Male mice engaging differently in emotional eating present distinct plasmatic and neurological profiles

Christine Heberden, Elise Maximin, Sylvie Rabot, Laurent Naudon

Note: Snapshot PDF is the proof copy of corrections marked in EditGenie, the layout would be different from typeset PDF and EditGenie editing view.

Author Queries & Comments:

Q1 : Please provide a short biography of the authors.

Response: Resolved

Q2 : Please modify the abstract into a structured abstract using the headings "Objectives; Methods; Results; Discussion", as per journal style.

Response: Resolved

Q3 : The abstract is currently too long. Please edit the abstract down to no more than 250 words.

Response: Resolved

Q4 : Please note that the Funding section has been created by summarising information given in your acknowledgements. Please correct if this is inaccurate.

Response: Resolved

Q5 : The funding information provided (INRAE) has been checked against the Open Funder Registry and we failed to find a match. Please check and resupply the funding details.

Response: Resolved

Q6 : Please check that the heading levels have been correctly formatted throughout.

Response: Resolved

Q7 : A Paranthesis seems to be missing following " ... with different functions". Please indicate where it should be placed.

Response: Resolved

Q8 : The disclosure statement has been inserted. Please correct if this is inaccurate.

Response: Resolved

Q9 : The reference [52] is listed in the references list but is not cited in the text. Please either cite the reference or remove it from the references list.

Response: Resolved

Q10 : The resolution of figures 2,3,4 is too low. Please resupply the figures in a resolution of at least 300 dpi.

Response: Resolved

CM1 : I do not know how to change the figures

Male mice engaging differently in emotional eating present distinct plasmatic and neurological profiles

Recto running head : NUTRITIONAL NEUROSCIENCE

Verso running head : C. HEBERDEN ET AL.

Christine Heberden[a], Elise Maximin[a], Sylvie Rabot[a], Laurent Naudon[b] **[Q1]**

[a] INRAE, AgroParisTech, Micalis Institute, Université Paris-Saclay Jouy-en-Josas, France

[b] INRAE, AgroParisTech, CNRS, Micalis Institute, Université Paris-Saclay Jouy-en-Josas, France

CONTACT Christine Heberden Christine.heberden@inrae.fr INRAE, AgroParisTech, Micalis Institute, Université Paris-Saclay, Jouy-en-Josas 78350, France

History :

ABSTRACT

Objective:Stressed individuals tend to turn to calorie-rich food, also known as 'comfort food' for the temporary relief it **[Q2]** provides. The emotional eating drive is highly variable among subjects. Using a rodent model, we explored the plasmatic and neurobiological differences between 'high and low emotional eaters' (HEE and LEE).

Methods:40 male mice were exposed for 5 weeks to a protocol of unpredictable chronic mild stress. Every 3 or 4 days, they were submitted to a 1-h restraint stress, immediately followed by a 3-h period during which a choice between chow and chocolate sweet cereals was proposed. The dietary intake was measured by weighing. Plasmatic and neurobiological characteristics were compared in mice displaying high vs low intakes.

Results:Out of 40 mice, 8 were considered as HEE because of their high post-stress eating score, and 8 as LEE because of their consistent low intake. LEE displayed higher plasma corticosterone and lower levels of NPY than HEE, but acylated and total ghrelin were similar in both groups. In the brain, the abundance of NPY neurons in the arcuate nucleus of the hypothalamus was similar in both groups, but was higher in the ventral hippocampus and the basal lateral amygdala of LEE. TSurprisingly in the lateral hypothalamus LEE had also more orexin (OX) positive neuronsbut no more melanin-concentrating hormone (MCH) neurons. Both NPY and OX are orexigenic peptides and mood regulators.

Discussion: Mice respond differently to eating after a stressful event. This Emotional eating difference was reflected in plasma and brain structures implicated in emotion and eating regulation. These results concur with the psychological side of food consumption **[Q3]**.

KEYWORDS

- Stress
- reward
- food intake
- brain

FUNDING

[Q4]This study was supported by funds from INRAE (ANSSD from **[Q5]**AlimH department).

Introduction[Q6]

Appetite, food consumption and dietary habits are closely linked to mood variations, stress perception and anxiety. Stress can impose a dual effect on appetite, either blunting or increasing it, sometimes uncontrollably. During the sanitary crisis experienced with Covid-19, overeating as well as hedonic reduction and anhedonia have been reported [1]. Under more common circumstances, overeating affects 35–60% of individuals while 25–40% confide that they eat less when experiencing stressful events [2].

If stress modifies eating behavior in both directions, a ruling shift towards calorie-rich food is acknowledged [2,3]. Indeed most individuals experiencing stressful situations, either in the usual or experimental settings, are turning to palatable food rather than nutriments more considered as healthy, and this so-called comfort food in turn mitigates the sensation of stress and anxiety [2]. The extent of the requirement to palatable food is variable among subjects, and depends on several factors, such as the gender [4], or the perception of stress [5]. Regarding this 'emotional eating' drive, the same result was obtained with rodent or primate models: when submitted to different stress protocols, experimental animals increase their food intake with a preference to high-calorie items [6–8]. Sugary and fat food dampens Hypothalamic–Pituitary–Adrenal (HPA) axis stress responses [9]. Similar to humans, this eating drive is variable, with animals more prone to it than others [10,11].

The brain circuitry controlling food intake is composed of two systems distinct and yet deeply intertwined: one, in the hypothalamus, controls the body's energy homeostatic requirements. The other, a corticostriatal hypothalamic circuitry, is involved in the hedonic eating and reward, and is part of the dopaminergic system [12]. The latter is crucial for motivation, and is also involved in emotive behavior, which is consistent with the fact that food intake go along with stress, and that eating is both physiological and psychological.

The eating impulse is mediated by peptides acting centrally. Ghrelin is the only known gut-derived peptide with orexigenic

properties. The acylated form, with an octanoyl residue on Serine 3, is active and binds to the 7-transmembrane G protein-coupled receptor, type 1a growth hormone secretagogue receptor (GHRS1a)[13]. Ghrelin acts in the arcuate nucleus (Arc) of the HT via blood circulation and vagal transmission. In the Arc, ghrelin signaling activates neuropeptide Y (NPY) and Agouti-related peptide (AgRP) neurons[14]. Ghrelin also activates the limbic dopaminergic system and modulate reward, motivation and anxiety. Several rodent models have demonstrated that ghrelin is both induced by stress and mitigating it [15–19].

