Dopamine 1 receptor antagonism prevents hyperactivity in rats hypersensitive to Activity Based Anorexia.

Boersma, GJ.1; Guidotti, S1; Benthem, L.2; Södersten P3; Van Dijk G1; Scheurink, AJW 1

1 Department of Neuroendocrinology, University of Groningen, The Netherlands
2 Department of Research and Development, AstraZeneca, Sweden
3 Anorexia Centre, Karolinska Institute, Huddinge, Sweden
Chapter 10

Abstract:

Hyperactivity is a seemingly contradictory and ill-understood symptom of Anorexia Nervosa. Risk factors for the development of hyperactivity during starvation are not extensively studied due to the large individual variation in the display of this symptom. We hypothesize that the personality, or coping style, of an individual might predict their risk to develop hyperactivity during starvation. To investigate this we selected rats with either a proactive or passive personality and subjected them to the activity-based anorexia paradigm, an animal model for Anorexia Nervosa. In this study we showed that rats characterized by an extremely passive coping style lost more weight and displayed higher levels of hyperactivity on the ABA paradigm than their proactive counterparts. In a second study we aimed to elucidate the mechanism behind the increase hyperactivity development in the passively coping rats. We hypothesized that differences in dopaminergic activity might be causal to the differential susceptibility. Therefore, we treated rats with a selective dopamine 1 receptor antagonist. This treatment partially attenuated hyperactivity in the passively coping rats, and this, in turn, delayed weight loss in these rats. Overall we may conclude that passively coping individuals are more prone to develop hyperactivity during ABA, which is possibly due to an over activation of the dopamine system in these rats.
Introduction:

Anorexia Nervosa (AN) is characterized by extreme hypophagia, low body weight, hypothermia, amenorrhea and hyperactivity (1). With a life time mortality rate between 5 and 20% this disease is among the most life-threatening disorders (2). One of the most intriguing symptoms of the disease is hyperactivity, which seems counterintuitive in energy deprived conditions. While hyperactivity is a common symptom of AN, there are clearly individual differences in the severity of the hyperactivity (3). Studies in humans and animal models for AN have shown that the occurrence of hyperactivity decreases survival rate significantly (4-6).

Over the course of primate history, our ancestors were probably frequently exposed to periods of famine, which may have lead to an adaptation in the physiology to enlarge changes of survival during famine. These physiological responses to starvation are well documented: lower body temperature, reduced heart rate, low body fat percentage and reduced levels of leptin and insulin (7;8); all responses aimed at saving energy under conditions of famine. However, as nicely reviewed by Charkravarthy (9), shortage of food is accompanied by an increased capacity for intermittently high levels of physical activity. For the original hunters and gatherers, the increased physical activity levels during periods of famine were crucial to increase foraging efforts, and thereby increase their survival chances (10), in addition to the above-mentioned physiological responses to famine. It seems that the hyperactivity observed in AN patients may result from adaptive evolutionary-based responses to starvation. In the case of AN patients, the adaptive hyperactivity does, however, not increase energy intake, like it did in our ancestors, since hypophagia in AN patients is not due to diminished opportunity to get access to food.

In a recent review, we introduced a framework for the development of hyperactivity in AN (11). We suggested that the hyperactivity in AN may have addictive properties to stimulate food searching behavior in times of famine. Several lines of evidence support a role of dopamine in this mechanism (12;13). This would mean that blockade of the central dopamine pathway should prevent behavioral hyperactivity in AN. We studied this in an animal model for AN and found indirect evidence for this (11).

More direct evidence is required to accept our hypothesis that dopamine plays a crucial role in the development of hyperactivity in AN. However, studying the contributors of AN objectively is challenging in patients, and therefore an animal model is necessary. The activity-based anorexia (ABA) model can be used for this purpose. The ABA model combines food restriction with availability of a running wheel, which results in a strong
Chapter 10

decrease in food intake and an increase in wheel running activity. The combination of hypophagia and hyperactivity results in extensive body weight loss. This model mimics several aspects of AN, such as weight loss, hyperactivity, hypothermia, and amenorrhea (5;14). However, it is important to note that within the human population there are large individual differences in personality profile which may affect the susceptibility to develop Anorexia Nervosa (15). Most studies that employ the ABA model or other animal models for eating disorders ignore this variation in personality by using standard rat strains such as Wistar or Sprague Dawley rats limiting the face validity of these studies for translation to the human situation.

