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THE INVOLVEMENT OF AMYGDALA NUCLEUS IN CHRONIC STRESS INDUCED CHANGES ON INGESTIVE BEHAVIOR OF WISTAR RATS

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ABSTRACT: Organisms are constantly subjected to stimuli that can be construed as stressors. Stress regulation is a highly integrated process controlled largely by the brain. The role of amygdala in stress tolerance has attracted continued interest because of its central role in processing emotional information. In the present study, the Wistar albino rats were subjected to chronic swimming (physical) stress and chronic immobilization (psychological) stress for 60 days with or without bilateral lesions of the nucleus of amygdala. Their food intake, water intake and body weight were measured. Exposure to stressors significantly decreased the body weight, food and water intake whereas amygdala lesioning significantly increased the body weight ($P<0.001$), food intake ($P<0.001$) and water intake ($P<0.001$).However, the stress induced decrease observed in the body weight and food intake of the amygdala lesioned groups was significantly more ($P<0.001$) during immobilization stress than swimming stress. It can be concluded that amygdala nucleus appears to play a prominent role in minimizing the stress induced changes in the food intake and body weight; and this role was more evident in immobilization stress than a physical stress. Thus, the present study support the notion that the amygdala nucleus play a definite role in minimizing stress induced changes in the ingestive behavior and its role in psychological stress is more prominent.

Key words: immobilization, swimming, amygdala, physical stress, psychological stress,

INTRODUCTION

In this modern world, stress is an unavoidable phenomenon. Stressful situations can lead to many physiological and psychological alterations. In terms of duration, stressors may be divided into two main categories as acute (single, intermittent and time limited exposure) and chronic (repeated and prolonged continuous-exposure) stressors. The duration and the frequency of the stress period are important determinants for the induction of the cascade of stress - triggered neurobiological processes [1].

In mammals, the circuit most closely linked with the stress response is the limbic-hypothalamic-pituitary-adrenal system. Several forebrain structures are known to influence stress responses and serve as transducers of glucocorticoid action in the brain [2]. Among them, the amygdala has attracted continued interest because of its central role in processing emotional information [3]. Like most parts of the brain, the amygdala is heterogeneous in both the structure and functions. Numerous lesion studies have shown the importance of the amygdala and its involvement in autonomic responses to stress [4, 5].

The amygdala is known to prompt cardiovascular and behavioral responses to stress [6]. The amygdala is involved in emotional responses especially in the case of fear and fear conditioning. These responses help in the release of stress hormones, and changes in blood pressure and heart rate are observed following activation of the autonomic and hormonal system.

How does the brain deal with stressors? The question has remained unanswered to this date. If the brain does deal with stressors categorically such as physical and psychological stress, one might predict that stressors should elicit category- specific patterns of neuronal activity in the brain. It is still a matter of debate that whether the brain recognizes two major categories of stressor, which were referred as 'physical' and 'psychological' based on the type of nucleus which is activated. A direct study based on the comparison of neuronal recruitment patterns elicited by different stressors from different categories would clearly be more reliable. However, only a few groups have tried to address this issue [7, 8, 9]. The present study was designed to elucidate the possible role of amygdala nucleus on stress by evaluating its role in physical & psychological stress and ingestive behavior separately and combinely.

MATERIALS AND METHODS

All procedures in this present study were performed in accordance with the guidelines established by the Institutional Animal Ethics Committee and of the Society for Neuroscience Policy on the Use of Animals in Research. Adult albino rats (150 to 250 g) of Wistar strain were used in the present study. The rats were procured from the central animal breeding center at our university. Animals were housed individually in polypropylene cages (29cms x 22cms x 14cms) during the experimental period at 28±2°C temperature and 50±5% humidity. The rats were maintained under standard laboratory conditions with 12h light: 12h dark cycle. Animals were fed on laboratory chow (Gold Mohur; Lipton India, Ltd) and tap water in drinking bottles were made available *ad libitum* .After 1 week acclimation to vivarium conditions, during which time they were handled extensively, rats were subjected to bilateral and sham bilateral lesions of amygdala nucleus and then allowed to recover for 1 week before undergoing the stress procedures. Rats were anesthetized (Pentobarbitone sodium, 40 mg/kg, i. p.) and then sacrificed by giving the lethal dose of Pentobarbitone sodium .The amygdala lesion was performed according to the stereotaxic coordinates prescribed in the Paxinos and Watson rat stereotaxic atlas [10]. The coordinates were as follows: anteroposterior (AP) = -2.8 mm posterior to bregma, lateral (L) = 4.8 mm from midline, vertical (V) = 7.8 mm from the surface of the skull. The lesion was produced using stainless steel electrode (gauge 22) which was insulated except for 0.5 mm at the tip. An anodal DC (direct) current of 2 mA was passed for 20 sec to produce the lesion. The cathode was connected to the tail. The procedure was repeated on either side to produce bilateral lesions. The surgical procedures performed in the sham lesioned control group were the same, except for the passing of the DC.