NPY, AgRP and Orexin (OX), neuropeptides activated centrally by ghrelin are also increased under stressful circumstances and display anxiolytic properties [20–24]. OX, as will be discussed below, demonstrates a more complex profile, and according to the timing and the target location, can be anxiolytic or anxiogenic [23].

To analyze the variability towards the urge to eat in humans, most studies are based on psychology and brain imaging. Rodent models open the possibility to examine the physiological origins of this difference. We have designed an experiment to examine the appetitive behavior of male mice immediately after being exposed to a restraint stress. We selected two groups of mice, referred to as either 'high emotional eaters' (HEE), based on their consistent high post-stress eating (PSE) level, or 'low emotional eaters' (LEE) because of their low PSE records. We then determined the plasmatic levels of corticosterone as a reflection of stress, and of ghrelin and NPY. We also analysed the neuronal organizations of a few structures involved in food intake, and emotive behavior.

Methods

Animals

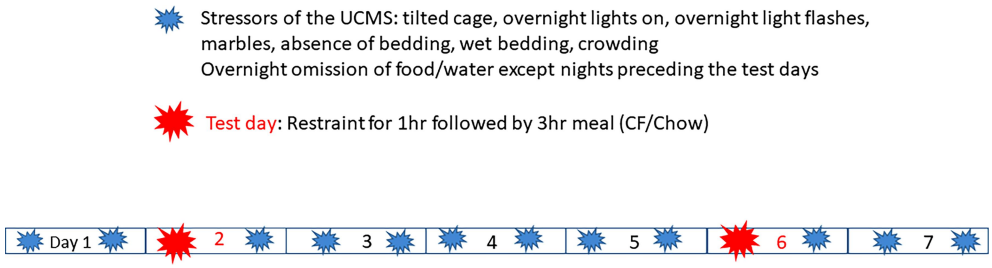
Two cohorts of 20 4-week-old C3H/HeN male mice were purchased from Janvier labs (Le Genest-Saint-Isle, France). The mice were maintained at 4 per cage under a 12–12 dark–light cycle (lights on at 7:30 am), fed ad libitum with a commercial rodent diet (RO 3/40 diet, Safe, Augy, France) and tap water was freely available. The experiment was carried out in compliance with the EU rules for animal experimentation, and was allowed by the French Ministry of Science under the reference APAFIS#10767-2017092915401597V1. The mice were weighed twice a week and examined for wellbeing throughout the experiment.

Experimental protocol: unpredictable chronic mild stress (UCMS) and PSE behavior

After a 2-week acclimatization, the mice were placed in individual cages and submitted to a protocol of 5-week UCMS[25] during the light phase, so that the mice would remain stressed on a consistent basis throughout the experiment. UCMS consisted of a succession of mild stressors such as tilted cage, wet bedding, omitting food or water overnight, foreign objects in the cage (marbles), 24-h exposure to light, or light flashes during the dark period, with 2 stressors per day. The succession of the stressors varied randomly so that the animals did not get used to it. We assessed the coat state once a week in eight body parts (head, neck, dorsal area, ventral area, genital area, tail, forepaws and hindpaws) before the beginning and until the end of the UCMS procedure. The mice received the same order of UCMS events, at the same time. For each body part, a score of zero was given for a well-groomed coat and a score of one for an unkept coat. Addition of those scores led to a weekly coat state score for each mouse [25].

Every 3 or 4 days, the sated mice were restrained for 1 h in 50-ml drilled Falcon tube and immediately after were proposed with a choice of the regular chow or commercial chocolate sweet cereals (Chocapic, Nestlé, France) for a 3-h period. A timeline representative of an experimental week is shown in Figure 1. The night before the test, food and water were provided ad libitum, under the usual conditions. Chow and the cereals were weighed before and after the 3-h period to measure the consumption. PSE index was calculated as the intake values (g) standardized by the metabolic mass (body weight (g) $^{2/3}$) [26]. Eight episodes of restraint-PSE were accomplished during the experiment. To avoid food neophobia before the first test, the mice were proposed with a couple of cereal flakes two or three times the week before, and the mice refusing to eat them were excluded from the experiment (2 out of 40 mice).

Figure 1. Linear timeline of one experimental week. All the mice received the same stressor at the same time.



In each test, the PSE values were sorted in descending order and divided into tertiles: mice assigned to the 'high emotional eaters' (HEE) group had to show PSE in the higher tertile in at least 5 of the 8 tests, and never in the lower one. Mice assigned to the 'low emotional eaters' (LEE) groups had to have PSE values in the lower tertile in at least 5 of the 8 tests, and never in the highest. In each cohort, four were identified as HEE, and 4 as LEE.

The mice were sacrificed at the end of the 5-week UCMS. Before the sacrifice, both groups were submitted to a 1-h restraint under the usual conditions, and decapitated immediately after. Mice not considered as HEE and LEE were decapitated without or with restraint, to determine the effect of the restraint on the levels of plasmatic hormones and peptides. Trunk blood and brains were collected. The brains were frozen in isopentane at −40°C and ultimately stored at −80°C until analysis.

Brain immunohistochemistry and in situ hybridization

The brains from HEE and LEE mice were sectioned at −20°C using a cryostat. Sections of 16 µm were collected on frost plus slides and allowed to dry out before storage at −80°C. The sections covered from bregma −1.22 to −2.70 mm.

For immunohybridization, the sections were fixed with 4% paraformaldehyde, permeabilized with 0.25% Triton and blocked with 3% of normal donkey and/or goat serum, according to the secondary antibody use. The antibody used were: anti-Orexin mAB763 from R&D systems (Biotechne, Lille, France) and anti-MCH from RayBiotech (Clinisciences, Nanterre, France).

In situ hybridization was performed using the fast Red labeling kit from ACD biotechnologies, according to the manufacturer's instructions. The sections after fixation by 4% formaldehyde were permeabilized by a 10-min incubation with protease at room temperature, and processed as recommended under the manual guidelines. Because of a strong expression for both probes, the fifth level of amplification was reduced to 5 min. The reference of the NPY probe was 313321. Following hybridization, the sections were counter colored with hematoxylin.

