TESIS DOCTORAL

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A mis padres

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Madrid, a 1 de octubre de 2019

"*A vague sense of order emerges from any sustained observation of human behavior*"

B.F. Skinner, 1953

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ABBREVIATIONS, ACRONYMS, AND SYMBOLS

%	Percentage
ºC	Celsius degrees
ABA	Activity-based anorexia
AN	Anorexia nervosa
ANOVA	Analysis of variance
APA	American Psychological Association
BMI	Body mass index
cm	Centimeter
DA	Dark Agouti rat
DSM	Diagnostical and Statistical Manual of Mental Disorders
FAA	Food-anticipatory activity
FD	Female diet
FT	Fixed-time
FW	Female wheel
g	Gram
g/day	Grams per day
h	Hour
I group	Immediate group
ICD	International Classification of Diseases
L group	Large interval group
M	Mean
M group	Medium interval group

MD	Male diet
MDMA	3,4-methylenedioxymethamphetamine
mg/kg	Milligram per kilogram
MHz	Megahertz
min	Minute
mL/kg	Millilitre per kilogram
MW	Male wheel
NaCl	Sodium Chloride
NIMH	National Institute of Mental Health
PFA	Post-food activity
PR	Progressive ratio
s	Second
SEM	Standard error of the mean
SIH	Semistarvation-induced hyperactivity
SPSS	Statistical Package for the Social Sciences
VT	Variable-time

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ABSTRACT

Anorexia Nervosa (AN) is a disorder characterized by the high failure of treatment approaches, both psychological and pharmacological. The low rate of clinical success may be due to a mischaracterization in most commonly used classifications, which implies a therapeutic approach focused on non-nuclear symptoms and delayed diagnosis. Activity-based anorexia (ABA) protocol has been widely accepted as an animal model of the disorder. Experimental evidence in ABA shows that excessive activity could be a crucial factor in the development of the phenomenon. The aim of this presentation is to review the results from animal research using the ABA model with an emphasis on the evidence and possible explanatory mechanisms of this excessive activity. Results obtained in our laboratory suggest that the combination of food restriction and exercise is the way to develop anorexia. Increased activity is a common foraging response in mammals subjected to food restriction. This activity in humans is expressed more frequently under diet, which facilitates its subsequent increase by mechanisms of reinforcement and induction. It has been proposed that cultural contingencies encourage people to be involved in diet regimes and exercise, which in some individuals may lead to the combination of strong food restriction and hyperactivity, initiating the cycle of anorexia. This analysis is in line with historical descriptions of the disorder and new clinical and research evidence that reports an excessive physical activity in a high proportion of diagnosed patients. Based on basic research data of several studies that point in the same direction, it is proposed a different framework that can guide future research and clinical approaches to AN.

RESUMEN

La anorexia nerviosa (AN) es un trastorno caracterizado por el alto fracaso de los enfoques de tratamiento, tanto psicológicos como farmacológicos. La baja tasa de éxito clínico puede deberse a una caracterización errónea en las clasificaciones más utilizadas, lo que implica un enfoque terapéutico centrado en síntomas no nucleares que resultan en un diagnóstico tardío. El protocolo de anorexia basada en la actividad (ABA) ha sido ampliamente aceptado como un modelo animal del trastorno. La evidencia experimental en ABA muestra que la actividad excesiva podría ser un factor crucial en el desarrollo del fenómeno. El objetivo de esta tesis es revisar los resultados de la investigación en animales utilizando el modelo ABA enfatizando de la actividad y sus posibles mecanismos explicativos. Los resultados obtenidos en nuestro laboratorio sugieren que la combinación de dieta y ejercicio es el camino para desarrollar anorexia. El aumento de la actividad es una respuesta común de forrajeo en mamíferos sujetos a restricción alimentaria. Esta alta actividad en los humanos que se expresa con mayor frecuencia bajo la dieta, facilita su aumento posterior mediante mecanismos de refuerzo e inducción. Se ha propuesto que las contingencias culturales alientan a las personas a participar en regímenes de dieta y realizar ejercicio intenso, lo que en algunos individuos puede conducir a la combinación de una fuerte restricción de alimentos e hiperactividad, iniciando el ciclo de la anorexia. Este análisis está en línea con las descripciones históricas del trastorno y la nueva evidencia clínica y de investigación que encuentra una actividad física excesiva en una alta proporción de pacientes diagnosticados. Basado en datos de investigación básica de varios estudios que apuntan en la misma dirección, se propone un marco diferente que puede guiar la investigación futura y los enfoques clínicos de la AN.

"*No es la conciencia del hombre la que determina su ser sino, por el contrario, el ser social es lo que determina su conciencia*."

Karl Marx, 1859

CHAPTER 1:

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Anorexia Nervosa

Anorexia nervosa (AN) is not a new disorder of our era. The behaviors that characterize this disorder, persistent restriction of food intake, accompanied by a pronounced weight loss that puts survival at risk, have been present throughout history. Fasting as a form of purification is part of human traditions since prehistory, being a common practice in all religions and cultures. This fact was especially evident during the middle ages, a period in which the suppression of food, anorexia mirabilis, was understood as a pathway to holiness (Bell, 1985), or from the Renaissance to the Victorian era, where it was considered a miracle that the languid and delicate “fasting girls” survived during a long period of starvation (Brumberg, 1988; Bemporad, 1996). However, these practices soon began to be seen as a symbol of demonic possession, later a fraud, and gradually became associated with mental illness. Already in the seventeenth century the observation of symptoms similar to modern anorexia began to attract medical attention. Morton (1689) described two cases that presented lack of appetite, weight loss, amenorrhea, constipation and hyperactivity without medical alterations that justify what he called "nervous consumption” (Pearce, 2004; Silverman, 1983). Its cause was attributed to "violent Passions of the Mind", which altered the nerves. The parallel works of Lasègue (1873) and Gull (1874) contributed to consolidate the idea that the origin of the disorder resided in psychological causes by coining its denomination, "Anorexia Nervosa", which is still used today (Walter & Van Deth, 1989).

Since the late nineteenth century, the predominant descriptions from the medical tradition considered the disorder as a disease related primarily to endocrine imbalances.

These descriptions were followed by psychoanalytic explanations that attributed the disorder to psychogenic causes, which would be both its distinctive mark and its cause. From this perspective, these clinical manifestations were attributed to conflicts in the family environment at an early age (Bergh & Södersten, 1998; Pearce, 2004; Richardson, 1939; Waller, Kaufman, & Deutsch, 1940). The 60s of the 20th century were marked by the works of Hilde Bruch, who suggested that the origin of AN would be an alteration in the perception of body image (Bruch, 1962, 1966). This approach coexisted with the rise of behaviorism and the application of operant schedules that established contingent rewards for weight recovery, as well as with the start of psychopharmaceutical treatments. The configuration of a multidimensional vision of AN would culminate around the 80s with the inclusion of sociocultural factors as determinants of its development, such as the pressure towards diet and thinness as an ideal of beauty (Garner & Garfinkel, Schwartz, & Thompson, 1980, Garner & Garfinkel ,1982). This evolution in the conception of AN is reflected in the diagnostic classification systems of greater use in the medical, psychological and psychiatric field. Thus, it appears in the first edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-I; American Psychiatric Association, APA, 1952) as a psycho-physiological reaction. The DSM-II (APA, 1968) moved it to "Special Symptoms - Feeding Disturbances".

In 1980, a new eating disorders section was finally created for the DSM-III (APA, 1980), an edition in which AN was presented as an adolescent onset syndrome with an operational criterion on an alleged empirical basis, under the influence of the Feighner (1972) criteria. The most commonly used editions, DSM-IV-TR (APA, 2002) and ICD-10 (International Classification of Diseases, World Health Organization, 1992) articulate the disorder around three essential characteristics: a) a reduction in intake that leads to a

dramatic weight loss (body weight less than 85% of that expected or BMI ≤ 17.5), b) the presence of a physiological alteration of endocrine system, such as the presence of amenorrhea in women, and c) intense fear to gain weight accompanied by a distortion in the perception of the body itself.

The amenorrhea criterion, which was necessary to obtain a diagnosis of AN in post-menarcheal women, has disappeared in the recent DSM-5 (APA, 2015) having found that women who fit the diagnosis of AN but have not lost their menstrual cycle do not differ clinically from those that have been diagnosed and do not menstruate (Attia & Roberto, 2009). In addition, this change responds to the fact that it is not a reliable indicator of weight status (Anderson, Bowers & Watson, 2001) and it is not applicable to males, prepubertal, postmenopausal women and women using hormone replacement therapy (Attia & Roberto, 2009). The remaining aspects that make up the syndrome remain virtually unchanged, with changes more to the form to the content. On the one hand, the minimum value of 85% has been eliminated to consider normal weight, thus trying to avoid the misunderstanding that a low body weight can be defined by a specific numerical value. On the other hand, the presence of behaviors evidencing the individual's willingness to lose weight is considered sufficient, even if they do not explicitly declare it (Call, Walsh, & Attia, 2013). These “aesthetic” changes possibly respond to the criticisms that have been made regarding the arbitrariness, the lack of predictiveness of the treatment results, and the insensitivity to aspects such as race, sex, age and the severity of the clinical diagnosis by the use in the DSM-IV-TR (APA, 2000) of a cut-off point with respect to the expected normal weight (Fairburn et al., 2007). Likewise, other authors have criticized that voluntary control of food intake and the distorted perception of body image and weight are essential factors for the maintenance of the disorder (Cooper, 2007;

Fairburn, Shafran, & Cooper, 1999; Russell, 2003). In previous research, it has been suggested that the common assumption that AN is a disorder caused by dissatisfaction with body image could be only axiomatic and the emphasis on this aspect would be a hindrance to recovery (Zanker, 2009). In addition, it has been claimed that the role of the thin ideal has been overrated and there is a widespread misperception about the volitional nature of the disorder (Striegel-Moore & Bulik, 2007). In this sense, it has been affirmed that the pursuit of thinness that would lead to rejecting food is determined by the Western culture, suggesting its contextual nature (Epling & Pierce, 1988; Goyal, Balhara, & Khandelwal, 2012; Rieger, Touyz, Swain, & Beumont, 2001; Rikani et al., 2013) and that could emerge in the therapeutic environment, during the search for reasons to refuse food (Gutierrez & Carrera, 2016). In that sense, some authors have argued that the cognitive symptoms expressed in AN may be the effect rather than the cause of the disorder. (Epling, Pierce, & Stefan, 1983; Keys, 1950; Kron, Katz, Gorzynski, & Weiner,1978).

Changes in diagnostic criteria over time, their cultural bias and methodological issues make it difficult to estimate the incidence and the true extent of the disorder, whose increase in recent decades has been called into question (Fombonne, 1995). It is known that adolescent girls and young adult women are particularly at risk (Zipfel, Giel, Bulik, Hay, & Schmidt, 2015). The lifetime prevalence is around 1% in women and less than 0.5% in men (Smink, van Hoeken, & Hoek, 2012), and the sex ratio in adults is 1:8, with more female individuals affected (Steinhausen & Jensen, 2015). Although AN is a rare disorder among the general population, its prognosis is not very encouraging. Steinhausen (2002) reports that less than a half of the patients recover from AN, while a third improve but maintain partial or residual symptoms and, in 20% of cases, the disorder follows a chronic course. In addition, AN has the highest mortality among all mental disorders

(Harris & Barraclough, 1998), with a crude rate of mortality of 5%, which can increase to 9.4% after more than 10 years of follow-up, and is higher in the adult than in the adolescent population (5.9% vs. 1.8%), (Steinhausen, 2002). Suicide is the second most common cause of death, after complications of the eating disorder, and the number of suicides was eight times higher in persons with AN than in the general population (Bulik et al., 2008; Pompili, Mancinelli, Girardi, Ruberto, & Tatarelli, 2004).

The truth is that, after 150 years of research on AN, the criteria on which the diagnosis is based, as well as the therapeutic strategies they have inspired, do not seem to have had the expected success (Chavez & Insel, 2007). In a recent review about psychological, pharmacological, and nutritional treatment, it has been concluded that new interventions are necessary to improve outcomes, especially in adults with the disorder (Zipfel, Giel, Bulik, Hay, & Schmidt, 2015). Related to psychological treatment, the evidence base in adults with AN is advancing, but no specific approach has shown clear superiority (Dare, Eissler, Russel, Treasure, & Dodge, 2001; Eisler et al., 2000; Gutiérrez & Carrera, 2018). In fact, a treatment conceptualized as placebo has had better results than specialized treatments (Gutiérrez & Carrera, 2018; McIntosh et al., 2005). Only Family-based treatment (FBT) and Maudsley family therapy (MFT) have shown any evidence of beneficial effect in adolescent population (Brockmeyer et al., 2018; Zeeck et al., 2018; Zipfel et al., 2015). Regarding pharmacological treatment, this mainly includes the administration of antidepressants and antipsychotics. There is no clear evidence on the contribution of these drugs to improve weight gain or their usefulness to prevent relapse (Attia, Haiman, Timothy Walsh, & Flater, 1998; Attia & Schroeder, 2005; Barbarich et al., 2004; Halmi , Eckert, LaDu, & Cohen, 1986;; Kaplan & Howlett, 2010). Although its contribution to reduce the symptoms of AN and other comorbid disorders

can be modest, in the case of antidepressants, its use is not recommended until it is specified whether these characteristics are due to starvation and disappear once the weight has been recovered; As for antipsychotics, more trials are needed to recommend their use (Aigner, Treasure, Kaye, & Kasper, 2011; de Vos et al., 2014).

In summary, the failure to develop more effective treatments seems to be due to the fact that they have been based on the symptoms established by the current classification systems, which give a central role to weight, concern for the figure, and body image distortions. Thomas Insel, former director of the National Institute of Mental Health (NIHM), following the launch of the DSM-5, warns of its lack of validity, criticizing that it would be a mere dictionary that is limited to creating labels and defining them, and recalls that a Diagnostic system should be based on the emerging research data. In this sense, one aspect repeatedly observed as forgotten is the excess of physical activity presented by patients suffering from AN and that, as we will see below, has generated a research corpus that may have important implications for a better characterization and treatment of this disorder.

Hyperactivity in anorexia nervosa and activity-based anorexia

High levels of physical activity have been systematically reported for patients that suffer AN since the disorder was described as a clinic entity by Gull (1874) and Lasègue (1873). This increase in physical activity is expressed in varied forms, from high intensity practice of some sport to the increase of physical activity during their daily homework. The frequency of hyperactivity in AN is estimated around 31 to 80% of patients (Gümmer et al., 2015; Hebebrand et al., 2003). If there are a common aspect on all descriptions

given for AN is the state of restlessness and the drive for activity. The clinicals were perplexed with the disproportionate energy expenditure and its consequent loss in weight showed by AN patients. However, the initial attention that was provided to this symptom (Janet, 1903) was not reflected in the classification systems of disease most widely used, and its appearance has been, in any case, as a secondary characteristic (Davis, Kennedy, Ravelski, & Dionne, 1994), probably due to the difficulty in offering a coherent explanation to this counterintuitive phenomenon. In Feighner's (1972) criteria and in the DSM-III, hyperactivity was considered a residual symptom of the disorder, which could appear with "periods of overactivity" or with extensive exercise".

The presence of excessive physical activity has recently been described in the DSM-5, but only as a symptom that could appear in a specific AN subtype, the presence of hyperactivity is not necessary to diagnose the disorder. This inclusion could be due to the recent vindications of the importance of hyperactivity in AN. However, both in the latest edition of the DSM, and in the ICD-10, hyperactivity appears conceptualized as a deliberate behavior realized by the patient to lose weight and achieve the ideal of beauty that is associated with thinness and not as an element central in the development of the pathology. This, as reported before, is characteristic of the shift of attention to psychological factors, that may be at the origin of AN for clinicians (Casper, 1998, 2006; Hebebrand, Casper, Treasure, & Schweiger, 2004).

Simultaneously with this development, in the conceptualization of AN throughout the twentieth century, experimentation with animals in the laboratory allowed to establish consistently that food deprived rats, as other mammalian species, show a significant increase in activity and a decline in food consumption days before the animals die from starvation (Campell, Smith, Misanin, Jaynes, 1966; Hall & Hanford, 1954; Richter, 1922;

Routtenberg & Kuznesof, 1967; Spatz & Jones, 1971). As Mabry and Campbell (1975) advanced "Overshadowed, if not overlooked, has been the analysis of a behavioral phenomenon of interest primarily to psychologists, namely the increase in locomotion activity that characteristically accompanies food deprivation in many mammals". However, the parallels between these observations in the laboratory and the clinical descriptions of AN had to wait almost a decade. Epling, Pierce and Stefan (1983) proposed to investigate the relationship between decreased intake, weight loss and physical activity in animal studies, and built a brilliant biobehavioral theoretical framework without resorting to cognitive constructs. (Pierce & Epling, 1988). Most interestingly, these authors reviewed clinical studies that suggested a crucial role of excessive activity in the development and maintenance of AN, and observed the results of the experiments performed by Routtenberg and Kuznesof (1967), in which rats were subjected to a restricted meal period per day (60 min) and had free access to a running wheel the rest of the time. Animals undergoing this procedure ran progressively more throughout the days, despite eating less, and died. However, while Routtenbeg and Kuznesof (1967) did not consider the activity as an essential factor, Epling, Pierce and Stefan (1983) established that the animals of the control group, with the same diet and with lack access to the wheel survived, suggesting that the most important factor in the development of the phenomenon was the excessive activity. Since then, the activity-based anorexia (ABA) has been the most promising animal model to reproduce the main characteristics observed in AN (reduction in food intake, weight loss and hyperactivity), ABA help us to understand its etiological factors and test the potential of new treatments (Carrera, Fraga, Pellón, & Gutiérrez, 2014; Chowdhury, Chen, & Aoki, 2015; Casper, 2008; Kim, 2012; Gutiérrez, 2013).

Overall, under the cultural and phenomenological manifestations of AN, an underlying general process is suggested, which involves restricted diet and excessive activity. These aspects would account for the development of the disorder in humans, as well as the observations made in various animal species through the ABA model. The different approaches to explain the animal phenomenon have been diverse, as we will see below.

Animal model: Definition and characteristics

ABA develops when rats are subjected to a single period of food (1 to 1.5 h per day) and have free access to a running wheel (23 to 22.4 h per day). Under these circumstances, rats show a gradually increase of running along days in procedure and a rapid weight loss (c.f. Carrera et al., 2014). Dramatic reduction in food intake and the development of stomach ulcers were reporter in experiments under the procedure (called sometimes Activity Stress). The development of stomach ulcers normally occurred when animals lose weight under the 70% of their free-feeding weights (Doerries, Stanley, & Aravich, 1991). Also, reduction in food intake was usually observed when animals were close to death in the procedure. Nowadays, according with new care regulations to promote health and reduce suffering, rats must be withdrawn from the procedure when reach the 75% of their free-feeding weights, before life is compromised (Dwyer & Boakes, 1997).

To check that the rapid weight loss observed in ABA is not only due to the restricted feeding schedule, a control group is usually added. Animals in control group (Diet or Sedentary group) have the same food restriction that the experimental group

(Wheel or Active group) but with lack of access to a running wheel. Under feeding restriction schedule, control rats show an initial reduction in their body weights but then, the rats can sustain their weights around the 85% of their free-feeding weights. Some experiments found a reduction in food consumption of animals in ABA procedure in comparison with animals in control group (Dwyer & Boakes, 1997; Paré & Houser, 1973), but other studies failed to observe these differences (Dixon, Ackert, & Eckel, 2003; Hampstead, LaBounty, & Hurd, 2003).

Regarding the temporal distribution of running, some studies found that in the first days on the procedure the activity levels are very low and are distributed throughout the whole interval, increasing slightly in the dark period, as would be expected in nocturnal animals. On the other hand, in the last sessions, the data usually show that the run is focus in the first hours of the session (right after the food exposition), then decreases during the dark period (time farthest from the time of access to food), and increase considerably during the hours before the intake period (Vidal et al., 2013; Fuentes et al., 2015; Pérez-Padilla et al., 2010). This last peak of running is called food anticipatory activity (FAA) (Gutiérrez y Pellón, 2002; Mistlberger, 1994; Wu et al, 2014). These data a show that ABA is a robust phenomenon and rats change its normal circadian rhythmicity, developing the running periods around the food exposition, that suggest that running pattern is controlled by the food delivery (Epling & Pierce, 1991).

There are some factors that can affect the development of ABA. Attending to subject characteristics, it is known that older rats are more resistant to ABA than young rats (de Paz, Vidal, & Pellón, 2019; Paré, 1975). Also, rats of same age but a lower body weight are more vulnerable than heavier rats to ABA procedure (Boakes & Dwyer, 1997; Boakes, Mills, & Single, 1999). Attending to sex-differences, most studies have found

that female rats show higher levels of activity (Boakes et al., 1999; Doerries et al., 1991; Hancock & Grant, 2009; Jones et al., 1990; Paré, 1975; Paré et al., 1978; Watanabe, Hara, & Ogawa, 1990). But in relation to the differences in body weigh loss, contradictory evidence was found. Some authors found greater vulnerability to ABA in females (Paré, 1978; Hancock & Grant, 2009), but in others studies, female rats showed greater resistance (Doerries et al., 1991) or similar resistance than males to ABA (Boakes et al., 1999).

Furthermore, prior experience with wheel running or feeding schedule can modulate the development of the phenomenon. Pre-adaptation to a similar feeding schedule could prevent (Dwyer & Boakes, 1997) or moderate (Cano, Gutiérrez, & Pellón, 2006; Lett, Grant, Smith, & Koh, 2001) the development of ABA. However, previous experience with a running wheel causes an acceleration in weight loss (Boakes & Dwyer, 1997).

Regarding FAA, data observed in our lab shows that the early development of FAA is related with greater vulnerability in ABA. Animals that reached the withdrawal criterion faster developed FAA earlier than “resistant” rats (Fuentes, Rick, López, Vidal, & Pellón, 2015). Unpublished analysis in our lab increasing the number of subjects are in line with this previous report: we analyze the data of 16 female Wistar rats under standard ABA procedure with 1 h (9.00 h) of food exposition and 22 hours of free access to a running wheel. The rats were divided into two groups depending on the time to reach the withdrawal criterion for statistical analysis. Vulnerable (n=8), with rats that reach the removal criterion in 8 days or less) and resistant (n=8), with rats that achieve the withdrawal criterion in more than 8 days). Rats were removed from the procedure when reached the 75% of the initial free-feeding weight. Figure 1 shows the total wheel turns

during FAA (2 h previous food delivery) along the first 6 sessions of the experiment for vulnerable and resistant rats. It can be seen that the vulnerable rats ran significantly more during the first 6 sessions of the experiment Statistical analysis of this results shown significant effects of Group [$F(1,14) = 20.143$, $p < 0.01$], Sessions [$F(5,70)=6.932$, $p<0.001$], and Days x Group interaction [$F(5, 70)=3.304$, $p<0.05$]. To verify if there was a relationship between the day that each rat reached the withdrawal criterion and the total rate of FAA during the first 6 sessions a Pearson R-test was carried out: $r= -0.507$, $p<0.05$. Vulnerable rats showed a sharp increase in FAA from day 3 while resistant rats began to increase the amount of FAA in the session 6.