The animals were divided into two major experimental groups apart from a normal control group. The experimental groups were sham lesioned groups and lesioned groups. Each experimental group was further divided into three subgroups as experimental control, two experimental stress groups. Each subgroup contains ten animals.

Normal Control (NC) - This subgroup of normal rats was not subjected to any kind of stress

Amygdala sham lesioned control (ASL-C)

This group of rats received the same surgical procedure for lesioning of amygdala as mentioned above except for passing of the DC (direct) current and these animals were not subjected to any kind of stress.

Amygdala sham-lesioned chronic swimming stress (ASL-SS)

This group of rats was subjected to sham lesion at amygdaloid nucleus and underwent chronic swimming stress daily for sixty days.

Amygdala sham-lesioned chronic immobilization stress (ASL-IS)

This group of rats was sham lesioned at amygdaloid nucleus and underwent chronic immobilization stress daily for sixty days.

Amygdala lesioned control (AL-C)

This subgroup of amygdala lesioned rats was not subjected to experimental stress.

Amygdala lesioned chronic swimming stress (AL-SS)

This subgroup of rats was lesioned at amygdala nucleus and subjected to chronic swimming stress for sixty days.

Amygdala lesioned chronic immobilization stress (AL-IS)

This subgroup of rats were lesioned at amygdala nucleus were subjected to chronic immobilization stress one hour per day for sixty days.

CHRONIC STRESS PROCEDURE:

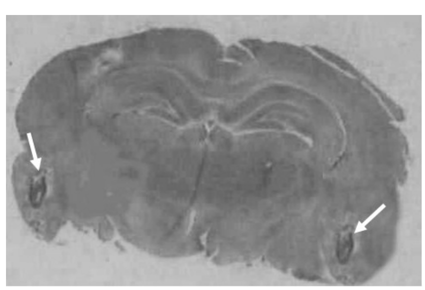
Chronic Immobilization stress: The immobilization chambers used in this study were plastic tubes of varying sizes to accommodate all sizes of rats (15cms long and 4cms diameter, 16cms long and 5cms diameter, 17cms long with 6cms diameter). The tubes had a conical head at one end. The conical head area contained numerous perforations which served as breathing holes. The rat was placed inside the tube with head in the conical end. The rats were totally restrained by packing the rear end of the tube and closing it firmly with a stopper. Rats were exposed to chronic stress in the form of immobilization for 2 hour per day for a period of 60 days.

Chronic swimming stress: The rats were allowed to swim in the plastic tubs containing tap water maintained at room temperature. The water level in the plastic tub was always kept at 30cms from the bottom. Rats were forced to swim in this tub until exhaustion. The point at which the animals became unable to stay at surface and showed signs of sinking was considered to be the point of exhaustion. After the stress session, the rats were towel dried and then placed back in their respective cages where water and food were available ad libitum. Animals were subjected to forced swimming daily for 60 days. All the experiments were done between 10AM to 12 Noon to minimize circadian variability.

HISTOLOGICAL ANALYSIS

On completion of the experimental procedures, all the rats were sacrificed and their brains, after transcardial formol saline injection, were dissected and processed for histological study. Serial sections 5µ thickness were taken and stained with Haematoxylin and eosin (H&E) [11] (Fig. 1). These sections were examined under dissection microscope to confirm the lesion and the lesion sites were again magnified under power 10X for a detailed view.

Figure-1



STATISTICAL ANALYSIS

The datas were summarized using mean ± SEM or median and interquartile range depending on the skewness [12]. For normally distributed data one way ANOVA was used and Kruskal Wallis test was used for skewed data. This was followed by multiple comparison tests for significant F value in ANOVA. The data of pre and post lesion was analyzed using two way ANOVA followed by post hoc tests in case of significant F value of ANOVA. P<0.05 were considered as statistically significant.

