Research report

High fat diet induced-obesity facilitates anxiety-like behaviors due to GABAergic impairment within the dorsomedial hypothalamus in rats

Sylvana Rendeiro de Noronha, Glenda Viggiano Campos, Aline Rezende Abreu, Aline Arlindo de Souza, Deoclécio A. Chianca Jr., Rodrigo C. de Menezes*

Department of Biological Sciences, Institute of Exact and Biological Sciences, Federal University of Ouro Preto, Ouro Preto, MG, Brazil

HIGHLIGHTS

• HFD facilitates the development of anxiety-related behavior.
• Muscimol intra-dorsomedial hypothalamus has an anxiogenic effect in obese rats.
• BMI intra-dorsomedial hypothalamus does not cause an anxiogenic effect in obese rats.
• Memory acquisition and locomotor activity are not altered by GABAergic manipulation.

ARTICLE INFO

Article history:
Received 11 May 2016
Received in revised form 18 August 2016
Accepted 22 August 2016
Available online 23 August 2016

Keywords:
Obesity
High-fat diet
Anxiety
Dorsomedial hypothalamus
GABA

ABSTRACT

Overweight and obesity are conditions associated with an overall range of clinical health consequences, and they could be involved with the development of neuropsychiatric diseases, such as generalized anxiety disorder (GAD) and panic disorder (PD). A crucial brain nuclei involved on the physiological functions and behavioral responses, especially fear, anxiety and panic, is the dorsomedial hypothalamus (DMH). However, the mechanisms underlying the process whereby the DMH is involved in behavioral changes in obese rats still remains unclear. The current study further investigates the relation between obesity and generalized anxiety, by investigating the GABA_A sensitivity to pharmacological manipulation within the DMH in obese rats during anxiety conditions. Male Wistar rats were divided in two experimental groups: the first was fed a control diet (CD; 11% w/w) and second was fed a high fat diet (HFD; 45% w/w). Animals were randomly treated with muscimol, a GABA_A agonist and brecculine methiodide (BMI), a GABA_A antagonist. Inhibitory avoidance and escape behaviors were investigated using the Elevated T-Maze (ETM) apparatus. Our results revealed that the obesity facilitated inhibitory avoidance acquisition, suggesting a positive relation between obesity and the development of an anxiety-like state. The injection of muscimol (an anxiolytic drug), within the DMH, increased the inhibitory avoidance latency in obese animals (focusing an anxiogenic state). Besides, muscimol prolonged the escape latency and controlling the possible panic-like behavior in these animals. Injection of BMI into the DMH was ineffective to produce an anxiety-like effect in obese animals opposing the results observed in lean animals. These findings support the hypotheses that obese animals are susceptible to develop anxiety-like behaviors, probably through changes in the GABAergic neurotransmission within the DMH.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Overweight and obesity have become a critical worldwide health problem on the last decades [1–3]. These conditions are associated with several adverse clinical health consequences, such as type 2 diabetes [4], cardiovascular diseases [5] and others comorbidities with an overall mortality increase [3,5]. Moreover, obesity has been linked to neuropsychiatric and anxiety disorders, including generalized anxiety disorder (GAD), panic disorder (PD), post-traumatic stress disorder (PTSD), emotional reactivity and cognitive dysfunctions [6–8].

Studies have shown a positive relation among obesity, psychiatric disturbance and physiological arousal. In fact, our group have recently shown that high fat diet (HFD; 45% lard fat) induced obesity rats, showed an exacerbated cardiovascular response during emotional stress [9,10]. Studies showed that, HFD consumption by
the mother during the perinatal period also promoted anxiety-like behaviors [11,12]. Moreover, premature exchange of breastfeeding to a HF diet in the postnatal period induced the development of anxiety-like behaviors in the offspring tested in the Elevated Plus Maze (EPM) [12]. However, the influence of adulthood obesity as a relevant cause for the dysregulation of brain circuits, which could lead to anxiety-like disorders, is not completely understood.

Converging lines of evidence suggest that the dorsomedial hypothalamus (DMH) is a crucial brain region involved in the regulation of behavioral responses, particularly fear, anxiety and panic-like disorders [13–16]. The medial hypothalamus is not only involved in behavior regulation, but also in physiological functions such as reproduction, food ingestion, metabolism and environmental threats [17,18]. In this context, the DMH has been shown to act as an important neuroanatomical substrate in coordinating behavioral changes [9,19]. Previous studies have shown that the DMH neuroactivity is essential in regulating anxiety conditions [13,14,20]. It is important to highlight that the activity level within this region depends on a balance of excitatory (Glutamate) and inhibitory (GABA) neurotransmitters [21,22]. In fact, injection of the GABA A agonist muscimol into the DMH evokes a panicky effect, which was evidenced by the increased escape latency on the Elevated T-maze (ETM), but was ineffective to change the learned conditioned anxiety-like response [17]. Notwithstanding, direct microinjections of GABA A antagonist bicuculline methiodide (BMI) into the DMH have an anxiogenic effect in animals tested in the Elevated Plus Maze (EPM) [13,20,23].