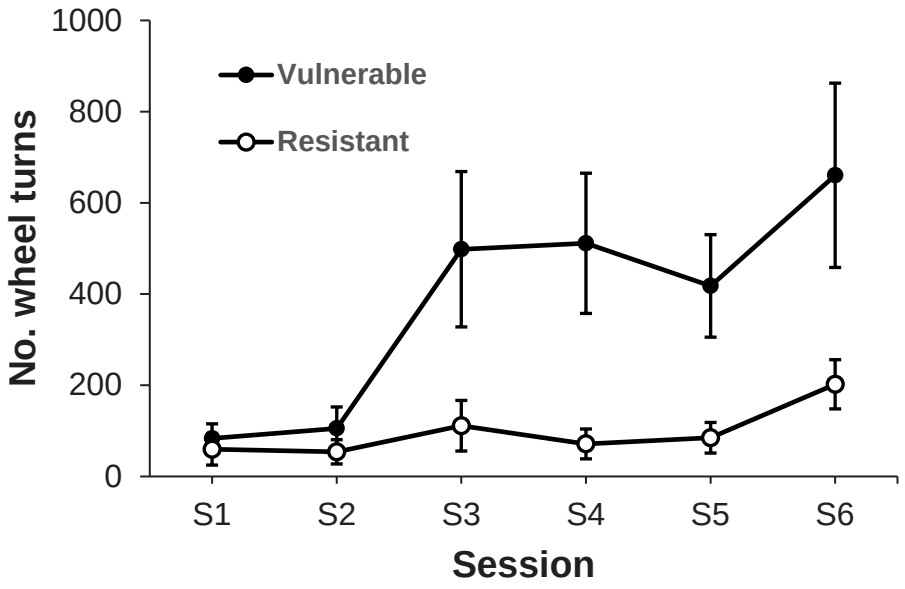

Figure 1. The mean (± SEM) of the total number of wheel turns during the last 2 h prior food exposition is show for vulnerable (black circles) and resistant animals (white circles)

Manipulations to the procedure have been effective to develop the phenomenon. Epling and Pierce (1998) found a comparable weight loss with the standard procedure (23 h of running wheel access) when the time for wheel running was up to 12 h. Other studies have found that animals could develop ABA with shorter access to a running wheel (2.5 to 4 h) (Boakes & Dwyer, 1997; Dwyer & Boakes, 1997). These results show that the phenomenon could develops with abbreviate ABA procedures.

Hypotheses to explain the animal model

Among the hypotheses that have been proposed to explain the ABA phenomenon, two approaches stand out: those that focus on meals and those that suggest a central role for the activity.

On the first hand, Dwyer and Boakes (1997) submitted a group of rats to a food adaptation schedule prior to exposure to the wheels. Rats during this pre-adaptation phase had same exposure to the food (1.5 h) than in the following experimental phase but without access to a running wheel. These authors found that the rats of the pre-adapted group did not develop ABA. In addition, pre-adaptation to a feeding schedule prevents the development of stomach ulcers until they reach a weight less than 70% (Morrow & Garrick, 1993). The underlying hypothesis suggests that the activity in the standard ABA procedure interferes with the adaptation to the meal schedule preventing rats from maintaining their weights, while the rats in control group with same food exposition but without free access to a running wheel can maintain their weights. Subsequent results have shown that prior exposure to the food schedule causes a delay in the development of ABA but it does not prevent its development (Lett & Grant, 2001; Cano, Gutiérrez, &

Pellón, 2006). These results show that although preadaptation to the food-schedule acts as a modulating factor, the fact that rats develop anorexia reduces the explanatory force of this hypothesis.

In the second group are the hypotheses that suggest that there is a relation between activity and food. Some authors have proposed that excessive activity produces a neurochemical response that acts as a satiety signal when food is presented (Aravich, 1996; Pierce, Epling, & Boer, 1986). It has also been suggested that the activity on the wheel can produce nausea and this can be associated with the food, resulting in taste aversion conditioning (Lett & Grant, 1996). The fact that differences in food intake do not occur in all experiments between ABA rats and the sedentary group without access to a running wheel limits these explanations (Vidal, Pérez-Padilla, & Pellón, 2013; Hampstead, LaBounty, & Hurd, 2003; Dixon, Ackert, & Eckel, 2003).

Giving a crucial role to the activity are also theories that suggests that rats run because exercise allows them to maintain the body temperature that decreases with the diet. Morrow et al. (1997) found that 60% of rats survived when they were exposed to heat after reaching a "nearly dead" criterion. From this observation, different experiments regarding temperature have been carried out. Hillebrand et al. (2005) found that animals during ABA with free access to a warm plate significantly reduced their hyperactivity and weight loss. In others studies, it has been found that elevating the ambient temperature causes a decrease in activity and rats are able to maintain or recover their body weights when the ambient temperature is elevated during the procedure (Gutiérrez, Byasari, Carrera, & Whitford, 2006; Gutiérrez, Cerrato, Carrera, & Vázquez, 2008; Cerrrato, Carrera, Vazquez, & Gutierrez, 2010). From this view, increase in exercise during ABA and AN could be a mechanism to maintain body temperature. In contrast, this view cannot

explain the findings regarding the temporal distribution of running (rats run near food episodes, changing their normal circadian rhythmicity) nor the increase in running next to feeding periods (Pérez-Padilla, Magalhães, & Pellón, 2010; Fuentes, Rick, López-Tolsa, Vidal, & Pellón, 2015).

Furthermore, other activity theories suggest that exercise is induced by the restricted feeding schedule, because in these circumstances running acquires a reinforcing value (Epling & Pierce, 1991). It is known that rats in sedentary confinement can press a lever to gain access to wheel running (Belke, 1996; Collier & Hirsch, 1971) suggesting that running has self-reinforcement properties. When rats were trained to press a lever to obtain 60 sec of wheel running access under a progressive ratio (PR) schedule, animals at 75% of their free-feeding weights worked for more ratios than animals maintained at 100%, suggesting that food restriction increases the reinforcing value of exercise (Pierce, Epling, & Boer, 1986). In this study, the authors also assessed whether the value of food as a reinforcer decreased with the possibility of exercise. Results found that animals reduced the lever presses to obtain food when they had access to a running wheel previously to the PR schedule (see also Epling & Pierce, 1984). These results are in contradiction with the observations during ABA, rats increase running on the previous hours to food exposition, which is called food anticipatory activity (FAA). These findings are difficult to explain if running interferes with the food reinforcing value by reducing it. In addition, de Paz, Vidal, and Pellón (2019) did not found a reduction in food value during ABA procedure, as these results will be discussed below. On this view, activity on wheel running is linked with foraging behavior. Animals increase their activity during shortage of aliment as a natural selection mechanism to obtain other sources of food (Epling & Pierce, 1991; Pierce & Epling,1994).

Epling and Pierce (1983; 1991) suggest that the excessive activity in ABA could be a schedule-induced behavior. The fact that the intensity of wheel running frequently increases in anticipation of food when it is given periodically (Bolles & Moot 1973; Bolles 1975), showing that running is maintained by its environmental consequences similarly to other operant or schedule-induced behaviors. Also, activity on a running wheel increases when the reinforcer is given intermittently rather than when it is given at the beginning of the experiment and is determined by the duration of the inter-food interval, as in other schedule-induced behaviors such as schedule-induced polydipsia (Gutiérrez-Ferre & Pellón, 2018; Levitsky & Collier, 1968). These similarities between activity in ABA and schedule-induced behaviors will be further discussed.

Objectives

The aim of this Doctoral Thesis was to observe the role of activity in the development of ABA. To achieve this goal, we conduct experiments in order to assess the crucial factors of the activity that can affect ABA. In the second chapter we studied the role of hyperactivity in the ABA phenomenon using a pharmacological preparation that causes an increase in activity. In the third chapter we studied the sex differences in activity and the temporal distribution of running in ABA. The role of running peaks observed on the temporal distribution of activity in ABA was evaluated in chapter 4. The objective of chapter 5 was to assess the relations between running and eating in order to evaluate the mechanisms that can maintain this phenomenon. After evaluating these results, a general conclusion is presented in chapter 6.

CHAPTER 2:

RAPID DEVELOPMENT OF SEMISTARVATION-INDUCED HYPERACTIVITY IN DARK AGOUTI RATS: EXCESSIVE WHEEL RUNNING AND EFFECT OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA)

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 2x2 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 emphasize the close relationship between activity and weight loss in the development of SIH and related phenomena such as ABA.

Keywords: Semistarvation-induced hyperactivity; Activity-based anorexia; MDMA; Dark Agouti rats.

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

Introduction

Increases in activity and mortality in rats deprived of food were early documented at different laboratories (Bolles & De Lorge, 1962; Finger, 1951; Hall & Hanford, 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 and Pierce (e. g. Epling, Pierce, & 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; M. 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; M. 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 levels in human anorexic patients (Holtkamp, Hebebrand, & Herpertz-Dahlmann, 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 (Gutierrez, Cerrato, Carrera, & Vazquez, 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, Ammar, Brodin, Bergström, Scheurink, and Södersten (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-Methylenedioxymethamphetamine (MDMA) (see Green, Mechan, 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 & Daumann, 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, Goubern, Laura, & 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 (Mechan et al., 2002).

Because acute administration of MDMA causes hyperactivity in DA rats (Callaway et al., 1990; Green et al., 2003; McNamara, Kelly, & Leonard, 1995; Rodsiri et al., 2011; Spanos & Yamamoto, 1989) that can last for 10 to 28 days (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 one 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 x 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., for Wistar 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 4 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 21ºC, 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 x 45 x 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. 8 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 x 32.5 x 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 one 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 minutes and, thereafter, at one-hour 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 one 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.

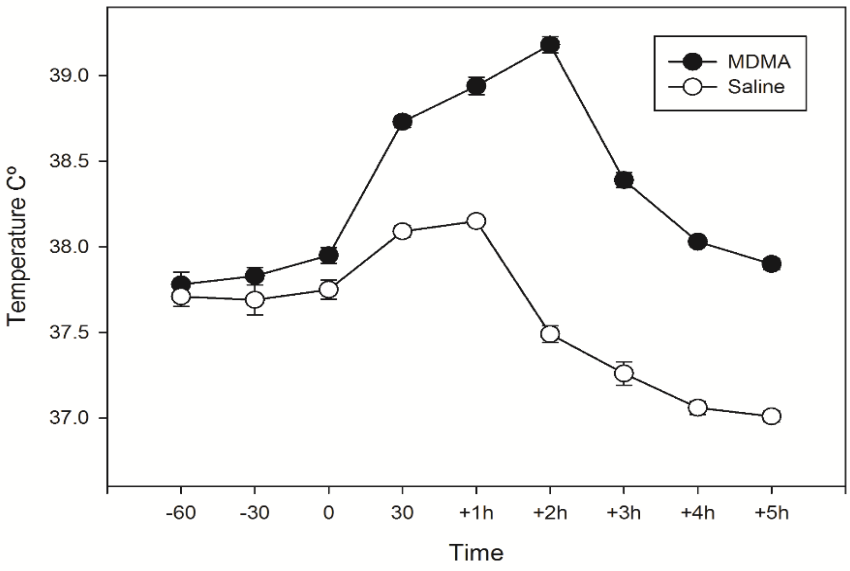

Figure 1. Mean rectal temperature in ºC (± SEM) measured for all animals by reference to treatment (MDMA or saline), across de pre- and post- injection periods (0 on the x- axis).

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 [$F(7,196)=610.20$, $p<.001$] and the Activity x Days interaction [$F(7,196)=16.51$, $p<.001$]. 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 ($p<0.001$).

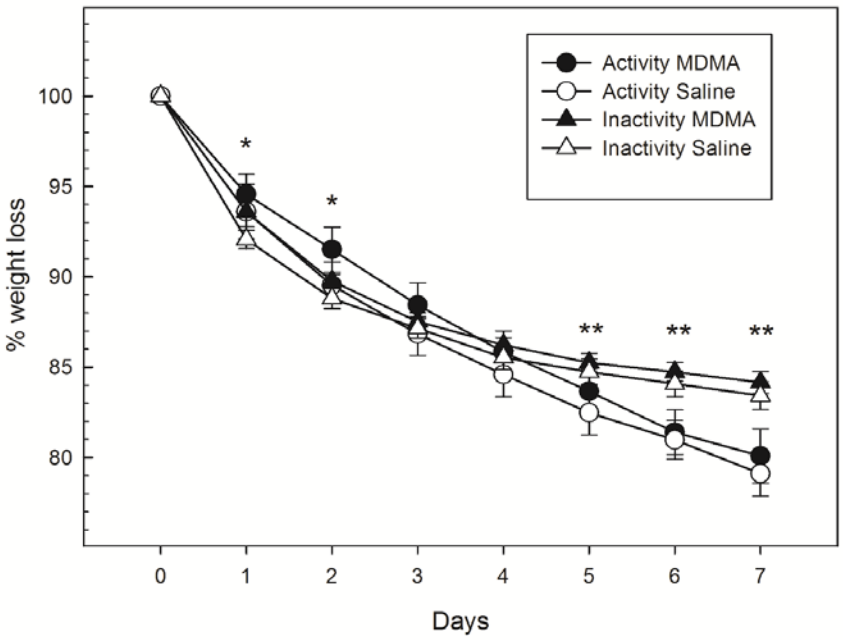

Figure 2. Mean percentage body weight (± SEM) of the four groups of animals in each day across the experiment vis-à-vis their weight at the commencement of the prodcedure. Circles denote animals with wheel access (activity), and triangles denote animals without wheel access (inactivity). Black symbols mark the scores of animals that received the MDMA dose, and white symbols mark those of rats that received the saline

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. While the statistical analysis revealed an effect for Days [$F(6,128)=178.24$, $p<.001$)], 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 [$F(6,128)=24.20$, $p<.001$] and Activity [$F(1,28)= 4.42$, $p<.05$].

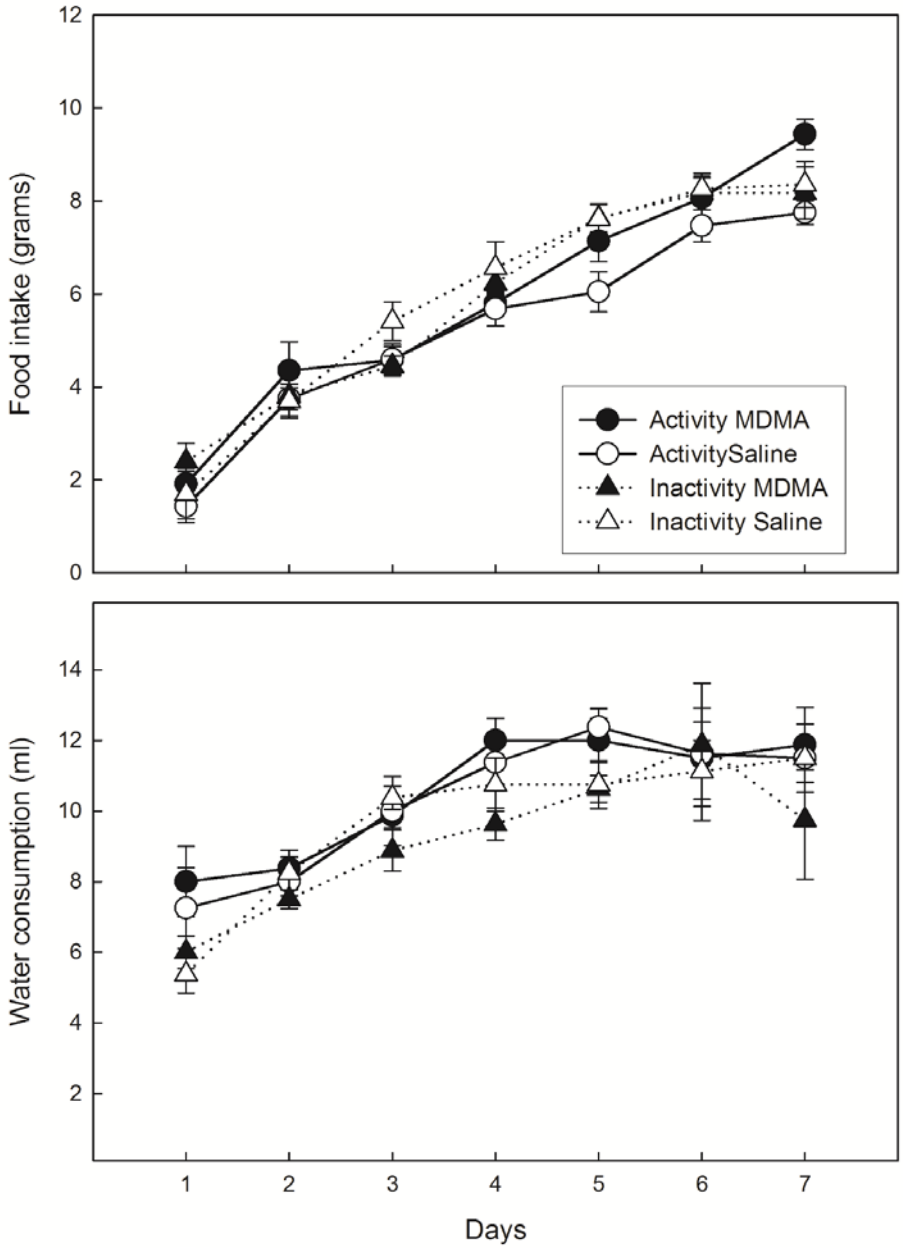

Figure 3. Shown, at top, the mean of grams of food (± SEM) and, at bottom, milliliters of water (± SEM) consumed during the feeding interval (3 h), both measurements having been taken during the experiment under all four conditions (activity vs. inactivity / MDMA vs. saline) and for each experimental day.

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 [$F(6,84)=42.66$, $p<.001$], and a trend towards statistical significance for Treatment [$F(1,14)=3.97$, $p=.06$] which reflects the general greater activity of MDMA versus saline animals counteracted by their similar final levels of activity.

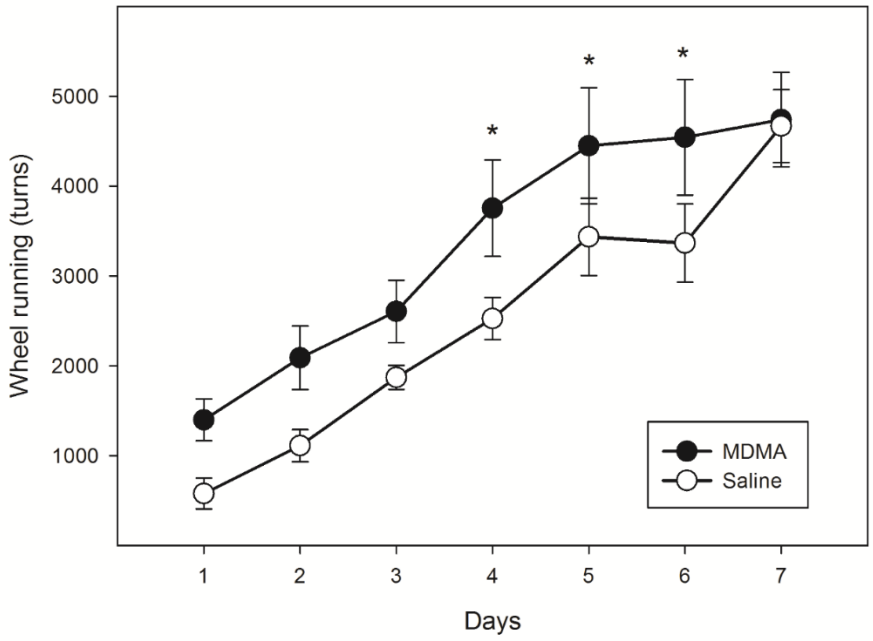

Figure 4. Mean (± SEM) wheel turns registered by groups with wheel access during each day (21 h), across the procedure. Black symbols denote animals injected with a dose of MDMA, and white symbols denote animals that received an injection of saline serum. * $p<.05$.

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 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 [$F(6,84)=24.24$, $p<.001$], no reliable effect was obtained with respect to Treatment or Days x Treatment interaction.

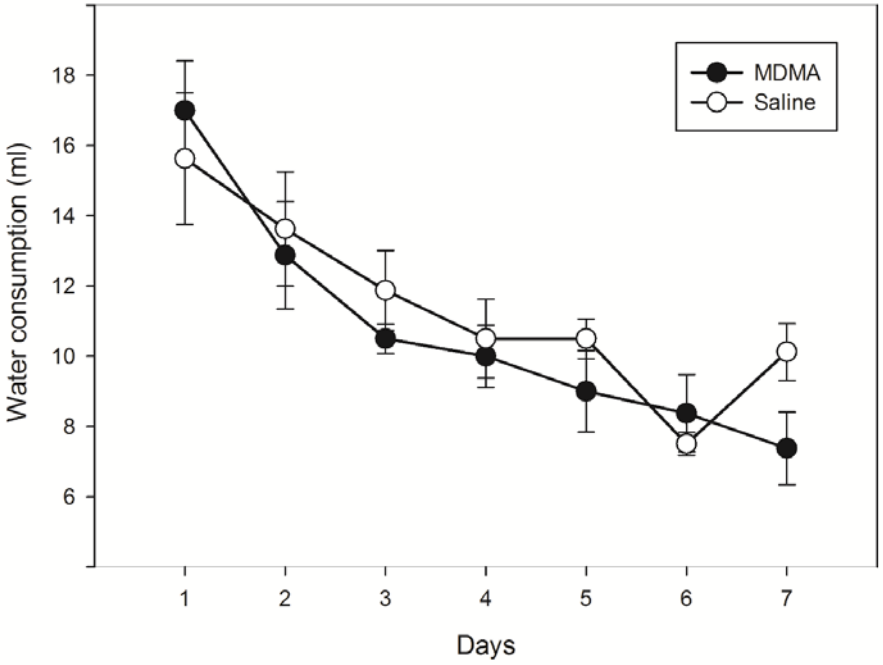

Figure 5. Mean (± SEM) milliliters of water consumed by animals under conditions of activity in each experimental day during times of free wheel running access (21 h).

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).