For this reason, we investigated the role of personality in AN, by subjecting rats to ABA that were 1) selectively bred for differences in personality or coping style (i.e., the Roman High and Low Avoidance rats), 2) passive and pro-active rats taken from the Wild type Groningen outbred population, which contains rats of varying coping styles/personalities (16-18). Rats from the Roman High avoidance (RHA) and Roman Low Avoidance (RLA) employ, respectively, extremely proactive and passive coping strategies, when exposed to psycho-physiological stimuli. This dissociation is more moderate when viewed in the proactively and passively coping rats obtained from the Wild Type Groningen population. Unselected standard Wistar rats obtained from Harlan Netherlands served as a references group for the ABA model (19). The aim of the second experiment is to obtain direct evidence for our hypothesis that dopamine may play a crucial role in the development of hyperactivity in ABA. This would mean that particularly rats that are (hyper)sensitive for the development of hyperactivity on the ABA model may benefit from blockade of the dopamine pathway. To this end, we administered a selective dopamine 1 receptor antagonist during the ABA paradigm in the RHA and RLA rat strains, the animals with the most extreme coping styles. Again standard Wistar rats serve as a reference.
Material and methods:

Animals

Female rats weighing 195-230 grams at the beginning of the study were used. The following strains participated in the study: Roman High and Low Avoidance rats, Wild Type Groningen rats, and Wistar rats. The Roman High and Low Avoidance (RHA and RLA, respectively) rats were obtained from a breeding colony at the Clinical Psychopharmacology Unit, University of Geneva, Switzerland. Wild Type Groningen (WTG) rats were derived from our own breeding colony at the University of Groningen, the Netherlands. Wistar rats were derived from a commercial breeding facility (Harlan). The animals were individually housed in Nalgene polycarbonate running wheel cages (50x27x36) with free access to the running wheel (diameter 27 cm, Mini Mitter, Oregon, USA). Food and water were available ad libitum unless mentioned otherwise. The room was controlled for temperature and humidity (T=20 °C, humidity 60%) and was kept at a twelve-hour light, twelve-hour dark cycle (lights switched on at CT 0). All animal experiments were approved by the local animal welfare committee (DEC, Groningen, the Netherlands).

Coping style selection.

The RLA and RHA rats originated from a different breeding colony, their coping style was known. The coping style of the WTG rats was determined by using a defensive burying test. The procedure of the defensive burying test used was first described by Pinel and Treit (20). In short, animals were housed in specialized defensive burying cages, standard cages (24x24x36 cm) with a hole of approximately 1 cm diameter. Through the hole an electric prod can be inserted. After a habituation period of at least a week the animals were tested. The test was performed in the middle of the light phase (CT4-CT10). The electric prod was inserted into their cage and after the first shock the behavior of the rat was monitored for 10 minutes (Eline software program). The time spent on exploration of the cage, exploration of the prod, burying the prod, and immobile behavior was scored. The percentage time spent burying the prod was the main criterion for selection of the coping style: animals burying ten or less percent of the time were characterized as passive, rats burying twenty or more percent of the time were characterized as active, and animals that were between the cut-off criteria (10-20% burying) were excluded from the study. Based on these criteria we selected 6 proactive WTG rats and 6 passive WTG rats. As expected, a complete segregation of burying behavior in RLA (no burying) and RHA (high burying) rats respectively, can be found (not shown).
Chapter 10

Protocol for Activity Based Anorexia (ABA):

The ABA experiments were similar to previous studies from our lab (11;21;22). After a period of baseline measurements, food was restricted for twenty-three hours per day. Food was available only during the first hour of the dark phase (CT12-13). Body weight was measured daily at CT5, seven hours before dark onset. Food intake was measured at 12 and CT 13 (see figure 1). Running wheel activity of the rats was continuously measured in Nalgene running wheel cages by recording the number of wheel revolutions using two magnets. One magnet was attached to the frame of the wheel; the other was attached to the wheel. The Vital View Data Acquisition System (Mini Ritter, Oregon, USA) counted the number of times the magnets passed each other. This magnetic switch counted only complete revolutions of the wheel.