In a previous work we tested the relation between the cardiovascular reactivity during emotional stress and GABAergic tonus within the DMH in obesity-induced rats. As reported in the literature, we observed that a muscimol injection into the DMH was effective to control mean arterial pressure (MAP) and heart rate (HR) increases caused by stress in control animals. Nevertheless our results have shown that muscimol was ineffective to reduce the increase in MAP and HR induced by air jet stress in rats fed a high fat diet. Moreover, the injection of BMI into the DMH of obese rats evoked only a transient increase in MAP and HR when compared to control animals. We believe that the ineffectiveness of these drugs (muscimol and BMI) in obese rats might be due to a blunted-GABAergic tonus within the DMH, which could be the main cause for the exacerbated response observed in these animals during emotional stress test (see Ref. [10]).

Mindful that obese animals seem to have a blunted inhibitory GABAergic tonus within the DMH, and that functional changes in the DMH are related to mood disorders, we hypothesized that obese rats could be susceptible to develop anxiety and/or panic-like disorders due to changes in GABA activity within this nucleus.

Thus, the current study further investigates the relation between obesity and generalized anxiety, by investigating the importance of the impairment of GABA A inhibition within the DMH observed in obese rats on anxiety conditions. For that, we used the elevated T-maze model (ETM), an apparatus capable to investigate two different behavioral responses. The ETM investigates the conditioned fear, associated with GAD, through inhibitory avoidance behavior. When the animal is placed in the enclosed arm of the ETM, they cannot see beyond the walls, unless it pokes its head outside the arm. Further, the ETM is capable to investigate an unconditioned fear, which is associated with PD, through measuring escape latency. Rats seem to have an innate fear of open and high spaces. Accordingly, we used this apparatus to assess anxiety-like and panic-like responses in obese and lean animals, during controlled conditions, and after the administration of the GABA A receptor agonist muscimol or GABA A receptor antagonist BMI into the DMH.

2. Methods

2.1. Animals

Male Wistar rats (CEUA #2013/33, Federal University of Ouro Preto) weighing 100 ± 10 g were housed in groups of four in a polypropylene cage during 9 weeks, under controlled temperature (23 ± 1 °C), and in a 12:12 h dark/light cycle with ad libitum water and food. After the surgical intervention, animals were individually housed until full recovery for the behavioral testing. The Ethical Committee for Animal Research at Ouro Preto Federal University has approved all the procedures in this study, according to Brazilian Society of Neuroscience and Behavior and the “National Institutes of Health Guidelines for the Care and Use of Laboratory Animals” (8th edition; 2011).

2.2. Diet

Animals were divided into two experimental groups, one received either a control diet (CD) composed by 11% w/w fat (Nuvilab, Brazil) and the other received a high fat diet (HFD), composed by 45% w/w fat [10] (based on a D12451 formula sold by Research Diets, Inc., New Brunswickwick, NJ, USA) [24,25]. Changes in body composition caused by HFD feeding during the diet protocol period of 9 weeks were defined according to Adiposity and Lee Indexes (AI; LI). To calculate the LI, the weight cubic root (g) was divided by nose- anal length (cm). For AI, the sum of visceral fat weight (took from the epididymal, retroperitoneal and inguinal tissues) was divided by final rat weight and the result was multiplied by 100.

2.3. Apparatus

To evaluate behavioral changes, animals were tested in the Elevated T-Maze (ETM). An apparatus made of wood and formed by three arms of equal dimensions (50 cm × 12 cm) 50 cm far from the floor. The perpendicular arm of the ETM was enclosed by a 40 cm high wall, and the two opposite open arms were surrounded by a 1 cm plexiglass rim (aimed to avoid animals’ falls). To evaluate the locomotor activity, animals were tested in an Open Field square arena (OF; 40 cm l × 32 cm h) made of polypropylene equally divided in 16 squares on the floor. Room luminosity was set at 60 Lux frequency on the ETM and OF center. Following each behavioral tests routine, the equipment was cleaned with 20% ethanol.

