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CHRONIC STRESS INDUCED CHANGES ON INGESTIVE BEHAVIOR IN PARAVENTRICULAR NUCLUES LESIONED WISTAR RATS

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ABSTRACT

The paraventricular nucleus of hypothalamus (PVN) is considered as a focal point in regulating stress responses. The idea that the brain categorizes stressors and uses response pathways that vary according to the category has gained significant support in the recent years. The present study was designed to elucidate the possible role of PVN nucleus on stress by evaluating its role in chronic physical & chronic psychological stress on some selective physiological parameters which depicts the ingestive behavior. Wistar albino rats were subjected to chronic swimming (physical) and chronic immobilization (psychological) stress for 60 days with or without bilateral lesions of nucleus of PVN. Food intake, water intake and body weight were measured. Exposure of stressors showed significant alterations in the body weight, food intake and water intake. Stress induced changes observed PVN lesioned rat was significantly more during ($P<0.001$) during swimming stress than immobilization stress. The data of the present study support the hypothesis that the brain recognizes at least two major categories of stressor, which has been referred to as physical and psychological. Further, our study also provides the supportive evidence that the response of a physical stress to a greater extent is regulated by paraventricular nucleus of the hypothalamus.

Keywords: Paraventricular nucleus, chronic stress, ingestive behavior, physical stress

Number of Figures: 2 **Number of Tables: 3** **Number of References:47**

INTRODUCTION

The paraventricular nucleus (PVN) of hypothalamus is an important area which plays a key role in the regulation of the HPA (hypothalamic-pituitary-adrenal) axis activation and is considered as a focal point in the complex interacting systems regulating stress responses[1]. In rat, the PVN, consisting of around 20,000 neurons is presumed to have more than 20 neuropeptides and putative neurotransmitters (Pacak K, 200, Meister B et. al., 1990**)**. Among them, the corticotrophin releasing hormone (CRH), oxytocin and vasopressin appear to play an important role in response to various stressors (Swanson LW et.al., 1986; Kiss JZ et.al., 1991, Rivest S and Rivier C, 1991). Extensive research has provided evidence for a crucial role of the paraventricular nucleus as an integrator of endocrine and autonomic functions (Kiss JZ et.al., 1991). The PVN neurons, receives various stress-related signals, which are involved in the control of autonomic outflow and the hypothalamo-pituitary-adrenal (HPA) axis (Benarroch EE. 2005). However, the mechanisms by which various stressors activate the PVN neurons remain unclear.

Organisms are constantly subjected to stimuli that can be construed as stressors. 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. Various types of acute and chronic stressors were employed in different stress studies by a number of investigators (Armario A et.al. 1991, Nayanatara AK et.al., 2005, Nagaraja HS and Jeganathan PS 1999, Pacak K and Palkovits M. 2001). The duration and the frequency of the stress period are important determinants for the induction of the cascade of stress - triggered neurobiological processes (Koolhaas JM , 1997).

A major question facing researchers is whether the brain deals with stressors categorically. Although not universally accepted (Pacak K et.al., 1998) , the idea that the brain categorizes stressors and uses response pathways that vary according to the category has gained significant support, particularly amongst groups investigating stress-induced hypothalamic-pituitary-adrenal (HPA) axis activation ((Sawchenko, PE et.al 1996, Cullinan WE et.al 1995). Most researchers would probably accept a classification of restraint as psychological stressors (Cullinan WE et.al 1995). Some researchers regard forced swim primarily as a physical stressor; others regard it primarily as psychological; yet others regard it as a

mixture of both (Neumann I et. al., 1998, Harbuz M S. & Lightman SL 1989**)** . It was our hope that, if the stress response elicited by our putative physical and psychological stressors were sufficiently distinct, the resulting data would settle this dispute by, in effect, allowing the brain itself to reveal its own decision.The present study was designed to elucidate the possible role of PVN nucleus on stress by evaluating its role in chronic physical & chronic psychological stress onsome selective physiological parameters which depicts the ingestive behavior.