In all experimental groups, the ABA protocol was stopped after ten days or when animals lost more than thirty percent of their body weight relative to baseline. The timing of food intake, body weight and activity measurements is schematically depicted in figure 1.

Figure 1: Experimental schedule of the standard ABA protocol. Black box indicates dark phase, white box indicates light phase, hatched box represents food access.
Study design

General:
All animals were habituated to the running wheel cages for at least ten days. Then food intake, body weight and baseline running wheel activity were measured for eight days; this included two estrous cycles.

Experiment 1:
Experiment 1 focused on the individual variation in the susceptibility for ABA by subjecting rats from various rat strains that differ in coping style to the ABA model. To this end, 10 RLA rats, 10 RHA rats, 6 proactive WTG rats (WTGa), 6 passive WTG rats (WTGp), and 6 Wistar rats were subjected to the standard ABA protocol (Experiment 1A). To control for handling effects, we included a sedentary group (sed), which was housed in similar cages but in which the running wheel was blocked. Food was only available during the first hour of the dark phase. This study (Experiment 1B) was performed with 8 RLA and 8 RHA rats. Finally, a second control group was included in which all animals, 8 RLA rats and 8 RHA rats, were pair-fed to the averaged daily intake of the RHA rats in Experiment 1A. This second control study (Experiment 1C) was included after we observed that there were differences in one hour food intake between the RHA and RLA rats in Experiment 1A. The food matched animals (fmc) received their food at the beginning of the dark phase at CT12 and there was no restriction in time.

Experiment 2:
Experiment 2 investigated the potential role of the dopaminergic system in the development of hyperactivity in the ABA model. To this end, the selective dopamine 1 receptor antagonist, SCH23390 or saline was administered daily during the ABA paradigm. This study was performed in the rat strains with the most extreme differences in coping style, the Roman High and Low Avoidance rats with standard Wistar rats as control. At day 0, all animals were subjected to the standard ABA protocol. On the next day, the animals received an intraperitoneal injection of 1 ml/kg of either SCH23390 (0.025mg/ml, n = 8 per strain) or saline (n = 8 per strain). The injections started at day 1 and were daily given until the end of the study (day 6). SCH23390 was purchased from Tocris Pharmaceuticals (Tocris Pharmaceuticals, Bristol UK) and was dissolved in saline. The dose of 0.025mg/kg per day was based on prior studies (Hyttel, 1983; Morelli 1985) and was sufficient to antagonize the D1 receptor for at least 6 hours without inducing cataplexy.
Data analysis

Data were calculated as averages with standard error of mean (SEM). The differences between the experimental groups in baseline body weight, food intake and differences in the defensive bury test were statistically tested with a one-way ANOVA with Tukey post-hoc analysis. Differences in the number of rats that have reached a body weight loss exceeding 25% on the third and tenth day of the ABA protocol were assessed using Chi-square analysis. Differences in body weight gain, food intake and wheel running activity during the standard ABA protocol were tested with a repeated measures ANOVA followed by an Tukey post-hoc test using the strain and the coping style as between subjects factors and the coping style*strain as an interaction effect. A confidence interval of five percent was used. Differences between the sedentary housed and running rats were statistically tested with a Repeated measures ANOVA with the coping style and treatment as between subjects factor, and coping style*treatment as interaction effect. Similarly, differences between the 1 hour restricted and the food matched control rats were statistically tested with a repeated measures ANOVA with the coping style and treatment as between subjects factor, and coping style*treatment as interaction effect. Differences between the SCH23390 treated and saline groups as well as strain differences were statistically tested with a repeated measures ANOVA with the coping style and treatment as between subjects factor, and coping style*treatment as interaction effect.