2.4. Surgery procedures

After 9 weeks of diet, animals were submitted to a surgical procedure to implant stainless steel guide cannulas, unilaterally or bilaterally, into the DMH to enable drugs microinjection [26]. Briefly, rats under anesthesia (80 mg kg −1 Ketamine; 11.5 mg kg −1 xylazine, i.p. supplemented if needed) were positioned in a stereotaxic frame, with the incisor bar positioned 3.3 mm below the interaural line. A small craniotomy was made near the bregma reference point to allow cannula insert (15 mm length; stereotaxic DMH coordinates: AP −3.2; LL ±0.6; DV −7.6 mm) according to Paxinos and Watson atlas [27]. The cannula was fixed in the skull with the aid of two stainless steel screws and acrylic resin. Following surgery rats received analgesics (ketoflex 4 mg/kg, 0.1 mL/300 g s.c., Mundo Animal, Brazil) and preventive treatment against infection (penthabetic, 0.2 mL/100 g s.c., Fort Dodge Animal Health, Brazil). Animals were re-allocated to individual cages and left quiescent for 6 days after surgery, until the experimental procedures, except for normal cage cleaning.
2.5. Experimental design

On the sixth and the seventh days after surgery, animals were gently handled for 5 min to familiarize with the experimenter. On the eighth day, rats were exposed, during 30 min, to one of the open arms of the elevated T-maze previously isolated from the rest of the apparatus by a wood board.

After 24 h, rats were randomly treated with vehicle (PBS; 100 nL), muscimol (GABA_A agonist; 100 pmol/100 nL; bilaterally) or BMI (GABA_A antagonist; 10 pmol/100 nL) bilaterally into the DMH. We used a dental needle (G30; 16 mm length) coupled to a polyethylene tube (PE-10 Intramedic, Clay Adams) attached to a 5 mL microsyringe Hamilton filled with Mili-Q water for drug injections. Following the injection, rats were tested in the ETM. This test started with the measurement obtained from inhibitory avoidance acquisition. For that reason, rats were placed at the distal part of the enclosed arm facing the central intersection of the ETM. The time taken by the rat to cross the central area boundary and leave the enclosed arm, with all four paws, were recorded. Three consecutive trials were performed, with a thirty-second break between trials; the first trial was called baseline, the second avoidance 1, and the third called avoidance 2. Thirty seconds after avoidance 2 rats were tested on the escape test. Then, rats were positioned in the distal part of the open arm (the same arm where the rats were pre-exposed), and the time taken by the rat to cross the boundary and leave this arm with four paws were recorded in three consecutive trials, called escape 1–3, also with a thirty-second break between trials. The cut off time for each trial was set up at 300 s. After the ETM test, the animal was placed in an OF to evaluate locomotor activity during 300 s, for that we counted the number of squares covered by each rat during this period. After 24 h, rats were re-exposed to the ETM test, for a single avoidance and a single escape trial, aiming to assess changes of inhibitory avoidance and escape memories acquisition. All the tests session was recorded, in case of need to answer future questions. The experimenter knew which treatment each animal received, because rats treated with BMI was cannulated only in the right side of the DMH, and the sessions were not blind. After vehicle and BMI injections, animals was immediately placed in the ETM, and after muscimol injections, we waited the drug time action of 20 min to test animals in the ETM. Animals that had the cannula positioned outside the DMH was submitted to the same procedure aforementioned, and used as negative control.

2.6. Experimental procedures

We investigated the impact of obesity (9 weeks HFD-feeding rats) on behavioral changes, such as inhibitory avoidance and escape conditions. To access behavior changes between groups, rats were fed a HFD (n = 16) or a CD (n = 15) and received the injection of vehicle (PBS; 100 nL) bilaterally into the DMH. Moreover, we tested if the GABA_A sensitivity to pharmacological activation/blockade in the DMH was compromised in obese animals. For that, rats fed a HFD (n = 10) or CD (n = 9) were treated with muscimol (GABA_A receptor agonist; 100 pmol/100 nL), bilaterally, into the DMH. In addition, rats fed a HFD (n = 9) or CD (n = 9) were treated with BMI (GABA_A receptor antagonist; 10 pmol/100 nL) unilaterally into the DMH. All animals were exposed to the ETM followed by OF test after the drugs injections. This dose of muscimol was chosen based on earlier studies demonstrating that this dose in the DMH effectively reduced the physiological responses evoked by air jet stress [10]. The BMI dose was chosen based on earlier studies demonstrating that this BMI dose injected into the DMH can lead to physiological responses that a similar to the ones observed during emotional stress [10,28,29].