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 PVN 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 PVN lesion was performed according to the stereotaxic coordinates prescribed in the Paxinos and Watson rat stereotaxic atlas (Paxinos G and Watson C, 1986). The coordinates were as follows: antero posterior (AP) = -1.3 mm posterior to bregma, lateral (L) = 0.3 mm from midline, vertical (V) = 8.0 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

PVN sham lesioned control (PVN SL-C)

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

PVN sham-lesioned chronic swimming stress (PVN SL-SS)

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

PVN sham-lesioned chronic immobilization stress (PVN SL-IS)

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

PVN lesioned control (PVNL-C)

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

PVN lesioned chronic swimming stress (PVNL-SS)

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

PVN lesioned chronic immobilization stress (PVNL-IS)

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

CHRONIC STRESS PROCEDURE (Nayanatara AK et.al., 2011)

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 trans cardial formol saline injection, were dissected and processed for histological study. Serial sections 5µ thickness were taken and stained with Haematoxylin and eosin (Culling CFA et.al., 1985) (**Fig. 1&2).**

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: *Paraventricular Lesion*

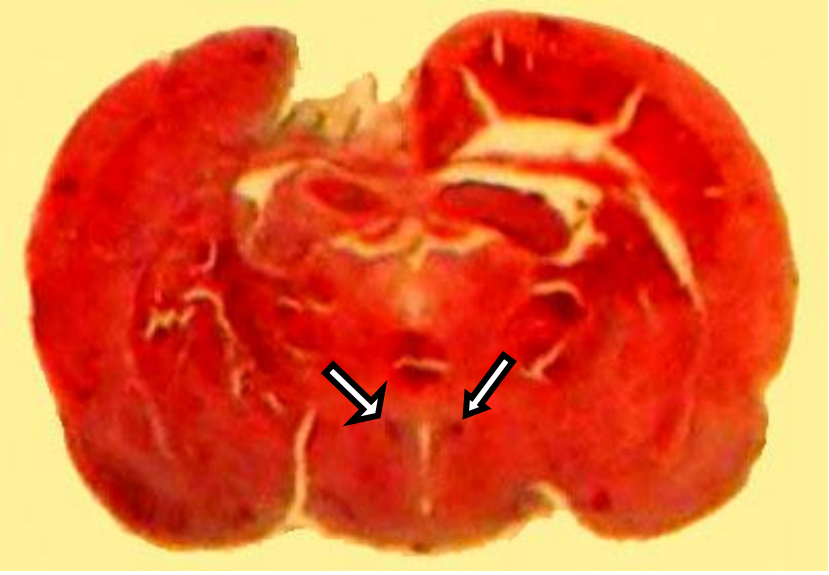
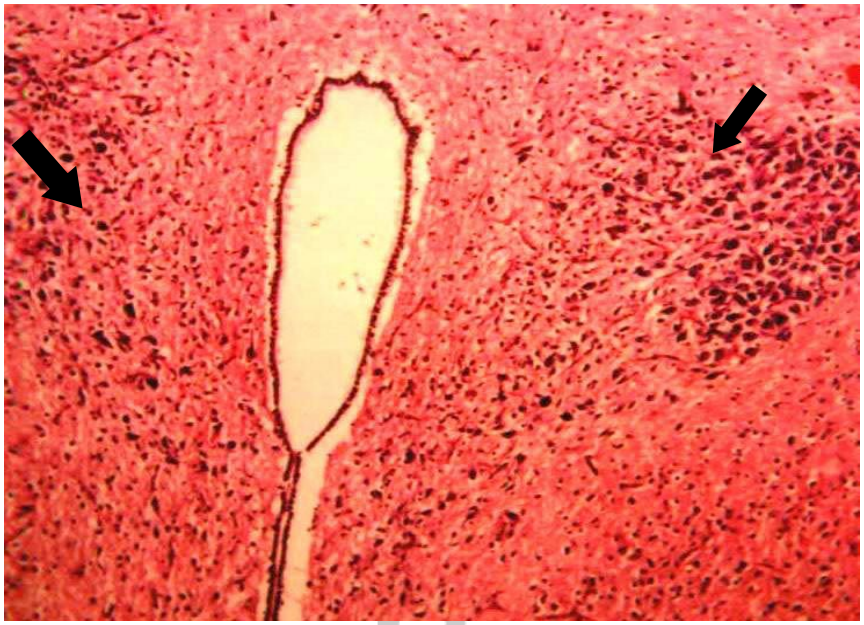


Figure 2: *Microscopic view of PVN Lesion*



STATISTICAL ANALYSIS

The data were summarized using mean ± SEM or median and interquartile range depending on the skewness (Hassard TH 1997). 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 ways ANOVA followed by post hoc tests in case of significant F value of ANOVA. $P<0.05$ were considered as statistically significant.

