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Chlorpromazine specifically prevents the wheel-induced feeding suppression in rats

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ABSTRACT

In rats, limited daytime wheel access suppresses feeding over the subsequent night [Lattanzio SB, Eikelboom R. Wheel access duration in rats: I. effects on feeding and running. Behav Neurosci 2003; 117:496–504.]. This phenomenon is known as the wheel-induced feeding suppression (WIFS). The classic antipsychotic, chlorpromazine, can minimize the severity of the related activity anorexia procedure, but is thought to act through a suppression of running [Routtenberg A. "Self-starvation" of rats living in activity wheels: adaptation effects. J Comp Physiol Psychol 1968; 66:234–8.]. We tested the effects of chlorpromazine (2 mg/kg IP) on the acute WIFS in 40 adult male rats by administering the drug before or after 3 h of daytime wheel access and measuring food consumption over the subsequent 24 h. Control groups received saline injections or were exposed to locked wheels. While chlorpromazine did not attenuate feeding or change wheel running alone, it blocked their interaction, the acute WIFS. This procedure might be useful in screening drugs for anorexia nervosa where exercise is often elevated and feeding is suppressed.

1. Introduction

Anorexia nervosa (AN) is a disorder that is characterized by excessive weight loss caused at least partially by reduced eating. Often, this condition is exacerbated by a constant general hyperactivity and compulsion to exercise (Attia and Walsh, 2009; Bergh and Södersten, 1996; Casper, 2006). It has been observed that up to 85% of AN patients exercise to excessive levels throughout the course of the disorder (Davis, 1997) and some argue that the relationship between excessive activity and reduced feeding is a key feature of AN, perhaps mediated by disruptions in leptin levels (Hebebrand et al., 2003; Hillebrand et al., 2008; Holtkamp et al., 2006). Given the importance of food intake and exercise in this disorder, the activity–feeding relationship needs to be explored in animal models to assess its role in the development and maintenance of AN.

When a rat is given continuous access to a running wheel, it will run and gradually escalate its running from about 1 km on the first day up to 5–6 km over a period of a few weeks (Eikelboom and Mills, 1988). One interesting consequence of wheel introduction is a 25% self-imposed suppression in feeding lasting about 7 to 10 days and a chronic reduction in weight (Afonso and Eikelboom, 2003). This phenomenon seems quite counterintuitive in that the animals are expending more calories in running yet are consuming less than non-wheel controls. It has been proposed that this could function as a model of an important aspect of AN (Lattanzio and Eikelboom, 2003).

The animal model that has attracted the most attention as a model of AN is the activity anorexia procedure (Casper et al., 2008; Epling et al., 1983). In this procedure, wheel introduction and food restriction (usually 1 h of food access a day) are introduced simultaneously and result in feeding reduction (relative to restricted non-wheel controls) and increased running (relative to non-deprived controls) which can prove fatal for rats within a few days (Routtenberg and Kuznesof, 1967). Its interpretation is complicated by a number of factors inherent to the procedure. Firstly, there is a learning complication in that animals must adapt to the experimenter-imposed feeding schedule. Thus, food intake generally increases over the first number of days and over cycles of this procedure in a manner that indicates learning (Boakes and Dwyer, 1997; Hampstead et al., 2003; Lett et al., 2001; Paré et al., 1985). Secondly, with food deprivation, wheel running increases significantly (relative to ad lib fed rats) and thus energy expenditure is increased (Routtenberg and Kuznesof, 1967). This occurs even if the wheel is not novel (Exner et al., 2000; Nergårdh et al., 2007). Lastly, at wheel introduction feeding is suppressed both with ad lib and restricted food access (Afonso and Eikelboom, 2003; Routtenberg and Kuznesof, 1967). The feeding suppression induced by the wheel is temporary, both with ad lib feeding (Afonso and Eikelboom, 2003) and in the activity anorexia procedure (Hampstead et al., 2003). The problem in the activity anorexia procedure seems to be that animals do not have enough energy reserves to have the time to adapt to the various changes before the onset of starvation. It is not clear how or if these three factors are connected, or how they interact in the activity anorexia procedure, but they ultimately may all be important in AN. What is apparent is that the wheel-induced feeding suppression (WIFS) seen in ad lib fed rats provides a simpler model that can address a specific aspect of the feeding–exercise relationship.