In the ABA model, the weight-loss induced hyperactivity occurs mainly in the end of the light phase, in the hours prior to the administration of food (Mistleberger 1994). This phenomenon is known as Food Associated Activity (FAA). FAA was calculated by dividing the running activity during the last 5 hours of the light phase by the total daily running activity and express this as a percentage (Hillebrand 2006). The difference in FAA between the groups was statistically tested with a repeated measure ANOVA with a Tukey post-hoc test using the coping style and the treatment as between subjects factors. To compare the differences on a specific day a multivariate ANOVA with a Tukey post-hoc test with the strain and the treatment as between subjects factors was performed. To display the circadian activity pattern the activity per hour was calculated. Statistical relevance of the observed differences were determined with a repeated measures ANOVA, followed by a multivariate ANOVA to determine difference on a specific time point. Strain and treatment were the between subjects factors and additionally the interaction effect of treatment and strain was determined. In all statistical tests a confidence interval of 5% was used.
Results:

Experiment 1:

During the baseline period there were no differences in body weight between the eight groups. There was however a slight but significant difference in daily food intake between the RLA and the RHA (RLA 22.6 ± 0.7, RHA 20.8 ± 0.6, WTGp 21.3 ± 0.7, WTGa 21.5 ± 0.8 and Wistar 20.1 ± 0.5) (Romans: RM-ANOVA coping style $F_{3, 27} = 3.465, P< 0.05$).

Figure 2A shows the body weights of all the groups before and during the ABA protocol. All animals lost weight during the ABA protocol. Overall, there was not a significant effect of strains (Roman vs WTG strains). There was, however, a significant interaction between the strain (Roman vs WTG) and the coping style (RM-ANOVA strain* coping style $F_{19,130} = 14.884, P< 0.01$). The source of significance came from the RHA/RLA lines: body weights of the RLA rats were significantly lower than those of the RHA rats (RM-ANOVA coping style $F_{3.27} = 14.884, P< 0.001$). Within the WTG strain no effect of coping style was observed. Daily food intake is presented in Figure 2B. Restricted food availability reduced the daily intake with approximately 70% on the first day to approx. 50% on days 3 to 6. Again, no differences were observed between strains, but there was a significant interaction between strain and coping style (RM-ANOVA strain* coping style $F_{21, 119} = 4.442, P< 0.01$). The source of significance was again found in the RHA/RLA lines: the RLAs ate significantly less than the RHAs on ABA (RM-ANOVA coping style $F_{3.27} = 3.465, P< 0.01$), no difference within the WTG line was found.
Figure 2: Body weights and food intake of rats of the Roman, Wistar and Wild Type Groningen strain subjected to ABA. Body weight and food intake are expressed as a percentages of baseline body weight. Open circles = RLA rats, closed circles = RHA rats, light grey triangles = WTGp rats, dark gray triangles = WTGa rats, and grey squares = Wistar rats. * indicates a significant difference between RLA and RHA.
From figure 2a, it can be observed that the curve of the RLA rats is interrupted after day 3, since most rats in this group were matching the criterion of weight loss from exceeding 30% at that day. To allow a comparison with other groups, Table 1 provides the number of rats that reached a weight loss of > 25% at day 3 and 10. Rats of the RLA lost more weight than the RHAs, and more frequently reached a body weight loss exceeding 25% on the third and tenth day of the ABA protocol, both in the 1 hr protocol as well as in the food matched controls.