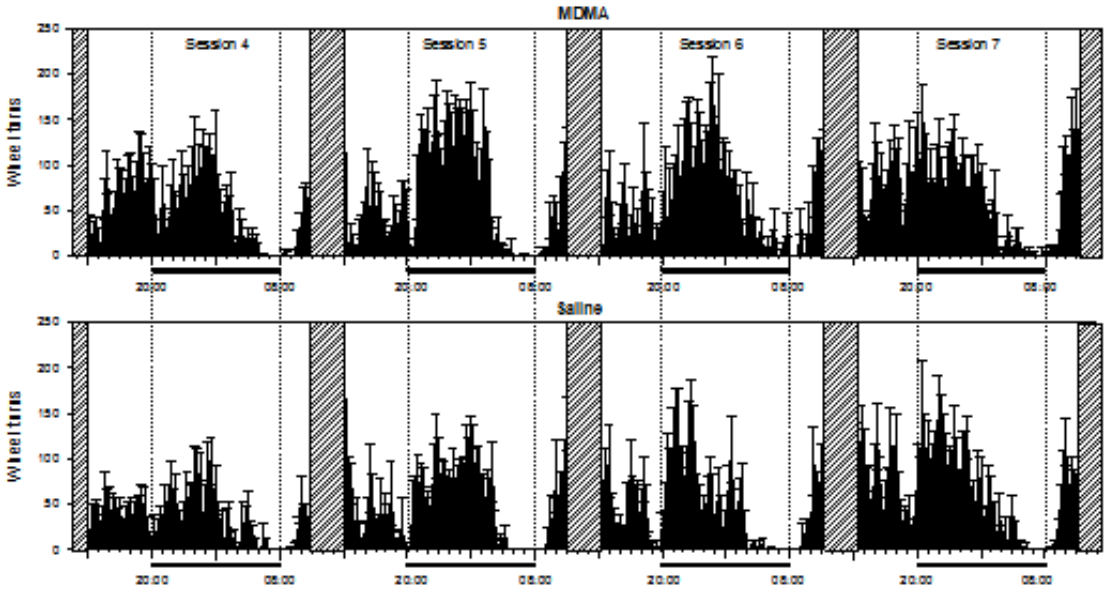

Figure 6. Temporal distribution of wheel turns of groups of animals exposed to the activity-based anorexia procedure. Each point represents the mean number of turns (± SEM) every 15 min, with only the data for the last four days being shown, since these were the days during which the highest activity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

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 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 Figure 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 analyzed, 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., M. 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 thought 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 Figure 3 upper panel, black versus white circles). The absence of differences between these two groups in terms of weight loss (Figure 2) despite differences in running (Figure 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 E. 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 contributed 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; Rodsiri 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.

CHAPTER 3:

SEX DIFFERENCES IN THE ACQUISITION AND TEMPORAL ORGANIZATION OF RUNNING IN AN ACTIVITY-BASED ANOREXIA PROCEDURE IN RATS

Abstract

Activity-based anorexia (ABA) is an animal model for anorexia nervosa (AN). ABA develops when rats are subjected to a single meal per day and have access to a running wheel the remaining time. Rats gradually increase their wheel running prevent to maintain their weights Clinical studies have found that patients with anorexia develop high activity levels, this are in line with the findings obtained with ABA model. Few studies have tested the differences in weight loss, food intake and activity between males and females and find conflicting evidence. The aim of this study is to test the sex-differences in ABA model and the temporal distribution of activity. Our results show that female rats are more vulnerable to develop the phenomenon than males. These results are in line with the greater incidence of AN in women. Female rats show higher levels of activity and lose weight faster than males. This high vulnerability seems to be caused by higher levels of activity in females than males. Regarding the temporal distribution of running, animals distribute activity around meals, showing a possible contribution of reinforcing and inducing mechanisms in the development of activity.

Keywords: Activity-based anorexia; animal models; hyperactivity; sex-differences; wheel running.

Sex differences in the acquisition and temporal organization of running in an activity-based anorexia procedure in rats

Introduction

Activity-based anorexia (ABA) is a widely accepted animal model of anorexia nervosa (AN) (Boakes, 2007; Gutiérrez, 2013). Usually the procedure consists on exposing rats to a restricted schedule of access to food, usually 60 or 90 minutes per day, and giving them free access to an activity-wheel for the rest of the day. When exposed to these conditions for several days, animals start to run on the wheel until they reach excessive levels of daily running and start to lose weight rapidly. If rats are maintained under these conditions, they eventually stop eating and die from self-starvation (Bolles & De Lorge, 1962; Finger, 1951; Paré, 1975; Paré, Vincent, Isom, & Reeves, 1978; Reid & Finger, 1955) or, following current ethical standards of care, until animals reach 75 % of their initial weight, moment in which they are removed from the procedure to prevent sickness and mortality (Dwyer & Boakes, 1997).

Routtenberg and Kuznesov (1967) were the first to systematically study this phenomenon. Later, this phenomenon was called Activity-based anorexia (cf. ABA) by Epling, Pierce and Stefan (1983), who also found similarities in the pattern of behavior between ABA and case reports of anorexia nervosa, where high activity seems to be a crucial factor in its onset and subsequent maintenance (c.f. Hebebrand et al., 2003), playing a decisive role in the etiology of the disorder (Epling & Pierce, 1988). Activity is

a factor that contributes significantly in hindering weight maintenance (Hebebrand et al., 2003). It has been observed that around 80 % of patients with anorexia have excessive physical activity (Holtkamp, Hebebrand, & Herpertz-Dahlmann, 2004). The similarities found between ABA and the human pathology have led to ABA being used as a model for the human pathology and has been used to test different therapeutic alternatives (Gutierrez, Cerrato, Carrera, & Vazquez, 2008; Hillebrand, Van Elburg, Kas, Van Engeland, & Adan, 2005).

Since then, many studies have been conducted using this procedure in order to assess the variables affecting its development and/or severity. Those variables include age at the beginning of the experiment (Carrera, Gutiérrez, & Boakes, 2006); initial body weight (Boakes & Dwyer, 1997); prior experience with a restricted food schedule (Cano, Gutiérrez, & Pellón, 2006; Dwyer & Boakes, 1997; Lett, Grant, Smith, & Koh, 2001); early maternal separation or handling (Carrera et al., 2006; Hancock & Grant, 2009); temperature in the room (Gutiérrez, Vázquez, & Boakes, 2002); number of meals during the day (Bolles & Moot, 1973; Kanarek & Collier, 1983); length of the period of access to food (Watanabe, Hara, & Ogawa, 1992); number of wheel turns required to gain access to food (Kanarek & Collier, 1979); phase of the estrous cycle (Dixon, Ackert, & Eckel, 2003); age, old rats expressed less activity and are more resistant (Boakes, Mills, & Single, 1999; de Paz, Vidal, & Pellón, 2019; Paré, 1975) and sex of the subject (Boakes et al., 1999; Doerries, Stanley, & Aravich, 1991; Hancock & Grant, 2009; Jones, Bellingham, & Ward, 1990; Paré et al., 1978)

Paré et al. (1978) found that female rats lost weight faster than male rats in the ABA procedure, but no age or free-feeding body weights were reported. Moreover, Hancock and Grant (2009) found similar results using rats of the same age . In the other

hand, Doerries, Stanley and Aravich (1991) compared males and females of the same age (41 and 42 days old, respectively) and with matched initial body weights, resulting in a rather small difference in the mean value of weight between males and females (10 g) (Doerries, 1996). Their results showed that female rats ran more but were more resistant than male rats to reach the withdrawal criterion, however these results could be attributed to the use of heavy females and light males (Boakes et al., 1999). Boakes, Mills and Single (1999) compared male and female young rats and old female rats with similar weights than young male rats. They found that young rats (both sexes) lost weight faster and ate proportionally more than old female rats. Also, old female rats took longer to achieve the removal criterion and ran less than young rats, yet, female young rats still ran more than male young rats, but no differences were found in reaching the withdrawal criterion between females and males.

One of the hypothesis regarding why ABA occurs is that it could be a consequence of failing to adapt to a feeding schedule (Dwyer & Boakes, 1997), although contradictory evidence on this account has been found (Cano et al., 2006; Lett et al., 2001). These authors had found that pre-exposure to the feeding schedule delays but does not prevent the development of the phenomenon. On the other hand, some authors have suggested that running in ABA could be a species-specific response to hunger (Boakes, 2007) that turns into a schedule-induced or operant behavior that could be reinforced by internal stimuli, such as a raise in corporal temperature (Gutiérrez, Baysari, Carrera, Whitford, & Boakes, 2006; Lambert, 1993); or external stimuli, such as the delivery of food (Epling & Pierce, 1991; Pierce & Epling, 1994).

Dwyer and Boakes (1997) found that rats with access to the wheel for only the 4 previous hours to the delivery of food (late group) reached the removal criterion, while

rats that had access to the wheel for 18.5 h after the delivery of food (early group) did not; thus suggesting that food-anticipatory running (FAA) is a critical condition for developing ABA. In Dwyer and Boakes' study, rats in the late group developed higher rates of running, probably because an operant conditioning relationship was established between running and the delivery of food by means of temporal proximity, as also happens with other 'operant' (lever pressing, key pecking) and 'schedule induced' behaviors (magazine entering, water drinking) (Ferster & Skinner, 1957; Killeen & Pellón, 2013; Skinner, 1948). Also, the fact that running in the ABA procedure increases when food is restricted (Pierce, Epling, & Boer, 1986; Premack & Premack, 1963), and its excessiveness (Beneke, Schulte, & Vander Tuig, 1995) makes it consistent with the reinforcement/schedule-induced behavior hypothesis (Levitsky & Collier, 1968; Pierce & Epling, 1991).

In accordance with the above, FAA could be the most potent indicator that running in the ABA procedure is a schedule-induced behavior, reinforced by the delivery of food; as well as an indicator that rats actually adapt to the feeding schedule quite rapidly; therefore helping to understand why running in ABA is maintained and even increases throughout the exposure to the procedure. Considering that from this view, running would be responsible for the development of ABA and male and female rats show different levels of activity, a more thorough approximation should compare rats of both sexes.

Therefore, the goal of this study was to observe and describe the acquisition and distribution of running throughout an ABA procedure, comparing between male and female rats.

Method

Subjects

Subjects were 32 (16 male, 16 female) Wistar Han rats, which were 8 weeks old at the beginning of the experiment. Rats were divided in four groups (n=8): Female-Wheel (FW); Male-Wheel (MW); Female-Diet (FD); and Male-Diet (MD). Rats in groups FW and MW were housed individually in transparent Plexiglas cages measuring 21 x 45 x 24 cm, that had access to an activity wheel (described in the apparatus section); rats in the FD and MD groups where housed in cages measuring 18 x 33.5 x 20.5 cm. All rats had access to water during the experiment. Subjects were housed in a room with temperature control (21 °C), with a 60 % relative humidity and a 12 h light-darkness cycle, light period beginning at 8:00 am. All applicable international and/or national guidelines for the care and use of animals were followed (European Union Council Directive 2010/63; Spanish Royal Decree 53/2013), and the procedures performed were in accordance with institutional ethical standards.

Apparatus

Home-cages previously described were used also as experimental cages. Cages of groups FW and MW had a round opening with a sliding metallic door that gave access to an activity wheel (9 cm wide; 34 cm diameter) in the left wall. The roof of home cages was a metallic grid that permitted access to food and water. Activity wheels were equipped with a brake that locked the movement of the wheel. The brake and wheel turns

were electronically controlled by MED-PC program for Windows installed in a conventional computer in an adjacent room.

Procedure

Subjects stayed in the home cages throughout the experiment. The experiment was divided in 24 h days, beginning at 9:00 a.m. every day. Experiments started with Day 0, when food was retrieved at 10:00 a.m. and access to the wheel was permitted for the wheel groups at 11:00 am. Day 1 started 24 h later, once the subjects had had 23 h of food deprivation. Rats stayed in the experiment, until each rat of the FW and MW groups reached 75 % of their base-line weight; at that point, the subject was removed from the experiment along with a rat from the corresponding diet group (FD for FW; MD for MW). Next, the details of experimental session are described for each group.

Wheel groups (FW and MW)

At the beginning of each daily session (9:00 a.m.), the brake was operated to stop the activity wheel and the door that gave access to it was closed, so that the subjects stayed in their home-cages. Each subject was individually weighed, every day in the same order. Once the subject was returned to its home-cage, 40 - 60 g of food was placed on the grid roof so that the rat could access it freely. After 60 min had elapsed, food remaining on the grid was retrieved and the quantity ingested by the subjects was calculated. Between 10:15 and 11:00 a.m., rats had a resting-period during which they did not have access to food or the running wheel. At 11:00 a.m., the brake was deactivated, and the door that gave access to the wheel was opened.

Diet groups (FD and MD)

After weighing the subjects from the Activity groups (approximately 9:15 a.m.), each subject from the Diet groups was weighed, and then returned to its home-cage and had access to food in the same way as the activity groups during 60 min. The remaining of the day, rats in these groups remained in their home-cages, without access to food.

Results

Table 1 shows the number of days in which each rat reached the criterion. Female rats achieved the criterion in less days ($\bar{X}$=10.1, range: 6-17) than male rats ($\bar{X}$=16.4, range: 9-27). A One-Way ANOVA was performed yielding significant differences between groups in terms of sessions to reach the criterion [$F(1,14)=6.1$, $p<.05$].

Table 1. Number of sessions in which each rat reached the criterion

	Groups	
Subjects	FW	MW
1	12	12
2	9	27
3	17	25
4	10	9
5	9	14
6	11	14
7	7	14
8	6	16
Mean	10.1	16.4

Since there were differences in weights among individuals and especially between male and female rats (see Table 2), the percentage of weight was calculated in accordance to each individual rat's weight in Session 0, to make the evolution of weight loss

equivalent among all subjects of the study. To verify whether there was a relationship between the initial weight and the number of sessions it took each rat to reach the criterion, a Pearson R-test was carried out. There was a weak tendency [r=0.56, $p<.05$] when analyzing data from both wheel groups together that suggests lower initial body is a vulnerability factor to develop ABA. Nevertheless, when performing Pearson R-tests for each group, that tendency was not found (r=-0.17) for FW; (r=0.19) for MW.

Table 2. Individual weights (in grams) and group mean in day 0.

	Groups			
Subjects	FW	MW	FD	MD
1	201	334	215	356
2	219	306	226	328
3	209	310	190	295
4	193	280	195	335
5	204	309	201	289
6	197	295	209	327
7	210	295	192	307
8	202	323	223	313
Mean	204.4	306.4	206.3	318.8

The mean percentage of weight was calculated for each group and every session. Once a subject reached the criterion stablished in 25 % of weight loss (Dwyer & Boakes, 1997), it was removed from the experiment, the data shown in this figure and throughout this paper includes until the first rat of each group was withdrawn (day 6 for FW and FD, and day 9 for MW and MD). Figure 1 shows the evolution of the percentage of weight throughout the experiment. It can be observed that weights of female rats (white circles and squares) decreased more rapidly than those of male rats (black circles and squares). Also, weights of diet groups (squares) tended to stabilize around 85 - 90 % of initial weight, whereas rats in ABA procedure continued to lose weight. FW rats showed a more

rapid decrease than FD rats, which began to stabilize their weights on Day 4. Regarding Male rats, MW showed a gradual reduction in their body weights compared to MD rats, MD rats began to reach a stabilization in their weights around Day 6. Comparing female and male rats, the progression in weight loss is more pronounced in FW than MW, as the first rat to reach the withdrawal criterion in FW did so 3 days earlier than the MW group. According to the diet groups (FD and MD), FD began to stabilize their weights faster than MD (Day 4 for FD and Day 6 for MD). A repeated measures ANOVA was performed with the data recorded until the first rat reach the withdrawal criterion (FW, 6th day). The ANOVA showed a significant effect for Days [$F(6, 168)=332.46$, $p<.001$, $\eta^2_{p=}.92$], Group [$F(3,28)=8.52$, $p<.001$, $\eta^2_p=.48$], and the Days x Group interaction [$F(6,18)=6.56$, $p<.001$, $\eta^2_p =.41$]. Post-hoc analyses using HSD tests show differences between FW with FD, MW and MD.

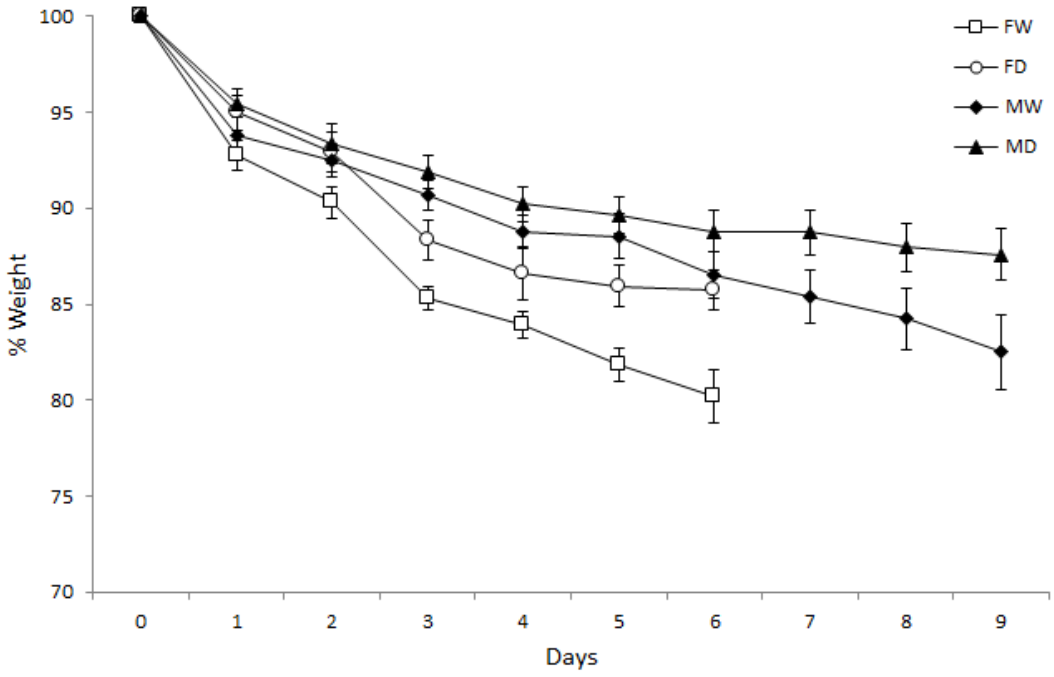

Figure 1. Mean (± SEM) of the weight percentage respect to Day 0 is shown for all groups. Animals in Wheel condition (squares) had 1 h of food exposition and access to a running wheel in terms described in Method section. Diet groups (circles) had the same diet schedule without access to a running wheel. The data is shown until the day on the first animal reached the withdrawal criterion in sex condition. Day 6 for Females (white) and Day 9 for Males (black).

When analyzing raw data from food intake, one could conclude that female rats lose weight more rapidly than male rats because they have a lower food intake: FW had a mean food intake of 5.6 grams per day (g/day) during the procedure, in contrast with the 9.6 g/day of MW; whereas FD ate a mean of 7.7 g/day, in contrast with the 10.9 g/day of MD. Nevertheless, taking into account the existing differences in initial weight of female and male subjects, we calculated the percentage of food intake compared to the weight of each individual subject, as suggested in Boakes et al., (1999). Data was

calculated as follows: ingested food (in grams) was multiplied by 100, then divided by the subject weight (in grams) for that session (Food*100)/weight) and is showed in Figure 2 (as with weight data, the figure shows values until Day 6 for females and until Day 9 for males). A repeated measures ANOVA was performed with the data collected until the first rat reached the withdrawal criterion (FW, 6th day). The ANOVA yielded significant differences for Days [$F(5,140=135.06$, $p<.001$, $\eta^2_p = .83$], and the interaction Days x Group [$F(15,140)=2.67$, $p<.005$, $\eta^2_p = .22$] but no effect for Group was found.

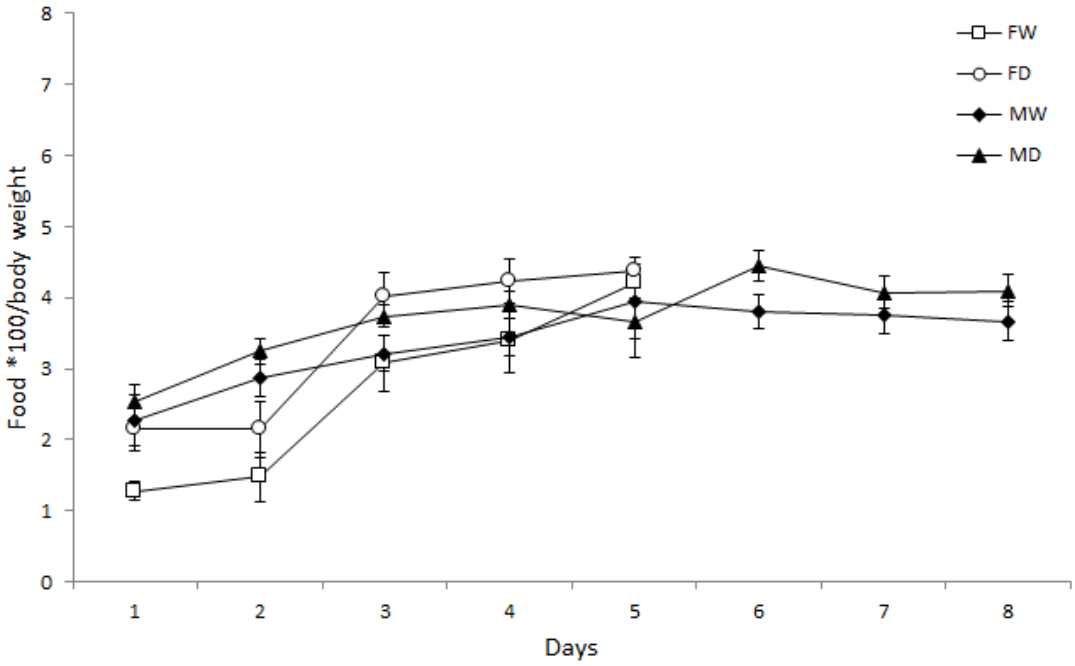

Figure 2. Shows the Mean (± SEM) of food intake in terms of percentage of body weight for each group. Animals in Wheel condition (squares) had 1 h of food exposition and access to a running wheel in terms described in Method section. Diet groups (circles) had the same diet schedule without access to a running wheel. The data is shown until the day on the first animal reached the withdrawal criterion in sex condition. Day 6 for Females (white) and Day 9 for Males (black).