The section once labeled were scanned using the Pannoramic Scan (3D Histech) and the slides were counted using Image J.

Corticosterone and peptide analyses

Trunk blood was collected in EDTA 0.5 mM coated tubes, immediately stored at 4° and spun at 300 rpm for 10 min. The dosage of corticosterone (Arbor Assays, Clinisciences, Nanterre, France) and total ghrelin (Sigma Aldrich Merck St Quentin Fallavier, France), acylated ghrelin (BioVendor, Euromedex, Souffelweyersheim, France) and NPY (Raybiotech, Clinisciences, France) were realized by Elisa, according to the manufacturers' guidelines.

Statistics

All statistical analyses were completed using Graphpad prism 7 (GraphPad Software, San Diego, CA, USA). All quantitative data are expressed as the mean ± standard error of the mean (SEM) for each group. The alpha risk for rejection of the null hypothesis was set to 0.05. All values met criteria for normality, unless otherwise specified. Groups were compared pairwise by using either *t*-test or nonparametric Mann Whitney-U test as appropriate. * $P < 0.05$.

Results

PSE determinations, weekly food consumption, body weights

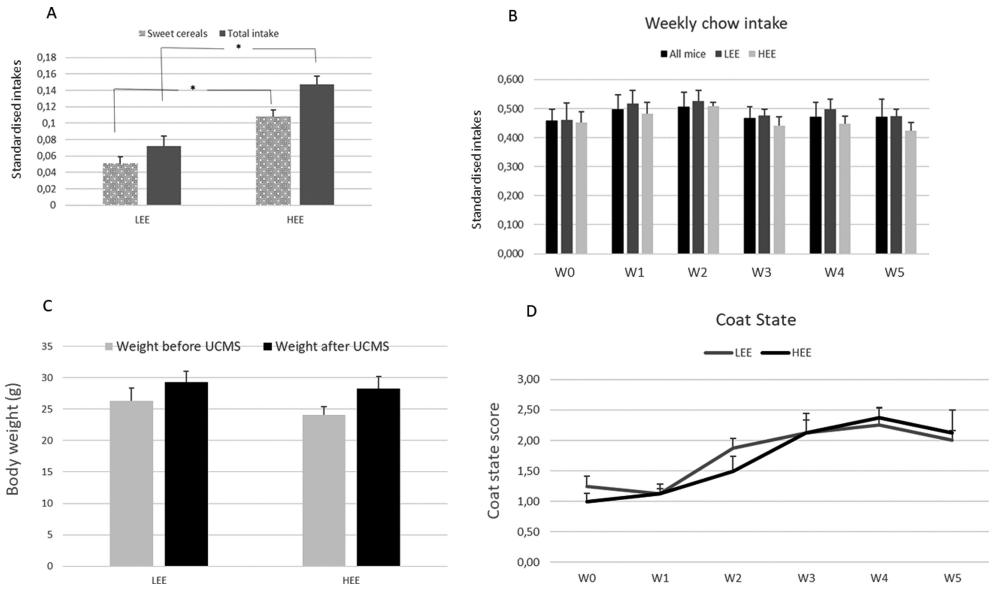
As indicated under Methods, during the whole experimentation, the mice were subjected to eight episodes of 1-h restraint stress immediately followed by a 3-h period in their own cage. During this time, sweet cereals and chow were freely available, and intakes were measured by weighing the food before and at the end of the 3 h.

Because the weights and food intakes were very coherent and similar between the 2 cohorts, the results were combined and

refer to all the HEE and LEE mice, defined according to the criteria described under Methods: in each cohort 4 mice were recognized as HEE and 4 mice as LEE.

Figure 2 reports the average PSE intakes. PSE intakes were significantly different between the two groups. In both groups, the sweet cereals were the preferred choice, as they represented about 75% of the total food intake during the 3-h. The fact that total food intake during the 3-h post-restraint period was also significantly lower in LEE shows that the drive to eat was less present in LEE, and that there was not a preference of chow over the sweet cereals in the LEE group. Yet average weekly chow consumption was similar and not significantly different from the mean intake registered for all the mice (Figure 2). The body weight evolution was similar in both groups, and so were the coat states (Figure 2).

Figure 2. **[Q10]**Food intake, body weights and coat state evolution during the 5-week experiment. (A) Intake of sweet cereals and chow after the 3-h restraint, The food was measured before and after restraint, and the difference was divided by (body weight) 2/3. (B) Weekly food intake, the weight of consumed food was divided by (body weight) 2/3. (C) Weight evolution of LEE and HEE during the 5-week experiment. (D) Coat state evolution in LEE and HEE. Student *t*-test *$p < 0.05$.



see attachment. Fig 2, 3, 4 and 5 are now 300 dpi minimum

1

Plasma peptides and hormone

Both HEE and LEE mice were placed under restraint before decapitation, so that the animals would be in the same physiological state as they were when proposed with the choice chow/sweet cereals. To check the effect of the restraint period, the rest of the animals (i.e. not qualified as HEE or LEE) were sacrificed with (R) or without restraint (NR). The results are reported in Figure 3. The restraint induced a significant rise in corticosterone, acylated ghrelin and NPY, but total ghrelin remained unchanged.

Figure 3. Plasma concentrations of peptides and corticosterone after 1-h restraint and in LEE and HEE. The mice not identified as LEE or HEE were submitted (R) or not (NR) to an 1-h restraint under the usual conditions, and were decapitated immediately after. The plasmas of LEE and HEE were analyzed after decapitation as described under methods. Student *t*-test ***$p < 0.01$; *$p < 0.05$.