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>CD (n = 35)</th>
<th>HFD (n = 33)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final Body Weight (g)</td>
<td>313.0 ± 5.22</td>
<td>363.6 ± 5.12</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Body Weight Gain (g)</td>
<td>225.6 ± 5.44</td>
<td>278.2 ± 5.52</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Lee Index</td>
<td>0.28 ± 0.001</td>
<td>0.28 ± 0.001</td>
<td>p = 0.5703</td>
</tr>
<tr>
<td>Adiposity Index</td>
<td>3.08 ± 0.135</td>
<td>6.15 ± 0.213</td>
<td>p &lt; 0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats.

* [Body weight (g)]/13 = nasoanal length (cm).

** Body fat mass (g) + final body weight (g) = 100.

* Significant difference between high-fat diet versus control diet group by unpaired Student’s t-test.

2.7. Histology

At the end of the experiments, rats were deeply anesthetized (80 mg/kg<sup>-1</sup> ketamine and 11.5 mg/kg<sup>-1</sup> xylazine, i.p.) and submitted to transcardiac perfusion with 60 mL of saline followed by 120 mL of a 4% buffered paraformaldehyde in 0.1 M phosphate-buffered saline. After that, the brains were removed and stored in 4% buffered paraformaldehyde overnight and then transferred to a 20% sucrose solution until saturation. Coronal brain sections were obtained by using cryostat equipment and the slides were mounted on PERMOUNT (Fisher Scientifics, Waltham, MA, USA). Sites of injections were confirmed with the aid of a microscope (LEICA DM LB Diagnostics Instruments Inc., Starling Heights, MI, USA) and according to the Paxinos and Watson reference atlas [27].

2.8. Statistical analysis

Avoidance and escape analysis in the ETM were made by repeated measures ANOVA, considering the treatment (drug or diet) as the independent factor, and the trial (baseline, avoidance 1 and 2, or escape 1–3) as the dependent factor. Memory trial acquisition, OF test for locomotor activity and body weight, were analyzed with unpaired Student’s t-test.

3. Results

Fig. 1 shows the positive DMH sites of microinjections from CD groups treated bilaterally with vehicle or muscimol, and unilaterally with BMI. Fig. 2 shows the positive DMH sites of microinjection from HFD groups treated bilaterally with vehicle or muscimol and unilaterally with BMI. The missed injections are demonstrated by bilaterally muscimol and unilaterally BMI nearby the dorsomedial hypothalamus nucleus.

3.1. Obesity induced by a HFD

Animals fed a HFD during 9 weeks presented higher final body weight gain ([t(28) = −4.97; p < 0.001] and higher elevated adiposity index ([t(26) = −7.35; p < 0.001] compared to CD group. Lee index revealed no difference between HDF and CD groups (Table 1). These results confirm the HFD efficiency in inducing obesity in rats [10].

3.2. HFD induces anxiety-like behaviors in rats

The effects of HFD on rats’ inhibitory avoidance and escape behavior are showed on Fig. 3. Repeated measures ANOVA revealed a significant effect of trials [F(2,58) = 29.35; p < 0.001] and diet [F(1,29) = 13.42; p = 0.001] and trials by diet interaction [F(2,58) = 5.33; p = 0.007]. On avoidance 1 [t(29) = −2.96; p = 0.006] and 2 [t(29) = −3.25; p = 0.003] student’s t-test showed that HFD group remains longer time in the enclosed arm of the ETM compared to CD group, characterizing an anxiety-like behavior.
Repeated measures ANOVA for escape measurements (Fig. 4) revealed no significant effect of trials \( [F(2,58) = 0.24; p = 0.787] \) and trials by diet interaction \( [F(2,58) = 0.067; p = 0.935] \), diet \( [F(1,29) = 0.093; p = 0.763] \) and trials by diet interaction \( [F(2,58) = 0.067; p = 0.935] \).

The effects of HFD on rats' memory acquisition for inhibitory avoidance and escape behavior after 24 h are showed on Fig. 5. Inhibitory avoidance of HFD/vehicle \( [t(29) = -2.75; p = 0.010] \) was significant, different from CD/vehicle group. No significant effects were found on inhibitory avoidance and escape when comparing avoidance 2 to avoidance memory trial, the same was observed when escape 3 was compared to escape memory trial between HFD and CD groups. Student's t-test revealed no difference on locomotor activity of HFD and CD group as shown on Table 2.

