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Chronic Restraint Stress Induced Neurobehavioral Alterations and Histological Changes in Rat

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Abstract

Several lines of research on human and rodent subjects have demonstrated that stress results in multiple negative outcomes, including increased incidence of psychopathologies. Restraint stress in rats is known to adversely affect the physiological, psychological and reproductive axis in rats. Male rats were subjected to restraint stress for 3 hours consecutively for 14 days. The behavioral studies include Elevated Place Maze, Open Field and Morris Water Maze tests. Our results show that chronic restraint stress involved a development of anxiety in EPM, reduced motor activity in OF, impaired memory spatial in MWM tests, and induced change in testicular function, as reflected by significant decrease in plasma level of testosterone, correlate well with the damages in testis. The Results of the present study confirm that chronic restraint stress induced cognitive dysfunction, enhance anxiety like behavior and induced testicular damage in male rats Wistar.

Keywords: Chronic restraint stress, Anxiety, Testis, Behavior, Memory, Rat

Introduction

Stress is a condition of highly individualized response of an organism to external and internal. Challenges which one can control with difficulties or can't control. It is one of the important factor acting upon a large human population in the entire country. It induces the strain upon both emotional and physical endurance which has been considered the basic factor in the etiology of a number of psychological and physicaldisorders[1,2]. Immobilization/restraint stress is believed to be the most severe type of stress in rodent models and has a comparative effect in humans; this type of stress was used in the present study. In response to stressors, a series of behavioral, neurochemical, and immunological changes occur that ought to serve in an adaptive capacity[3,4].

The hypothalamic-pituitary-adrenal (HPA) axis is a key endocrine adaptor against stressors and plays an important role in the pathophysiology of stress-related psychiatric diseases such as depression and anxiety disorders[5]. Hyperactivity of HPA axis induced important secretion of catecholamine and glucocorticoid which are believed to underlie the onset of many physiological changes, among these: the psychiatric disorders. Exposure to chronic restraint stress in rats has been shown to alter cognitive functions such as learning and memory and impaired performance in Morris water maze test[6,7] and alter some behavioral parameters in mice as anxiety like disorders[8]. On the other hand, some studies were demonstrated that reproductive functions are suppressed under various stress conditions[9].

This study was undertaken to investigate the effects of chronic restraint stress in neurobehavioral and reproductive axis in male Wistar rat.

Results

Physical Development

Application of restraint stress during 2 weeks significantly decreased the body weight in 2nd Week that 1st Week (Stressed 1st week 220.29 ± 7.93 vs Stressed 2nd week: 199.43 ± 13.95, $p \leq 0.01$) which were increased significantly in control groups (Control 1st week: 305.86 ± 11.63 vs Control 2nd week: 321.43 ± 9.31, $p \leq 0.01$) (Figure 1).

Anxiety-like Behavioral Development

The statistical analysis of results revealed a very sig-

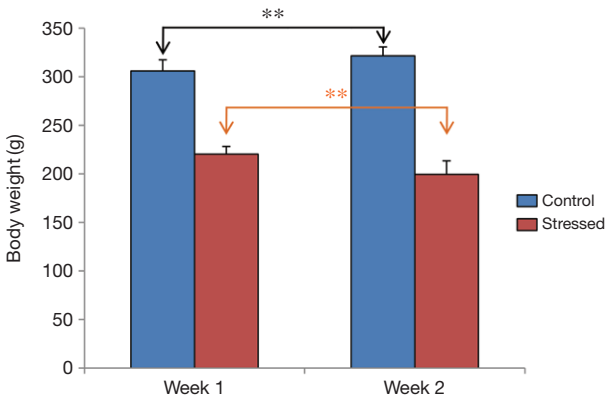

Figure 1. Evolution of body weight in control and stressed rats for 2 weeks (**$p < 0.01$).

Table 1. Changes in the parameters of the PM test following controls and stressed groups.

Parameters/batches	Control	Stressed
Time spent in center (sec)	23.00 ± 15.61	29.20 ± 30.49 Ns
Time spent in open arms (sec)	80.00 ± 29.52	21.00 ± 13.87**
Time spent in closed arms (sec)	190.75 ± 29.36	249.80 ± 39.77**
Number of entries in open arms	2.25 ± 1.16	1.00 ± 0.00*
Number of entries in closed arms	2.50 ± 1.06	1.40 ± 0.54*

Indicate a significant difference between the 2 groups ($p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

nificant decrease ($p \leq 0.01$) of the time spent in the open arms of the dispositive in the stressed rats compared to the control rats and consequently a very significant increase ($p \leq 0.01$) of the time spent in the closed arms in the stressed rats compared to the control rats. Concerning the number of entries in open arms and closed arms, we observed that there are a significant decrease ($p \leq 0.05$) in stressed rats compared to control rats (Table 1).