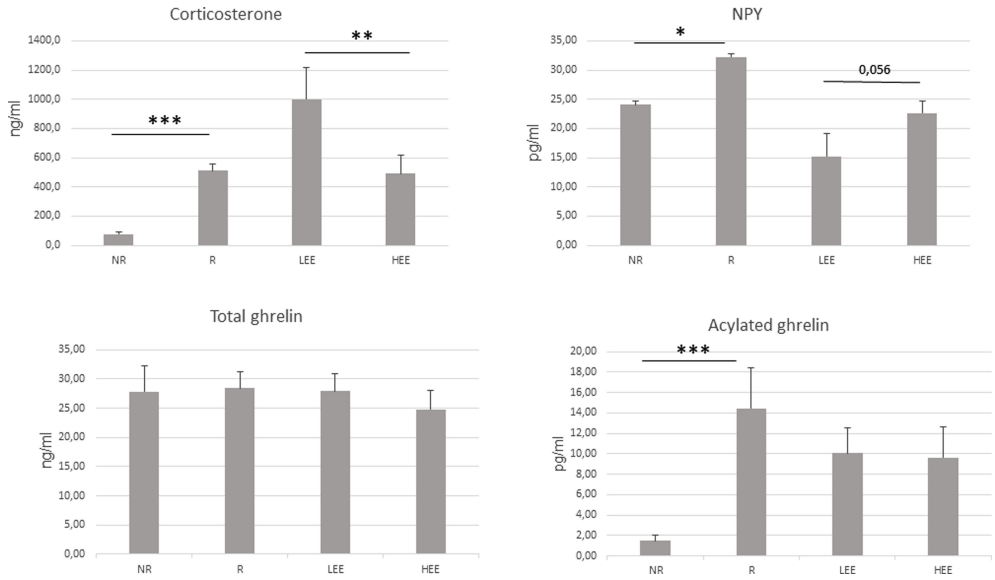


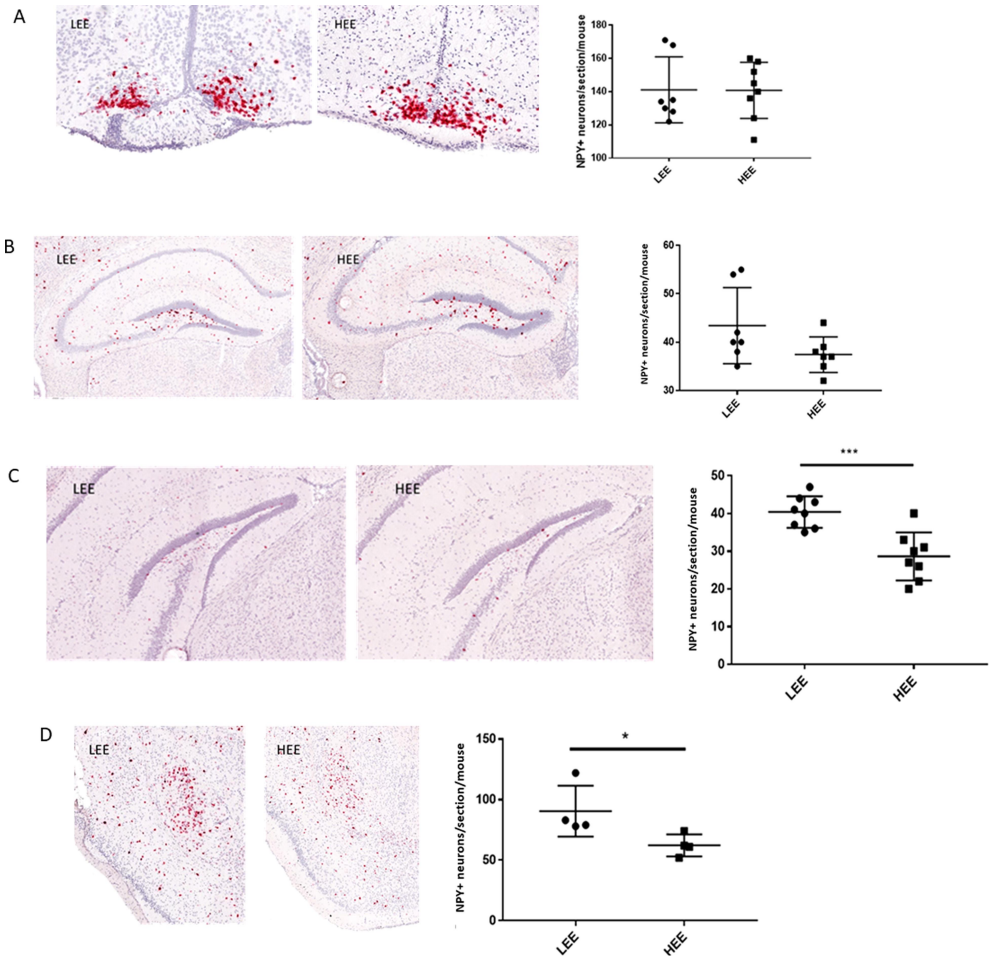
Figure 3 shows the results of the same determinations for HEE and LEE. Corticosterone was higher in LEE, but total and acylated ghrelin were similar in both groups. The orexigenic peptide NPY was slightly lower in LEE, not significantly though.

In light of the differences in the plasmatic NPY profiles, we examined its expression pattern in cerebral structures involved in emotion and eating.

Food behavior- and stress-related peptides occurences in the brain

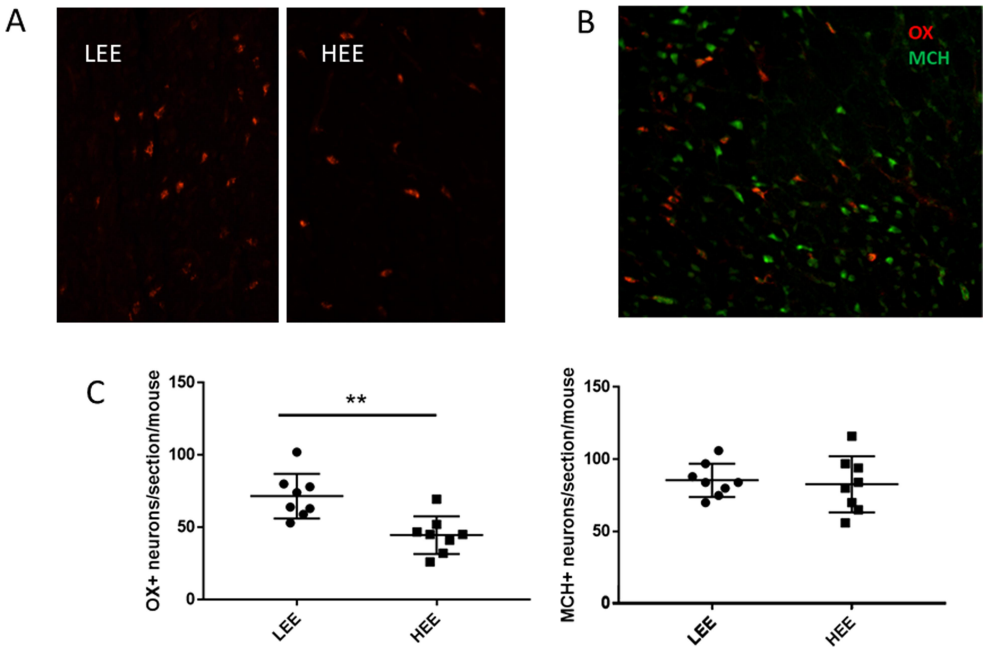
Because peripheral NPY was lower in LEE, we examined its expression in the arcuate nucleus (Arc) of the hypothalamus, the structure involved in energy homeostasis, and main site of expression of NPY. We also checked the expression in the hippocampus, both dorsal and ventral, and in the amygdala. Because the numbers of counted neurons were very similar in the 2 cohorts, the data were combined. The expression was visualized by in-situ hybridization, allowing a very clear imaging of the positive neurons. In the Arc, there was no difference in the number of NPY+ neurons. Neurons expressing NPY were significantly more abundant in LEE in the ventral hippocampus and in the basal lateral amygdala (BLA) (Figure 4).