Table 1: The number of rats that have reached a body weight loss exceeding 25% on the third and tenth day of the ABA protocol. The symbols indicate differences that are significant \( a \) between RLA rats and all other strains, \( b \) between RLA rats and all other strains, \( c \) between the sedentary and the 1hr ABA within the same strain (\( p<0.05 \)). (day 3: \( \chi^2 (100, N=9) = 4.287 \), \( p<0.001 \); day 10: \( \chi^2 (100, N=9) = 2.716 \), \( p<0.001 \))

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 3</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># BW&lt; 75% / total # rats</td>
<td># BW&lt; 75% / total # rats</td>
</tr>
<tr>
<td>RLA (1hr)</td>
<td>7/10 ( a )</td>
<td>10/10 ( a )</td>
</tr>
<tr>
<td>RHA (1hr)</td>
<td>0/10</td>
<td>0/10 ( b )</td>
</tr>
<tr>
<td>WTGp (1hr)</td>
<td>1/6</td>
<td>4/6</td>
</tr>
<tr>
<td>WTGa (1hr)</td>
<td>0/6</td>
<td>3/6</td>
</tr>
<tr>
<td>Wistar (1hr)</td>
<td>0/6</td>
<td>3/6</td>
</tr>
<tr>
<td>RLA (sed)</td>
<td>0/8 ( c )</td>
<td>0/8 ( c )</td>
</tr>
<tr>
<td>RHA (sed)</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td>RLA (fmc)</td>
<td>5/8</td>
<td>8/8</td>
</tr>
<tr>
<td>RHA (fmc)</td>
<td>0/8</td>
<td>0/8</td>
</tr>
</tbody>
</table>
Figure 3 depicts the wheel running activity over the day of the different rat strains at baseline and on the third and sixth day of the ABA protocol. During ABA, increased running occurred mainly at the end of light phase, a phenomenon known as Food Associated Activity (FAA). At day 3, FAA was markedly increased in the passive but not in the proactive animals, the differences between the RLAs and the RHAs were significant (RM-ANOVA coping style $F_{3, 19} = 16.792, P < 0.01$). The RHA showed almost no FAA at all during the ABA protocol.

Figure 3: Circadian running wheel activity patterns of rats of the Roman and WTG strains on the standard ABA paradigm. A: wheel running of Roman rats. B: Wheel running of WTG rats. Data are expressed as average running activity per group ± SEM for day 0, 3 and 6 of the ABA protocol. Note the increased running (Food Associated Activity) in the light phase during ABA at day 3 and 6 when compared to day 0.
Figure 4 presents the body weight changes and daily food intake in Experiment 1B: 1 hour food availability of the two Roman strains with or without access to running wheel. The body weights of the sedentary RLA and RHA rats remained above 85 percent of baseline during the whole experiment and were significantly higher those of the animals that had access to the wheel (RM-ANOVA treatment \( F_{3, 27} = 5.976, P < 0.01 \)). Food intake of the sedentary housed RLA rats was significantly higher than that of the running RLA rats (RM-ANOVA treatment*coping style \( F_{3, 27} = 4.536, P < 0.05 \); RLA : RM-ANOVA treatment \( F_{1, 11} = 7.648, p < 0.01 \)). Within the RHAs there were no difference in food intake between running and sedentary rats.

\[ A \]

Figure 4: Food intake and body weights of RLA and RHA rats on the standard ABA paradigm with or without running wheel. \( A \): Body weight expressed as a percentage of baseline at day 0. \( B \): Food intake expressed as a percentage of the baseline at day 0. Open circles = running RLAs, closed circles = running RHAs, open triangles = sedentary RLAs, closed triangles = sedentary RHAs. * indicates a significant difference between RLA and all other groups (interaction between treatment and coping style) \( p < 0.01 \).
Figure 5 presents the body weight changes and daily food intake in Experiment 1C: 1 hour restricted rats versus food matched controls. There were no significant differences in body weight between the restricted and food matched controls for both the RLAs and the RHAs. The differences in body weight between the RLAs and RHA are were significant, both for the restricted and the food matched controls (RM-ANOVA coping style $F_{3, 19} = 16.948$, $P < 0.01$). The food matched control RLAs had a significantly higher food intake compared to RLA rats on 1 hour of food restriction (RM-ANOVA treatment* coping style $F_{3, 19} = 8.425$, $P < 0.05$; RLA: RM-ANOVA treatment $F_{1, 11} = 6.897$, $P < 0.01$) (figure 4B).