Locomotion-like Behavioral Development

The results Shows a highly significant decrease of the distance travelled in stressed rats (control: 920.6 ± 511.1 vs stressed: 310.0 ± 200.2) ($p \leq 0.01$) and significant increase of the immobility time (Control: 117.72 ± 33.2 vs Stressed: 256.40 ± 29.45) ($p \leq 0.001$). No difference significant in the number of entries in center (Figure 2).

Cognitive Development

The results of Figure 4 shows that the spatial memory of stressed rats was impaired in memory task but not in learning task because the latency time was increased in 4th day of test compared to control rats that the latency time was decreased (Figure 4).

Histological Parameter

The testis of controls groups showed a normal structure and seminiferous tubules were replete with germ cells at different stages of spermatogenesis and abundant spermatozoa (Figure 3A), whereas the seminiferous tubules of stressed rats (Figure 3B) was shrunken with vacuolization in the seminiferous epithelium (*) and contained fewer spermatozoa (flesh) compared to control group.

Assessment of Testosterone Levels (ng/mL)

The application of restraint stress in male Wistar rats during 14 days, results a highly significant ($p \leq 0.01$ (decrease in the testosterone level (stressed: 0.5748 ± 0.2729 vs control: 1.0260 ± 0.1488) compared to the control group (Table 2). This results confirm with the histological study the damages induced by restraint stress in testis.

Discussion and Conclusion

Stress has been associated with onset or precipitation of mood-related disorders[10,11]. Numerous studies have been carried out to understand the role of various lifestyle factors contributing to stress and the development of the anxiety-like behavior[12]. Anxiety is one of the major mental disorders affecting a large number of the population, which disturbs normal physiological equilibrium of the body by producing adverse effects on the nervous, endocrine, biochemical and immune systems[13]. Exposure to acute and repeated restraint stress was reported to induce anxiety like behavior[8,12,14-16]. However, the investigating of many conditions of stress intensity depending period may lead to understand more the responses associated anxiety and whether this reaction could persist later.

According to our results, stressed rats displayed a significant increase in anxiety-like behavior compared to control group in EPM test. Following exposure to 3 h/day for 14 days of restraint stress, rats spent relatively less time in the open arms and more time in the closed arms of the EPM. In addition, the reduction in the traveled distance and the increase of immobility time of stressed rats in OF test represents a decrease in the exploratory activity. In our experiment, anxiety symptoms continue even within the 14 days after stopping the application of restraint stress, suggesting the presence of alteration on neurotransmission and neuronal