The WIFS, seen with voluntary running and ad lib feeding, has many attributes that make it a useful animal model of this particular aspect of AN. The WIFS highlights an aspect of the activity anorexia procedure,

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which has been suggested as the most suitable model of AN (Casper et al., 2008). Our model is considered to have obvious face validity for AN (Willner, 1990), as it reflects the negative relationship between exercise and feeding found in the human condition (Attia and Walsh, 2009; Bergh and Södersten, 1996; Casper, 2006). This isomorphic model of AN (Smith, 1989) reflects many aspects of the human disorder without commenting on its etiology or mechanisms, which remain largely unknown. The most obvious benefit to the WIFS model is that all of the changes in feeding and running are intrinsically motivated, not externally imposed by an experimenter. As well, an important characteristic of the WIFS model is that it can be elicited when rats are given short-term access to a wheel. It has been shown that 2 h of wheel access during the day is enough to trigger this feeding suppression over the next night (Lattanzio and Eikelboom, 2003). Since in rats most eating occurs at night, it appears that daytime wheel access has effects lasting many hours, suppressing the next night's feeding. This acute WIFS may prove a valuable procedure for determining the neuro-chemical systems involved in this paradoxical exercise–feeding relationship. Using an acute WIFS model, a drug can be given to an animal before, after, or in the absence of, short-term wheel access, permitting an evaluation of the drug's effect on running, feeding, and the running–feeding interaction without the complication of constant wheel access. As drug tests in animal models have long been suggested as useful for preclinical drug evaluations (Mc Kinney, 1974), this procedure could ultimately lead to suggestions for pharmaceutical interventions for AN.

It has long been evident that antipsychotic drugs, both typical and atypical, commonly carry weight gain as a side effect (Allison et al., 1999). There has been speculation as to whether these drugs could help in the initial phases of AN treatment, because of their weight gain inducing qualities and the possible alleviation of psychological symptoms. Recently, a randomized, double-blind, placebo-controlled trial of olanzapine, an atypical antipsychotic, was conducted using female AN patients as participants (Bissada et al., 2008). Olanzapine use resulted in increased weight gain and lessening of obsessive symptoms and has been suggested as a valuable option for the initial, short-term phase of treatment. In light of these results, it would be prudent to test antipsychotics in the acute WIFS procedure.

In the current study, chlorpromazine (CPZ), the classical typical antipsychotic, was evaluated using the acute WIFS procedure. This was the first of many drugs tested in the activity anorexia procedure (Routtenberg, 1968; Routtenberg and Kuznesof, 1967; Woods and Routtenberg, 1971). Chronic administration of CPZ in the activity anorexia paradigm reduces wheel running and so indirectly decreases the severity of the procedure. While these studies do provide a model in which to test drugs, the model is limited by the aforementioned complications of the activity anorexia procedure. As wheel access is continuous (except for feeding time) it is not clear when the drug should be administered to have the most effect. In these early studies by Routtenberg's group CPZ or saline was injected immediately after the 1 h feeding period. Thus, administration was far removed from the next meal and could have resulted in learned taste avoidance. This could indirectly prevent a recovery of feeding, making it difficult to distinguish the avoidance from drug-induced reduction.