### 3.3. Effects of GABA<sub>A</sub> activation within the DMH on inhibitory avoidance and escape behavior

The effects of muscimol (100 pmol/100 nL) on rats' inhibitory avoidance behavior are showed on Fig. 3. Three-way ANOVA revealed a significant effect on trials \( [F(2,92) = 20.80; p < 0.0001] \), trials by treatment \( [F(2,92) = 6.30; p = 0.003] \), diet \( [F(1,46) = 15.50; p < 0.001] \) and treatment \( [F(1,46) = 18.70; p < 0.001] \). No significant
Hypothalamus; of CD/BMI Values

Table 2

Locomotor activity of rats tested on the Open Field.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of covered squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD/vehicle</td>
<td>46.70 ± 3.96</td>
</tr>
<tr>
<td>CD/muscimol</td>
<td>59.80 ± 7.01</td>
</tr>
<tr>
<td>CD/bicuculline</td>
<td>60.90 ± 7.32</td>
</tr>
<tr>
<td>HFD/vehicle</td>
<td>56.40 ± 3.89</td>
</tr>
<tr>
<td>HFD/muscimol</td>
<td>43.91 ± 4.57</td>
</tr>
<tr>
<td>HFD/bicuculline</td>
<td>60.22 ± 7.9</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SE; n, number of rats: CD/vehicle = 16; HFD/vehicle = 15; CD/BMI = 9; CD/muscimol = 9; HFD/muscimol = 10; HFD/BMI = 9; significance level, p < 0.05; unpaired Student’s t-test.

Fig. 3. Effect of vehicle muscimol and BMI on inhibitory avoidance behavior of rats tested on the ETM. Effects of vehicle (100 nl), muscimol (100 pmol/100 nl) and BMI (10 pmol/100 nl) microinjections into the DMH on inhibitory avoidance measures of rats tested on the ETM. The time spent by the animals to leave the enclosed arm of the ETM on baseline, avoidance 1 and avoidance 2 are shown in three trials (with 30s intervals and a cutoff time of 300s). Student’s t-test and repeated measures ANOVA with Tukey post-test expressed difference as mean ± SEM; *p < 0.05 different from control group; **p < 0.05 different from vehicle. Abbreviation: DMH – dorsomedial hypothalamus; BMI – bicuculline methiodide.

Fig. 4. Effect of vehicle muscimol and BMI on escape behavior of rats tested on the ETM. Effects of vehicle (100 nl), muscimol (100 pmol/100 nl) and BMI (10 pmol/100 nl) microinjections into the DMH on escape behavior measures of rats tested on the ETM. The time spent by the animals to leave the open arm of the ETM on escape 1–3 are shown in three trials (with 30s intervals and a cutoff time of 300s). Student’s t-test and repeated measures ANOVA with Tukey post-test expressed difference as mean ± SEM; *p < 0.05 different from control group; **p < 0.05 different from vehicle. Abbreviation: DMH – dorsomedial hypothalamus; BMI – bicuculline methiodide.

The effect of muscimol (100 pmol/100 nl) on rats’ escape behavior is showed on Fig. 4. Three-way ANOVA revealed a significant effect on the treatment [F(1,46) = 16.55; p < 0.001]. No significant effect was found on trials [F(2,92) = 0.01; p = 0.988], trials by diet [F(2,92) = 1.73; p = 0.183], trials by treatment [F(2,92) = 0.26; p = 0.771], trials by diet by treatment [F(2,92) = 1.13; p = 0.327], diet [F(1,46) = 2.23; p = 0.142] and diet by treatment [F(1,46) = 3.09; p = 0.085]. Tukey post-hoc test revealed that HFD/muscimol group on escape 2 (mean difference: −9.87; standard deviation: 3.18; p = 0.017) and escape 3 (mean difference: −13.52; standard deviation: 3.35; p = 0.001) spent more time to leave the open arm when compared to HFD/vehicle group. Further, Student’s t-test revealed
that CD/muscimol group on escape 2 \[ t(22) = -2.02; \ p = 0.038 \], spent a longer time to leave the open arm when compared to CD/vehicle group.

The effect of muscimol on rats’ memory trial for inhibitory avoidance and escape behavior after 24 h is shown on Fig. 5. No significant effects were found for inhibitory avoidance and escape in memory trial. Although, when we compared the escape 3 to the memory trial of the HFD/muscimol group \[ t(18) = -3.75; \ p = 0.001 \], it was observed that rats treated with muscimol spent a shorter time to leave the open arm of the ETM after 24 h. No significant difference was found between avoidance memory trial and avoidance 2. Student’s t-test revealed no difference on locomotor activity of HFD/muscimol and CD/muscimol groups as shown at Table 2.