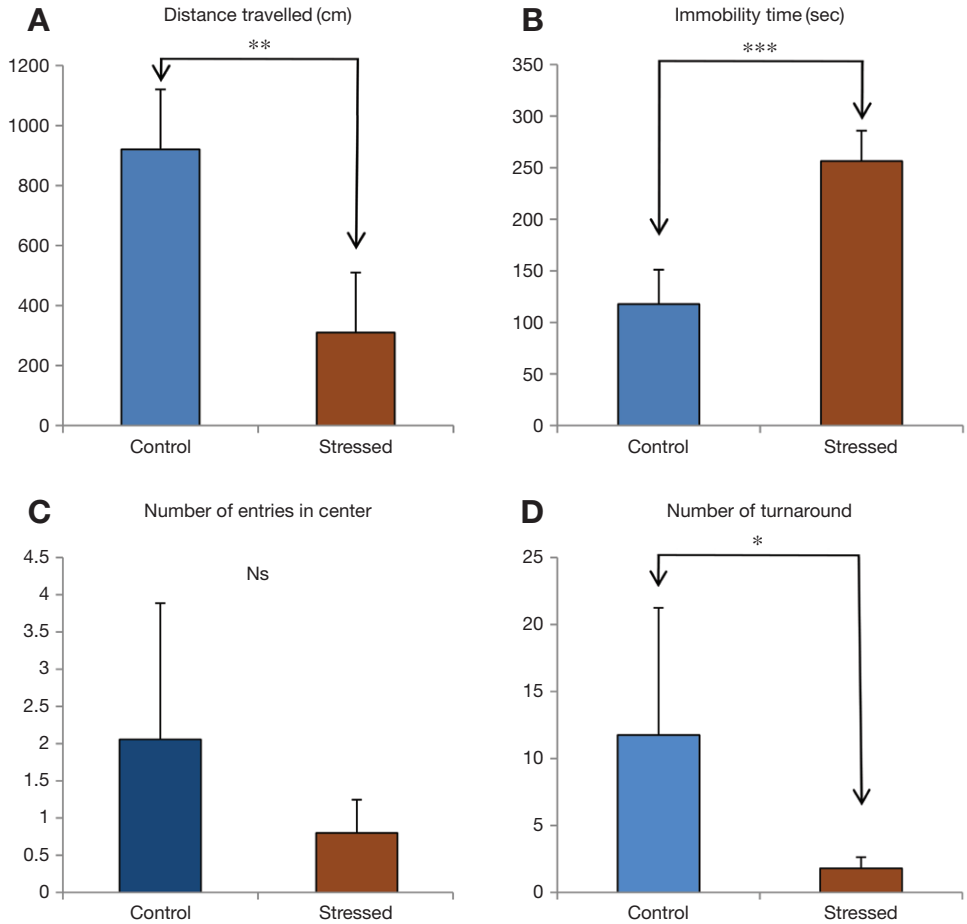

Figure 2. Changes in the parameters of the OF test following controls and stressed groups. A. Travelled distance (cm), B. Immobility time (sec), C. Number of entries in center, D. Number of turnaround (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

plasticity of brain.

The same observation for the reduction of body weight which may refer to the decrease in food intake and the consumption of organism reserve. Many mechanisms of actions were established to explain stress related anxiety such as oxidative stress, release of glucocorticoids and alteration in Gabaergic and serotonin system[17].

Stress seems to be a potential risk factor for reproductive function. Reduced male fertility is one of the known consequences of psychological stress[18]. In males, physical and psychological stressors may inhibit reproductive function mainly through the suppression of hypothalamus-pituitary-gonadal (HPG) axis and activation of hypothalamus-pituitary-adrenal (HPA) axis[19]. Many studies have shown decrease in sperm production, sperm count and motility of spermatozoa, increase in percentage of morphologically abnormal spermatozoa, impaired spermatogenesis, decrease in levels of serum testosterone and LH levels[20,21].

In the present study, chronic exposure to restraint stress induced changes in testicular function, as reflected by significant decrease in plasma level of testosterone, correlate well with histological damage in semenifereus tubules (necrosis in germinal cells).

In accordance with our study there are other reports indicating that immobilization/restraint stress reduces serum levels of testosterone and LH[13,22-25]. Other studies also have found that immobilization stress alters testes function and reduced spermatogenesis[23,26].

Various hypotheses have been proposed to explain the fall in serum testosterone level following immobilization stress. Mayfield (1980)[27] explained that neuroendocrine effect of stress is mediated by hypothalamus. Corticotrophin releasing hormone (CRH) neurons present in hypothalamus summate a large variety of neuronal and hormonal signals which arise in various regions of nervous system and eventually this specific hypothalamic neurohormone either stimulate or inhibit the hypophysal activity in response to stress. Knol