Table 1: *Swimming stress and Immobilization stress induced changes on body weight (BW) changes in PVN lesioned rats. Values are expressed as Mean ± SEM; grams (g).*

Groups	Day-One	Day –Sixty
NC (n=10)	149.33± 0.42	163.33 ± 0.42*^{NS}
PVN SL-C (n=10)	150.33± 0.61	163.67± 0.33*
PVN-C (n=10)	150.33± 0.33	202.67± 3.04**‡‡
PVN SL-SS (n=10)	150.33± 0.61	140.67± 1.03*
PVNL-SS (n=10)	152.0± 0.73	122.33± 0.8**¶¶
PVNSL-IS (n=10)	147.83± 1.51	126.0± 1.9**
PVNL-IS (n=10)	147.0± 2.23	168.67± 3.10$^{**§§\#\#}$

n= number of rats

NC versus PVN SL-C – Non significant (NS)

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

‡‡ P < 0.001; PVN SL-C versus PVNL-C

¶¶ P < 0.001; PVN SL-SS versus PVNL-SS

§§ P < 0.001; PVN SL-IS versus PVNL-IS

$^{\#\#}$ P< 0.001; PVNL-SS versus PVNL-IS

Table 2: *Swimming stress and Immobilization stress induced changes on food intake changes in PVN lesioned rats when compared to PVN 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}
PVN SL-C (n=10)	9.3± 0.54	9.52± 0.54
PVN-C (n=10)	8.33± 1.03	28.17 ± 1.47** ‡‡
PVN SL-SS (n=10)	10± 0.63	8.63± 0.52
PVNL-SS (n=10)	18.0 ± 1.41	6.33± 0.75 **
PVNSL-IS (n=10)	9.5± 0.55	7.8 ± 0.75
PVNL-IS (n=10)	18.5± 1.76	12.1± 2.11 ** $^{§§\#\#}$

n= number of rats

NC versus PVN SL-C – Non significant (NS)

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

‡‡ P < 0.001; PVN SL-C versus PVNL-C

¶¶ P < 0.001; PVN SL-SS versus PVNL-SS

§§ P < 0.001; PVN SL-IS versus PVNL-IS

P< 0.05; PVNL-SS versus PVNL-IS

Table 3: *Swimming stress and Immobilization stress induced changes on water intake changes in PVN lesioned rats when compared to PVN 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}
PVN SL-C (n=10)	18.18± 3.46	18.8± 2.49
PVN-C (n=10)	20.33± 2.5	46.33 ± 1.86 ** ‡‡
PVN SL-SS (n=10)	18.17± 1.03	14.05± 1.05*
PVNL-SS (n=10)	22.83± 2.99	10.33 ± 1.06**¶¶
PVNSL-IS (n=10)	18.83±1.17	14.5±1.05*
PVNL-IS (n=10)	22.23± 0.42	14.67± 0.42$^{** §§\#\#}$

n= number of rats

NC versus PVN SL-C – Non significant (NS)

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

‡‡ P < 0.001; PVN SL-C versus PVNL-C

¶¶ P < 0.001; PVN SL-SS versus PVNL-SS

§§ P < 0.001; PVN SL-IS versus PVNL-IS

P< 0.05; PVNL-SS versus PVNL-IS

RESULTS & DISCUSSION

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

Body weight (Table 1): PVN lesioning (PVN-C) significantly increased ($P < 0.001$) the body weight when compared to PVNsham lesioned control group (PVN SL-C). A significant decrease in the body weight was observed in all the stress groups (PVN SL-SS ($P < 0.05$); PVNL-SS, PVN SL-IS ($P < 0.001$ for both), except in the lesioned group (PVNL-IS)exposed to chronic immobilization stress there was a significant increase in the body weight. The changes in the body weight was more significant ($P < 0.001$) in the PVN lesioned stress groups (PVNL-SS, PVNL -IS) when compared to respective PVN sham lesioned stress groups (PVN SL-SS, PVN SL-IS). In the lesioned stress groups, exposure to swimming stress (PVNL-SS) showed more significant ($P< 0.001$) changes in the body weight when compared immobilization stress (PVNL-IS).