Figure 5: Body weight change (A) and food intake (B) during the ABA protocol expressed as a percentage of the baseline of 1 hour restricted RLA and RHA rats and their food matched controls. Open circles = RLAs 1 hour food restricted, closed circles = RHAs 1 hour food restricted, open squares = RLAs food matched control, and closed squares = RHAs food matched control. $^{a}$ indicates significant difference between both groups RLA rats and between both groups RHA rats (coping style effect) ($p < 0.01$). $^{b}$ indicates significant difference between 1 hour food restriction RLAs and food matched control RLAs (treatment effect).
Experiment 2

Figure 6 depicts the body weights and food intake of RLAs, RHAs and Wistar rats treated with SCH23390 or saline. All rats lost weight during the ABA protocol (RM-ANOVA time $F_{18,204} = 518.249, p<0.001$). Weight loss of the saline treated RLA rats was significantly higher than that of all other groups (RM-ANOVA time*coping style* treatment $F_{18,204} = 24.750, p<0.001$). There were no significant differences in food intake between the groups.

**Figure 6:** Body weight change (A) and food intake (B) during ABA expressed as percentage of baseline of RLA, RHA and Wistar rats treated with SCH23390 or saline. Open Triangles = saline treated RLA rats, black triangles = saline treated RHA rats, grey triangles = saline treated Wistar rats, open circles = SCH23390 treated RLA rats, black circles = SCH23390 treated RHA rats, and grey circles = SCH23390 treated Wistar rats. “a” indicates that saline treated RLA rats differ significantly from all other groups (treatment * coping style interaction) (P<0.01). “b” indicates saline treated rats differ significantly from SCH23390 treated rats (treatment effect) (p<0.01).
Figure 7 presents wheel running activity in the dark phase. There were significant differences effects of coping style (RM-ANOVA time* coping style $F_{69,644} = 3.199$, $P<0.001$), with RLA rats having significantly elevated running wheel activity compared to RHA rats, but not between saline and SCH23390 treated animals of the coping style.

![Figure 7](image_url)

**Figure 7:** Wheel running activity during the dark phase in saline or SCH23390 treated RLA, RHA and Wistar rats on the third day of the ABA protocol. Activity is expressed as the total number of wheel revolutions during the dark phase (12 hours). White bars = saline treatment, black bars = SCH23390 treatment.

Figure 8 presents the Food Anticipatory Activity (FAA) of RLA, RHA and Wistar rats treated with either saline or SCH23390. An effect of coping style was observed on FAA (with significantly higher FAA in the RLA rats when compared to the RHA rats: RM-ANOVA coping style $F_{19, 44} = 8.564$, $p<0.01$). Overall, treatment with SCH23390 did not lower FAA. However, an interaction between treatment and coping style was observed (RM-ANOVA treatment* coping style $F_{18,204} = 24.750$, $p<0.001$), with differences between saline and SCH23390 treated animals in the RLA group specifically contributing to this effect. (RM-ANOVA treatment $F_{16,58} = 13.78$, $p<0.01$). In the Wistar, an effect of treatment was found (RM-ANOVA treatment $F_{12,48} = 11.564$, $p<0.01$), which appeared analogous to those found in the RLA coping style.
Figure 8: Food Anticipatory Activity (FAA) in saline or SCH23390 treated RLA, RHA and Wistar rats during ABA. FAA was calculated as the total number of wheel revolutions ran in the 5 hours prior to food access divided by total daily revolutions times 100%. **A:** FAA on the fourth day of ABA. White bars = saline treatment, black bars = SCH23390 treatment. * indicates a significant difference. **B:** FAA during the first 6 days of ABA. Open circles = saline treated RLA rats, black circles = saline treated RHA rats, grey circles = saline treated Wistar rats, open triangles = SCH23390 treated RLA rats, black triangles = SCH23390 treated RHA rats, and grey triangles = SCH23390 treated Wistar rats. "a" indicates that saline treated RLA rats differ significantly from all other groups (treatment * coping style interaction) (P<0.01). "b" indicates that RLA's differ significantly from RHAs (coping style effect) P<0.05. "c" indicates that all RLA's differ significantly from all RHA rats and that saline treated RLA rats differ significantly from SCH23390 treated RLA rats (coping style effect and interaction effect)(P<0.01).
Chapter 10