Figure 4. In situ hybridization of NPY in cerebral structures of HEE and LEE. (A) Arcuate nucleus of the hypothalamus; (B) Dorsal hippocampus (bregma −1.34 to −2.30); (C) Ventral hippocampus (bregma −2.46 to −2.92); (D) Basal lateral amygdala. The labeled cells from 4 to 6 sections were counted using Image J. Student *t*-test ***$p < 0.01$ *$p < 0.05$.



OX is a peptide induced by Ghrelin in the lateral hypothalamus (LH), so we checked the abundance of OX neurons in this structure by immunohistology. OX neurons were significantly more abundant in LEE (Figure 5). OX is not the only orexigenic peptide in the LH, since the melanin-concentrating hormone (MCH) is also expressed in this structure and is associated with food intake and impulsivity [27]. MCH labeling showed no difference between both groups, indicating that OX+ neurons were specifically more abundant in LEE.

Figure 5. Immunohistochemistry of Orexin (OX) and melanin-concentrating hormone (MCH) of neurons in the lateral hypothalamus of HEE and LEE. The labeled cells from 4 sections were counted using Image J. Student *t*-test $^{**}p < 0.02$.



Discussion

Discussion

Not all individuals are equal in front of comfort food. Our objective here was to study this variability in a rodent model. We subjected male mice to a protocol of chronic mild stress, to generate a constant level of anxiety and examined food intake following an acute stress, realized by a restraint period. Two categories of mice could be identified, differing in their interest to consuming food immediately after restraint. It is to be underlined here that the mice were sated and not experiencing hunger at the time the choice sweet food/chow was proposed, since the test was performed in the morning (from 9 am to 12 pm), and that chow had been freely available during the dark section. Yet all the mice consumed some amount of food during the 3-h period. Although LEE and HEE differ in post-restraint intake, both groups consumed the same weekly amount of chow under the rest of the time (Figure 2). We did not measure the chow intake in the 24 h immediately preceding the tests, so it could be assumed that elevated intake of the cereal in the HEE mice could be driven by mice that routinely did not consume much food in the sated feeding window. However, this would be unlikely, since the assignment to either group HEE/LEE relied on the consistency of the intakes during 8 tests. Furthermore, we found no difference in the weekly intake, and no correlation between the body weights, the amount of weekly food, and the food intake during the tests for both groups. This suggests that there was no difference in the metabolism of HEE and LEE, which is also shown by their similar weight evolution during the whole experiment.

Plasmatic determinations showed distinct profiles regarding NPY and corticosterone. Although significance was not reached, NPY results were consistent with the PSE behavior of LEE and HEE, since NPY concentrations were slightly higher in HEE mice. Corticosterone concentrations were also significantly different between the two groups, the higher value in LEE suggesting a higher level of stress. Therefore, the plasmatic profiles of the LEE seem consistent with the literature since they showed higher Corticosterone and lower NPY: the plasmatic levels of NPY are indeed higher in animals consuming higher amounts of food [22], and higher corticosterone levels signal that the HPA recation to stress was not dampened by food [6–9].

In spite of the demonstrated role of ghrelin in the escalation of food intake and food reward [19,28], in our hands, ghrelin, either total or acylated, was similar in both groups. Therefore, the difference in post-stress intake could not be attributed to a difference in ghrelin activation or synthesis.

We hypothesized that acylated ghrelin was similar in both groups because the mice were subjected to a robust stressful event before euthanasia, resulting in maximal activation in both groups, therefore hiding a possible difference. Indeed, the control assay realized with the unselected mice clearly show the strong effect of the restraint on the plasmatic levels of the active form, consistent with published data [29]. Therefore, the orexigenic drive was similar in HEE and LEE, and this could suggest that LEE mice could display a resistance to ghrelin. This hypothesis could be considered in light of the fact that anorectic patients develop very high levels of ghrelin but fail to respond to them [30–32].

This possibility led us to inspect some structures known to respond to ghrelin signaling, and the secondary neuropeptides tied to it, such as NPY and OX.

NPY is a neuropeptide abundantly present in the arcuate nucleus of the hypothalamus where it is co-expressed with AgRP and GABA. NPY and AgRP are important in the homeostatic regulation of energy intake and are activated by acylated ghrelin [33]. That plasmatic NPY was lower in LEE, was consistent with a lower food intake, but plasmatic NPY levels are not always commensurate with the expression of NPY in the brain[34,35]. In the brain, we checked the expression of NPY by in situ hybridization. We found the highest expression in Arc, consistent with other studies. In Arc, NPY did not differ between both groups (Figure 4). This is in line with the fact that weekly food consumption was similar in both groups. NPY was also abundantly expressed in the hippocampus and the BLA, two structures involved and emotive behavior. The BLA is at the interface of perception, emotion, and motor behaviors. This structure plays an important role in the processing of fearful and rewarding stimuli, as well as emotional memory [36] and acute stress impacts BLA neurons[37]. The hippocampus is characterized by a dorsal-ventral axis, with different functions **[Q7]**. The dorsal part (D hipp) processes spatial and fear memory, while the ventral pole (V hipp) is involved in emotional and affective states [38]. The hippocampus is also a structure implicated in food control [39] and lesions in V hipp increase food consumption [40].