RESULTS

The normal control group (NC) did not show any significant changes in the body weight, food intake & water intake when compared to amygdala sham-lesioned control group (ASL-C)

Body weight (Table 1): Amygdala lesioning (AL-C) significantly increased (P < 0.001) the body weight when compared to amygdala sham lesioned control group (ASL-C). A significant decrease (P < 0.05, P < 0.001) in the body weight was observed in all the stress groups (ASL-SS, AL-SS, ASL-IS and AL-IS) after 60 days of exposure to swimming stress and immobilization stress. The decrease in the body weight was more significant (P < 0.001) in the amygdala lesioned stress groups (AL-SS, AL-IS) when compared to respective amygdala sham lesioned stress groups (ASL-SS, ASL-IS). Exposure to immobilization stress (AL-IS) in the amygdala lesioned group showed a significant decrease (P < 0.001) in the body weight when compared to exposure to swimming stress (AL-SS).

Table 1: Swimming stress and Immobilization stress induced changes on body weight (BW) changes in amygdala lesioned rats when compared to amygdala sham lesioned rats. Values are expressed as mean ± SEM; grams (g).

Groups	Day-One	Day –Sixty
NC (n=10)	148.67± 0.66	160 ± 0.56^{NS}
ASL-C (n=10)	149.33± 0.66	163 ± 0.68*
AL-C (n=10)	149.30± 0.42	190.33± 1.20 **‡ ‡
ASL-SS (n=10)	151.67± 0.65	140.67± 0.42*
AL-SS (n=10)	141.17± 1.62	126.33± 2.02 **¶ ¶
ASL-IS (n=10)	158.33± 0.94	127.67± 9.80*
AL-IS (n=10)	158.67± 2.01	76.33± 2.02 **§§#

n= number of rats
NC versus ASL-C – Non significant (NS)
* P < 0.05; ** P < 0.001; compared between Day- One and Day-Sixty in each individual group (ASL-C, AL-C, ASL-SS, ASL-IS, AL-SS, AL-IS)
‡ ‡ P < 0.001; ASL-C versus AL-C
¶¶ P < 0.001; ASL-SS versus AL-SS
§§ P < 0.001; ASL-IS versus AL-IS
# P< 0.05; AL-SS versus AL-IS

Food intake (Table 2): Amygdala lesioning (AL-C) significantly increased (P < 0.001) the food intake when compared to amygdala sham lesioned control group (ASL-C). Exposure to stressors in amygdala lesioned groups significantly decreased (AL-SS; P < 0.05, AL-IS; P < 0.001) the food intake. Chronic immobilization stress in the amygdala lesioned group (AL-IS) showed a significant decrease (P < 0.001) in the food intake when compared to its respective sham lesioned group (ASL-IS) and amygdala lesioned swimming stress group (AL-SS).

Table 2: Swimming stress and Immobilization stress induced changes on food intake changes in amygdala lesioned rats when compared to amygdala sham lesioned rats. Values are expressed as mean ± SEM; g / 100 g of BW.

Groups	Day-One	Day –Sixty
NC (n=10)	8.60± 0.18	8.20± 0.2 NS
ASL-C (n=10)	8.67± 0.21	8.17± 0.4
AL-C (n=10)	8.60± 0.59	26.50 ± 0.71 ** ‡‡
ASL-SS (n=10)	9.17± 0.30	9.67± 0.59
AL-SS (n=10)	8.87 ± 0.21	4.67± 0.4 *
ASL-IS (n=10)	8.83± 0.3	8.80 ± 0.4
AL-IS (n=10)	8.0± 0.54	2.0± 0.83 ** §§# #

n= number of rats in each group

NC versus ASL-C – Non significant (NS)

* P < 0.05; ** P < 0.001; compared between Day- One and Day-Sixty in each individual group (AL-C, AL-SS, AL-IS)

‡ ‡ P < 0.001; ASL-C versus AL-C

§§ P < 0.001; ASL-IS versus AL-IS

P< 0.001; AL-SS versus AL-IS

Water intake (Table 3): Amygdala lesioning (AL-C) significantly increased (P < 0.001) the water intake when compared to amygdala sham lesioned control group (ASL-C). Exposure to chronic stressors significantly decreased the water intake (ASL-SS; P < 0.05, AL-SS; P < 0.05, AL-IS; P < 0.001). Chronic immobilization stress (AL-IS) in the amygdala lesioned group showed a significant decrease (P < 0.001) in the water intake when compared to its respective sham lesioned group (ASL-IS) and swimming stress group (AL-SS)

Table 3: Swimming stress and Immobilization stress induced changes on water intake changes in amygdala lesioned rats when compared to amygdala sham lesioned rats. Values are expressed as mean ± SEM; ml / 100 g of BW.