Discussion:

The present paper addressed the role of personality in the susceptibility to develop activity-based anorexia (ABA) in rats. ABA in rats has a high face-validity for the processes that underlie Anorexia Nervosa in humans. Important for consideration of the presented data is that we included rats from a Wistar reference population with known susceptibility for ABA development (11;21). The most conspicuous findings in our studies on the role of personality in ABA development were: 1) passively coping Roman Low Avoidance (RLA) rats subjected to ABA have a much larger decline in body weight and suppression of food intake than proactively coping Roman High Avoidance (RHA) rats and reference Wistar rats; 2) relative to the reference Wistar rats, RHA rats are less susceptible for body weight loss and suppression of food intake in the ABA model; 3) treatment with a selective dopamine 1 antagonist reduces hyperactivity and weight loss in the ABA model.

Since body weight loss in the ABA model is the result of a complex interplay between hyperactivity and suppression of food intake, we performed food-matching experiments to tease out cause-effect relations. From the food-matched control groups, we can conclude that even though passive RLA rats have a lower food intake than proactive RHA rats, this is not the determining factor for their hypersensitivity to develop ABA. Since running activity is essential for escalation in the body weight loss during food restriction as seen during ABA, it is more likely that the hyperactivity arm of the model could predict differences between RLA and RHA rats with respect to ABA development, which indeed appears to be the case. Interestingly, within the strains there was no correlation between total 24-hours running activity and the amount of body weight loss on the ABA regime. However, there was a positive correlation between the amount of food associated activity (FAA), expressed as a percentage of the total activity, and the amount of body weight loss. It thus suggests that not the total amount of running activity determines the decline in body weight, but the amount of activity during periods that the animal would normally not be active.

Food associated activity (FAA) was increased in the RLA. An explanation for this might be the rewarding properties of running wheel activity. There are several lines of evidence that support the rewarding aspects of running. First, rats are willing to press a lever to get access to a running wheel (23;24). Secondly, running can reinforce reward responses to drugs of addiction. In other words, running reinforced ethanol addiction in a similar way as cocaine administration does (25). Finally, running wheel access and drug of abuse were shown to be interchangeable; access to a treadmill reduced morphine self administration in rats (26). Rats that are prone to drug addiction were shown to develop high levels of

"
D1 receptor antagonism in rats hypersensitive for ABA

voluntary running activity as compare to non-drug preferring rats (27). Since dopamine may be the neurobiological substrate of reward, we hypothesized that the hyperactivity, as observed in the ABA model, can be blocked with a dopamine 1 receptor antagonist, and this hypothesis was tested in the second experiment in this paper. We therefore treated RLA rats, hypersensitive for the development of ABA, with a selective dopamine 1 receptor antagonist in the attempt to block the development of FAA during the ABA regime. Consistent with previous findings we have shown that saline treated Wistar rats display higher levels of FAA and consequently loose more weight than SCH23390 treated Wistar rats (11). In line with this, in the current study we were able to attenuate the development of FAA in the RLA rats by treatment with the d1 receptor antagonist. The decrease in FAA levels in these rats lead to an attenuation of body weight loss, although this was only a partial attenuation. Interesting, the course of body weight loss of the SCH23390 treated RLA rats was remarkably similar to that of saline treated normo-sensitive Wistar rats.

The efficacy of dopamine antagonism to attenuate FAA development seems receptor type dependent. In our studies a selective dopamine 1 receptor antagonist proved effective in blocking FAA. In contrast, Mistleberger and Mumby showed that Haloperidol, a non-specific dopamine 2 receptor antagonist, did not affect FAA development in male rats, a finding that was later confirmed by Verhagen and colleagues in female rats (28;29). It thus seems that specifically the dopamine 1 receptor is involved in the development of FAA. The dopamine 1 receptor is known to be more involved in motor control and may therefore be more effective. This raises the question whether treatment with SCH23390 induced motor function impairment, which then may have resulted in the observed reduced physical activity levels. Since we used a dose known to be below the threshold to induce motor dysfunction or cataplexia (30;31), which is consistent with the fact that dark phase activity was not affected by this dose of SCH23390, we are confident that the observed decrease in physical activity is not caused by an inability to be physically active.