In V hipp and BLA, NPY neurons were more numerous in LEE than in HEE, which seems paradoxical because of the orexigenic nature of this peptide. Yet in addition to its orexigenic role, NPY is also a key regulator in emotional behavior and is associated with resilience. NPY neurons coexpress GABA, and are inhibitory neurons. In different stress protocols, NPY blunts stress and confers resilience. In humans, depressed or bipolars patients show lower plasmatic NPY levels. NPY is a gene that shows polymorphisms, and higher levels seem to be protective towards mental health disorders [41]. Rodent models provide examples of the anxiolytic properties of NPY: for instance, the anxiolytic effect of exercise is associated with higher expression of NPY in the hippocampus[35]. NPY is also upregulated in response to the induction of stress[42]. It is

therefore very interesting that NPY neurons are more abundant in two structures involved in emotive behavior. Our hypothesis is that the higher number of neurons in Vhipp and BLA could represent a compensatory mechanism to counteract a higher level of stress perception in LEE.

We also inspected the presence of OX neurons in the lateral hypothalamus. OX is indeed a neuropeptide relay to ghrelin in the brain [43,44]. Again and surprisingly, we counted more OX neurons in the LH of LEE than in HEE. This result was counter-intuitive, as OX is known for not only its orexigenic property, but is also involved in reward seeking [45]. Consistently, it has been recently demonstrated that OX neurons were more abundant in Cocaine-addicted rats in LH[46]. Here, the more abundant OX neurons could be viewed as a compensatory mechanism, once more under the hypothesis of a resistance to ghrelin in LEE.

Yet another interpretation relies on the fact that a higher abundance of OX neurons has been reported in other rodent models of stress [47,48], and that generally a higher abundance in OX neurons is associated with higher stress and emotivity. This, in our experiment, could be connected with the higher corticosterone level in LEE. Indeed, OX, similar to NPY, is a key regulator of stress reactivity, and OX neurons can be activated by a ghrelin surge, but also by stress [49]. While NPY is associated with resilience, OX displays a complex and ambivalent profile towards stress management, and can be tied either to resilience or to depression and anxiety. This dual profile can be interpreted in view of the opposite properties of its receptors: OXr1, and OXr2. While OXr1 mediates an anxiogenic and pro-depression response, OXr2 is involved in the induction of resilience and is anxiolytic [50]. LH orexigenic output to the lateral habernula have been shown recently to confer an anxiolytic and anti-depressant effect after social defeat stress [51]. Yet OX is also known to activate central and peripheral parts of the HPA axis [52,53]: a cerebroventricular injection of OX can increase Corticotroprin Releasing hormone in the Para Ventricular Nucleus and ultimately lead to an increase in adrenocorticotropin hormone (ACTH) and corticosterone plasma levels [23]. The OX system connects with multiple regions of the brain involved in emotivity and stress reactivity, and confers distinct responses depending on the target location.

Indeed, the OX neurons of the LH also project to VTA and activate the dopaminergic neurons in the VTA, which project into the NAc. This can lead to the promotion of reward-seeking behaviors[53,54]. But on another hand, it has also been shown that, on the contrary, high frequency phasic optogenetic stimulations of the DA neurons in the VTA could lead to anhedonia and a decrease in motivation [55–57]. It was also shown that the hyperactivity of the axis VTA-NAc increases social defeat stress susceptibility and social avoidance [57]. We could thus hypothesize that the higher numbers of OX neurons in LEE drive a dopaminergic hyperactivity of the VTA-NAc axis, leading ultimately to a down stimulation of the NAc. If this hypothesis were correct, then the surplus of OX neurons could lead to the motivation deficit seen in the LEE. Interestingly, one can mention that anorexia nervosa is also considered as a condition linked to a disorder of reward circuitry and a dysfunctional axis VTA-NAc [31,58].

Conclusion

Our interpretation of these results is that LEE mice could experience a higher stress (as shown by the higher corticosterone concentrations), either due or related to a higher OX content, counterbalanced by a compensatory higher NPY number of neurons in areas promoting resilience.

A limitation comes from the fact that we restricted the study to male mice, and it would be necessary to investigate female mice, since the drive towards comfort food seems to be higher in women [4]. We also performed our measurements during the light phase, and further experimentation would require data generated during the dark phase.

Stress modifies the brain, and by these modifications, the brain finds a way to cope and stay protected [37,59]. This in turn has an impact on behavior. Our results show that structures involved in emotion and eating display different organizations of neurons expressing peptides linked to coping and eating.

Acknowledgements

We are grateful to the staff of the animal facility Anaxem (INRAe, Jouy en Josas, France) for excellent care to the animals, and their help and support, and to the staff of the @BRIDGE imaging facility (INRAe, Jouy en Josas, France).

Disclosure statement

No potential conflict of interest was reported by the author(s **[Q8]**).

Data availability statement

Data are available upon request from the corresponding author.

References

1 Moccia L, Janiri D, Giuseppin G, Agrifoglio B, Monti L, Mazza M, et al. Reduced hedonic tone and emotion dysregulation predict depressive symptoms severity during the COVID-19 outbreak: an observational study on the Italian general population. Int J Environ Res Public Health. 2021;18:255.

2 Zellner DA, Loaiza S, Gonzalez Z, Pita J, Morales M, Pecora D, Wolf A. Food selection changes under stress. Physiol Behav. 2006;87:789–93.

3 Dallman MF. Stress-induced obesity and the emotional nervous system. Trends Endocrinol Metab. 2010;21:159–65.

4 Linde JA, Jeffery RW, Levy RL, Sherwood NE, Utter J, Pronk NP, Boyle RG. Binge eating disorder, weight control self-efficacy, and depression in overweight men and women. Int J Obes Relat Metab Disord. 2004;28:418–25.

5 Tomiyama JA, Dallman MF, Epel ES. Comfort food is comforting to those most stressed: evidence of the chronic stress response network in high stress women. Psychoneuroendocrinology. 2011;36:1513–9.