Regarding the activity data, Figure 3 shows the mean (± SEM) of wheel running turns throughout the experiment until the first rat reached the withdrawal criterion (Day 6 for FW and Day 9 for MW). Animals in the FW group showed a higher level of activity throughout the procedure than MW rats and the increase in activity was more pronounced. Male rats showed less activity from the beginning of the experiment and, in the data presented (until day 9), it can be observed that they did not reach the activity levels of FW rats. A repeated measures ANOVA until Day 6 showed significant effects for Days [$F(5,70)=12.13$, $p<.001$, $\eta^2_p=.46$], Group [$F(1,14)=16.77$, $p<.005$, $\eta^2_p=.54$], and the Days x Group interaction [$F(5,70)=6.42$, $p<.001$, $\eta^2_p=.31$]

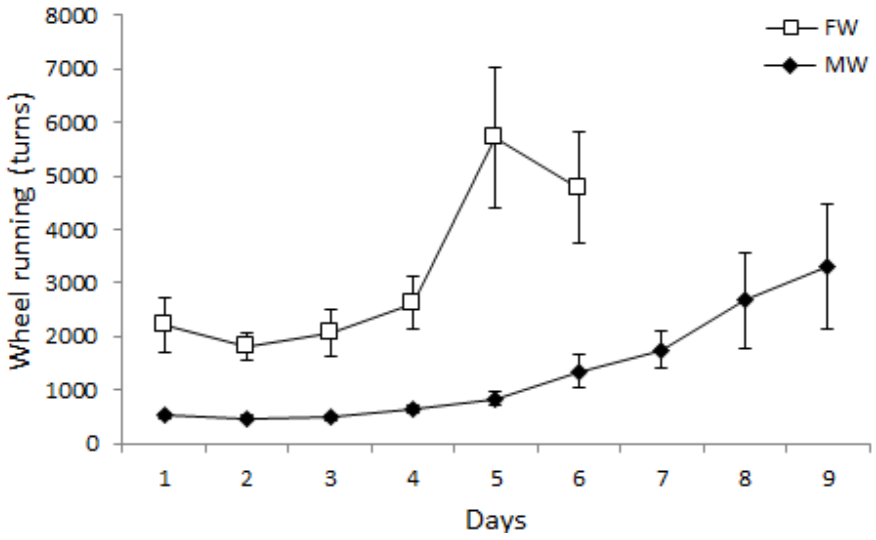

Figure 3. The mean (± SEM) of the total number of wheel turns is shown for animals in Wheel condition. The data is shown until the first rat of each sex condition reached the withdrawal criterion. Day 6 for Females (white squares) and Day 9 for Males (black squares).

In this study, we considered FAA as the number of wheel turns that occur in the two hours previous to the delivery of food. The development of anticipatory running for each group is shown in Figure 4. FW rats showed higher levels of FAA than MW in Sessions 2 and 3, but the differences disappeared from Day 4. A repeated measures ANOVA using data until Day 6 yielded significant differences for Days [$F(5, 70)=4.31$, $p<.005$, $\eta^2_p =.23$], but not for Group or the Days x Group interaction. To test whether there was a relation between the development of FAA and the time to reach the criterion, a Pearson R-Test was performed using the total amount of FAA of Days 2 to 4 (as on Day 1, running could only take place after food administration) showing no significance. The same test was performed to analyze each group separately, showing a tend towards significance in FW rats ($r=-.68$, $p=.06$) and no significant correlation for MW.

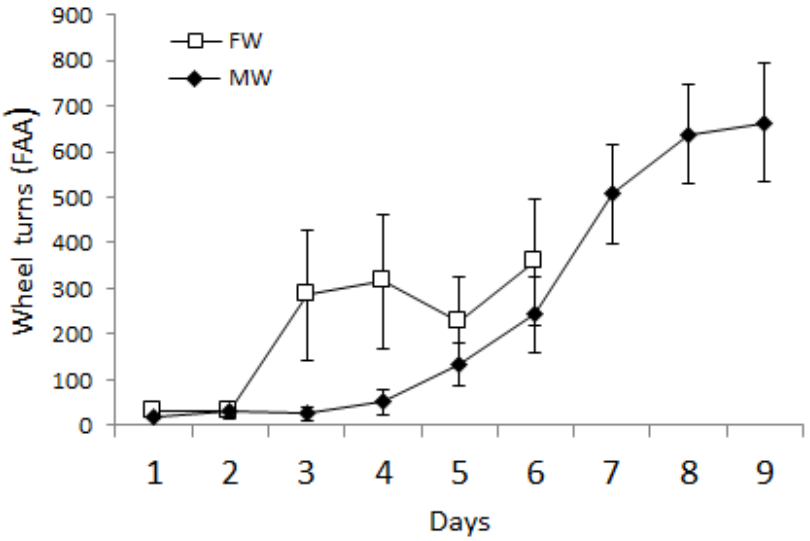

Figure 4. The mean (± SEM) of the total number of wheel turns during the last 2 h prior food exposition is shown for animals in Wheel condition. The data is shown until the first rat of each sex condition reached the withdrawal criterion. Day 6 for Females (white squares) and Day 9 for Males (black squares).

In order to obtain more information about activity during this procedure, the running distribution was analyzed. The number of wheel turns in 15 min bins in the first three and last three sessions for each subject in FW (upper panel) and MW (lower panel) are shown in Figure 5 for females and Figure 6 for males. FW and MW rats developed similar patterns. In the first three sessions, wheel turns were distributed throughout the entire session, showing higher running levels during the dark period (night) of each session. In contrast, in the last three sessions, the distribution changed: most of the running took places during light periods, right after the delivery of food and resting period (11:00 a.m.), decreased during the dark periods, and increased again during the last two hours before access to food (7:00 to 9:00 a.m.). These patterns were observed for both wheel groups, although MW rats showed lower levels than FW rats and this difference appeared from the first sessions. MW rats showed lower levels than FW rats and this difference appeared from the first sessions.

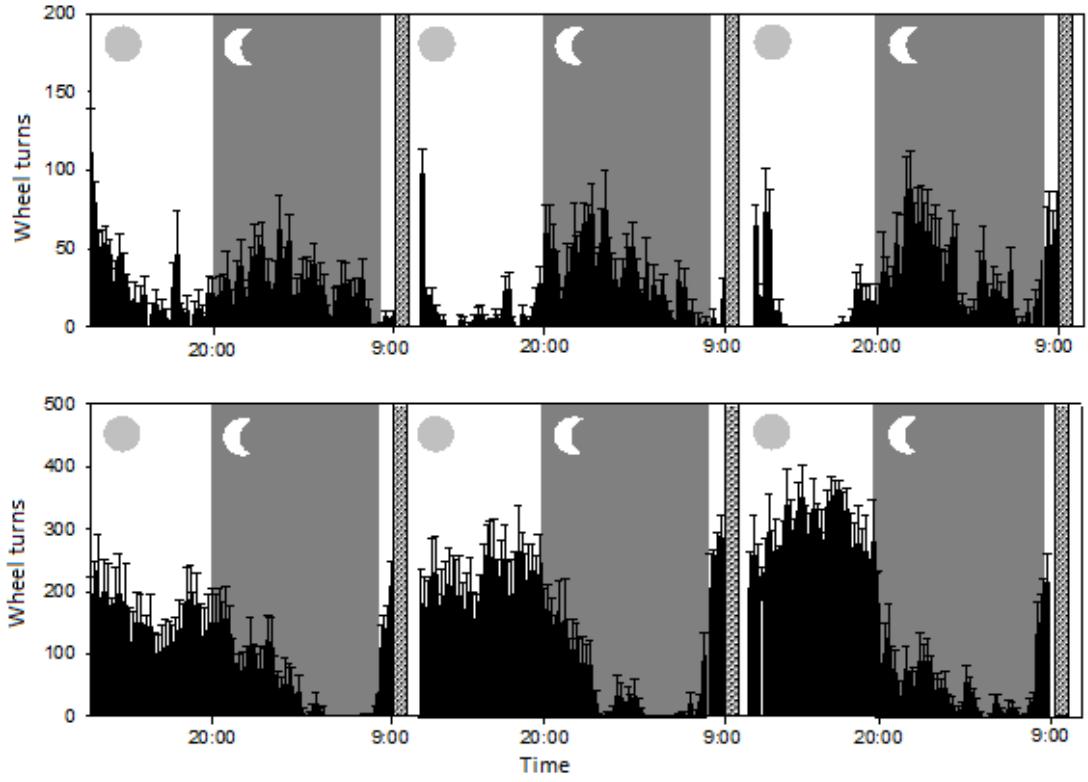

Figure 5. Temporal distribution of wheel turns for female rats exposed to the activity-wheel (FW). The data was collected every 15 min of the experimental procedure. Each shaded segment represents the time that rats were exposed to the food (1 h). The gray background represents the dark-cycle period. Periods with no plot lines mark that rats had no access to a running wheel

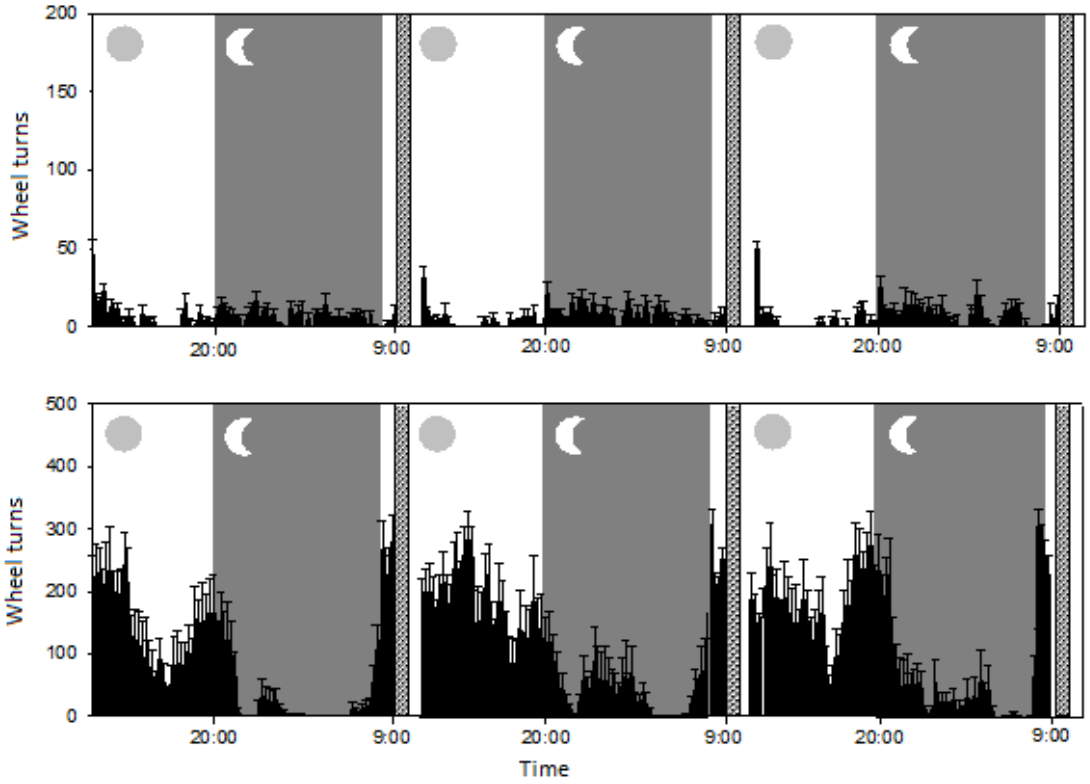

Figure 6. Temporal distribution of wheel turns for male rats exposed to the wheel running (MW). The data was collected every 15 min of the experimental procedure. Each shaded segment represents the time that rats were exposed to the food (1 h). The gray background represents the dark-cycle period. Periods with no plot lines mark that rats had no access to a running wheel.

Discussion

In this study, male and female rats were exposed to a traditional ABA procedure. Mean daily measures such as body-weight, food intake and number of wheel turns, as

well as a more detailed observation of the distribution of running and the development of FAA were reported.

The most significant finding in this study is that female rats exposed to an ABA procedure developed the phenomenon faster than male rats with same age with no differences on food intake in proportion to their weight. Female rats subjected to ABA (FW) expressed higher levels of wheel running than males (MW), highlighting the crucial role of hyperactivity in the development of the phenomenon. The fact that female rats were more vulnerable to ABA than males is congruent with studies of human anorexia nervosa, where a greater vulnerability in women to suffer the disorder has been consistently observed (DSM-IV-TR).

Along the studies using the ABA model, multiple similarities with human AN have been found, such as the development of stomach ulcers (Hall & Beresford, 1989; Paré, 1975; Paré et al., 1978), hormonal cycle mismatch (Watanabe et al., 1992), the loss of gray and white matter (Frintrop et al., 2018). Finding sexual differences in the model that fits what has been observed in humans reinforces ABA as a good animal model for this pathology. Attending to the no differences in food intake between males and females, we think that the high hyperactivity expressed for female rats could be the cause behind their faster weight loss. The higher vulnerability for female rats could be linked with a greater level of activity expressed than males also under *ad libitum* conditions (Eikelboom & Mills, 1988; Tokuyama, Saito, & Okuda, 1982).

Rats subjected to ABA (FW and MW) gradually reduced their body weight throughout the procedure, reaching a starvation criterion (Dwyer & Boakes, 1997). This did not occur in diet rats that had the same food restriction schedule but lacked access to

a running wheel (FD and MD). Rats of the diet groups, after an initial weight loss, showed a stabilization around 85 - 90 % of their initial weights.

The differences between diet (FD and MD) and wheel (FW and MW) groups showed that the effect in body weight reduction was due to the exercise expressed, as the food schedule was the same for all groups. These results are in line with those generally found using ABA procedure (c.f. Carrera, Fraga, Pellón, & Gutiérrez, 2014). Regarding sex-differences, FW rats showed a more abrupt weight loss than males, reaching the starvation criterion faster. This high vulnerability to ABA is in line with the findings obtained in other studies (Hancock & Grant, 2009; Paré 1978), but are in contradiction with other results that found no differences between males and females (Boakes et al., 1999) or high vulnerability in males (Doerries et al., 1991).

Boakes & Dwyer (1997) suggested that the initial body-weight might account for female rats' vulnerability to the ABA procedure, and greater vulnerability to ABA for lower initial body weights has been found using male rats (Pare, 1980; Woods & Routtenberg, 1971). We found a weak correlation between initial body weight and days to reach the withdrawal criterion, but this correlation could be an artefact because female rats weighed less than male rats at the same age. Also, we did not find any correlation within group.

Seemingly contradictory evidence has been found when comparing food-intake of male and female rats. Doerries, Stanley, and Aravich (1991) found that that female rats ate more than male rats of the same age, but these differences could be explained because the authors selected heavy females and light males (Boakes et al., 1999; Doerries, 1996). Jones et al., (1990) failed to find differences in body intake between males and females, but these authors only provided the mean of weights for all animals (200 g, range 180 -

205 g) and did not report age. In our study, as reported in the results section, female rats showed less food consumption than male rats, that is consistent with previous results (Hancock & Grant, 2009). But these results are difficult to interpret as male rats are heavier than female rats of same age. In order to compare males and females with different initial weights, the percentage of body weight was used as reported in the Results section. Analyzing the results, we did not find significant differences between groups in terms of percentage of food intake. Similar results were found in Boakes, Mills, and Single (1999), using the same transformation to compare food consumption between males and females. Our results seem to replicate those findings, except in the case of Jones et al., (1990) and Hancook and Grant (2009), because when analyzing absolute values, male rats ate more than female rats, but when analyzing the percentage of food intake to each rat's body weight, there were no differences between male and female rats in the wheel groups. These two exceptions could be due to the commented reasons (use of heavy females and light males or age differences).

Some authors found that animals with access to a running wheel ate less than the control diet animals and these results were explained in terms of interference between activity and food intake, using different explanatory mechanisms, such as running interfering with the adaptation to the feeding schedule (Dwyer & Boakes, 1997), or producing a neurochemical signal that causes satiety which interferes with food intake (Aravich, 1996; Pierce et al., 1986), or producing nausea, which could provoke taste aversion conditioning (Lett & Grant, 1996). The fact that this and other experiments (Boakes & Dwyer, 1997; de Paz et al., 2019; Dixon et al., 2003; Hampstead, LaBounty, & Hurd, 2003; Vidal, Pérez-Padilla, & Pellón, 2013) did not find differences between active and sedentary groups invalidates these hypotheses.

Regarding activity, as shown in Figure 3, all animals in active groups gradually increased their running along the procedure, which is consistent with data normally obtained in the ABA standard procedure (c.f. Carrera et al., 2014). Comparing males and females of the ABA group, it can be observed that females (FW) expressed higher levels of activity than males (MW), and this exercise was increased more abruptly and faster than males. These higher levels of activity for female rats have been observed systematically in other studies that compare males and females using ABA procedure (Boakes et al., 1999; Doerries, 1996; Doerries et al., 1991; Hancock & Grant, 2009; Jones et al., 1990; Paré, 1975; Paré et al., 1978; Watanabe, Hara, & Ogawa, 1990). The fact that the rats run, despite the fact that the higher energy expenditure makes it impossible for them to maintain weight during the food restriction regime, shows us the dysfunctionality of this behavior

Furthermore, some studies take into account the importance of FAA (Dwyer & Boakes, 1997; Wu et al., 2014) in the ABA procedure. A previous study in our lab, using female rats, showed that the early development of FAA is related with a higher vulnerability to weight loss in ABA (Fuentes, Rick, López, Vidal, & Pellón, 2015). All animals in our experiment gradually increased their FAA levels. As noted in Figure 4, the levels of FAA were higher for females on Days 2 and 3, males reached similar levels on Day 4. Despite this visual inspection, no differences between groups were found when the first 6 days were analyzed. Analyzing the correlation between FAA levels on the first days and reaching the withdrawal criterion, we found a moderate negative correlation that tends towards significance in females, which is in line with the study of Fuentes et al., (2015), commented in Chapter 1 of this Doctoral Thesis. The fact that we did not find the same results regarding males could be because we analyzed only the first few days

and FAA develops slower in male rats. Anyway, the effect of food-anticipatory running in males requires further research. FAA suggests that the animal has adapted to the feeding schedule; nevertheless, being able to anticipate food in this procedure, could make organisms more vulnerable to develop anorexia.

The distribution of wheel running is presented in Figures 5 (for females) and 6 (for males). As informed before (Fig. 3), female rats showed higher levels of activity both in the first and last days of the procedure, but the pattern of running was the same for males and females, despite females showing higher FAA than males from Day 3 on visual inspection (but no statistical differences were found). It can be observed that all animals showed activity during the first days, but running levels gradually increased along the procedure. Furthermore, animals during the first days ran more during the night-cycle but as the days progressed, they tended to express higher levels of running around the time when food was delivered. This pattern of activity, with one peak of running before food (FAA) and another peak after food exposition (post-food activity, PFA) are in line with other studies that have reported the time distribution of wheel running (Pérez-Padilla, Magalhães, & Pellón, 2010; Vidal et al., 2013), and show that ABA is a robust phenomenon in which rats change their normal circadian rhythms, developing running during the periods around food exposition, which suggests that the running pattern could be controlled by food delivery (Epling & Pierce, 1991). The emergence of FAA and PFA resembles the behavior pattern observed in fixed interval schedules. When organisms are exposed to a fixed interval schedule, they develop a scalloped behavior pattern that consists on a gradual increase of the behavior towards the end of the interval until subjects receive the reinforcer (Ferster & Skinner, 1957). This pattern is similar to that found in magazine entries studies (Boakes, Patterson, Kendig, & Harris, 2015). In relation with

the PFA, this pattern is similar to those obtained in studies of "interim behaviors" as schedule-induced drinking (Falk, 1966; López-Crespo, Rodríguez, Pellón, & Flores, 2004; Pellón & Pérez-Padilla, 2013).

Premack and Premack (1963) showed that when animals were food deprived, they expressed an increment on wheel running, and this increase could be caused because the time that rats spent in eating is readjusted when food is not available increasing other possible behaviors. Also, other studies have found that activity could be a self-reinforced behavior (Belke, 1996; Belke & Heyman, 1994; Belke, Pierce, Magee, & Laffoley, 2016; Collier & Hirsch, 1971; Iversen, 1993) or that it increases to prevent hypothermia caused by food restriction (Gutiérrez et al., 2002). These mechanisms could explain that running in ABA is expressed on high rate. Killeen and Pellón (2013) suggested for adjunctive behaviors (as schedule-induced behaviors) that when subjects make the responses and then receive the programmed reinforcer, a non-explicit contingency emerges that causes the response to increase its probability of occurrence, and this process is not different from that in other operant behaviors in which the contingency is explicit; behaviors followed by appetitive consequences will increase. These progressive increases of running throughout the sessions have also been found in other schedule-induced behaviors (or adjunctive behaviors). Findings obtained regarding food being an effective reinforcer for running (de Paz et al., 2019) and the fact that higher rates of running were found under variable-time schedules in comparison with fixed-time (Pérez-Padilla et al., 2010) is consistent with what has been observed with other operant behaviors.

We propose that the activity in ABA is a schedule-induced behavior and that it is maintained by the intermittency of food schedule. FAA and PFA activity are the two faces of the mechanism involved: reinforcement and induction. The contiguity between activity

and food delivery develops FAA, and then, the correlation between food and the activity of FFA induces the PFA. The wheel running distribution data support this view, as it can be observed that FAA develops earlier and then the pattern of activity is moved from the night-cycle to the post-food period. The higher levels of activity in female rats could facilitate the association between food and running, with higher activity levels making it more probable that female rats run near food delivery from the first sessions, showing this more pronounced increase in activity earlier than males.

CHAPTER 4:

FOOD-ANTICIPATORY ACTIVITY IS SUFFICIENT BUT NO NECESSARY TO GENERATE ACTIVITY-BASED ANOREXIA IN RATS

Abstract

Activity-based anorexia (ABA) is a robust phenomenon that develops when rats are subjected to a single meal per day (1 - 1.5 h) and have access to a running wheel the rest of the time (22.5 - 23 h). Under these conditions, animals show a progressive increase in wheel running and gradually weight loss. Due to the parallelism with the clinical manifestations of anorexia nervosa, the ABA procedure is considered an adequate model of this human pathology. When the ABA procedure progresses, animals tend run in two periods around the meal; one period before food, called food-anticipatory activity (FAA) and another period after food delivery, called post-food activity (PFA). Previous experiments have shown that animals running only during FFA develop ABA. Studies with shorter times of wheel access without FFA have found conflicting results. The aim of these study is to asses the contribution of these peaks of activity in the development of the phenomenon. In experiment 1, animals had a variable time schedule (VT) of food delivery on daytime, and the access to the wheel was impeded from 8.00 a.m. to food delivery in order to prevent FAA (between 13 to 22 h of wheel access per day). In experiment 2, animals had the meals in same VT schedule and had lack of access to the wheel from the food delivery to 8.00 a.m. (between 1 and 10 h of wheel access). Our results show that ABA can develops with shorter periods of activity. Animals can develop ABA in the two conditions, showing that both FFA and PFA activity contribute to the development of the phenomenon.

Keywords: Activity-based anorexia, food-anticipatory activity, hyperactivity, animal model.