In the current study, the acute WIFS model is used to evaluate the impact of CPZ on feeding, running, and WIFS. It may be that CPZ (and similar drugs) directly simulate or suppress feeding which would then be evident in non-wheel controls. Alternatively, it may directly reduce activity and thus indirectly prevent the WIFS, tested by comparing groups given the drug before or after the limited wheel exposure. A third more interesting possibility is that the exercise–feeding suppression dyad may be prevented by this drug. Such a drug would then target the relationship between running and feeding without increasing eating in non-exercising control animals or decreasing exercise, specifically removing the threat of this harmful relationship. If this dyad is an important aspect of the etiology of AN, it would suggest this class of drugs might prove useful in the treatment of this puzzling disorder. While future work will need to look at both male and female rats, because males have been used in most previous work, this initial study was also done with male rats.

2. Method

2.1. Subjects

40 male Sprague–Dawley rats (Charles River Canada, St. Constant, Quebec, Canada) weighing 200–225 g (47–49 days old) upon arrival were kept on a 12 h light–dark cycle, with lights on at 0700. They were housed individually in standard shoebox cages (20×24×45 cm). Colony conditions were kept stable (50% relative humidity, 21–22 °C), and food and tap water were available *ad libitum* throughout the experiment. All experimental procedures were approved by the Wilfrid Laurier University Animal Care Committee which follows the policies and guidelines of the Canadian Council on Animal Care.

2.2. Apparatus

Wheel access was given in Nalgene™ running wheels (33 cm diameter and 11 cm wide) inserted in standard shoebox cages. These wheels could be locked using clips on the outside of the cage to prevent wheel turning. Wheel turns were counted to a resolution of 1 s using a magnetic closure system and the VitalView™ Minimitter Co. Ltd. software package.

2.3. Drug and doses

Chlorpromazine solution (CPZ, as chlorpromazine hydroxide, Sigma Aldrich, St. Louis, MO) was prepared fresh on injection day in sterile isotonic saline. CPZ, 2 mg/kg, was injected intreperitoneally at a volume of 1 ml/kg. The control rats that did not receive CPZ received equivalent injections of saline.

2.4. Procedure

Baseline measures of food consumption, water consumption and weight were taken daily at about 1430 throughout the experiment. Food consumption was measured by calculating the differences in food weight from one day to the next. Small crumbs and food particles were ignored, as in previous work they have been previously found to weigh less than 1 g, and not to vary across conditions. After 7 days of baseline, all 40 rats were given 24 h of wheel access to establish a baseline running level, measure the WIFS seen with one day of ad lib wheel access and provide rats familiarity with the wheel. The rats were then assigned, based on wheel running and feeding suppression, to 5 groups of 8 rats: drug before wheel access (DW); drug after wheel access (WD); drug with locked wheel access (DNW); saline with locked wheel access (SNW); and saline with wheel access (SW). Half of each of the three control groups (DNW, SNW and SW) received their injections before wheel access and half were injected after the wheel access period (a 3 group by 2 injection time ANOVA revealed no significant differences in feeding on the critical day due to injection time, so the two sub-groups for each group were combined for the final analysis).

Three days after the 24 h baseline wheel exposure, animals injected before wheel access received the appropriate injections, either saline or CPZ, at approximately 1500, 4 h before lights out. Thirty minutes after their injection (about 1530), all rats were placed in the wheel cages, with the wheel unlocked for wheel access or locked for novel environment only groups, and remained there for 3 h. The rats were then placed back in their home cages. Groups that received their injection after wheel access were injected immediately after being removed from the wheel cage. Each rat received only one injection. Food consumption over the next 24 h was measured as the critical dependent variable.

3. Results

3.1. Initial 24 h baseline access

A 5 group analysis of variance (ANOVA) revealed that the experimental groups did not differ significantly in distance run over the 24 h baseline running period suggesting that assignment to groups was effective. Wheel turns in this period did not correlate with later 3 h running, or with the decrease in feeding induced by the 24 h of this baseline wheel access.

Food consumption for the day before and the day after 24 h wheel access was analyzed using a 5 group by 2 day mixed ANOVA. This revealed an overall significant feeding suppression $F(1,35) = 52.26$, $p < .001$, but again, because rats were assigned to groups based on their feeding suppression, the groups did not differ. Rats ate $29.8 \pm .52$ (SEM) g the day before the 24 h wheel access and $25.0 \pm .58$ g over the 24 h of wheel access.

