140) = 8.650$ ,  $p < .01$ , indicating that ST animals lost weight faster than IS animals. The Arrangement  $\times$  Activity over days interaction was not significant,  $p > .05$ .

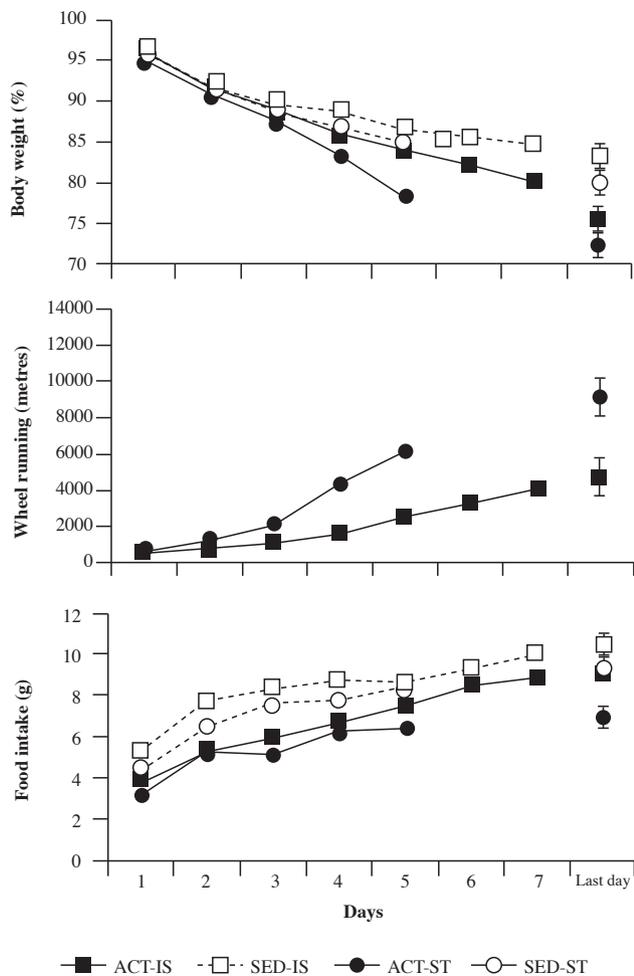
Concerning the univariate ANOVA for the Last Day, the Activity factor was significant,  $F(1, 29) = 34.437$ ,  $p < .001$ , indicating that ACT animals lost more weight than SED animals. The Arrangement factor (ST vs. IS) and the Activity  $\times$  Arrangement interaction did not yield significant differences in Last Day body weight, both  $p > .05$ .

As for the running activity, a repeated measures ANOVA revealed significant results for the Arrangement factor, main effect  $F(1, 14) = 10.167$ ,  $p = .007$ , and interaction  $F(4, 56) = 9.503$ ,  $p = .004$ , indicating that overall running activity, and its rate of increase, were higher for ST animals. Thus, ST animals showed a twofold increase in running over the last five days in comparison to the IS animals. Figure 2 (medium panel) shows the daily mean distance ( $\pm$ SEM) run by these two groups of males with wheel access during days before the first animal was removed, and for the Last Day of participation when animals reached the removal criterion. This difference in running was maintained on the Last Day,  $t(13) = 2.81$ ;  $p = .015$ .



**Figure 1.** Body weight, wheel running and food intake over days for female rats.

Abbreviations: Active Isolated (ACT-IS); Active Standard (ACT-ST); Sedentary Isolated (SED-IS); Sedentary Standard (SED-ST). For Last Day  $n = 7$  for the ACT-IS group



**Figure 2.** Body weight, wheel running and food intake over days for male rats.

Abbreviations: Active Isolated (ACT-IS); Active Standard (ACT-ST); Sedentary Isolated (SED-IS); Sedentary Standard (SED-ST). For Last Day  $n=7$  for the ACT-IS group

Similar to female animals on Day 0, food intake for ACT males after the three-day period of pre-exposure to the wheel (2h/day) was lower than for SED animals as shown by the univariate ANOVA,  $F(1, 31) = 4.747$ ,  $p = .038$ . The Arrangement factor and the Activity  $\times$  Arrangement interaction did not reach significance, both  $p > .05$ . Figure 2 (lower panel) shows the mean daily food intake ( $\pm$ SEM) for male rats, during days before the first animal was removed in the active condition and for Last Day of participation when the animals reached the removal criterion. The higher food intake of SED animals was maintained throughout the five days shown in Figure 2, as shown by the  $2 \times 2$  repeated measures ANOVA, main effect  $F(1, 28) = 17.323$ ,  $p < .001$ ; interaction  $p > .05$ . During this period, ACT animals ate almost 25% less than their SED counterparts (24.9% and 22.3% for the IS and ST arrangements respectively). Food intake over days did not differ between ST and IS groups as shown by the non significant results for the Arrangement condition for both the main effect and interaction,  $p > .05$ . The Activity  $\times$  Arrangement interaction was not significant,  $p > .05$ . For the Last Day, a univariate ANOVA showed a significant effect for both the Arrangement,  $F(1, 30) = 4.761$ ,  $p = .038$ , and the Activity condition,  $F(1, 30) = 7.364$ ,  $p = .012$ , indicating that

Last Day food intake was greater for the IS and the SED animals respectively. The Activity  $\times$  Arrangement interaction was not significant,  $p > .05$ .