Food-anticipatory activity is sufficient but not necessary to generate activity-based anorexia in rats

Introduction

Activity-based anorexia (ABA) is a phenomenon that is caused experimentally when food intake is reduced to a single period in the day (1-1.5 h) and free access to an activity wheel is allowed the rest of the time. To compare the results, a control group is typically used subject to the same food restrictions but without access to exercise. After the first few days in which the rats of both groups lose weight, the rats in the control group (without wheel access) tend to stabilize at around 85% of their weight ad libitum. The rats in the ABA group, however, continue with a constant and progressive loss of their weight that forces them to be withdrawn from the experiment when they reach 75 % to prevent death during the procedure (Dwyer & Boakes, 1997; for review of the experimental protocol, see Carrera, Fraga, Pellón, & Gutiérrez, 2014).

The first studies that found an increase in activity after subjecting animals to reductions in intake were conducted in the 50s and 60s (Bolles & De Lorge, 1962; Finger, 1951; Hall & Handford, 1954; Reid & Finger, 1955), but Routenberg and Kuznesof (1967) were the first to study it systematically, finding an increase in activity and mortality accompanied by reduced intake during periods of access to food in the

experimental group. In the case of controls, they managed to stabilize their weight and survive the same restrictive diet.

The alterations produced by the combination of running and food restriction are similar to those found in human patients with anorexia nervosa. These signs include the development of stomach ulcers (Hall & Beresford, 1989; Paré & Houser, 1973), disruption or mismatch of the estrous cycle (Watabe, Hara, & Ogawa, 1992) or the loss of gray and white matter (Frintrop et al., 2017). This phenomenon was later called activity-based anorexia (cf. ABA) by Epling, Pierce and Stefan (1983), who also found similarities in the pattern of behavior between ABA and case reports of anorexia nervosa where high activity seemed to be a crucial factor in its onset and subsequent maintenance (for a review, see Hebebrand et al., 2003), playing a decisive role in the etiology of the disorder (Epling & Pierce, 1988). Activity is a factor that contributes significantly towards hindering weight maintenance (Hebebrand et al., 2003). It has been observed that around 80% of patients with anorexia have excessive physical activity (Holtkamp, Hebebrand, & Herpertz-Dahlmann, 2014). The similarities found between ABA and anorexia nervosa have led to ABA being used as a model of human pathology (Boakes, 2007; Gutiérrez, 2013) and has been used to test different therapeutic alternatives (Gutiérrez, Cerrato, Carrera, & Vázquez, 2008; Hillebrand, van Elburg, Kas, van Engeland, & Adan, 2005).

In order to explain the development of ABA, two theoretical frameworks have been proposed that we can divide into two blocks: those theories that focus on the food schedule and those that focus on the activity.

Taking into account the former, Dwyer and Boakes (1997) subjected a group of animals to an adaptation period prior to the food schedule (1.5 h of access to food per

day) without access to the activity wheel. This period of adaptation to the food regime prevented the development of ABA in the animals. The suggested hypothesis is that access to the wheel interferes with the adaptation to the food regime and prevents animals from maintaining weight as is the case with rats in the control group. Subsequent studies have qualified these conclusions, finding that the pre-adaptation to the food regime causes a delay in the development of the phenomenon but does not prevent it (Cano, Gutiérrez, & Pellón, 2006; Lett & Grant, 2001).

In relation to the second theoretical framework, many studies have found that high activity plays a decisive role in ABA (Pérez-Padilla, Magalhães, & Pellón, 2010; Pierce, Epling, & Boer, 1986; Vidal, Pérez-Padilla, & Pellón, 2013). Some of the hypotheses that take activity into account explain the mechanism of weight loss through the fact that activity interferes with the reinforcing value of food, either because intense exercise causes nausea and leads to taste aversion (Lett & Grant, 1996) or because activity produces a neurochemical signal of satiety (Aravich, 1996; Pierce et al., 1986). In line with the crucial role of activity, Epling and Pierce (1992) suggested that excess activity in ABA could be a schedule-induced behavior. The fact that running on the wheel increases in anticipation of food (FAA) shows that running could be maintained by its environmental consequences such as operant behavior in general, including schedule-induced behavior (see Killeen & Pellón, 2013). Recent studies in our laboratory have shown that food in ABA does not lose reinforcing value with the progress of the phenomenon, thus it could act as a running reinforcer (de Paz, Vidal, & Pellón, 2019), which is consistent with the development and maintenance of anticipatory running throughout the sessions (Fuentes, Rick, López-Tolsa, Vidal, & Pellón, 2015).

Running in the ABA phenomenon has a characteristic pattern that consists of a gradual development of the activity, which increases as the procedure progresses. This activity also goes from being centered in the night period to being distributed in relation to meals, modifying the normal pattern of nighttime activity of the rat when intake occurs during the daytime period (Pérez-Padilla et al., 2010; Vidal et al., 2013). Two fundamental moments are observed as days progress: a peak of post-food activity (PFA), in which the rats develop a high level of activity just after the time of exposure to food; and a very high period of activity prior to the delivery of the next meal, which some authors call food-anticipatory running (FAA) (Mitslberger, 1994; Dwyer & Boakes, 1997; Wu, van Kuick, Tambuyzer, Luyten, Aerts, & Nuttin, 2014). Therefore, this pattern of wheel running distribution shows a post-meal peak (PFA) similar to that observed in other schedule-induced behaviors such as polydipsia (called interim by Staddon, 1977) and another FAA peak that resembles classical operants such as lever pressure. In induced behaviors, the response that occurs in the interval between meals can be related to the reinforcer generating an unexplained contingency that increases its probability of occurrence (cf. Killeen & Pellón, 2013); ABA studies show this gradual increase of running throughout the sessions.

Epling and Pierce (1984) used different running times (2, 6, 12, 18 or 22 of wheel access per day) with 1.5 h of daily access to food. The results showed greater weight loss the longer the access times to the activity wheel were, being comparable after 12 h of access.

Dwyer and Boakes (1997, Exp. 4) compared two groups, one with early access to the wheel and one with late access. The group with early access had the possibility of exercising on the wheel for 18.5 h, the remaining hours, the wheel was locked to prevent

the development of FAA. The late group had access to the wheel only during the four hours before the meal, preventing the PFA. The results showed greater weight loss in the late group. These results, which seem to indicate a greater contribution of anticipatory running to the development of ABA than the post-food activity, are in line with observations that early development of FAA running favors reaching the withdrawal criterion earlier (Fuentes et al., 2015). However, qualifications can be made to Dwyer and Boakes (1997) study. Figure 5 (upper panel) shows a stabilization in the weight of the early group, which contrasts with what was observed in procedures with similar running times (Boakes, Mills, & Single, 1999; Carrera, Gutiérrez, & Boakes, 2006; Epling & Pierce, 1984; Pérez-Padilla et al., 2010). In addition, the fact that in the late group the availability of the wheel is reduced prevents the observation of FAA, as animals run during the entire time of availability of the wheel, which could suggest that the exercise on the wheel of this group is partly due to access to the wheel after a sedentary period and not to the development of FAA. Several studies have found that rats with limited access to the activity wheel press the lever to gain access to it, which suggests a reinforcing value of exercise in confinement conditions (Belke, 1996; Belke & Heyman, 1994; Belke & Pierce, 2016; Iversen, 1993).

The purpose of the present work was to analyze separately the contribution to weight loss of the two components of the temporal distribution of running in ABA improving the previous experimental designs. For this purpose, two experiments were carried out in which, with a variable temporal distribution in the administration of the food, access to the activity wheel was blocked to prevent the development of FAA (in the first experiment) and the development of the PFA (in the second experiment).

Experiment 1

The objective of this first experiment was to analyze the contribution to weight loss in ABA of PFA. Dwyer and Boakes (1997) found a stabilization of the weights in a group with 18.5 h of running that prevented the development of food-anticipatory running. However, Epling and Pierce (1984) found that rats that ran 12 h a day or more achieved a weight loss similar to the usual ABA procedure. In the design of this experiment, a variable schedule was used for the delivery of food similar to that used in Pérez-Padilla et al. (2010). The rats had access to the activity wheel after the intake period and until 8:00 a.m. the following day. As food delivery took place at variable hours, the rats had access to the wheel between 13 and 22 h each day throughout the experiment.

Method

Subjects

A total of 16, 9-week old, experimentally naïve, female Wistar strain rats from the Charles-River laboratories (Lyon-France), with an average weight of 206 g (between 190 and 222 g) at the beginning of the experiment, were used. Upon arrival at 7 weeks of age, they were housed in groups of four rats each, having free access to water and food throughout the entire time. At the beginning of the experiment, the animals of the experimental group were placed in individual boxes with an activity wheel and the animals in the control group were placed in individual boxes without an activity wheel. The room where the rats were housed was kept at a temperature between 17 and 23 ºC, with a relative humidity of 60 % and a 12 h light-dark cycle, starting the day cycle at 7:00 a.m. and the night cycle at 7:00 p.m. All applicable international and/or national

guidelines for the care and use of animals were followed (European Union Council Directive 2010/63; Spanish Royal Decree 53/2013), and the procedures performed were in accordance with institutional ethical standard.

Apparatus

The experimental subjects were placed in 21 x 45 x 24 cm transparent methacrylate boxes, with activity wheels arranged on one side and with a grid at the other end, where the food was placed and a water bottle. The wheels had a device to record the number of turns made by each animal and a brake that was activated daily at 8:00 a.m. and remained activated until after the time of food access. The experimental boxes were controlled by a Pentium II computer at 233 MHz, programmed in MED-PC, which were located together with the communication interfaces in a separate room. The subjects of the control group were placed in transparent methacrylate boxes of 18 x 32.5 x 20.5 cm with a metallic grid to place the food and the water bottle.

Procedure

At the beginning of the experiment, the subjects were randomly assigned to one of the two groups, the experimental group with access to the activity wheel (n=8) and the control group without an activity wheel (n=8). Access to food was established through an intermittent schedule of variable hours to which the subjects were exposed, having only one daily hour of access to food. Four possible meal schedules were randomly established, all within the day cycle (9:00 a.m. to 10:00 a.m., 12:00 noon to 1:00 p.m., 3:00 p.m. to 4:00 p.m. and 6:00 p.m. to 7:00 p.m.). The activity wheels were programmed in such a

way that at the beginning of each experimental session, that is, after the time of access to the food, the wheels were in operation until 8:00 a.m. the following day, in order to prevent the possibility of animals exercising before the meal period. On the first day, the rats were weighed and placed in their individual boxes, having an hour of access to the food without access to the activity wheel, then the food left by the animals of both groups was removed and weighed, activating the activity wheels for the experimental group and the turns being recorded every 15 min throughout the session. Regardless of the time of access to food, the wheels were stopped at 8:00 a.m., which resulted in variable intervals also for the time of access to the activity wheel. The amount of food consumed by each subject during the exposure periods was also recorded. The rats were removed from the experiment once the criteria proposed by Dwyer and Boakes (1997) were fulfilled, after which animals were allowed free access to food and access to the wheel was prevented once their weight was maintained below 75 % of its initial weight for two consecutive days. The experiment had a total duration of 7 sessions, day in which the first experimental rat had to be removed from the procedure. The data were analyzed using the statistical package SPSS 24.0. The post-hoc analyzes were Newman-Keuls tests performed with that same computer program. The minimum level of statistical significance was set at $p<.05$.

Results

The percentage of body weight reduction throughout the days is shown in Figure 1, calculated for each rat in proportion to its weight at the beginning of the experimental sessions (Day 0), with Day 1 being the first session of exposure to food restriction. It can

be observed how the weight of all animals progressively decreased over the course of the days, with Day 5 being the first day in which statistically significant differences were found between the groups (p<.01). After Day 5, the reduction of weight was more pronounced for the experimental group (with access to the wheel) compared to the control group (without access to the wheel). Statistical data showed a main effect of Group [F(1,14)=6,24; p<.05], a main effect of Days [F(7,98)=269,46; p<.001], and a significant interaction between Group x Days [F(7,98)=9,25; p<.001].

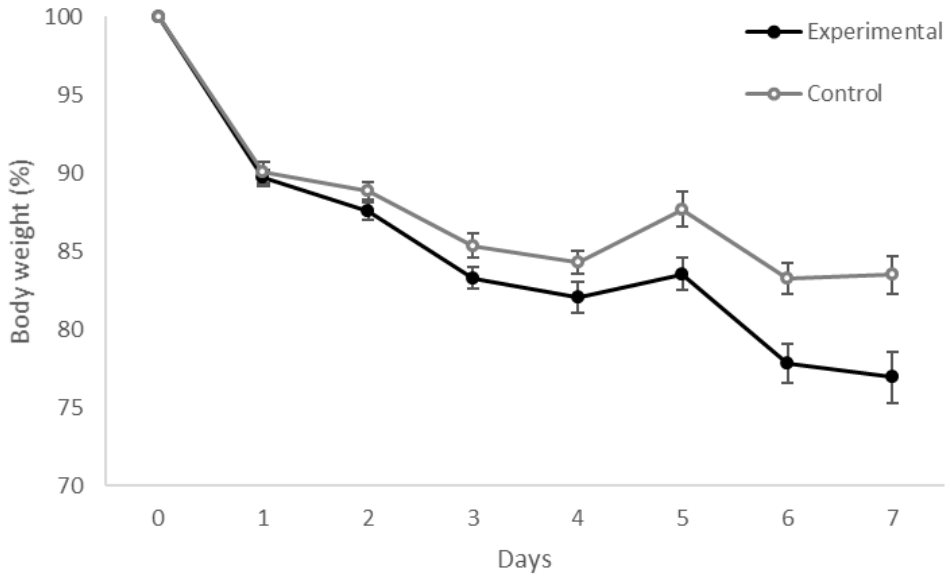

Figure 1. Mean (± SEM) of the weight percentage respect to Day 0 is shown for Experimental and Control groups. Animals in Experimental group had 1 h of food exposition and access to a running wheel in terms described in Method section. Control group had the same diet schedule without access to a running wheel

The amount of food consumed by both groups during the time of food access is shown in Figure 2. The amount of food ingested increased as the experimental procedure

progressed, the consumption of the animals in the experimental group being slightly lower compared to that of the control group, a difference that was statistically significant only on Days 3, 5 and 6 (p<.05). The ANOVA showed a Group effect close to the minimum level of statistical significance established [F(1,14)=3,93; p=.067], a main effect of Days [F(7,98)=110,89; p<.001], and a significant interaction Group x Days [F(7,98)=2,26; p<.05].

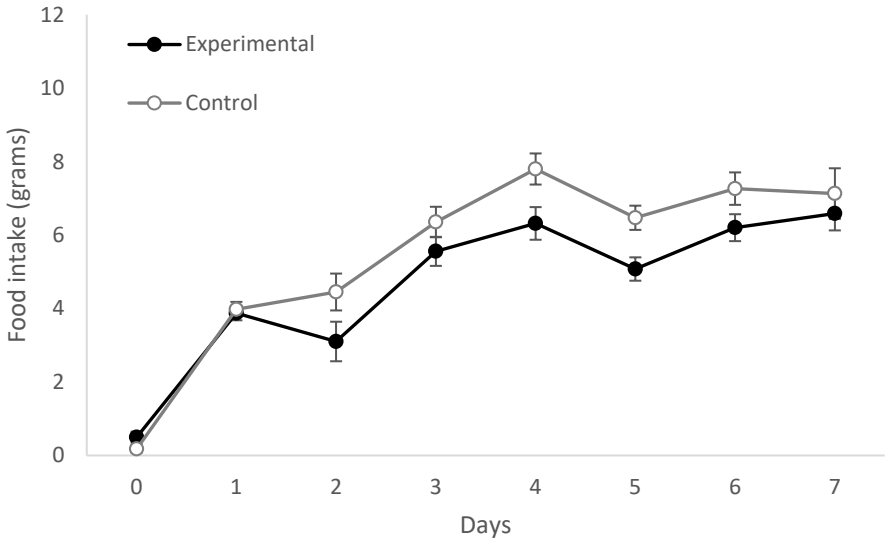

Figure 2. Shows the mean (± SEM) of food consumption during the 1 h of food exposition for each group. Experimental (activity and diet) and Control (diet only) were exposed to the same feeding schedule.

The activity on the wheel of the subjects of the experimental group can be observed in Figure 3, which shows an increase in activity as the days progressed. The activity shown is the mean number of turns per hour that the subjects carried out each day (once the food was removed after having had access to it for one hour) and until 8:00 a.m.

the following day, thus preventing activity during the rest of the day and at the time of access to food. A gradual increase in activity can be observed throughout the days [$F(6,42)=7,80$, $p<.001$], until it stabilized after Day 5.

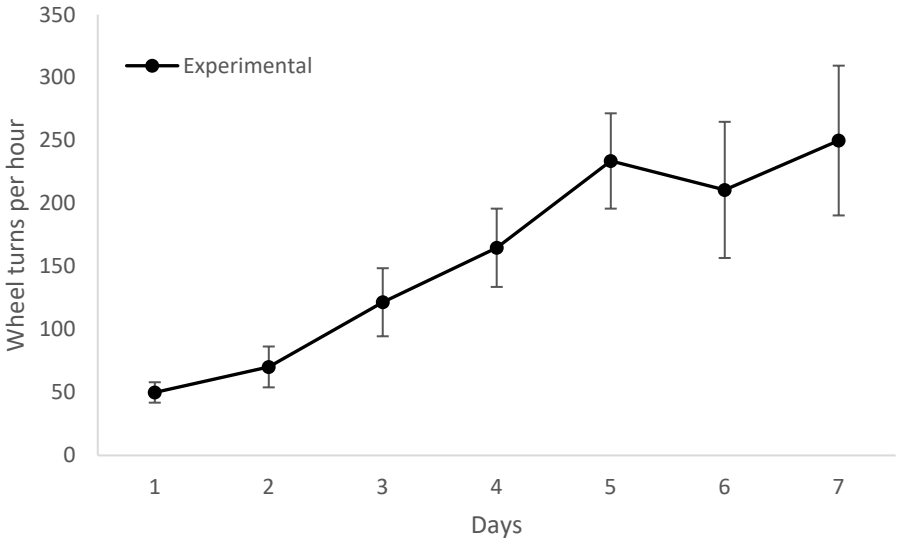

Figure 3. The mean (± SEM) number of wheel turns per hour is shown for animals in Experimental group. The time exposition to a running wheel varied along days between 13 and 22 h (see Method section).

Figure 4 shows the mean number of turns of all subjects in the experimental group recorded every 15 minutes during the last 4 days of the experiment. It can be observed that from the end of the period of access to food (vertical bars), the subjects began to show activity (black circles) regardless of the meal schedule of the day, although on Day 6, a period of inactivity is observed after running immediately after food. Every day, there was more activity during the dark cycle (indicated by a horizontal black bar on the abscissa axis), which was greater at the beginning of the cycle on Days 5 and 6. The

periods of inactivity at the beginning of the Light cycle are due to the activation of the wheel brake to prevent activity on it.

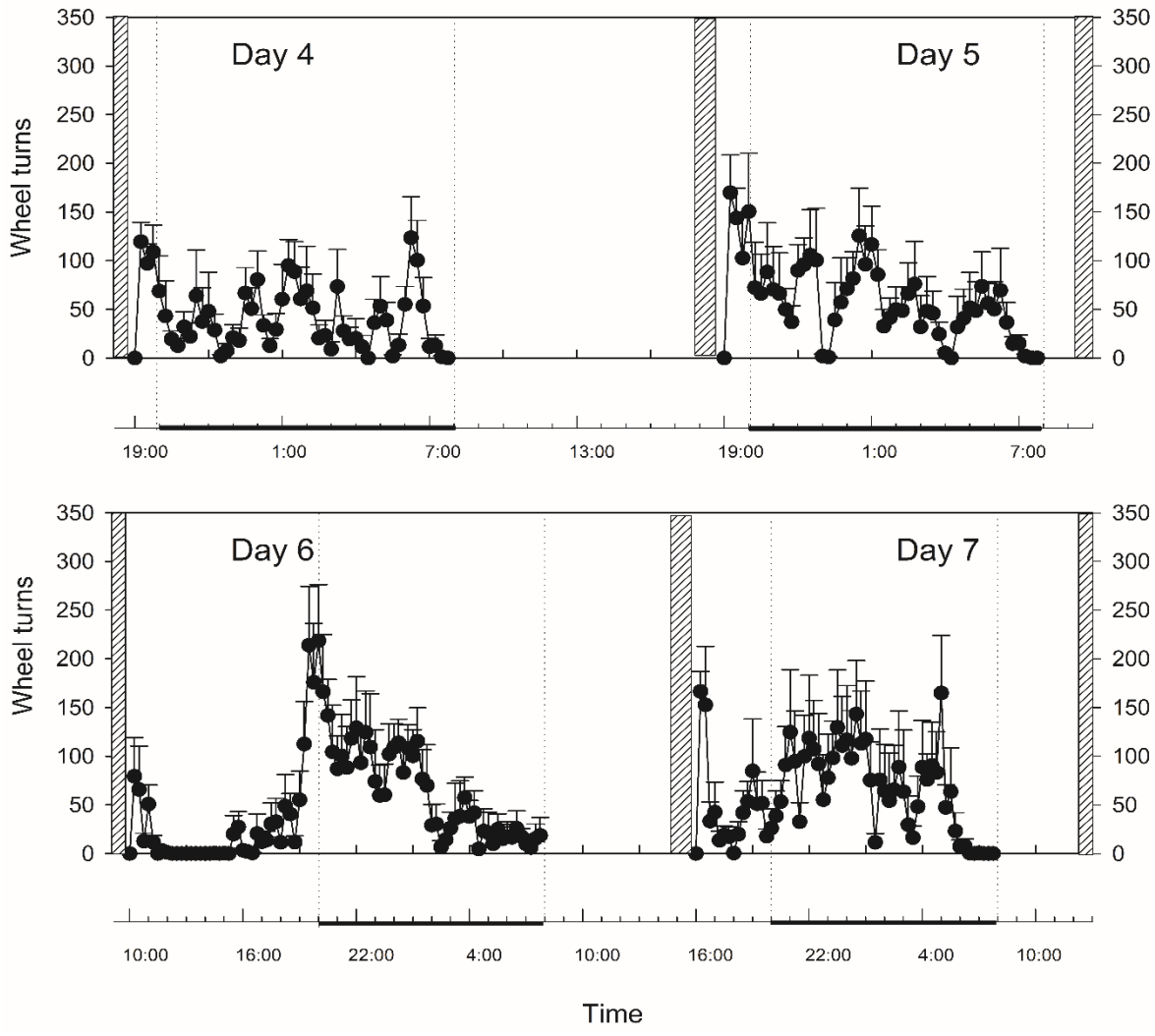

Figure 4.Temporal distribution of wheel turns of groups of animals exposed to the activity-wheel. Each point represents the mean number of turns (± SEM) every 15 min during the four last days of the experimental procedure. Each shaded segment represents the time that rats were exposed to the food (1 h). Black lines in X axis mark the dark-cycle period. Periods with no plot lines mark that rats had no access to a running wheel.