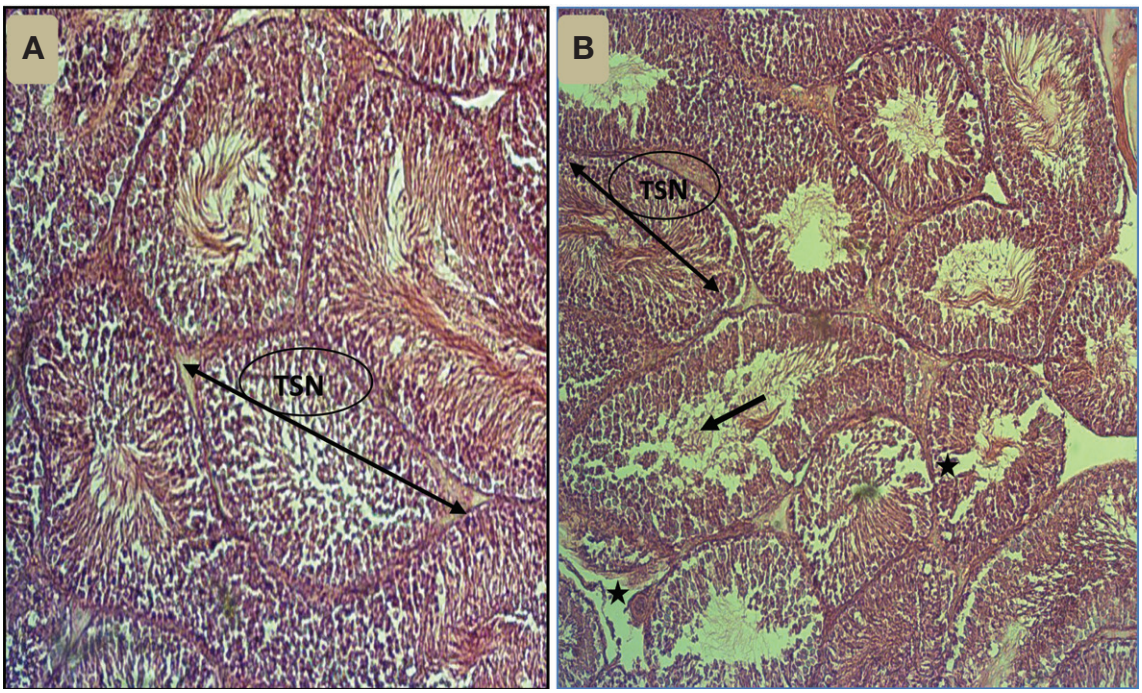

Figure 3. Histological appearance of testis sections obtained from control group (A) and stressed group (B) in male rat Wistar (H&E, ×100).

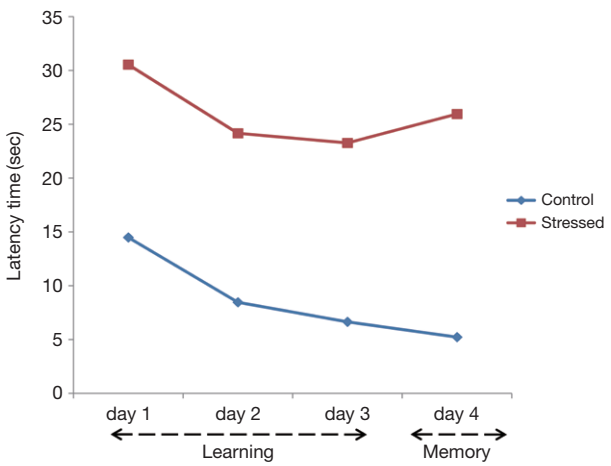

Figure 4. Chang of latency time in MWM test in control and stressed rats.

(1991)[28] proposed that stressors generally induce depression of hypothalamo-pituitary-testis system, resulting in fall in plasma LH and testosterone levels. CRH induces the release of endo genous opioids from hypothalamus, which along with corticosteroids suppresses the secretion of hypothalamic gonadotrophin releasing hormone (GNRH). Suppression in secretion of GNRH causes reduced secretion of LH & FSH

Table 2. Change in testosterone level in control and stressed groups.

Parameters/batches	Control	Stressed
Testosterone (ng/mL)	1.0260 ± 0.1488	0.5748 ± 0.2729**

*Indicate a significant difference between the 2 groups (**$p < 0.01$).

from pituitary, which in turn causes decrease in testosterone level and spermatogenesis. Orr *et al.* (1990)[22] found that restraint stress causes increase in plasma level of glucocorticoids,decrease in testosterone level without any effect on LH level. He suggested that increase in plasma level of glucocorticoids act via glucocorticoid receptors on testicular interstitial cells to suppress the testicular response to gonadotropins. Mc Givern and Reddi (1994)[29] supported the primary role of glucocorticoids in stress-induced inhibition of reproductive function in rats.

Numerous research reports have shown that stress exposure has a complex effect on learning and memory[30,31]. However, the literature in this area lacks consistency, with studies reporting that stress can enhance, impair, or have no effect on learning and memory[32,33]. Emotional and stressful experiences, via the activation of specific hormonal and brain systems, alter brain function and regulate memory storage. The response to

stress involves the activation of glucocorticoids. These notably bind to mineralcorticoid and glucocorticoid receptors (MRs and GRs) in the hippocampus[34,35]. For example, a high dose of corticosterone or stress prior to training or testing resulted in impaired spatial performance and memory[36-38]. Similarly, the removal of endogenous corticosterone (by adrenalectomy or GR antagonist) impaired performance in a spatial task and fear conditioning[39-41]. It has been reported that Twenty one days of restraint stress impaired spatial performance of male but not female rats[42].