3.2. Feeding after injection and 3 h access

Fig. 1 shows feeding over the 24 h after injection of chlorpromazine or saline before or after 3 h access to a locked or unlocked wheel. Overall, the 5 group ANOVA revealed the food consumption of the groups differed significantly $F(4,35) = 3.82$, $p < .05$. A Newman–Keuls post hoc test found that only the saline injected, wheel exposed rats (Group SW) ate less than the rats in the other four groups, $p < .05$.

In the three wheel groups, feeding suppressions (change from the 24 h before injection and wheel access to the 24 h afterwards) differed; 3 group ANOVA, $F(2,21) = 18.49$, $p < .001$. Again, post hoc tests show that the SW group was responsible for this effect, being the only wheel group that showed a feeding suppression.

3.3. Running on injection days (3 h access)

The 3 group ANOVA revealed the wheel groups (DW, WD, and SW) did not differ significantly in their running over the 3 h of wheel access (as measured in wheel turns). The DW group ran an average of 325.37 ± 59.50, the WD group ran 425.62 ± 91.92, and the SW group 567.50 ± 119.10 wheel turns. There was also no significant correlation between the number of wheel turns and feeding in the 24 h after the 3 h wheel exposure (for the 24 rats with wheel access the Pearson $r = -.039$).

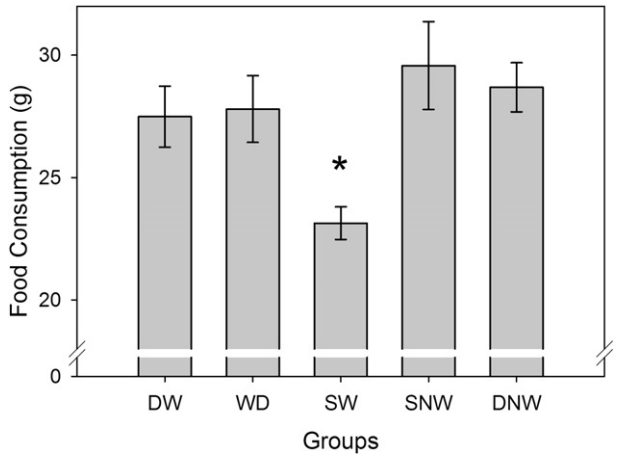

Fig. 1. Mean (±SEM) food consumption (g) in the 24 h following chlorpromazine (D) or saline (S) injection and 3 h of wheel (W) or locked wheel (NW) access. Group DW was injected before, whereas group WD was injected after wheel access. *Significantly different from other four groups (Newman–Keuls $p < .05$).

4. Discussion

Using the WIFS, a simple model that focuses on a specific aspect of the activity anorexia procedure, we are able to dissect some of the links between exercise and feeding. In particular this procedure can be used as a simple screen to assess some of the acute effects of drugs and elucidate how the drug works to impact feeding in the WIFS. In this procedure the direct effect of drugs on feeding are evident in the comparison between the saline and drug groups that did not have wheel access. The comparison between the two saline groups with and without wheel access demonstrates that the procedure supports a feeding suppression. Further comparison of rats receiving drug before or after wheel access demonstrates the drug effect on acute daytime wheel running. Finally the comparison of the drug wheel groups with the drug no wheel group permits a demonstration that the drug prevents the WIFS.