Discussion

The rats in the experimental group reduced their weight throughout the days until reaching the withdrawal criterion, unlike the rats subject to a food schedule without access to the activity wheel. The rats in the control group, after a sharp drop in initial weight at the beginning of the food restriction, reduced the decrease in weight, reaching a stabilization that was not observed in rats subjected to ABA. These differences shown indicate that the effect of the decrease in the weight of ABA rats is due to the exercise carried out, as food restrictions were identical for both groups. These results are consistent with previous studies that show that ABA can be obtained with wheel access values lower than the standard procedure from 10:30 p.m. to 11:00 p.m. (cf. Carrera et al., 2014; Epling & Pierce, 1984). The percentage of weight loss and the fact that the first rat was withdrawn from the procedure on the 7th day is consistent with what was observed in other studies with the standard ABA procedure (Carrera et al., 2006; Epling & Pierce, 1991; Pérez-Padilla et al., 2010).

However, these results contrast with Dwyer and Boakes (1997, Exp. 4) because these authors found that animals with 18.5 h of access to food reduced their weight more slowly and no animal met the criteria until reaching Day 14. In our study, a variable time schedule (VT) was used for food delivery, in contrast to the fixed-time scheduele (FT) used by Dwyer and Boakes. VT schedules in ABA produce greater activity and therefore greater vulnerability to the procedure (Pérez-Padilla et al., 2010). However, in this case, the differences in the sample could better explain the differences found.

One of the differences with this study is the sex of the animals, the aforementioned work by Dwyer and Boakes (1997) used males, who have been reported to be more resistant to the procedure, needing on average more sessions to reach the withdrawal

criteria and showing in general lower running rates (Jones, Bellington, & Ward, 1990; Paré, Vincent, Isom, & Reeves, 1978).

As for other critical variables that can explain this variation in the data, the starting weight of the animals was considerably greater in the case of the aforementioned study (M= 299.6 gr). The weight of the animals is one of the factors that affect the speed of the ABA procedure, with animals with lower weight being more vulnerable to the procedure (Boakes & Dwyer, 1997). In addition, this greater weight would indicate an older age, a factor that has also been found relevant in several studies (Paré, 1975). In adult rats, the ABA procedure is slower because animals are less active than adolescent or young rats traditionally used in ABA studies (de Paz et al., 2019). Our results, however, are in line with what was found in Experiment 3 of the work of Dwyer and Boakes (1997), in which rats with wheel access for 21 h reached the withdrawal criteria in a range of 7 to 10 days. In such experiment, the weights of the animals at the beginning of the experiment were comparable to that of our experiment (M=216.4 g).

As for food consumption, a lower amount of intake was observed as the days progressed in the ABA group. These results are compatible with those found in other studies in which it seems that wheel access interferes with the amount of food eaten (Carrera et al., 2006; Dwyer & Boakes, 1997; Paré & Houser, 1973). However, other studies have not shown these differences (Dixon, Ackert, & Eckel, 2003; Hampstead, LaBounty, & Hurd, 2003; Vidal et al., 2013). In the current case, it can be observed that the lowest consumption of food was at the beginning of the development of ABA but not when the animals approached the final ABA development criterion, which somehow makes previous contradictory data compatible. In addition, direct measures of motivation

for food, using a progressive ratio schedule, have not shown that wheel access reduces the incentive of food in the ABA procedure (de Paz et al., 2019).

As for running (Fig. 3), a gradual increase in the activity of the rats with wheel access was observed, which is compatible with studies in the general ABA procedure (for reviews see: Boakes, 2007; Carrera, Fraga, Pellón, & Gutierréz, 2014) but also with Dwyer and Boakes' Experiment 4 (1997), in which the development of FAA was also prevented. This increase in running was accompanied by a decrease in the weight of the animals, which is consistent with previous studies. The prevention of FAA did not seem to impede the development of running after the food delivery.

Regarding the distribution of activity (Fig. 4), it can be observed that the rats did not develop FAA, as access to the wheel was prevented in the moments prior to the delivery of food. However, the fact of not developing anticipatory running did not prevent the rats from reducing their weight until reaching the withdrawal criteria. These data, in contrast to Dwyer and Boakes' Experiment 4 (1997), show that FAA is not indispensable for the development of ABA. With respect to the distribution of the PFA, it can be seen in the graph that the greatest amount of running occured in coincidence with the night period. In contrast to what was observed in other ABA experiments where running tends to establish its peaks of maximum activity around meals (PFA and FAA) (Fuentes et al., 2015; Pérez-Padilla et al., 2010; Vidal et al., 2013), in the present experiment, running was distributed mostly during the night period. That is, the normal nocturnal distribution of activity of the animals was not altered by preventing FAA, unlike what happens when animals are allowed to run in the previous period close to food delivery. These data could indicate that preventing the development of FAA influences the distribution of activity periods, perhaps by hindering the relationship between running and food administration,

but could also be explained by the association of the availability of wheel with contextual keys: the fact that the wheel was locked at the beginning of the daytime period, preventing animals from using it, can make turning off the lights for nighttime period more salient as discriminative of the availability of wheel access. Although in general, the results of the ABA procedure show that running is developed around meals, this pattern of greater running during the night period has been found in some studies (Dwyer & Boakes, 1997; Pérez-Padilla et al., 2010). It is possible that issues related to the schedules of running and meals, as well as other characteristics of the animals (age, initial weight) may also influence the distribution of the response.

Experiment 2

Experiment 1 showed that rats undergoing an ABA procedure without the possibility of developing FAA lose weight until they reach the anorexia criterion if they exclusively run mainly during the night period of the light-dark cycle, attending to an irregular schedule of food administration. Experiment 2 tests the ability to develop anorexia in animals that are allowed to run only in the moments before a meal, keeping a variable schedule of food administration, complementing the results of the previous experiment and previous studies on FAA with regular cycles of food administration (e.g., Dwyer & Boakes, 1997).

Method

Subjects

The same 16 rats from Experiment 1 were used, now 11 weeks old. After the animals were removed from the procedure, they were given ad libitum food for 7 days until they recovered 100 % of the weight that they had at the beginning of Experiment 1, so that there were no differences in weight between the two experiments, as it is a determining variable for ABA development (Boakes & Dwyer, 1997). Accommodation conditions and animal maintenance were as described for Experiment 1.

Apparatus

The boxes for the experimental rats and for the control rats were the same as those used in Experiment 1. The wheels available in the boxes of the experimental rats were active from 8:00 a.m. until food administration. As in Experiment 1, the experimental control was carried out through a Pentium II computer at 233 MHz, programmed in MED-PC and was located in a separate room.

Procedure

The animals were assigned to the groups so that the rats of the experimental group of Experiment 1 now constituted the control group (n=8), while the experimental group (n=8) was formed by the rats from the control group of Experiment 1. The time of access to food was maintained according to the variable schedule established for Experiment 1 (9:00 a.m. to 10:00 a.m., 12:00 noon to 1:00 p.m., 3:00 p.m. to 4:00 p.m. and 6:00 p.m. to 7:00 p.m.), always within the daytime part of the light-dark cycle. The activity wheels

were programmed to be stopped from the beginning of each daily session (just after food administration) until 8:00 a.m. the next morning, at which time the brake was released and remained active until the following food administration (between 1 and 10 h of wheel access per day). On the first day, the animals were weighed and placed in their individual boxes, having an hour of access to the food without access to the activity wheel, then the food left by the rats of both groups was removed and weighed. For the experimental group, the activity wheel remained with the brake activated until 8:00 a.m. the following morning, registering, every 15 minutes, the number of wheel turns from the time of access to the wheel until food administration (however, these results will not be shown here). The amount of food consumed by each subject was also recorded. The rats were removed from the experiment once the criteria proposed by Dwyer and Boakes (1997) were met, according to which animals were allowed free access to food and the possibility of wheel activity was prevented once their weight was kept below 75 % of its initial weight for two consecutive days. The experiment lasted a total of 11 sessions, which was the day on which the first experimental rat had to be removed from the procedure. The data were analyzed through a two-way ANOVA using the statistical package SPSS 24.0. The post-hoc analyses were Newman-Keuls tests performed with that same computer program. The minimum level of statistical significance was set at $p<.05$.

Results

The percentage of body weight reduction through the days is shown in Figure 5, calculated for each rat in proportion to its weight at the start of the experimental sessions (Day 0), with Day 1 being the first session of exposure to food restriction. It can be

observed how the weight of all animals progressively decreased over the course of the days, being from Day 4 when statistically significant differences were found between the groups (p<.01). From this day onwards, the reduction of weight was more pronounced for the experimental group (with access to the wheel) compared to the control group (without access to the wheel). Statistical data showed a main effect of Group [F(1,14)=23,63; p<.001], a main effect of Days [F(11,154)=238,05; p<.001], and a significant interaction Group x Days [F(7,98)=26,29; p<.001].

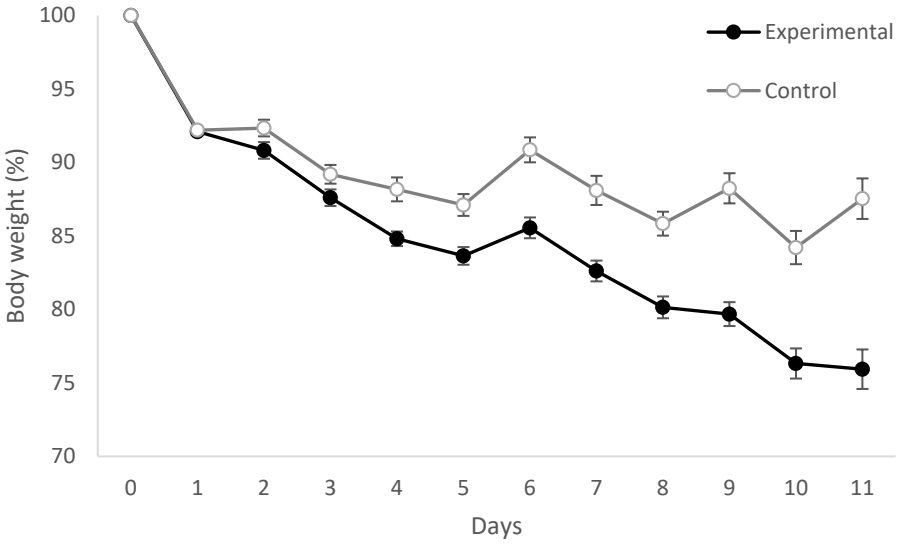

Figure 5.Shows the mean (± SEM) of the body weight percentage respect day 0 for Experimental and Control groups. Experimental animals had 1 h to food exposition each day and access to a running wheel between 1 and 10 h Control animals had the same feeding schedule without access to a running wheel.

The amount of food consumed by both groups during the time of food access is shown in Figure 6. The animals in the experimental group consumed a smaller amount of food compared to the animals in the control group, this difference being significant most days. In both groups, the consumption of food gradually increased as the days passed, stabilizing consumption after approximately day 5. Statistically significant effects were found for Group [$F(1,14)=14,17$; $p<.05$], Days [$F(11,154)=70,76$; $p<.001$], and for the interaction Group x Days [$F(11,154)=2,67$; $p<.05$].

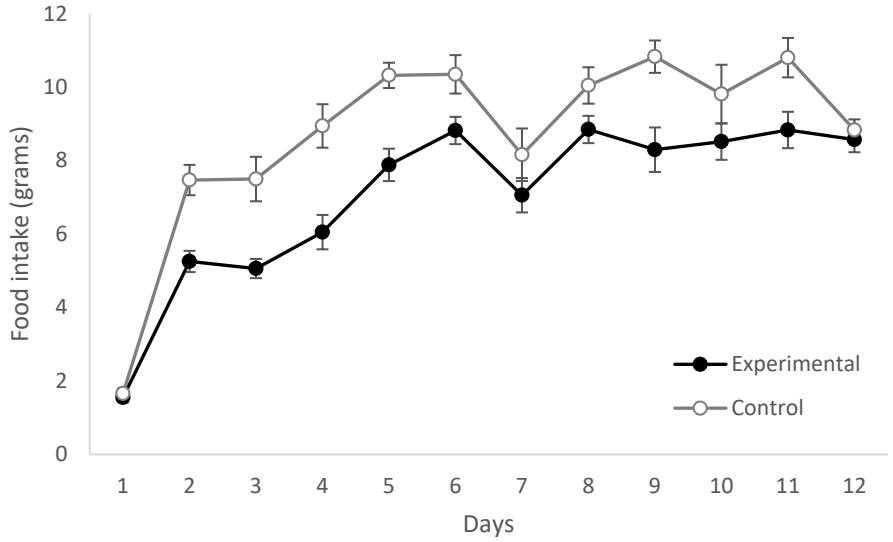

Figure 6. Mean (± SEM) of food consumption during the feeding schedule each group. Experimental and Control group had the same time of food exposition each day (1 h) Experimental animals had access to a running wheel before the food exposition between 1 and 10 h each day.

Figure 7 shows the turns per hour of the animals with access to the activity wheel. The rats had access to the activity wheel from 8:00 a.m. until the beginning of the intake period, which was at variable times as indicated above. Throughout the days, a gradual

increase in activity was observed, reaching relatively high and stable values from Day 7. The statistical analysis carried out showed a main effect of Days [F(10,70)=12,12, p<.001].

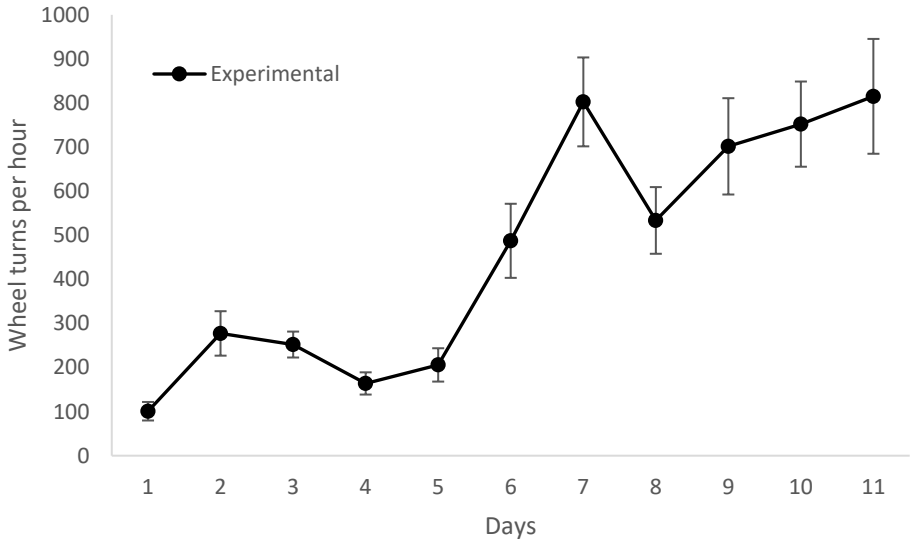

Figure 7. Shows the mean (± SEM) number of wheel turns per hour for Experimental animals. The time exposition to a running wheel varied along days between 1 and 10 h (see Method section).

The presentation of the distribution of wheel turns every 15 minutes during the period before the meal was omitted because the animals could only run during that limited time of availability of the wheel, which began shortly after the lights were turned on. This result has, in a way, already been reported by Pérez-Padilla et al. (2010) when using an identical food schedule.

Discussion

As can be seen in Figure 5, the rats in the experimental group gradually reduced their weight throughout the procedure, until the first rat reached the withdrawal criterion on Day 11. The animals in the control group, however, after a weight loss on the first day of food restriction, gradually reduced their losses and achieved stability in their weights. This experiment shows that the ABA phenomenon develops with shorter exposure times to running than the general procedure, as discussed in Experiment 1. The intermittences observed in animal weight (e.g. weight gain on Day 7) are probably due to the variable duration of the programs that, in this case, shortened the duration of the periods between meals and meant a shorter access time to the activity wheel. These data are consistent with the decrease in intake seen in Figure 6, and are due to shorter times between intakes, as mentioned above.

The results of this experiment are consistent with those observed in Dwyer and Boakes' (1997) Experiment 4, where the rats reached the withdrawal criteria with an average of 13 days (although in this case, males of a greater weight were used as experimental subjects). The differences between the present study and the aforementioned study are the variable duration of access to the wheel, which in the present study depended on the variable times of access to food while in the aforementioned study it was 4 h. There are no differences observed between both procedures.

Regarding food consumption, the animals showed the usual pattern observed in ABA studies, with a very low intake on the first day of food restriction that increased until stabilization was achieved. In this study, the animals of the experimental group were found to eat less food than the animals of the control group, in line although more

pronounced than the results observed in Experiment 1. These data are consistent with what was found in other ABA studies, however, as commented in the previous experiment, there are studies that do not find these differences (Dixon et al., 2003; Hampstead et al., 2003; Vidal et al., 2013). In addition, it is observed that the rats of the control group maintain higher levels of intake, with the difference in food consumption between the experimental group and the control group being higher than in Experiment 1. This may be due to the experience of the previous experiment with food restriction, so that in the second exposure to food restriction, the intake per meal period increases (see General Discussion). With regard to the decrease in intake on Day 7, as commented, is due to the shorter distance between exposure to food. As can be observed, this occurs in a similar way in both groups, therefore, it is not possible to think that could be due to differences in the interaction with the activity wheel.

In relation to the activity developed by the rats of the experimental group (Figure 7), it is observed that their level of activity gradually increased, which is congruent with previous studies and is consistent with what was found in Dwyer and Boakes' (1997) Experiment 4, in which the rats of one of the groups had 4 h of previous access to food. The fluctuations observed in the amount of running may be due to the differential duration of the periods of access to the wheel, with the fact of having longer periods resulting in the measurement of turns per hour being lower as there would be longer periods of inactivity This is not found in the aforementioned study, as the period used was of a constant duration (4 h). The rats gradually decreased their weight as an increase in activity was observed, as reported by other studies with the ABA model (Carrera et al., 2014). This study shows that the development of ABA is possible with short exposure times to the wheel, as other studies have found (Dwyer & Boakes; 1997; Epling & Pierce, 1984)

and, in addition, that the development of FAA is sufficient to develop anorexia, without the need for nocturnal activity or PFA. The importance of food-anticipatory running in ABA has already been pointed out by other studies that found greater vulnerability to the procedure when it appears early (Fuentes et al., 2015). The present study extended this to when a variable instead of fixed schedule of food is used.

Some interesting qualifications can be made to the results mentioned above. The rats used in this study were those that were used in Experiment 1 (see Method section). The rats of the experimental group are the same that were part of the control group in the previous experiment and therefore had experience with a meal schedule but not with the activity wheel.

The fact that rats submitted to a food schedule previously did not differ in their compliance with the withdrawal criteria of Dwyer and Boakes' (1997) Experiment 4 suggests that prior exposure to the food schedule does not impede ABA development. Dwyer and Boakes (1997, Exp. 2) subjected rats of the "pre-adapted" group to a food schedule for 14 days, subsequently introduced the activity wheel and observed that the pre-adaptation to a food schedule prevented the development of the ABA phenomenon. Other authors, however, have found that, although pre-adaptation to a food schedule does delay the phenomenon, it does not prevent it (Cano et al., 2006; Lett & Grant, 2001). In the present study, the procedure was different, as after 7 days of pre-adaptation, the rats had 7 days of recovery without restriction of food. The present experiment found no effect of pre-adaptation to the food schedule using short exposure times to the wheel instead of the standard procedure, although it apparently could have occurred, given the re-exposure of the animals to the same food deprivation schedule previously experienced. It may be that the effect of pre-adaptation is diluted with the return to the period without restriction

of food. In this case, it can be observed in Figure 3 that the animals, when they were subjected again to the food restriction, acted as the non-pre-adapted groups, with a drop in the levels of intake that increased progressively, contrary to what occurred in the pre-adapted group that maintained their intake levels (Dwyer & Boakes, 1997). Therefore, it can be concluded that pre-adaptation to the food schedule does not seem to maintain a long-term effect of impeding or delaying ABA development after mere exposure to it in the past.

General Discussion

In light of the experiments presented here, we can confirm that the ABA phenomenon can develop with shorter exposure times to the wheel than those of the standard protocol from 10:30 p.m. to 11:00 p.m. Both the animals of Experiment 1, with between 13 and 22 h of possible activity, and the animals of Experiment 2, with between 1 and 10 h, developed the typical pattern found in these studies, which consists of a gradual reduction of body weight as the experiment progresses, in contrast to the food restriction control groups, which after an initial descent, were able to maintain their weights. These results corroborate those found by Epling and Pierce (1984) and show that ABA experiments can be performed using wheel access times shorter than usual. In relation to Experiment 1, the most important conclusion is that the phenomenon can be developed by preventing the development of FAA, contrary to that reported by Dwyer and Boakes (1997, Exp. 4). As mentioned, the characteristics of the sample used can explain this apparent contradiction in the data: in the aforementioned experiment, male rats were used, which show less vulnerability to the phenomenon than females (Jones et

al., 1990; Paré et al., 1978) and both the age and weight of the animals were higher than those used in this study, both factors very sensitive to both, activity rates and weight loss (Boakes & Dwyer, 1997; Paré, 1975). In relation to the distribution of the activity, it developed mainly during the night period, not observing the change in circadian patterns that is usually present in ABA studies with the standard procedure, in which running around meals is observed regardless of the light/dark cycle. As mentioned above, this may be due to the fact that the switched off light may be acting as a discriminative stimulus of access to the wheel (and, in addition, the period of light as associated with food). Future experiments should examine this issue.

Experiment 2 is compatible with other studies that have used abbreviated ABA procedures with short periods of exposure to the wheel prior to food delivery. These results are compatible with those found for Dwyer and Boakes' (1997) Experiment 4 FAA group, although some qualifications can be made. In our case, the study was developed with females, which could explain why the withdrawal criteria was reached a few days earlier. Another difference with the aforementioned study is that our animals had a 7-day food pre-adaptation schedule, followed by 7 days of weight recovery. Several studies have shown that prior exposure to a food schedule prevents (Dwyer & Boakes, 1997) or delays (Cano et al., 2006; Lett & Grant, 2001) the development of the phenomenon. In our case, we did not observe this pre-adaptation effect. In our study, however, there was a recovery period between pre-exposure to the regime that had not been used in previous studies. The results indicate that the effects of pre-exposure are not maintained in the medium term.