6 La Fleur SE, Houshyar H, Roy M, Dallman MF. Choice of lard, but not total lard calories, damps adrenocorticotropin responses to restraint. Endocrinology. 2005;146:2193–99.

7 Arce M, Michopoulos V, Shepard KN, Ha QC, Wilson ME. Diet choice, cortisol reactivity and emotional feeding in socially housed rhesus monkeys. Physiol Behav. 2010;101:446–55.

8 Patrono E, Di Segni M, Patella L, Andolina D, Valzania A, Latagliata EC, et al. When chocolate seeking becomes compulsion: gene-environment interplay. PLoS One. 2013;10:e0120191.

9 Christiansen AM, Dekloet AD, Ulrich-Lai YM, Herman JP. Snacking causes long term attenuation of HPA axis stress responses and enhancement of brain FosB/deltaFosB expression in rats. Physiol Behav. 2011;103:111–6.

10 Klump KL, Racine S, Hildebrandt B, Sisk CL. Sex differences in binge eating patterns in male and female adult rats. Int J Eat Disord. 2013;46:729–36.

11 Sinclair EB, Culbert KM, Gradl DR, Richardson KA, Klump KL, Sisk CL. Differential mesocorticolimbic responses to palatable food in binge eating prone and binge eating resistant female rats. Physiol Behav. 2015;152:249–56.

12 Rossi MA, Stuber GD. Overlapping brain circuits for homeostatic and hedonic feeding. Cell Metab. 2018;27:42–56.

13 Sun Y, Wang P, Zheng H, Smith RG. Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. Proc Natl Acad Sci USA. 2004;101:4679–84.

14 Schellekens H, Finger BC, Dinan TG, Cryan JF. Ghrelin signalling and obesity: at the interface of stress, mood and food reward. Pharmacol Ther. 2012;135:316–26.

15 Lutter M, Sakata I, Osborne-Lawrence S, Rovinsky SA, Anderson JG, Jung S, et al. The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. Nat Neurosci. 2008;11:752–3.

16 Chuang JC, Perello M, Sakata I, Osborne-Lawrence S, Savitt JM, Lutter M, Zigman JM. Ghrelin mediates stress-induced food-reward behavior in mice. J Clin. Invest. 2011;121:2684–92.

17 Dickson SL, Egecioglu E, Landgren S, Skibicka KP, Engel JA, Jerlhag E. The role of the central ghrelin system in reward from food and chemical drugs. Mol Cell Endocrinol. 2011;340:80–7.

18 Huang HJ, Zhu XC, Han QQ, Wang YL, Yue N, Wang J, et al. Ghrelin alleviates anxiety- and depression-like behaviors induced by chronic unpredictable mild stress in rodents. Behav Brain Res. 2017;326:33–43.

19 Al Massadi O, Nogueiras R, Dieguez C, Girault JA. Ghrelin and food reward. Neuropharmacology. 2019;148:131–8.

20 Yam KY, Ruigrok SR, Ziko I, De Luca SN, Lucassen PJ, Spencer SJ, Korosi A. Ghrelin and hypothalamic NPY/AgRP expression in mice are affected by chronic early-life stress exposure in a sex-specific manner. Psychoneuroendocrinology. 2017;86:73–7.

21 Yang Y, Babygirija R, Zheng J, Shi B, Sun W, Zheng X, et al. Central neuropeptide Y plays an important role in mediating the adaptation mechanism against chronic stress in male rats. Endocrinology. 2018;159:1525–36.

22 Hassan AM, Mancano G, Kashofer K, Fröhlich EE, Matak A, Mayerhofer R, et al. High-fat diet induces depression-like behaviour in mice associated with changes in microbiome, neuropeptide Y, and brain metabolome. Nut Neurosci. 2019;22:877–93.

23 James MH, Campbell EJ, Dayas CV. Role of the orexin/hypocretin system in stress-related psychiatric disorders. Curr Top Behav Neurosci. 2017;33:197–219.

24 Fang X, Jiang S, Wang J, Bai Y, Kim CS, Blake D, et al. Chronic unpredictable stress induces depression-related behaviors by suppressing AgRP neuron activity. Mol Psych. 2021;26:2299–315.

25 Yalcin I, Belzung C, Surget A. Mouse strain differences in the unpredictable chronic mild stress: a four-antidepressant survey. Behav Brain Res. 2008;193:140–3.

26 Heusner AA. Body size and energy metabolism. Ann Rev Nutr. 1985;5:267–93.

27 Noble EE, Wang Z, Liu CM, Davis EA, Suarez AN, Stein LM, et al. Hypothalamus-hippocampus circuitry regulates impulsivity via melanin-concentrating hormone. Nat Commun. 2019;10:4923–27.

28 Valdivia S, Cornejo MP, Reynaldo M, De Francesco PN, Perello M. Escalation in high fat intake in a binge eating model differentially engages dopamine neurons of the ventral tegmental area and requires ghrelin signaling. Psychoneuroendocrinology. 2015;60:206–16.

29 Kristenssson E, Sundqvist M, Astin M, Kjerling M, Mattsson H, Dornonville de la Cour C, et al. Acute psychological stress raises plasma ghrelin in the rat. Regul Pept. 2006;134:114–7.

30 Dardennes DM, Zizzari P, Tolle V, Foulon C, Kipman A, Romo L, et al. Family trios analysis of common polymorphisms in the obestatin/ghrelin, BDNF and AGRP genes in patients with anorexia nervosa: association with subtype, body-mass index, severity and age of onset. Psychoneuroendocrinology. 2007;32:106–13.

31 Gorwood P, Blanchet-Collet C, Chartrel N, Duclos J, Dechelotte P, Hanachi M, et al. New insights in anorexia nervosa. Front Neurosci. 2016;10:256.

32 Schalla MA, Andreas Stengel A. The role of ghrelin in anorexia nervosa. Int J Mol Sci. 2018;19:2117.

33 Liu CM, Kanoski SE. Homeostatic and non-homeostatic controls of feeding behavior: distinct vs. common neural systems. Physiol Behav. 2018;193(Part B):223–31.