The results of our work showed that chronic restraint stress impaired spatial memory but not learning in MWM test that reveled in the increased of latency time in memory task and the reduction in learning task compared to control groups that the latency time was decreased in learning and memory tasks.

In conclusion, chronic restraint stress produces inescapable physiological and mental disorders, in our study there is a significant alteration in neurobehavioral and reproductive axis. It was affected the locomotors activity, altered behavioral and cognitive functions (anxiety and memory) and induced testicular damages in male Wistar rats.

Materials and Methods

Animals

20 male Wistar rats obtained from Pasteur Institute (Algiers, Algeria) were housed in transparent cages at a constant temperature (23 + 1°C) with a 12 h/12 h light/dark cycle. Rats had access to standard rodents chow and tap water *ad libitum*. The rats were divided into two groups. Control group (n = 10) and stressed groups (n = 10).

Chronic restraint stress protocol: Male rats Wistar were subjected to restraint stress in a plastic cylinder 3 h/day (8:00 am to 11:00 am) for 14 consecutive days.

Behavioral test: On the 15th day, rats were tested in an EPM and OF for 5 min.

Open Field Test (OF): The open field can be considered as a non conditioned anxiety test based on the creation of conflict between the exploratory drive of the rat and its innate fear of exposure to an open area[43]. The 0F test was performed to measure changes in exploratory behavior and emotionality. Briefly, the apparatus, as previously described[44] consist of a gray square (70 cm × 70 cm × 40 cm) divided into 16 equal squares that had been drawn in the floor of the arena. Each rat was placed in the arena individually and allowed for freely explore it for 5 min. upon completing the task, the rat was removed from the arena by the experimenter and returned to the home cage. After each test, the apparatus was cleaned with an alcoholic solution followed by wet and dry paper towels to avoid transfer of olfactory cues between animals.

Elevated Place Maze Test (EPM): The elevated place maze test is a widely used paradigm to investigate anxiety-related behavior in rats[45]. PM was made of pained wood cross (arms 50 cm long × 10 cm wide) elevated by walls (10 cm × 50 cm × 45 cm high) and two arms were open. The arms extended from a central platform (10 × 10 cm)[46]. The open arms in the maze that we use to not have a railing. But addition of a 3-5 mm high railing on the open arms of the place maze has been used with success to increase open arm exploration. The rat was placed in the center of the apparatus facing one of the open arms, for a free exploration of 5 min. entry into an arm was defined as the animal placing all four paws on the arm. After each test, the rat was returned to its home cage and maze was cleaned with an alcoholic solution followed by wet and dry paper towels.

Morris Water Maze Test (MWM): The Morris Water Maze test is used to identify and evaluate rodent spatial learning and memory[47]. It's a circular pool (120 cm in diameter and 0.47 and 60 cm deep) made of polypropylene and installed on a support. This is divided into four quadrants; One of them has a slightly submerged platform; 1 cm below the surface of the water (target quadrant 3). It is filled to a depth with 30 cm with water (22°C-32°C). The MWM consists of 4 test/days with 5 passages per day (3 days with learning platform) and in the 4th day 2 passages with platform (learning) and 3 without platform (memorization). The rat is deposited in the water at the periphery from different places; he's swims to find the platform and then is removed from the water. The test is redone with only a passage of 60 seconds If the rat does not find the platform after 60 seconds, The passage is completed and the experimenter places the animal on the platform for 10 seconds The platform is removed on the 4th day of the test and the test lasts 60 seconds. All the tasks are filmed and we calculate the latency time (The time spent by the rat to find the hidden platform.

Assay of Testosterone

The taking away is done at the end of 14th days of chronic restraint stress. The blood samples are collected in the heparinized tubes then centrifuged at 1500 rpm for 10 minutes. Plasma was used for progesterone assay. The testosterone is proportioned by the conventional ELISA method[48]. Measurement is done using a reader ELISA TECAN Magellan provided with data-processing software which calculates the range standard automatically and the value of the progesterone to the desired unit gives us directly.

Statistical Analysis

MINITAB (Minitab® 13.31) was used for statistical analysis. All values are presented as mean ± S.E.M. Statistical significance was evaluated by one-way analysis of variance (ANOVA) test. Significance was measured using Fisher's least significant for the exact P values and significant differences are noted in the results. The difference between groups was considered significant when $\alpha < 0.05$.

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