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Original Article

Assessment of Coercive Stress on the Behavioral, Exploratory and Metabolic Response in the Wistar Rats

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ABSTRACT

Background: Stress is a strain on our mental and physical functions caused by various aversive and harmful events, inducing adaptive biological responses. However, if a stressful situation is too intense or persists over time, its repercussions on the individual become pathogenic. Stress also affects the animal world and represents an ethical and economic problem for farm animals and their welfare. **Aim:** The aim of our study is to evaluate the effect of restraint stress and its consequences. **Materials and Methods:** Twenty-four male and female *Rattus rattus* rats of the Wistar strain were exposed to two daily sessions of restraint stress over a period of 5 days. **Results:** Following this, locomotors exploratory anxiety-like behaviors were quantified in the elevated plus maze test and the open field test, and compared to control groups. **Conclusion:** The existence of a correlation between the behavioral consequences and the variation of the lipid profile following the induction of restraint stress was noted.

Key words: Stress, Anxiety, Lipid profile, Locomotion, Elevated plus maze.

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INTRODUCTION

According to the World Health Organization and the Health Programme in 2018, mental and neurological disorders, including various types of stress, are common worldwide, affecting every community and age group in countries with varying levels of wealth. These disorders account for 14% of the global burden of disease.[1]

Stress not only attributes a negative impact on human life, but also affects other living organisms that are frequently exposed to variants of intrinsic and extrinsic stress stimuli.[2] The brain is the organ that interprets bodily information, whose triggers are internal or external to the body, imposing the activation of stress mechanisms when warranted. The value given to the perceived information (valence), as well as the feeling of control, explains the difference between the intensity of the stress perceived by the individual and the actual aggressiveness of the stressor.[3]

Restraint and immobilization stress are stressors that include limiting the movement of animals. Restraint stress involves placing the animal in a plastic tube, wire mesh or soft plastic cone that fits its body,[4] and has been widely used as an emotional stress.[5] Itis considered as a condition that leads to a disruption of body homeostasis,[6] producing endocrine and autonomic responses characterized by increases in glucocorticoids levels, blood pressure and heart rate.[7,8] These responses are accompanied by various physiological, emotional and behavioral changes that may be related to adaptations of the hypothalamic-pituitary-adrenal (HPA) axis.[9-11]

Stressful events activate the hypothalamic-pituitary-adrenal (HPA) axis and increase the release of corticotrophin hormone (CRH) from the par ventricular nucleus of the hypothalamus, which causes the secretion of adrenocorticotropin (ACTH) from the anterior pituitary. The latter stimulates the secretion of corticosterone from the adrenal cortex from cholesterol.[12,13]

Glucocorticoids play many roles in the body's metabolism, inducing changes in energy storage, carbohydrate and lipid metabolism and feeding behavior in animals.[14,15] Furthermore, studies suggest that these stress hormones can influence the lipid profile.[16]

In order to assess the concept of stress, one should look at life events that have long been central and that would be likely to be a source of stress. These correspond to important events that occur in the life of the subjects underlining the hypothesis that stress would result from the accumulation of changes that impose an adaptation.[17]

Increasingly, experimental animal models are being developed to identify the neurobiological disturbances that underlie vulnerability to these disorders. However, modeling in animals remains relative.[18,19] They may not adequately reproduce human disease, and instead model specific symptoms. The development of animal models of behavioral disorders linked to this type of stress (Plus maze test, open field test etc.) may contribute to a better understanding of the physiological mechanisms linked to gestation and improve therapeutic strategies.[19,20]

The aim of this study was to establish an inter-sexual comparison following chronic restraint stress in Wistar rats through the respective evaluation of their anxiety and exploratory activity levels as well as their lipid profile variations.

MATERIALS AND METHODS

Animals

White rat of the Wistar strain were obtained from the Pasteur Institute in Algiers, Algeria and used as the experimental model in this study. The animals were 5±1 months old and had a body weight of 200±10g. They were reared in the animal house of the Biology Department, Faculty of Sciences, Badji Mokhtar, Annaba University, Algeria.

Male and female rats were housed in standard polyethylene cages, lined with wood shavings. The cages were cleaned and the bedding changed every second day. The animals were acclimatized to the conditions of the animal house, at an average temperature of 18°C and subjected to a seasonal light-dark cycle. The food provided to the animals is in the form of sticks made up of corn, barley, milk and vitamin supplements, while the drinking water is presented in bottles adapted to the cages. Food and water are served *ad libitum*.