In this experiment animals that received a saline injection and were given 3 h of wheel access were the only animals that showed the anticipated feeding suppression, at this dose CPZ prevented the feeding suppression when injected either before or after wheel exposure. That the non-wheel CPZ injected controls did not eat significantly more than the saline control animals indicates that the drug does not simply work to increase appetite overall. In this experiment running did not differ significantly between the groups so it appears this acute CPZ administration did not decrease activity and in this way circumvent the WIFS phenomenon. This study and the preliminary results of a dose–response pilot study (unpublished results), suggest that there may be an optimal dose that does not significantly reduce running with acute application, but does prevent the WIFS. The dose used in this study, 2 mg/kg, appears to be on the upper threshold of this 'optimal' dose and thus there is a non-significant trend towards activity reduction in the DW rats. This dose was chosen to mirror the previous work with the drug in activity anorexia, in which 2 mg/kg were used (Routtenberg, 1968; Routtenberg and Kuznesof, 1967; Woods and Routtenberg, 1971). It appears that CPZ targets the relationship between feeding and activity in the running wheel, specifically uncoupling them to avoid the counterintuitive reduction in feeding that often accompanies this activity. In this experiment it is also interesting how little running, around 500 wheel turns, is necessary to suppress feeding. This is consistent with our previous work that 2 h daytime wheel access can suppress feeding as much as ad lib wheel access (Lattanzio and Eikelboom, 2003) and also with the lack of correlation between running and the feeding suppression in an ad lib wheel and feeding model (Afonso and Eikelboom, 2003). Chlorpromazine was previously studied in the context of the feeding–exercise relationship in a series of studies (Routtenberg, 1968; Routtenberg and Kuznesof, 1967; Woods and Routtenberg, 1971), where it was found that chronic administration of this drug reduced activity in the wheel under the activity anorexia procedure. Most recent work has focused on the deprivation induced running increase evident in the activity anorexia procedure. Many drugs have been tested and are discussed in a recent review (Hillebrand et al., 2008); here we will mention only drugs that act on the dopamine system.

Olanzapine, the atypical antipsychotic, and pimozide, the dopamine D2 receptor blocker, both reduce the running increase seen when food restricted rats are given chronic wheel exposure (Hillebrand et al., 2005; Lambert and Porter, 1992). The effect of dopamine blockers on the running seems to be quite complex, as running is reduced by the D2 blocker haloperidol, but the D1 antagonist SCH23390 changes the dark:light running ratio in activity anorexia, in this way reducing the daytime increase (Nomura et al., 1995). As these studies were focused on the running increase seen with deprivation, they did not include controls to look at how these drugs influenced the feeding suppression caused by wheel access alone. As well, rats had prior exposure to the wheel and there were no food restricted non-wheel controls to see how baseline feeding was effected. Thus it will be interesting to look at these drugs, particularly olanzapine, in the acute WIFS procedure, both as a preclinical animal model to determine the mechanism by which it

works in human AN patients, and as a measure of predictive validity for the model.

Overall, the WIFS model shows great promise as a testing apparatus for AN drugs that may change exercise's direct effect on feeding. There are however many questions that remain. In humans, females are much more likely to develop AN. While the activity anorexia is evident in females (Hampstead et al., 2003; Nergårdh et al., 2007) and the WIFS can be seen in females (Adams and Eikelboom unpublished observations), there are sex differences in running, weight regulation and the sensitivity of the animal to the stress of restricted feeding (Hebebrand et al., 2003), which suggest females should be tested in this model.

The current experiment using the WIFS model involved acute, one time exposure to the drug, prior to the application of an AN model. While the acute condition is valuable for screening this and other drugs for effectiveness, in reality, these drugs would be given chronically to humans. Thus further study involving chronic access to chlorpromazine and repeated access to the wheel is needed. Prior work has suggested that even with only a few hours a day of wheel access a pronounced feeding suppression lasting days is evident (Lattanzio and Eikelboom, 2003) so this model can be used to test drugs in a more chronic application. With CPZ a dose–response study would be beneficial, as to determine if the effects seen here are dose dependant, and to assess optimal dosage. Based on the early work of Routtenberg's group (Routtenberg, 1968; Routtenberg and Kuznesof, 1967; Woods and Routtenberg, 1971) it might be expected that with a higher CPZ dose or chronic administration of the drug, the drug before and the drug after wheel groups might differ in wheel running. If these studies were to reinforce this model's validity, it could be used to test other drugs (such as olanzapine and related drugs) in a preclinical setting.

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