## Discussion

There have been contradictory reports on whether sound attenuation prevents or has no effect on the activity of starved rats. The present study presents advantages in terms of procedure (isolation) and selection of subjects (age) in comparison to previous reports, documenting the effect for both male and female rats. The main finding of this study was that running was significantly reduced in both male and female IS rats. However this fifty percent reduction in running activity did not prevent progressive weight loss in both males and females given that all the animals but one reached the 25% weight-loss removal criterion during the 2-week period of ABA exposure. The higher running displayed by the ST animals in comparison with IS animals lends support to the view that external stimulation is a factor modulating the intensity of wheel running, and that noise generated by the turns of the contiguous wheel has a facilitation effect on running. These experimental findings corroborate the lower levels of running reported in rats when wheels and cages were isolated in a wooden incubator providing an attenuation of the noise produced during wheel running by other animals in the same room (Gutiérrez et al., 2008, 2009; Cerrato, 2007; Cerrato et al., 2011) in comparison to activity reported in the ABA literature with the conventional wheel setup in a row. Bearing in mind that the ST arrangement employed in our study is the standard wheel setup employed in practically all ABA studies performed up to date, this facilitation effect is inherent to the excessive running reported in these studies.

In spite of their poorer meal efficiency, animals in the ST arrangement notably exceeded the running observed in IS animals. It is worth noting that on the day the first animals were removed, Day 5 and 6 for males and females respectively, body weight loss and running activity for ACT-IS animals were significantly below the levels for ACT-ST animals (all  $p < .05$ ), while food intake for ACT-IS animals was higher than in their ACT-ST counterparts, and even matched that of female SED-ST animals. Similarly, the effect of the IS arrangement was evident in both female and male animals in terms of running activity and food intake on the Last Day, when the animals reached the removal criterion (100% in the ST groups and 7 out of eight animals in the IS group).

Besides the effect of Activity, the Arrangement factor also had a main effect on body weight on the Last Day in females, but not in the case of males. Isolation facilitated food intake in both female and male active rats (Fig. 3 and 6), particularly on females which on Day 6 paralleled the food intake of SED-ST rats, showing their better protection to cope with the energy demands arising from voluntary wheel running (Carrera, Cerrato, Vázquez, Sineiro, & Gutiérrez, 2011). However, in spite of the slower weight loss trend, the lower running activity levels and the higher food intake of IS rats in comparison to the ST animals did not prevent IS rats from reaching the removal criterion.

Despite the low running levels of IS animals, it appears that the limited access to food imposed by the restricted feeding schedule did not enable the animals to escape from the negative energy imbalance brought on by the unlimited access to the wheel. This negative net energy imbalance was higher for the ACT-ST females due to their poorer meal efficiency which in conjunction with the

sudden jump in running activity and the corresponding accelerated weight loss had deleterious consequences for six of the eight animals which had to be removed from the study on Day 6. This exponentially increase in activity in the context of a reduced food intake is indicative of a “strong anorexia effect” (Epling & Pierce, 1984) in which thermoregulatory needs seem to play a central role (Gutiérrez et al., 2002).

Thus, deficient food consumption might not involve so much a failure to adapt to the restricted feeding schedule (Boakes & Dwyer, 1997), but a ceiling effect that impedes greater food ingestion during the 1.5 h/day feeding time. The consequences of this ceiling effect with respect to the amount of food that rats are able to digest during the feeding time can be appreciated in the evolution of food intake and body weight loss of Sedentary rats. Both Sedentary males and females lost weight throughout the study despite reduced activity in the stationary cage.

In summary, our findings suggest that the attenuation of auditory stimulation provided by the isolated wheel arrangements had a marked effect on the running activity of rats, although isolation did not prevent rats from the ABA outcome (25% body weight loss) and only one rat survived in each group of males and

females. This low rate of survival contradicts the 100% survival rate reported by Spatz & Jones (1971) in their isolated animals, and the 50% survival of animals in “communal” arrangement, similar to our ST arrangement. In all likelihood, the discrepancy with the results of Spatz & Jones (1971) is due to the fact that these authors employed older and heavier rats than those used in our study, factors which are crucial for ABA endurance. Furthermore, half of the male rats surviving the “communal” arrangement in Spatz & Jones (1971) showed a much lower running activity levels than our younger males whilst communal rats reaching the removal criterion exhibited running activity levels similar to those found in our IS animals, revealing that the outcome of rats subjected to ABA is primarily dependent on running.

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