Experimentation

Stress induction

The restraint stress induction procedure was carried out after identifying the rats (marks on the tail of each rat) following two daily sessions

(09:00 "morning" and 14:00 "afternoon") of 30 min each, over a period of 5 days. Rats were placed in an 8cm diameter, 12cm long box with their tails and snouts exposed to air. At day 1 (D01), day 3 (D03) and day 5 (D05), each stress session was followed by a behavioral assessment of anxiety levels, locomotors and exploratory activity.

Behavioral tests

The tests used in animal experiments are similar to constructions or enclosures adapted to the size of the animal with the aim of observing its exploratory behavior, or subjecting it to a constraint or task, for a defined time. The observation of each passage in a test thus makes it possible to identify variables, which inform the experimenter about the behavioral register of the animal.

For these different reasons, we measured the behavior of rats on the emotional status observed in two unconditioned conflict tests: a test space open field and raised cross maze (elevated plus maze).

Biochemical parameters

At day 6 (D06), the rats were sacrificed by decapitation. This took place at the level of the animal facility of the Department of Biology of UBMA. The blood was collected into test tubes for biochemical determination of total cholesterol.[18]

Statistical data

The results were represented as a mean plus or minus the mean standard error standard error of the mean (SEM). The comparison between the different groups was performed using student t test analysis. Statistical analysis of the data was performed using XLSTAT software (2016 version)

The differences are considered to be;

- * Significant when ($p< 0.05$)
- ** Highly significant when ($p<0.01$)
- *** Highly significant when ($p<0.001$)

RESULTS

The anxiety level of the rats in the elevated cross test (Figure 1), reveals that in session 1 and session 2 more time was spent in the open arms of the test system, as well as the time spent in the center in female rats compared with the male rats The time spent in the closed arms was higher for males rats, with significant differences noted between the averages of the three days of group evaluation.

Exploration and locomotion performance in the open field test. In session 1 and 2, there was a higher time spent in the periphery and a highly significant difference between the means of the male rats evaluated over three days, every other day it was lower in the female rats

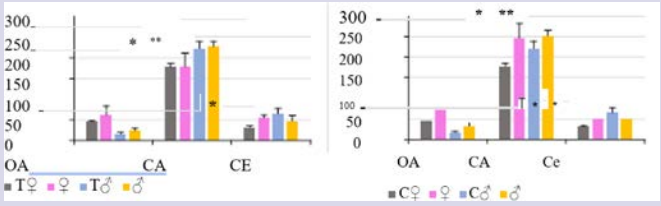

Figure 1: The Elevated plus maze parameters of the rats of the test groups (n=24) for (a) session 1 and (b) session 2.
Expressed as Mean ± SEM. OA: Open Arms, C: Control, CA: Closed Arms and Ce: Center.
* $p \leq 0,05$, ** $p \leq 0,01$, *** $p \leq 0,001$.

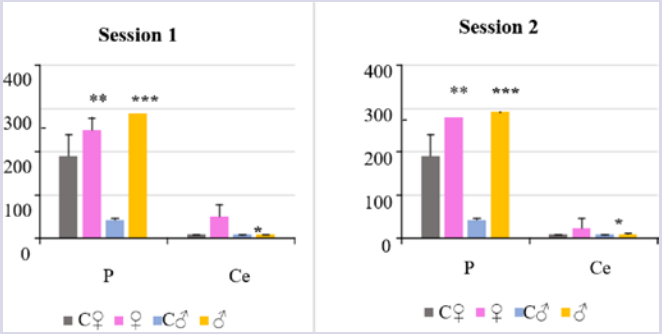

Figure 2: The open field parameters of rats of the 04 groups (n=24). Expressed as Mean ± SEM. C: Controls, P: Periphery and Ce: Centre. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