Comparing both procedures, we can observe that the animals that had access to the wheel during the post-meal period met the criteria earlier than the animals in the

second experiment, which had access to the activity wheel in the period prior to the meal. These results do not seem to be due to the previous exposure to food restriction of the animals of Experiment 2, since this effect was not observed. The results of the animals of Experiment 2 are comparable to those obtained by other animals with similar procedures without a pre-adaptation program and, in Figure 6, it can be observed that the intake maintains the typical pattern of the non-pre-adapted groups. In contrast, this severe reduction in intake is not found in pre-adapted groups after the start of the procedure. Although this comparison should be considered with certain nuances, as the animals in Experiment 2 were 2 weeks older at the beginning of the procedure, a factor that could be important in the resistance/vulnerability to the ABA phenomenon. Another important qualification is that the animals of Experiment 2 had a shorter total exposure time to the activity wheels. Although, if the activity figures are compared (Figures 3 and 7), it is observed that the running rate (turns per hour) is higher in the animals of Experiment 2, thus, the total running rates can be comparable or even lower due to the lower exposure time to the wheel.

An interesting observation arises when comparing both control groups. The animals of the control group in Experiment 2 maintained their weight at higher levels than those in Experiment 1 (Figures 1 and 5) and consumed more food during the food schedule (Figures 2 and 6). The control group in Experiment 2 was formed by the animals of the experimental group of Experiment 1. These animals had a 7-day phase of the ABA procedure (with access to the wheel and food schedule). This higher intake could suggest that animals exposed to the ABA procedure develop changes in the effectiveness of the intake that are maintained beyond 7 days of recovery after the procedure; Similar results have been found with rats exposed to cyclic diets (Archambault, Czyzewski, Cordua Y

Cruz, Foreyt, & Mariotto, 1986). These changes could be due more to the effect of the activity or a combination of activity and food restriction, as this increase in intake was not observed in the experimental group previously pre-exposed to food restriction, or in other pre-adaptation experiments (Cano et al., 2006; Dwyer & Boakes, 1997; Lett & Grant, 2001).

As for the activity, it developed gradually in both groups, coinciding with the weight loss of the animals subjected to the procedure. Animals without access to the activity wheel were able to stabilize their weights, thus these results are congruent with other experiments by assigning a fundamental role to the activity in the development of the phenomenon (Pérez-Padilla et al., 2010; Vidal et al., 2013). We can conclude, therefore, that both activity peaks, the post-food activity and food-anticipatory activity, seem to be sufficient to develop ABA.

CHAPTER 5:

ACTIVITY-BASED ANOREXIA IN RATS AS A FUNCTION OF THE INTERVAL BERWEEN RUNNING AND EATING

Abstract

Activity based anorexia (ABA) develops when laboratory rats have food access restricted to a single period in the day and are given access to a wheel running. Clinical studies have found that patients with anorexia develop high activity levels. These data suggest a possible implication of exercise in the etiology of anorexia and are in line with findings obtained in animals during experimental procedures to model interactions between activity and weight loss. One hypothesis is that in ABA exercise acquires reinforcing properties by its association with food. In order to test this idea an experiment was designed in which the potential association of running in a wheel and food consumption was manipulated by interposing time intervals between both activities. Three groups of 8 male Wistar rats had 2 h of wheel running access per day and 1 h of consumption. We found that level of activity was directly related to proximity to food, being this not related to food intake nor to circadian activity, thus favoring the interpretation that running in ABA is somehow maintain by the food regimen.

Keywords: Activity-based anorexia; food; schedule-induced behavior; wheel running

Activity-based anorexia in rats as a function of the interval between running and eating.

Introduction

ABA is a robust phenomenon provoked experimentally when food is restricted and rats had access to a running wheel. The classical design consists in granting access to food in a single period of the day (1-1.5h) and free access to a running wheel the rest of the time (Carrera, Fraga, Pellón, & Gutiérrez, 2014). In order to check that the results are not exclusively due to the meal restriction, it is common to use a control group exposed to the same feeding schedule but without access to a running wheel (Cano, Gutiérrez, & Pellón, 2006; Carrera et al., 2014; Pérez-Padilla, Magalhães, & Pellón, 2010). The results typically show that both groups lose weight gradually during the first days. After a few days, the control group slows down the rate of weight loss until it stabilizes at around 80-85% of its free-feeding weight. Animals in the experimental condition do not show this stabilization and weight loss continues until life is in danger and rats are removed from the procedure (Dwyer & Boakes, 1997).

The first studies that found an increase in activity when rats are subjected to a food restriction (Bolles & De Lorge, 1962; Finger, 1951; Hall, 1955; Reid & Finger, 1955; Routtenberg & Kuznesof, 1967) found also that this increase in activity increased

mortality in rats subjected to these conditions. Rats ran until death and showed a decrease in food consumption during the last days of the procedure. The results regarding a decrease in food intake have not been replicated recently as, in accordance with the new animal care regulations to promote health and reduce suffering, rats must be withdrawn from the procedure before life is compromised (Carrera et al., 2014; Dwyer & Boakes, 1997). This phenomenon was later called ABA (Epling, Pierce, & Stefan, 1983) attending to the relevant role that activity seems to play in its development. These authors also found similarities between the animal model and case reports of AN, in which hyperactivity seems to be a crucial factor in its onset and subsequent maintenance. Activity in AN plays a crucial role and contributes towards hindering weight maintenance (Hebebrand et al., 2003). It has been observed that around 80 % of patients with anorexia have excessive physical activity (Holtkamp, Hebebrand, & Herpertz-Dahlmann, 2004). Furthermore, there are similarities between the pathological signs in ABA and Anorexia nervosa, such as the development of stomach ulcers (Hall & Beresford, 1989; Paré & Houser, 1973), the disruption or mismatch of the estrous cycle (Watanabe, Hara, & Ogawa, 1992), or the loss of gray and white matter (Frintrop, Liesbrock, et al., 2018). Theses similarities have led to the use of ABA as an animal model of the human pathology (Boakes, 2007; Gutiérrez, 2013) and it has been used to test different therapeutic alternatives (Gutierrez, Cerrato, Carrera, & Vazquez, 2008; Hillebrand, Van Elburg, Kas, Van Engeland, & Adan, 2005).

On the explanations of the phenomenon, there are two main frameworks: those that focus on an adaptation to the meal regime and those that give a fundamental role to activity. Regarding the meal adaptation view (Dwyer & Boakes, 1997), these authors suggest that running in ABA procedure interferes with the adaptation to the food schedule

preventing rats from maintaining their weights like the subjects of the control group with the same exposition to food but without free access to a running wheel. Subsequent results have shown that prior exposure to the food schedule causes a delay in the development of ABA but it does not prevent its development (Cano et al., 2006; Lett, Grant, Smith, & Koh, 2001).

In relation to the second framework, other authors find that the interference in food consumption is caused by the activity either because intense exercise causes a satiety signal that interferes with food consumption (Aravich, 1996; Pierce, Epling, & Boer, 1986), or because it provokes nausea that could develop in a taste aversion conditioning to the food (Lett & Grant, 1996). Similarly, other activity theories suggest that activity is induced by the restricted feeding schedule because in these circumstances running acquires a reinforcer value (Pierce & Epling, 1991), which could interfere with the food's reinforcer value by reducing it (W. F. Epling & Pierce, 1984; Pierce, Epling, & Boer, 1986).

Alternative explanations to the "activity" hypothesis suggest that exercise on wheel running is linked with foraging behavior. Animals increase their activity during shortage of food as a natural selection mechanism to obtain other sources of food. (W. F. Epling & Pierce, 1996; Morse et al., 1995; Pierce & Epling, 1991). Other authors suggest that activity contributes towards maintaining the body temperature that is decreased by food restriction (Cerrato, Carrera, Vazquez, Echevarría, & Gutiérrez, 2012; Gutiérrez, Baysari, Carrera, Whitford, & Boakes, 2006; Gutierrez et al., 2008). Thus, this would suggest that activity increases its probability of occurrence as the days of food restriction progress.

Epling and Pierce (1991) also suggest that activity in ABA could be a schedule-induced behavior. The temporal distribution of the standard ABA procedure shows a pattern of two peaks of running during the inter-meal interval that occur around food delivery (Pérez-Padilla et al., 2010; Vidal, Pérez-Padilla, & Pellón, 2013). This distribution of running changes the normal circadian activity of the rat (rats normally show more activity during the night-cycle period). Activity is expressed in two peaks, one post-meal peak similar to that observed in other schedule-induced behaviors (post-food activity, PFA), such as schedule induced polydipsia (Falk, 1966; López-Crespo, Rodríguez, Pellón, & Flores, 2004; Pellón & Pérez-Padilla, 2013) and another peak before the delivery of food called food-anticipatory activity (FAA) (Dwyer & Boakes, 1997; Mistlberger, 1994; Wu et al., 2014), which is similar to other operant behaviors, such as lever press. Thus, activity on a running wheel increases when the reinforcer is given intermittently rather than when it is given at the beginning of the experiment and it is determined by the duration of the inter-food interval, similarly to other schedule-induced behaviors such as schedule-induced polydipsia (Gutiérrez-Ferre & Pellón, 2019; Levitsky & Collier, 1968). In relation, de Paz, Vidal, and Pellón (2019) showed that the reinforcer value of food increases in ABA and hence, food could be an effective reinforcer for wheel running.

It has been proven that weight loss can occur with shorter expositions to wheel running (Boakes & Dwyer, 1997; Dwyer & Boakes, 1997; W. F. Epling & Pierce, 1984) (see previous experiment of this Doctoral Thesis). In fact, 2.5-4 h of wheel running access a day, immediately before food exposition (FAA), is sufficient to provoke a weight loss comparable to the ABA standard procedure. In the present experiment, we aimed to assess the importance of running immediately before the delivery of food (FAA), rather than

running with less contiguity by using different intervals to maintain or break the contiguity between the two behaviors. If activity interferes with food intake by reducing it (Aravich, 1996; W. F. Epling & Pierce, 1984; Lett & Grant, 1996; Pierce et al., 1986), we expect that the animals that run immediately before feeding will reduce their food consumption; However, if running is reinforced by food delivery, then rats that feed immediately after wheel running will increase their activity.

Method

Subjects

A total of 24 male, experimentally naïve Wistar rats obtained from the Charles River Laboratories (Lyon, France) with an average weight of 215.7 g were used in this experiment. On arrival, the rats were 6 weeks old and were housed in groups of four until they were placed individually at the start of the behavioral procedure, 2 weeks after arrival. All subjects were monitored daily and maintained on an ad libitum food and water regime. The animals were kept in a temperature-controlled room at 21±2 °C, 60 % of relative humidity, and with a 12 h light–dark cycle (8.00 a.m. to 8.00 p.m.). 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 care and use procedures were in accordance with the European Communities Council Directive 2010/63 and the Spanish Royal Decree 53/2013.

Apparatus

During the behavioral procedure, experimental animals were housed in individual transparent Plexiglas chambers measuring 21 x 45 x 24 cm., which had a hole in the left wall where an activity wheel (9 cm wide and 34 cm diameter) was attached. Access to the wheel was granted by sliding manually the flap used to open and close the hole. Each chamber was provided with a water receptacle, inserted into the right-hand side of the roof and permanently accessible. Along the water container there was a concave area in which the food was deposited.

The wheel had a brake mechanism to prevent movement during the food and break times; the brake mechanism and the number of completed laps were controlled through an MED-PC-IV program for Windows (MED Associates Inc., Georgia, VT, USA).

Procedure

After 1 week of acclimatization, rats were randomly separated into 3 groups that differed in the time of wheel access. All animals received one hour of unlimited food exposition at 6.00 p.m. each day. The Immediate group (I group) had access to a running wheel between 4.00 p.m. and 6.00 p.m. Animals in the medium interval (M group) and the large interval (L group) groups had access to the wheel from 12.30 p.m. to 2.30 p.m. and from 9.00 a.m. to 11.00 a.m., respectively. The experiment finished for each animal when the percentage of body weight fell below 75 % during 2 consecutive days, in order to prevent death (Dwyer & Boakes, 1997), or after 15 days in the procedure. Water was freely available at all times. All rats were weighed daily immediately before food exposition.

Recovery phase: When rats reached the withdrawal criterion, they were fed with ad libitum food until each animal reached the expected weight according to their theoretical weight curve and were maintained at that weight for 2 weeks.

Second phase: In order to test whether there could be differences in running due to circadian rhythms, rats were exposed in a second phase to the same exposition to wheel running and with same schedule but with ad libitum food during the 15 days of experiment. This phase was performed 2 weeks after the first experimental procedure had ended.

Data analysis

The computer recorded the wheel turns each second during the running sessions using MPC IV program for Windows. We analyzed all data using a repeated-measures analysis of variance (ANOVA) to assess differences based on between group and within-subject factors. The rejection criterion was fixed at $p<.05$ for all tests. Effect size was estimated by η^2_p. All statistical analyses were performed using the SPSS 24.0 software package.

Results

In figure 1, we can observe the mean (± SEM) percentage of body weight during the first three and the last three sessions of each animal. The 100 % was the animal weight on Day 0, immediately before the beginning of the experimental phase. We can observe that animals in all groups reduced their body weights during the experiment. A repeated-

measures ANOVA (Group x Days) showed an effect for Days [F(6,126)=526,02, p<.001, η^2_p =.96], but no effect for Group or its interaction with Days.

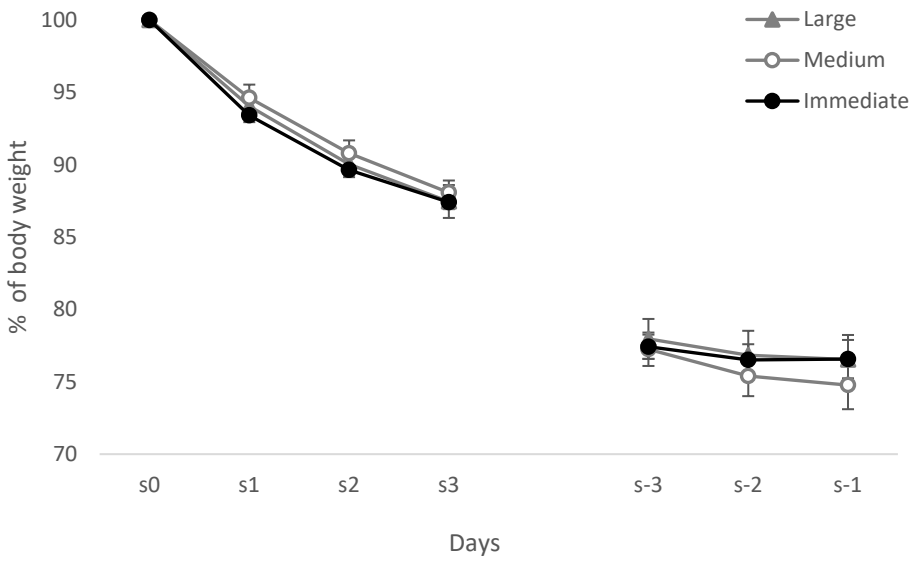

Figure 1. Mean (± SEM) of the weight percentage respect to Day 0 is shown for each group for the three first and the three last days of each rat. All animals had 1 h of food exposition and 2 h of access to a running wheel in terms described in Method section.

Data on Figure 2 shows the mean (± SEM) of the grams of food consumed by each group during the first three and the last three days in the experimental procedure for each rat. All animals had 1 h of food access at the same time (6:00 p.m. to 7:00 p.m.). Rats in the three groups showed an increase in food consumption during the procedure, the total of food intake per day was significantly higher for the rats that ran immediately before the exposition to food (I group). The repeated measures ANOVA yielded a significant effect for Days [F(5, 105)=66.11, p<.001, η^2_p =.76], and Group [F(2, 21)=7.68,

$p<.005$, $\eta^2_p = .42$], but showed no effect for the interaction. Post hoc analyses revealed differences between the I and L groups ($p<.0050$) and between the I and M group ($p<.05$). No differences were obtained between the L and M groups.

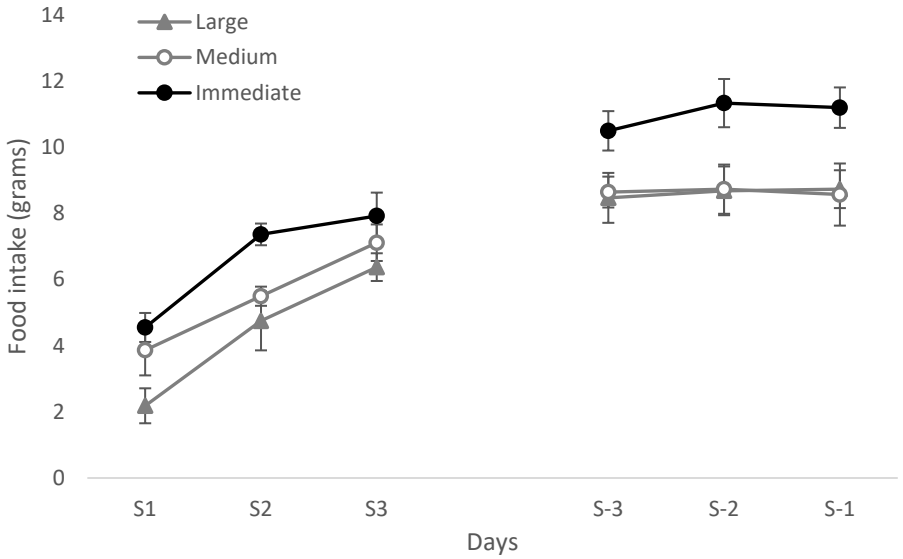

Figure 2. Shows the mean (± SEM) of food consumption during the 1 h of food exposition for each group for the three first and the three last days of each rat. All animals had 1 h of food exposition and 2 h of access to a running wheel in terms described in Method section.

Figure 3 shows the mean (± SEM) of wheel turns per group for the first three and the last three sessions in the procedure for each rat. As can be observed, after starting with a low number of wheel turns, all animals gradually increased their activity throughout the experiment, reaching a high level of activity. Rats that ran immediately before food exposition (I group) increased their activity significantly more than the other groups that had an interval between running and eating (L and M groups) during the procedure. The

repeated measures ANOVA yielded significant effect for Days [F(5, 105)=132.51, p<.001, η^2_p =.86], Group [F(2,21)=4.65, p<.05, η^2_p =.31], and for the Days x Group interaction [F(10, 105=4.97, p<0.001, η^2_p =.32]. Post hoc comparisons showed significant differences between the I and L group (p<.05), between the I and M group (p<.05), and no differences between the L and M group.

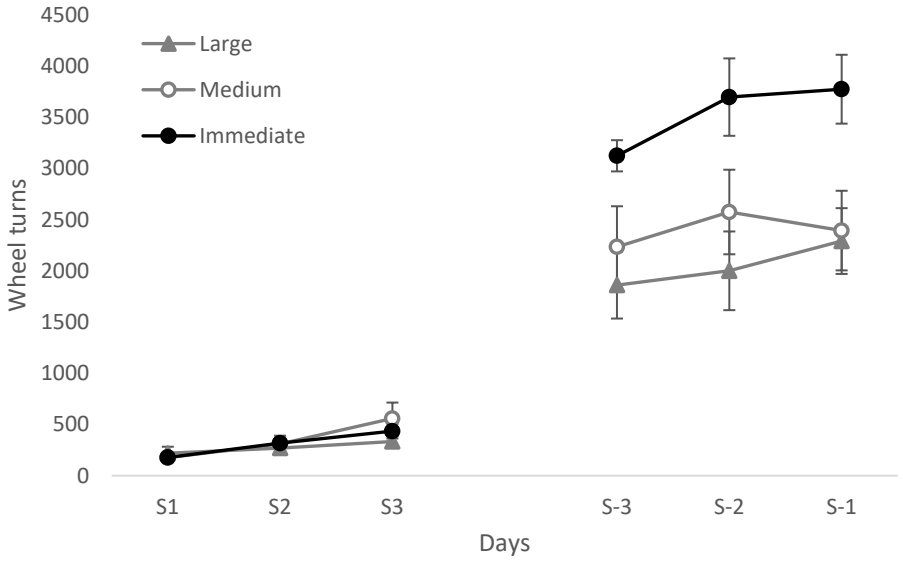

Figure 3. The mean (± SEM) of the number of wheel turns during the 2 h of wheel access is shown for each group for the three first and the three last days of each rat. All animals had 1 h of food exposition. Groups are described in Method section.

The results of Figure 4 show the mean (± SEM) of wheel turns for the first three and the last three days of the second phase procedure. This phase was carried out with animals exposed to an ad libitum food regime and the same time intervals for wheel access that were used in the experimental procedure. It can be observed that all rats reached low values of running and that these values decreased throughout the procedure. A repeated

measures ANOVA yielded significant differences for Days [F(5, 105)=12.38, $p<.001$, η^2_p =.37] and an interaction effect [F(10, 105)=6.41, $p<.001$, η^2_p=.38], yet no effect for Group was found.

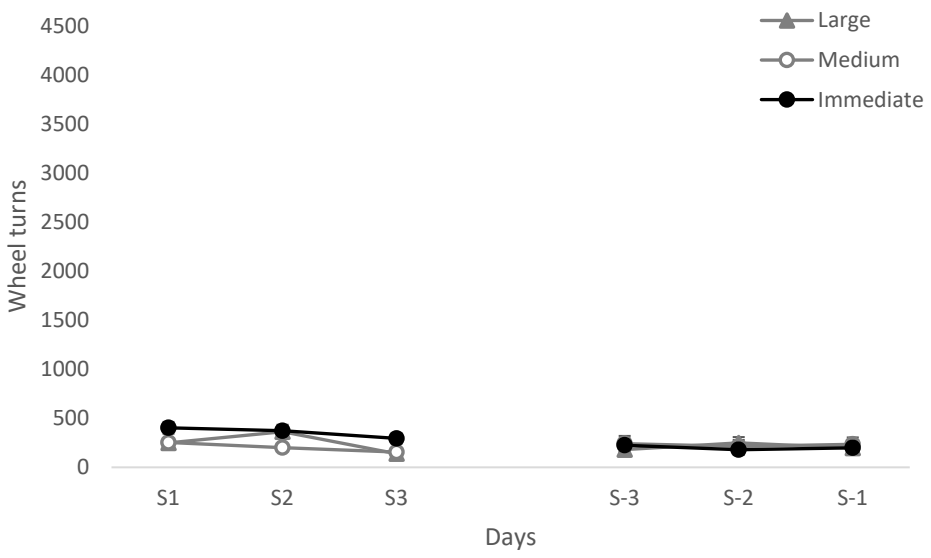

Figure 42. Shows the mean (± SEM) of the number of wheel turns during the 2h of wheel access for each group for the three first and the three last days of each rat. All animals in this condition were maintained ad-libitum. Groups are described in Method section.