Groups	Day-One	Day –Sixty
NC (n=10)	20.0± 0.77	21.0± 0.83 NS
ASL-C (n=10)	21.0± 0.77	21.0± 0.83
AL-C (n=10)	22.33± 0.55	32.33 ± 0.33 ** ‡‡
ASL-SS (n=10)	22.33± 0.5	16.66± 0.42*
AL-SS (n=10)	22.33 ± 0.42	16.33± 1.08 *
ASL-IS (n=10)	22.33± 0.50	24.66 ± 0.42
AL-IS (n=10)	22.23± 0.42	11.67± 0.42** §§ ##

n= number of rats

NC versus ASL-C – Non significant (NS)

* P < 0.05; ** P < 0.001; compared between Day- One and Day-Sixty in each individual group (AL-C, ASL-SS, AL-SS, AL-IS)

‡ ‡ P < 0.001; ASL-C versus AL-C

§§ P < 0.001; ASL-IS versus AL-IS

P< 0.001; AL-SS versus AL-IS

DISCUSSION

Stress responses, an integral part of an adaptive biological system, and are initially developed as adaptive responses that enable the organisms to respond to environmental challenges. When it becomes excess, it can lead to pathological responses. Even though, the entire CNS is involved in the maintenance of internal homeostasis and participates in the reorganization of stress response some areas may have specific role in the regulatory mechanism [13]. Limbic dysfunction and hypothalamo – pituitary – adrenocortical [HPA] axis dysfunction could be the key features of affective disorder[14]. Studies have also showed that amygdala nucleus is the "window of limbic system" plays an important role in the organization of stress response [15, 16, 17].

Ingestive behavior and body weight are the variables sensitive to stress These particular variables are interesting in stress research not only because of the impact of food on growth and health but also because it can be measured with minimal disturbances to animal [18]. Stress pathway may involve and consolidate the ingestive behavior of an individual.

In the present study, no significant changes were observed in body weight, food intake, and water intake in between the normal control group and amygdala sham control group ·This finding confirms that surgical procedure did not affect the studied parameters of the present study. Further, lesioning of amygdala increased the food intake, water intake and body weight suggest that amygdala nucleus have an important role in the regulation of food intake, water intake and body weight. The involvement of amygdaloid centers in ingestive behavior has been confirmed by many researchers [19,20,21,22]. The amygdala nucleus probably 'modulates' the more basic regulation of ingestive behavior by hypothalamic sites through fibre system (stria terminalis) from the hypothalamus [23, 24, 25] The result of the present study also supports the above findings and suggests that the modulatory effect of Amygdala may be by its inhibitory influence on ingestive behavior.

In the present stress experiment, the effects of chronic stress were studied by using two different stressors namely swimming which is more of a physical stress and immobilization stress which is a psychological type of stress.

In this study, the exposure to swimming stress and immobilization stress showed no significant changes in the food intake in the sham lesioned stress (experimental control) groups though the body weight decreased. Stress is known to increase protein catabolism [26, 27]. Therefore, rats subjected to chronic stress continued to lose weight despite of normal food intake. However, in contrast to our results stress induced alterations in the food intake were also observed in some other studies [28, 29].

But with amygdaloid lesion, though exposure to both type of stresses showed a significant decrease in the food & water intake and bodyweight; the stress induced changes was significantly more during the exposure to immobilization stress than the swimming stress. This showed that amygdala nucleus appears to play an important role in minimizing the stress induced changes in these ingestive behaviors during an exposure to the psychological type of stress rather than a physical type of stress like swimming.

The results of the present study suggest that the amygdala nucleus has a definite role in regulating the stress induced changes in the ingestive behavior. Further, it emphasizes that the role of amygdala in the stress is more evident in psychological type of stress. Thus, the data of the present study support this hypothesis that the brain recognizes at least two major categories of stressor, which has been referred to as 'physical' and 'psychological'. There might be the presence of an intricate web of reciprocal independent connections of amygdala nucleus to the brain areas regulating these responses designed for the psychological stress. However, the precise role of these nuclei and their interaction among themselves and other brain areas in regulating homeostasis warrants further study.

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