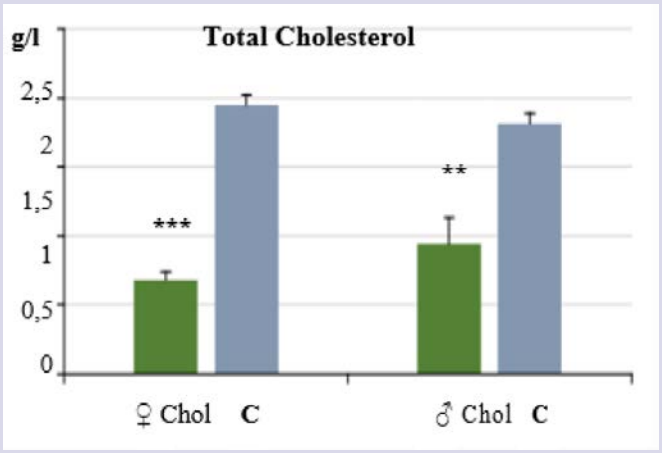

Figure 3: Total cholesterol changes in rats of the 04 groups (n=24). Expressed as Mean ± SEM. C: Controls. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

The time spent in the center of the arena was greater for females than for males (Figure 2).

The total cholesterol level (g/l) measured on the sixth day of the experiment was very low in female rats, and highly elevated in male rats (Figure 3).

DISCUSSION

Stress causes complex physiological and behavioural changes in the body, which adjust homeostasis.[21,22] Changes in physiological and behavioural responses to chronic stress may be related to adaptations of the HPA axis. In rodents, these adaptations have been monitored, mainly by measuring corticosterone levels in response to stress.[23-25]

Life stress and genetic predisposition are major contributors to the development of psychiatric illnesses, including major depression and anxiety.[26,27] Anxiety is often comorbid with other psychiatric disorders, particularly depression. Gender differences in the clinical manifestations, morbidity and prevalence of these psychiatric disorders are well established.[28] In rodents, anxiety can be defined by a quantification of exploratory behaviour, defecation and urination.[29] Indeed, the different tests used are based on the ability of the rat to explore a new field

(usually aversive situation) and to stay in a protected field (usually safe situation) to escape a predator. Studies on anxiety behaviour use different paradigms (protocols) including open field testing,[30] and elevated cross maze "elevated Plus maze".[31]

Our study reveals that Wistar rats undergoing restraint stress show disturbances, including an increase in anxiety represented by a high amount of time spent in closed arms compared to open arms of the test system, and in the centre, both sexes.

Open field activity is the oldest and simplest measure of anxiety behavior in rodents.[32,33] The open field test is used to assess the effects of environmental manipulations or genetic factors on rodent anxiety.[34] The device used in our study is known to present a natural conflict for the rodent to actively explore a novel environment in order to establish cues to form a representation of its environment, and its difficulty in venturing away from a wall that offers relative safety from predators.[35] As a result, anxious rats tend to spend more time in the corners and periphery of the apparatus rather than in the center.[36]

From these studies and our results, it was noted that restraint-stressed rats spent more time near the walls than in the central arena, indicating increased anxiety levels in the latter.

Activation of the sympathetic nervous system during acute or episodic stressors could lead to a cascade of events, beginning with increased catecholamine and cortisol release, elevated blood pressure (BP) and altered lipase activity, to alter blood lipid concentrations producing increased lipid energy mobilisation and higher metabolic demand, potentially leading to altered sensitivity to long-term stressors.[37]

The use of cholesterol as a essential substance for the synthesis of glucocorticoids, thus causing a decrease in cholesterol levels.[38] Furthermore, studies suggest that these stress hormones can influence the lipid profile. Ronsein *et al.* (2004)[39] suggested that stressed individuals have increased TC and low-density lipoprotein (LDL) and decreased HDL level.

A relationship between stress level and lipid profile disorders. demonstrated by Shahnam and colleagues in 2010[40]As well as a decrease in cholesterol and the development of mental status disorder has been suggested by Nagaraja and his team in 1999.[41]

Mossner and colleagues in 2007[42] indicate that the biochemical profile of depression is often characterized by low blood cholesterol levels and elevated triglycerides.

These studies and our results show a significant decrease in cholesterol levels in both male and female rats that were subjected to chronic restraint stress, indicating increased levels of anxiety in these rats.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ACTH: Adrenocorticotropin; **BP:** Blood pressure; **CRH:** Corticotrophin hormone; **HDL:** Low-density lipoprotein; **HPA:** Hypothalamic-pituitary-adrenal; **LDL:** Low-density lipoprotein.

SUMMARY

A remarkable decrease in cholesterol levels in both male and female rats that were subjected to chronic restraint stress, indicating increased levels of anxiety in these rats.

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