Discussion

All animals in the three conditions gradually reduced their body weights along the experimental procedure. This data is consistent with the findings obtained in other ABA experiments with the standard ABA procedure (Cano et al., 2006; Carrera, Gutiérrez, & Boakes, 2006; Pérez-Padilla et al., 2010). In this case, we found a reduction in body

weight with 2 h of running wheel access that are in line with the reports of other studies using shorter exposure times to a running wheel (Epling & Pierce, 1984; see previous experiment of this Doctoral Thesis). Experiments with a similar procedure (2 h of running wheel access) showed similar results in terms of body weight reduction (Boakes & Dwyer, 1997; Dwyer & Boakes, 1997), despite our feeding period being 1 h rather than 1.5 h long. In the articles cited above, the experimental group had 2.5-4 h of wheel access immediately before the feeding period, which is comparable to our I group. In our results, there are no differences in terms of body weight reduction for animals that ran 6 h (L group) or 4.5 h (M group) before the food exposition, which suggests that exercise is a crucial factor in terms of reduction of body weight, regardless of the time schedule. These results are in conflict with those reported by Dwyer and Boakes (1997, Expt. 4). These authors found that animals that had access to running wheel 18.5 h a day, which was prevented for the 4 h prior to the delivery of food in order to prevent the development of FAA, stabilized their weights in contrast with animals that had access to the running wheel during the 4 h before the feeding period. In our experiment, animals with only 2 h of wheel access did not differ in terms of body weight loss, suggesting that 2 h of running is sufficient to provoke the experimental phenomenon. Differences in body weight at the beginning of the experiments could explain these results, in the cited experiment, the average weight of the rats was higher (299.6 g). Rats with a higher body weight are more resistant to the ABA procedure (Boakes & Dwyer, 1997; see previous experiment of this Doctoral Thesis). Our results are in line with previous experiments that have found reductions in body weights similar to standard ABA procedure with less time of wheel running access (W. F. Epling & Pierce, 1984), and with prevention of FAA (see previous experiment of this Doctoral Thesis).

Regarding food intake, all animals increased their consumption along the procedure, this data is consistent with other experiments using the standard ABA procedure (c.f. Carrera et al., 2014) and others experiments using abbreviated wheel running access procedure (Boakes & Dwyer, 1997; Dwyer & Boakes, 1997; see previous experiment of this Doctoral Thesis). Some authors have suggested that animals lose weight because excessive activity produces a neurochemical signal that causes satiety, interfering with food intake (Aravich, 1996; Pierce et al., 1986) or that an increase in the reinforcer value of activity could interfere with the food reinforcer value (Epling et al., 1983; W. F. Epling & Pierce, 1988). Similarly, Lett and Grant (1996) suggested that exercise on the wheel can produce nausea and that this can be associated with food, resulting in a taste aversion conditioning. In this experiment, rats that had access to a running wheel immediately before feeding ate more than rats that had an interval between running and food exposition. We can conclude that intense exercise before eating does not seem to interfere with food consumption by reducing it. Some studies had found that access to a running wheel reduces the motivation for food (Levitsky & Collier, 1968; Pierce et al., 1986) but this does not seem occur in rats undergoing an ABA procedure. This pattern of increase in food intake when rats have access to a running wheel was also found in other studies (Tokuyama, Saito, & Okuda, 1982).

All animals increased their exercise throughout the days, which is congruent with previous studies on both, the standard ABA procedure (Carrera et al., 2014) and the abbreviated with 2.5-4 h of wheel running access (Boakes & Dwyer, 1997; Dwyer & Boakes, 1997). Some studies have found that rats that ate during the night-cycle were more resistant to the ABA procedure (Dwyer & Boakes, 1997; Hara, Manabe, & Ogawa, 1981). In this study, all rats started the feeding period 2 h before the start of the dark-

cycle, the difference between the groups was the time exposition to a running wheel, always during the day-cycle, comparable with other ABA studies with abbreviate procedures (Boakes & Dwyer, 1997; Dwyer & Boakes, 1997) (see previous experiment of this Doctoral Thesis). Rats that ran just before the food exposition ran more than rats that had an interval between wheel running access and feeding. Our results suggest that food can reinforce running. Previous studies in our lab have found that rats subjected to ABA do not reduce the food motivation (de Paz et al., 2019), and are compatible with food in ABA acting as an effective reinforcer for activity. The fact that the rats that ran more (I group) did not reduce their weights significantly more that the rats with less turns in the running wheel could be explained by these rats showing a higher food consumption during the feeding schedule, which could alleviate the higher energy expenditure.

Furthermore, we can show that when animals ran during the no feeding restriction schedule (Fig. 4), they ran significantly less than animals in the ABA procedure and no differences were found regarding the time schedule of running. Also, we can see a reduction of the time spent in wheel running in all groups. These results could suggest two important explanations: First, the excessiveness of running is related with food restriction. Second, breaking the correlation between activity and food delivery, and reducing the food reward value (by giving unrestricted food) could affect the expression of exercise, as other operant behaviors can be affected by its consequences.

Our results evidence the relevancy of the study of FAA and the relation between food and running in animal models of anorexia nervosa. As discussed before, the fact that the rats that ran immediately before eating gradually increased their exercise reaching higher values in the last days of the experiment could be explained through the correlation

with food delivery. De Paz, Vidal, and Pellón (2019) showed that animals during ABA do not reduce the food reinforcer value, which is congruent with our results.

In induced behaviors, the response that occurs in the interval between meals can be related to the reinforcer, generating a non-explicit contingency that increases its probability of occurrence (c.f. Killeen & Pellón, 2013). Several studies have found that rats with limited access to a running wheel press the lever to obtain it , suggesting a self-reinforcing value of exercise (Belke, 1996; Belke & Heyman, 1994; Belke, Pierce, Magee, & Laffoley, 2016; Collier & Hirsch, 1971; Iversen, 1993). As reported before, running could also be expressed to maintain body temperature when it is reduced by food restriction (Cerrato et al., 2012; Gutiérrez et al., 2006; Gutierrez et al., 2008). Furthermore, activity during no food periods could be a phylogenetically prepared behavior in order to increase the probability to obtain other sources of food (W. F. Epling & Pierce, 1996; Morse et al., 1995; Pierce & Epling, 1991; Sherwin, 1998). These findings are in line with activity during ABA being expressed on a high rate. These high baseline levels of activity increase the probability that running occurs near to food periods and facilitate activity being associated with food, thus increasing its expression through reinforcement. We suggest that two mechanisms are involved in this phenomenon: induction and reinforcement. In relation to the PFA, the correlation between activity and food could explain that rats run after eating. The correlation between behavior and a Phylogenetically Important Event (such as food) is the mechanism where, when food is presented, it induces the behavior related with it (Baum, 2015), showing the PFA found in ABA experiments with the standard procedure (Fuentes, Rick, López, Vidal, & Pellón, 2015; Pérez-Padilla et al., 2010; Vidal et al., 2013). We characterize ABA as a

biobehavioral phenomenon that appears when both, a high probability of expressing activity and a limited exposition to food occur.

The fact of not consistently finding a "loss of appetite" in studies with ABA suggests that this is a good model of pathology acquisition. As we discussed previously, the decrease in intake in rats occurs in the final stages of the phenomenon, when the rats were close to death. Current studies, for ethical reasons, do not allow to observe this process, as the rat is removed from the procedure before its life is in danger (Carrera et al., 2014; Dwyer & Boakes, 1997). Regarding human AN, some authors suggest that voluntary refusal to eat has been exaggerated in the literature (Zanker, 2009), and in that step that goes from the diet to the extreme restriction, the volition of the patients has been magnified in the literature without any evidence (Striegel-Moore & Bulik, 2007). In this line, the criteria of voluntary refusal to eat has recently disappeared from the new version of the DSM (5th ed., APA, 2013) after criticism of the DSM-5's provisional criteria (e.g. Hebebrand & Bulik, 2011). Some authors have suggested that the core symptoms of AN in a classical view could be a consequence rather than a cause of neurological changes induced by malnutrition (Epling et al., 1983; Franklin, Schiele, Brozek, & Keys, 1948; Keys, Brožek, Henschel, Mickelsen, & Taylor, 1950) .

Modifications to the procedure have been proposed that may be interesting to observe what happens in a longer period of time (Frintrop, Trinh, et al., 2018) -similar manipulations were also reported in Pirker (1996)- because the phenomenon under usual conditions (standard or abbreviated ABA procedure) develops very quickly. The novel contribution of chronic ABA looks promising to study the effects of maintaining a low weight over time. The differences regarding the food schedule make us think that the standard procedure is more appropriate as an AN model. In chronic ABA, the

experimenters calculate the amount of food that is dispensed to each rat to reach (and then maintain) the weight criterion. Animals can eat along the day the food that was previously calculated and this amount of food is different each day between animals and is different from day to day for the same animal. For the same reason (maintaining the weights at 75 %), there are differences in food given by experimenters between diet (food restriction without wheel access) and the ABA group because ABA rats spend more energy during running. In the standard ABA procedure, diet and ABA rats have the same exposure to food (1 h) and the differences observed in intake (ABA rats show less food consumption) are due to the differences in procedure (access to a running wheel or not), this point is crucial and has greater ecological validity with human AN (patients consume less food under same availability).

CHAPTER 6:

GENERAL DISCUSSION

General discussion

In Chapter 2, we analyzed the effect of MDMA, a drug that induces locomotor hyperactivity in Dark Agouti rats, an effect that can last from days to weeks. Guided by results that suggest a crucial role of hyperactivity in the development of ABA, we wanted to check whether showing more activity is a vulnerability factor to weight loss. We found a trend towards significance (p=.06) for the treatment effect, that is, rats treated with MDMA one week before the experimental procedure expressed more activity than Saline rats. Despite this difference, we did not find greater weight loss in treated rats. These results could be explained by the fact that the phenomenon developed very fast in Dark Agouti rats, which showed high levels of activity in comparison to Wistar rats, and there was little room to observe the effects of the treatment. The hyperactivity caused by MDMA seemed to lead treated rats to reach the maximum levels of wheel running earlier than untreated rats. In relation with behavioral differences on the temporal distribution of running, we showed that MDMA could affect the pattern of activity; showing a more flattened distribution with greater night activity for MDMA-treated rats. Furthermore, this study confirms that ABA is a robust phenomenon that develops in different strains of rats.

The aim of Chapter 3 was to assess differences in ABA between female and male rats, and to characterize their pattern of activity. Several studies have previously found conflicting results (Boakes et al., 1999; Doerries, Stanley, & Aravich, 1991; Hancock &

Grant, 2009; Jones, Bellingham, & Ward, 1990; Paré et al., 1978), but in some case these differences can be explained by methodological reasons. Female rats showed greater vulnerability to ABA, reaching the withdrawal criterion faster than males. This vulnerability does not seem to be due to a lower initial body weight or a lower food intake (in percentage of body weight) in females. These results are in line with the greater vulnerability found in women than men in AN, giving an important ecological validity to ABA as a good model of the human pathology.

Regarding the pattern of wheel running, as found in previous studies (Chapters 1 and 2), wheel running tends to be expressed in two peaks related to food delivery, post-food activity (PFA) and FAA. No differences were found between males and females in terms of temporal distribution of wheel running except for a higher level of activity in females and earlier development of FAA. The fact that female rats show higher wheel running than males seems to be the cause of their rapid weight loss, emphasizing the role of hyperactivity in ABA. This high activity in the ABA phenomenon may be related to the fact that female rats generally show higher levels of activity than males (Eikelboom & Mills, 1988; Tokuyama, Saito, & Okuda, 1982). These high levels of wheel running, increase the probability of its association with food under reinforcing and inducing mechanisms. This could explain the more abrupt increase in running and the earlier development or FAA shown in female rats.

The experiments described in Chapter 4 had the objective of assessing separately the contribution to weight loss of the two peaks of activity normally found in ABA studies (Chapters 2 and 3; Pérez-Padilla, et al., 2010). For this purpose, we used a variable-time (VT) schedule for the delivery of food. Our results showed that ABA can be developed using abbreviated procedures with less access time to the wheel, as previously reported

(Boakes & Dwyer, 1997; Dwyer & Boakes, 1997; Epling & Pierce, 1984). As opposed to the results described in Dwyer and Boakes (1997), when access to the wheel was prevented before eating, in order to impede the development of FAA, animals developed ABA and showed similarities in weight loss with other studies using the standard ABA procedure (c.f. Carrera et al., 2014). Regarding FAA, we found that animals developed ABA with very short periods of exercise when the rats lacked access to the wheel right after the delivery of food, in order to prevent PFA. This evidenced the importance of FAA in the development of ABA and the results are in line with previous experimental data (Dwyer & Boakes, 1997; Boakes & Dwyer, 1997). Due to limitations derived from the methodology of this study (animals in Experiment 2 were the same used in Experiment 1, which were older and had pre-exposure to the food schedule during Experiment 1) we cannot compare the greater or lesser relevance of each peak in the vulnerability to the phenomenon. Nevertheless, the effect of pre-exposure to the food schedule seems not to be maintained after two weeks of weight recovery with ad-libitum food regimen. Regarding the temporal distribution, the rats without FAA showed a shift in wheel running; the activity was expressed mostly during the night period. This effect was previously reported using also a variable-time procedure (Pérez-Padilla, Mgalhães, & Pellón, 2010).

The aim of Chapter 5 was to study the relation of the temporal distance between running and eating in an abbreviate ABA procedure. As previously found (Boakes & Dwyer, 1997; Dwyer & Boakes, 1997; Chapter 4), animals can develop the phenomenon with very short exposure to a running wheel. We found that animals that ran immediately before food exposition ran more and ate more than animals that had an interval between running and eating. The fact that the rats that ate immediately after running showed an

increased food consumption, allows us to invalidate the hypotheses that suggest that the effect of weight loss in ABA is due to an interference between running and eating caused by different mechanisms (Aravich, 1996; Dwyer & Boakes, 1997; Epling et al., 1983; Epling & Pierce, 1988; Lett & Grant, 1996; Pierce & Epling, 1986). Also, the greater activity levels expressed in the Immediate group allows us to support the idea that activity in ABA could be reinforced by food delivery. Wheel running in ABA seems to be similar to other schedule-induced behaviors that are maintained by its environmental consequences (c.f. Killeen & Pellón, 2013). These results are in line with those found in de Paz et al., (2019), which suggests that food can be an effective reinforcer for activity in the ABA procedure.

Analyzing the data of these experiments together, we can reach conclusions about the role of activity in the development of activity-based anorexia. The fact that rats run even when the higher energy expenditure prevents them from maintaining their weights during the food restriction regime, shows the dysfunctionality of this behavior. It has been found that a higher activity rate contributes to a greater vulnerability to weight loss in the development of the phenomenon. This is the case of the studies discussed above in which the higher exercise level of females is related to reaching the withdrawal criteria faster (Chapter 3). The vulnerability related to high levels of running was not found in Chapter 2, but this could be due to the use of a hyperactive strain of rat, which could mask the effect of hyperactivity caused by MDMA. Also, in the case of experiments within the same sex, manipulations that produce a greater running make the weight loss faster and reach the withdrawal criterion earlier (Pérez-Padilla et al., 2010).

In Pérez-Padilla et al. (2010), animals subjected to a VT food schedule ran more than rats under a comparable fixed-time schedule (FT). Exposure to the activity-based

anorexia procedures resulted in significant increases of running on the wheels as sessions progressed. The greater running rate in the VT procedure resulted in a higher percentage of body-weight loss than in the FT procedure. It is well known that, in operant behaviors, variable schedules produce a higher and more stable response rate than fixed schedules (Ferster & Skinner, 1957). In addition, these results indicate that variations in the form of food delivery affect the development of wheel running and are compatible with the analysis that activity in ABA is similar to other operant behaviors.

As has been discussed, when FAA develops early, rats reach the starvation criterion faster (see Introduction section; Fuentes et al., 2015; Chapter 3). Rats gradually increase their running rate close to food delivery occurrence. This pattern is similar to other operant or schedule-induced behaviors, such as magazine entries or no-contingent lever pressing. As previously mentioned, it is known that a preadaptation to the meal schedule, prior to introducing the activity, makes the animal more resistant to the development of activity-based anorexia (Cano et al., 2006; Dwyer & Boakes, 1997, Lett et al., 2001). However, once the procedure is started, the FAA reflects that the animal is adapted to the meal schedule (rats ran “in anticipation” to food), and this adaptation, rather than making the animal resistant, makes them more vulnerable. Therefore, there is a difference between whether the adaptation to the meal schedule is done before, or after the activity is introduced. The presentation of food without the opportunity to run could interfere with the learning of the running-food contingency that emerges in the procedure. As it has been suggested in other studies with schedule-induced behaviors, the introduction of the intermittent food schedule without the possibility of making the response, prevents further development of the behavior when the access to the response is available.

According to previous results, there is evidence that activity plays a crucial role in the development of ABA. Rats exposed to standard ABA procedures show a peak of running immediately after eating (PFA) and before food delivery (FAA). This pattern modifies their normal circadian activity across the procedure. In the first sessions, rats run mostly during the night part of the cycle, as it is expected in nocturnal animals, but when ABA develops, these running periods are established around feeding, breaking the normal circadian activity. Some experiments have found that PFA shifts to the night when rats are subjected to a VT schedule (Chapter 4; Pérez-Padilla et al., 2010). These results could be an effect caused by the discriminative stimuli present during wheel access or food exposition. Also, the failure or difficulty in developing FAA could affect induction, showing a flattener pattern of activity.

We have previously reviewed the importance of FAA, the fact that the high rate of wheel running during previous hours to food exposition makes rats more vulnerable to the ABA procedure (see Introduction; Chapter 3) is in line with this hypothesis. In addition, ABA can be developed by exposing rats only to a FAA (Chapters 4 and 5; Boakes & Dwyer, 1997; Dwyer & Boakes, 1997). The temporal distribution of running shows a consistent pattern throughout experiments (Chapters 2 and 3; Pérez-Padilla, Magalhães, & Pellón, 2010; Dwyer & Boakes, 1997).

Rats in ABA may run until death, but recently, attending to a greater care of animal suffering, rats are removed from the experiment after reaching a critical point that threatens their lives. This excessiveness, coupled with the location of running, shows similarities with other adjunctive behaviors, such as SIP (López-Crespo et al., 2004; Castilla & Pellón, 2013). In adjunctive behaviors, the response emerges during inter-food interval with a non-explicit contingency between response and its consequences. When

subjects make the responses and then receive the programmed non-contingent reinforcer, a non-explicit contingency emerges which causes the response to increase its probability of occurrence; we can see this progressive increase of running throughout sessions similar to those found in other schedule-induced behaviors (c.f. Killeen & Pellón, 2013). Results which report that schedule-induced running occurs during FT food schedules support this view (Gutiérrez-Ferre & Pellón, 2019).

Traditionally, schedule-induced behaviors are divided into two groups depending on their distribution along inter-food intervals: behaviors that were expressed during a post-food period were called “interim behaviors” and those that emerged near food delivery were called “terminal behaviors” (Staddon, 1977).

Animals that live in confinement conditions, after having access to a running wheel, show high levels of activity. As discussed before, exercise on a running wheel could be a self-reinforced behavior (Belke, 1996; Belke & Heyman, 1994; Belke & Pierce, 2016; Iversen, 1993). Furthermore, as aforementioned, activity when food is not available could be a phylogenetically prepared behavior in order to increase the probability to obtain other sources of food (Sherwin, 1998) or to maintain body temperature (Cerrato, Carrera, Vazquez, Echevarría, & Gutiérrez, 2012; Gutiérrez, Baysari, Carrera, Whitford, & Boakes, 2006; Gutierrez et al., 2008). This high baseline level of activity results in a high probability that running would occur near to food periods, thus increasing its rate and excessiveness during ABA. We suggest that two mechanisms might be involved: induction and reinforcement. We believe that running in ABA seems to act as a “terminal” and “interim” behavior. The aforementioned experiments prove that activity can be reinforced by food exposition (Chapter 5). In relation to the PFA, the correlation between activity and food could explain why rats run

after eating. The correlation between behavior and phylogenetically important events (PIE; e.g., food) is the mechanism which provokes that when a PIE occurs, it induces the behavior related with it (Baum, 2015). Relevant results are in line with this hypothesis: Bolles and Moot (1973) showed that when an expected meal is not provided, rats run before the meal in its anticipation (FAA) but not after the absence of the scheduled meal, showing no post-food activity. These results are in line with the induction hypothesis for PFA. We characterize ABA as a biobehavioral phenomenon that appears when these circumstances occur.

Regarding similarities of AN and ABA (see Chapter 1), the high vulnerability of females to ABA correlates with data reported for women in AN. Regarding age, adolescent and young adult rats show more vulnerability to the phenomenon, in line with what is reported in epidemiological studies of human population. This effect could be due to the higher level of activity shown by adolescents and young humans. It is known that activity decreases as age progresses. Furthermore, attending to the relation between running and eating in ABA, the fact that adolescent and young people are normally subjected to a FT feeding schedule (meal schedules imposed by the family) could increase their vulnerability to develop AN.

Taking into account the results mentioned in the previous sections, the symptoms of body dysmorphia and voluntary rejection of food could be imposed by culture. The importance given to body image factors seem to be exaggerated in AN because, despite many adolescents being on a diet, only 1% develop anorexia (Casper, 2002) and this symptomatology is not found in all cultures (Woodside & Twose, 2004). The importance given to the fear of gaining weight in diagnostic classifications occurred during the twentieth century. These symptoms could be related to modern changes in the

phenomenology of AN characteristic of the Western culture (Fairburn, Shafran, & Cooper, 1999; Keel & Klump, 2003; Lee, 2001). The rejection of food that appears in the diagnostic manuals is a characteristic that did not appear in previous descriptions of AN, which could suggest that this symptom may be the result of an acculturation process in both the media and the context of therapy (Gutierrez, 2011).

Both animal experiments with the ABA model and human studies seem to indicate that the combination of food restriction and exercise can lead to the development of anorexia. As Epling, Pierce and Stefan (1983) commented, it is the contingencies of reinforcement established in Western culture that stimulate and encourage people to exercise and go on a diet, with which some individuals can perform the combination of food restriction and exercise, path through which the AN begins.

Based on the advances in the study of ABA and AN, the therapeutic failure (see Chapter 1) could be due to a poor characterization of the phenomenon in which cognitive aspects have been exaggerated and mechanisms such as hyperactivity have not been sufficiently attended. In addition to this, the diagnosis criteria (see Chapter 1) based on diagnostic guidelines, which require a certain body weight and cognitive symptoms that may appear when the disorder is consolidated could results in patients receiving therapeutic help in late stages of the pathology, when the starvation is extreme and entails probable neurological damage (Epling et al., 1983). We believe that progress must be made by adding hyperactivity criteria together with the food restriction. This would help to make an early diagnosis in order to promote better management for clinicians. Likewise, it would be interesting for public authorities to carry out awareness campaigns so that the social environment can be attentive to behavioral changes such as the progressive increase and excessiveness in exercise. These could be helpful to take into

account the firsts signs of the disease in order to promote early interventions that improve success in therapeutic management.

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