We have also analyzed the effects of muscimol treatment outside the DMH on inhibitory avoidance conditions as negative control. Three-way ANOVA revealed a significant effect for inhibitory avoidance on trial \[ F(2.70) = 10.27; \ p < 0.001 \], trial by drug \[ F(2.70) = 3.55; \ p = 0.034 \] and diet by drug \[ F(1.35) = 9.50; \ p = 0.004 \]. No effects were found on trial by diet \[ F(2.70) = 2.71; \ p = 0.073 \], trial by diet by drug \[ F(2.70) = 0.37; \ p = 0.687 \], diet \[ F(1.35) = 0.19; \ p = 0.667 \] and drug \[ F(1.35) = 0.748; \ p = 0.393 \]. Tukey post-hoc test revealed that CD/vehicle group was different from CD/muscimol group treated outside the DMH on baseline (mean difference: -136.68; standard deviation: 30.94; \ p = 0.001), indicating impairment on locomotor activity from animals treated with muscimol outside the DMH. Three-way ANOVA for escape analysis revealed a significant effect on drug \[ F(1.35) = 11.58; \ p = 0.002 \]. No differences were found on trial \[ F(2.70) = 0.40; \ p = 0.672 \], trial by diet \[ F(2.70) = 0.18; \ p = 0.837 \], trial by drug \[ F(2.70) = 0.65; \ p = 0.526 \], trial by diet by drug \[ F(2.70) = 0.39; \ p = 0.674 \] and diet \[ F(1.35) = 1.00; \ p = 0.324 \]. Tukey post-hoc test revealed that HFD/vehicle group was different from HFD/muscimol group treated outside the DMH on escape (mean difference: -10.12; standard deviation: 2.94; \ p = 0.008), indicating that muscimol could acted as an anxiolytic drug when injected in nuclei nearby the DMH, however, this effect could be due to the drug spreading to the DMH.

### 3.4. Effects of GABA\(_A\) blockade within the DMH on inhibitory avoidance and escape behavior

The effect of BMI (10 pmol/100 mL) on rats’ inhibitory avoidance behavior is showed on Fig. 3. Three-way ANOVA revealed a significant effect on trials \[ F(2.88) = 28.93; \ p < 0.001 \], avoidance by trial \[ F(2.88) = 10.83; \ p < 0.001 \] and diet by treatment \[ F(1.44) = 6.07; \ p = 0.018 \]. No significant effect was found on avoidance by treatment \[ F(2.88) = 2.27; \ p = 0.109 \], avoidance by diet by treatment \[ F(2.88) = 0.32; \ p = 0.724 \], diet \[ F(1.44) = 0.20; \ p = 0.652 \], and treat-
4.4 and [F(1.35) = 6.31; p = 0.017]. No effects were found on trial by drug [F(2.70) = 0.68; p = 0.510], trial by drug by drug [F(2.70) = 0.35; p = 0.702], diet [F(1.35) = 0.01; p = 0.950] and drug [F(1.35) = 2.83; p = 0.101]. Tukey post-hoc test revealed that CD/vehicle group was different from CD/muscimol group treated outside the DMH on baseline (mean difference: −128.18; standard deviation: 37.81; p = 0.009), indicating that locomotor activity from animals treated with BMI outside the DMH is altered. Three-way ANOVA for escape analysis revealed a significant effect on trial by drug [F(2.70) = 3.35; p = 0.041], diet [F(1.35) = 4.58; p = 0.039], drug [F(1.35) = 18.14; p < 0.001] and trial by drug [F(1.35) = 5.10; p = 0.030]. No differences was found on trial [F(2.70) = 2.76; p = 0.070], trial by diet [F(2.70) = 0.91; p = 0.411] and trial by diet by drug [F(2.70) = 0.72; p = 0.489]. Tukey post-hoc test revealed that HFD/vehicle group was different from HFD/vehicle group treated outside the DMH on escape 1 (mean difference: −16.62; standard deviation: 4.98; p = 0.010), escape 2 (mean difference: −19.12; standard deviation: 4.02; p < 0.001) and escape 3 (mean difference: −41.66; standard deviation: 12.66; p = 0.011). Animals treated with BMI outside the DMH spent more time to perform the escape trial, indicating that the possible anxiogenic effect evoked by BMI requires the DMH blockade.

4. Discussion

The present study further examined the relation between obesity and generalized anxiety, by investigating the importance of GABAergic neurotransmission within the DMH in obese rats under anxiety conditions. Our data showed that obese rats exhibit significant behavioral changes in inhibitory avoidance, which has been associated to an anxiety-like behavior. Obese animals did not exhibit behavioral changes in the escape task, suggesting that these animals do not present panic-like behaviors in standard conditions. DMH inhibition, by the activation of GABA\(_A\) receptors, induced an anxiety-like behavior in obese animals but not in lean animals. Activation of the GABA\(_A\) receptors also evoked a panicolytic effect in obese animals. DMH disinhibition, induced by the blockade of GABA\(_A\) receptors, caused an anxiety-like behavior in lean animals but it was ineffective to induce this condition in obese rats. Our results showed that obese animals presented anxiety-like behavior, which might be the result of a change in GABAergic neurotransmission within the DMH. Although recent studies have reported that obese rodents present an anxiety-like behavior condition when they were tested in different paradigms [6,30], our study is the first to demonstrate that the behavioral changes observed in obese animals, tested in the ETM, are associated to a pharmacological GABAergic resistance within the DMH.