34 Morris MJ, Chen H, Watts R, Shulkes A, Cameron-Smith D. Brain neuropeptide Y and CCK and peripheral adipokine receptors: temporal response in obesity induced by palatable diet. Int J Obes. 2008;32:249–58.

35 Joksimovic J, Selakovic D, Jovicic N, Mitrovic S, Mihailovic V, Katanic J, et al. Exercise attenuates anabolic steroids-induced anxiety via hippocampal NPY and MC4 receptor in rats. Front Neurosci. 2019;26:172.

36 Janak PH, Tye KM. From circuits to behaviour in the amygdala. Nature. 2015;517:284–92.

37 McEwen BS, Nasca C, Gray JD. Stress effects on neuronal structures: hippocampus, amygdala, and prefrontal cortex. Neuropsychopharmacology. 2016;41:3–23.

38 Fanselow MS, Dong HW. Are the dorsal and ventral hippocampus functionally distinct structures? Neuron. 2010;65:7–19.

39 Kanoski SE, Grill HJ. Hippocampus contributions to food intake control: mnemonic, neuroanatomical, and endocrine mechanisms. Biol Psychiatry. 2017;81:748–56.

40 Davidson TL, Kanoski SE, Chan K, Clegg DJ, Benoit SC, Jarrard LE. Hippocampal lesions impair retention of discriminative responding based on energy state cues. Behav Neurosci. 2010;124:97–105.

41 Enman NM, Sabban EL, McGonigle P, Van Bockstaele EJ. Targeting the neuropeptide Y system in stress-related psychiatric disorders. Neurobiol Stress. 2015;1:33–43.

42 Zhang Z, Li N, Chen R, Lee T, Gao X, Yuan Z, et al. Prenatal stress leads to deficit in brain development, mood related behaviors and gut microbiota in offspring. Neurobiol Stress. 2021;15:100333.

43 Toshinai K, Date Y, Murakami N, Shimada M, Mondal MS, Shimbara T, et al. Ghrelin-induced food intake is mediated via the orexin pathway. Endocrinology. 2003;144:1506–12.

44 Hsu TM, Hahn JD, Konanur VR, Noble EN, Suarez AN, Thai J, et al. Hippocampus ghrelin signaling mediates appetite through lateral hypothalamic orexin pathways. Elife. 2015;4:e11190.

45 Harris GC, Wimmer M, Aston-Jones G. A role for lateral hypothalamic orexin neurons in reward seeking. Nature. 2005;437:556–9.

46 James MH, Stopper CM, Zimmer BA, Koll NE, Bowrey HE, Aston-Jones G. Increased number and activity of a lateral subpopulation of hypothalamic orexin/hypocretin neurons underlies the expression of an addicted state in rats. Biol

Psychiatry. 2019;85:925–35.

47 Jalewa J, Wong-Lin KF, McGinnity TM, Prasad G, Hölscher C. Increased number of orexin/hypocretin neurons with high and prolonged external stress-induced depression. Behave Brain Res. 2014;272:196–204.

48 Clifford L, Dampney BW, Carrive P. Spontaneously hypertensive rats have more orexin neurons in their medial hypothalamus than normotensive rats. Exp Physiol. 2015;100:388–98.

49 Peleg-Raibstein D, Burdalov D. Do orexin/hypocretin neurons signal stress or reward? Peptides. 2021;145:170629.

50 Sargin D. The role of the orexin system in stress response. Neuropharmacology. 2019;154:68–78.

51 Wang D, Li A, Dong K, Li H, Guo Y, Zhang X, et al. Lateral hypothalamus orexinergic inputs to lateral habenula modulate maladaptation after social defeat stress. Neurobiol Stress. 2021;14:100298.

~~**52** Russell SH, Small CJ, Dakin CL, Abbott CR, Morgan DG, Ghatei MA, Bloom SR. The central effects of orexin-A in the hypothalamic-pituitary-adrenal axis in vivo and in vitro in male rats. J Neuroendocrinol. 2001;13:561–6~~**[Q9]**~~.~~

52 Samson WK, Taylor MM, Follwell M, Ferguson AV. Orexin actions in hypothalamic paraventricular nucleus: physiological consequences and cellular correlates. Regul Pept. 2002;104:97–103.

53 Thomas CS, Mohammadkhani A, Rana M, Qiao M, Baimel C, Borgland SL. Optogenetic stimulation of lateral hypothalamic orexin/dynorphin inputs in the ventral tegmental area potentiates mesolimbic dopamine neurotransmission and promotes reward-seeking behaviours. bioRxiv. 2021. doi:10.1101/2020.09.10.291963.

54 Tunisi L, D'Angelo L, Fernández-Rilo AC, Forte N, Piscitelli F, Imperatore R, et al. Orexin-A/hypocretin-1 controls the VTA-NAc mesolimbic pathway via endocannabinoid-mediated disinhibition of dopaminergic neurons in obese mice. Front Synaptic Neurosci. 2021;13:622405.

55 Chaudhury D, Walsh JJ, Friedman AK, Juarez B, Ku SM, Koo JW, et al. Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. Nature. 2013;493:532–6.

56 Carlton CN, Sullivan-Toole H, Ghane M, Richey JA. Reward circuitry and motivational deficits in social anxiety disorder: what can be learned from mouse models? Front Neurosci. 2020;14:154.

57 Cao JL, Covington 3rd HE, Friedman AK, Wilkinson MK, Walsh JJ, Cooper DC, et al. Mesolimbic dopamine neurons in the brain reward circuit mediate susceptibility to social defeat and antidepressant action. J Neurosci. 2010;30:16453–8.

58 Duriez P, Ramoz N, Gorwood P, Viltart O, Tolle V. A metabolic perspective on reward abnormalities in anorexia nervosa. Trends Endocrinol Metab. 2019;30:915–28.

59 McEwen BS. Protective and damaging effects of stress mediators: central role of the brain. Dialogues Clin Neurosci. 2006;8:367–81.

Attachment Files

1 Fig 3.tif *:*

2 Fig 2A.tif *:*

3 Fig 2B.tif *:*

4 Fig 2C.tif *:*

5 Fig 2D.tif *:*

6 Fig 4 B.tif *:*

7 Fig 4 C.tif *:*

8 Fig 4 D.tif *:*

9 Fig 5.tif *:*

10 Fig4 A.tif *:*