Different from what would be expected from the increased susceptibility of the RLA to the development of ABA, Corda et al (32) have shown that the RHA, not the RLA, are more susceptible for the development of drug addiction. Furthermore, RHA rats display an elevated dopamine release in response to both stress and drug of abuse. We hypothesized that the running during the ABA regime would elicit an elevation of the mesolimbic dopamine pathway, leading to an elevated dopaminergic input into the Lateral Hypothalamus. This in turn leads to elevated orexin release, which leads to increase physical activity. The amount of dopamine released to orexin neurons might be crucial in this event. Alberto and co-workers (33) have showed that dopamine has a differential effect on the firing rate of orexin
neurons, dependent on the ambient concentration of dopamine. When orexin neurons are stimulated with a low dose of dopamine (1 µM) the firing rate of the orexin neurons increase. However, when the neurons were exposed to a higher dose (10 µM), the firing rate of the orexin neurons decrease. The dose-dependent response seems to result from activation of the different subclasses of dopamine receptors. The dopamine 1 receptor elicits a stimulatory effect, whereas the dopamine 2 receptor elicits an inhibitory effect, therefore a low dose of dopamine solely activates the d1 receptors. When a higher dose is administered both the d1 and d2 receptor subtypes are activated (33). The mechanism for this dose-dependent d1 and d2 receptor-mediated modulation is not known (34), but differences in affinity state of the receptors might explain the observed differences between the drug and running induced responses within the Roman rat strains.

In the first experiment, the role of personality in susceptibility for ABA was tested in RHA/RLA rats lines selectively bred for avoidance behavior, as well as in rats obtained from our Wild Type Groningen outbred population, which were selected for coping style (active/passive) of burying behavior. From previous studies, we have learned that RHA rats selected for proactive avoidance behavior have a high degree of burying behavior, whereas the RLA rats (selected for passive behavior in avoidance tasks) do not engage in burying behavior. Despite compatibility of the RHA/RLA and active/passive WTG coping styles, only the RHA/RLA lines differed considerably in body weight loss and food intake suppression (figure 2), and behavioral hyperactivity (figure 3). There are several explanations for this divergence between strains. Firstly, we may not have selected extremely passively coping rats in the WTG population, comparable to the passiveness that exists in the RLA line. It is at this point not clear whether the difference between the Roman and the WTG strains are due to the degree of passivity or due to co-selection of a genetic trait within the RLA line. A second possibility is that avoidance behavior is regulated by a set of genes which affect ABA development as well as burying behavior, but these genes do not necessarily overlap to the extent that selection of differences in burying automatically influences avoidance behavior and ABA development. Future studies are required to clarify these issues.

Summarizing the data presented here show that an extremely passive coping style might be a risk factor for hyperactivity development in AN. Secondly, we showed an attenuation of hyperactivity by a dopamine 1 receptor antagonist in these rats which suggest a role for the dopaminergic reward system in the hypersensitivity of RLA rats to ABA. Based on these results, we hypothesize that hyperactivity in AN develops as a result of evolution inspired adaptations to starvation. To sustain food searching behavior, physical activity
should be rewarding under conditions of famine, this results in activation of the mesolimbic system to reinforce hyperactivity. Since a passive coping style originated to have a higher survival rate under unstable environmental conditions (18), the adaptive hyperactivity response to starvation might more strongly be reinforced in extremely passively coping individuals. As a result these individuals may experience hyperactivity more rewarding under starved conditions, making them more susceptible to AN development.

Acknowledgements:
We would like to thank Jan Bruggink for his excellent technical support. These studies were supported by an unrestricted research grant by AstraZeneca.
Chapter 10

Reference List


D1 receptor antagonism in rats hypersensitive for ABA