The HFD model used in this study was effective to increasing body weight, adiposity index and visceral fat in rats after nine weeks of diet protocol (Table 1), characterizing an obesity condition, reinforcing the finds of our previous study [10,30,31].

Earlier studies have established obesity as an important cause to increased cardiovascular risk [32], insulin resistance [33] and diabetes [4]. Recently, obesity has been shown as an important factor in developing psychiatric disorders, such as anxiety, depressive behavior and mood symptoms [6,7,30,34]. An important finding from the present study is that obese rats had a significant increase in latency by trial to leave the enclosed arm during the trials on the ETM, demonstrating an intense learning capacity to avoid an aversive open space (open arm), probably due to their susceptibility to develop an anxiety-like behavior condition. Accordingly, these obese animals showed a longer latency to leave the enclosed arm, during the one trial test of memory acquisition (Fig. 5), suggesting a relation between memory learned-fear to sustained anxiety-like behavior. Animals fed a CD also sustained their level of anxiety-like behavior on memory trial, however their retained anxiety-like memory was less pronounced than in HFD rats (Fig. 5).

In this regard, neurobehavioral studies, have showed that independent neuronal structures mediate different forms of memory, such as hippocampus, amygdala and dorsal striatum [35]. However, obesity appears to have a role on cognitive disruption through neuronal inflammatory processes, affecting hippocampal-dependent contextual and spatial memory [36]. Despite that, our obese rats showed improvement on memory acquisition, probably due to a robust emotional modulation factor caused by the ETM, which could be promoting the activation of brain areas related to fear memory retention.

We believe that obesity, especially the visceral type, is probably the main cause in the development of anxiety-like behaviors in rats. Obesity activates pro-inflammatory process and induces increases in peripheral cytokine (e.g. IL-1β, IL-6 and TNF-α), which could lead to neuroinflammation [37,38]. Some studies have pointed out brain inflammation, especially when occurred in the hypothalamus, as an important cause for neuronal injury, highlighting how over-fat nutrition can damage a range of neuronal interactions [38–40]. Importantly, neuroinflammation can lead to neurotransmitters imbalance, particularly in GABA [41], and that GABAergic neurotransmission appears to be impaired in obese animals [10].

Based on these information, we hypothesized that the development of an anxiety-like condition in obese rats could be related to a GABAergic neurotransmission impairment in the DMH.

In order to investigate if the behavior differences between HFD and CD animals were due to changes in GABA\(_A\) activity within the DMH, we activated or blocked these receptors and examined animals’ behavior during the ETM test. Our findings showed that, in the control group, GABA\(_A\) activation increased the latency by trial on inhibitory avoidance 1, but this effect was refrained in avoidance 2 (Fig. 3) and in avoidance memory trial (Fig. 5). The changes observed on inhibitory avoidance among trials (baseline vs. avoidance 1 and 2) indicate that CD animals were capable of learning to avoid an aversive open and high space, but the lack of increase on latency during the avoidance 2 and memory trial (after 24 h) corroborates the expected anxioylicic muscimol effect in lean animals, as described in the literature [42]. In obese animals, activation of DMH GABA\(_A\) receptors caused an increase in latency during avoidance 1 and 2, suggesting that obesity could be the cause for this intensive learning along the trials. We believe that the muscimol inability to control an anxiety-like condition, in the HFD group, contributed to this intense learning during the trials. In fact, in the course of our experiments, we observed that muscimol injection into the DMH of obese animals acted in an unexpected way,
increasing the latency during the avoidance trials in HFD/muscimol group. This result suggests that \( \text{GABA}_A \) activation in the DMH of obese rats might evoke an anxiogenic effect, or that obesity condition could induce a pharmacological resistance to muscimol action over this nucleus. Muscimol, in the first fifteen minutes of experiment, also compromised the locomotor activity from CD and HFD. Previous studies described the baseline trial as capable to measure alterations in animal’s locomotor activity, and not to predict behavior condition such as anxiety-like [17,43]. In fact, a previous study demonstrated that the temporary inactivation of the DMH with 1 nmol of muscimol was capable to induce a sedative effect in lean animals, and was capable to evoke a panicolytic effect (increasing the latency that animals spent to leave the open arm of the ETM) in these animals. These data corroborate our finds, since we observed an increase in baseline (inhibitory avoidance) and escape latency (a panicolytic effect) [17]. However, we believe that a persistent impairment in the locomotor activity is unlikely, because we could not find differences in the analysis obtained from the OF test (Table 2).

As mentioned above we believe that nine weeks of HFD treatment was capable to induce, in our model, a low-grade inflammation. Previous studies indicated that low-grade inflammation could be one of the causes of changes in neuron balance, specific \( K^+/Cl^- \) cotransporter KCC2, affect the \( \text{GABA}_A \) receptors function mechanism. The change in this cotransporter could be responsible for enabling the \( Cl^- \) ions to flow into the neuron, therefore changing \( Cl^- \) equilibrium potential, causing the \( Cl^- \) exit from the cell through \( \text{GABA}_A \) receptors, instead of entering the cell, thus prompting depolarization [44,45]. Therefore, muscimol could be acting to increase an anxiety-like condition (despite being described as an anxiolytic drug) in those HFD-obese rats, depolarizing instead of hyperpolarizing the DMH. However, this aforementioned mechanism it is only a speculation, since this \( Cl^- \) imbalance may or may not occur as a consequence of obesity state, and need to be carefully investigated in future studies. On the other hand, muscimol was effective to control escape behavior, decreasing the panic-like behavior of obese animals. This result is in line with panicolytic effects observed in HFD/muscimol group, already described in the literature [17]. In addition, escape memory trial was improved, suggesting that the panicolytic effect of muscimol was due to drug administration, and did not comprised rat innate fear of heights and open spaces [46]. Regardless of muscimol effects [10,47], our results demonstrated that the injection of muscimol into the DMH did not alter locomotor activity (Table 2).

The acute \( \text{GABA}_A \) blockade, with BMI, was effective to compromise the inhibitory learning during the three trials (Fig. 3), suggesting an increased and sustained anxiety-like behavior in lean animals. A range of studies demonstrated that \( \text{GABA}_A \) receptor blockade, through the injection of antagonists into DMH induce neurovegetative responses [13,20,48–50], such as: tachycardia, tachypnea, hypertension [48,51] and anxiety-like behaviors [20]. The relation between escape to panic-like behavior and inhibitory avoidance to anxiety-like behavior, is exemplified in our results, since we demonstrated that BMI acted mostly as an anxiogenic drug. Moreover, the BMI injection was efficient to block a possible increase in escape latency (Fig. 4), which could happen when the animals are continuous tested in the ETM. On the other hand, we observed that lean animals treated with muscimol showed an increase on the escape 2 trial, indicating that this drug acted, in CD animals, as a panicolytic/anxiolytic drug [52]. The \( \text{GABA}_A \) blockade also changed the avoidance memory trial, increasing the latency on this one trial, suggesting that emotional conditions, such as fear and aversion, can be an important factor to modulate the memory formation [53]. Otherwise, the acute drug treatment with BMI was ineffective to alter inhibitory avoidance and escape behavior on HFD animals.

In our previous work, we demonstrated that \( \text{GABA}_A \) acute blockade in obese animals increased the HR during a transient period compared to lean animals, suggesting a reduction of \( \text{GABA}_A \) modulation in the DMH of obese animals [10]. This last finding corroborates ours, as HFD-obese rats appear to show a pharmacological resistance to BMI, and their inhibitory avoidance latency, under a threatening ETM stimulus, was similar to CD-vehicle group (Fig. 3). Meanwhile, the injection of BMI in lean animals led to sustained anxiety-like behavior (Fig. 3). Despite of the agitation observed in animals after the BMI injection, the locomotor activity remained similar between HFD and CD-vehicle group (Table 2).

The present study shows that HFD-induced obese rats are prone to anxiety-like behaviors, possibly because of a change in \( \text{GABA}_A \)–mediated inhibition within the DMH. We have also shown that obese animals present a longer latency to leave the enclosed arm, during the one trial test of memory acquisition, suggesting a relation between memory learned-fear to the sustained anxiety-like behavior. Our work provides further insight on how obesity can lead changes in brain neurotransmission, particularly in the hypothalamus, which could be the main mediator for the development of anxiety-like behaviors in obese animals.

Conflicts of interest

The authors declare no conflicts of interest.

Funding and disclosure

This work was supported by FAPEMIG (Grant number: APQ-01543-14), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), UFOP, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Acknowledgments

The authors are thankful to Claudia Carneiro and to Immunopathology Laboratory (UFOP); to Marly Lessa and Milton de Paula for their technical assistance; and to CNpq, FAPEMIG, CAPES and UFOP, for their financial support.

References


学霸图书馆

www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：

图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具