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

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

DISCUSSION

Stress regulation is a highly integrated process controlled largely by the brain. The brain is capable of translating a wide range of stress related inputs into a general class of

neuroendocrine/autonomic responses designed to enable the organism to efficiently cope with environmental change elicited by variety of stressors (Herman JP et. al., 1996). Extensive research has provided evidence for a crucial role of the paraventricular nucleus as an integrator of endocrine and autonomic functions ((Herman JP et.al., 2002, Whitnall MH 1993. Herman JP and Cullinan WE1997) . Different types of stressors trigger a core set of endocrine, autonomic and behavioral responses, the brain pathways that drive these responses might differ according to the stressor category. The nature of the stressor itself is important in the pattern of expression of stress responses.

Ingestive behavior and body weight are the variables sensitive to stress (Marti O et. al., 1994). 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 (Valles A et. al., 2000). In the present study, no significant changes were observed in body weight, food intake, and water intake in between the normal control group sham control group ·This finding confirms that surgical procedure did not affect the studied parameters of the present study. Energy homeostasis which is the balance among energy intake, metabolism, and storage and energy expenditure is a complex regulatory function which is mainly performed by the hypothalamus (Bray GA et. al., 1990). Disruption of any of these components of energy balance can lead to the pathologies ranging from wasting disorders or anorexia to obesity, which is the major health problem emerging in the modern societies (Hill JO and Peters JC 1998). In the present study, PVN lesion increased the food intake, water intake and body weight suggest that PVN nucleus have an important role in the regulation of food intake, water intake and body weight. PVN through its connections with nucleus of solitary tract (NST) and dorsal motor nucleus of vagus modulates the activity of viscerosensory, orosensory and autonomic motor system (Kirchgessner AL and Sclafani A 1988). This modulatory activity was evidenced by the earlier findings that electrical stimulation of PVN excites vagal neurous (Rogers RC and Nelson D O 1984), alters gastric secretion (Rogers RC et. al., 1980) and gastric

motility (Sakaguchi T and Ohtake M 1985). Based on the above evidences, it appears that one of the functions of PVN is to modulate the processing of visceral and perhaps gustatory afferent information. Herman GE and Rogers RC have demonstrated the direct convergence of visceral and gustatory inputs within brain stem sites that receive PVN projection (Hermann GE and Rogers RC 1985). Lesioning of PVN interrupts its projections to nucleus of solitary tract which may alter the normal integration of visceral and gustatory inputs; as a result, the animal may over respond to oral stimuli and over eating (Hermann GE and Rogers RC 1985). Previous study of Tokunaga et al showed that the extent of PVN damage was directly related to the weight gain (Tokungo K et. al., 1986). The observed hyperphagia and obesity after PVN lesion in the present study supports the findings of various other studies. (Tokungo K et. al., 1986 , Sims JS and Lorden JF1986).In the present study, PVN lesioning increased water intake. Based on the previous studies (Tokungo K et. al., 1986 , Sims JS and Lorden JF1986) , it has been suggested that the pathways arising from subfornical organ, medial preoptic nucleus, organum vasculosum of lamina terminalis project to the medial PVN neurons and the subfornical organs also directly activates the hypothalamic CRH secretion (Plotsky PM et. al.,1988). This response may itself be mediated through direct interaction, by circulating angio tension II via osmo receptors (Fitzsimons JT 1998). Therefore, in the present study after PVN lesion, the loss of CRH secretion might have resulted in the increased water intake. The active involvement of PVN has been implicated in regulation of homeostasis function such as co-ordination of energy balance by regulating the food consumption and energy expenditure (Billington CJ et. al 1994, Glass MJ et. al., 2000) supports this findings.

Body weight is the index of the energy balance. Body weight undergoes significant changes when experimental animals are exposed to different types of stressors (Nagaraja HS and Jeganathan PS 2003, McLaren GW et. al., 2004). The present results showed that exposure to both; the swimming stress and immobilization stress caused a significant decrease in body weight in all the control groups. This decrease in the body weight might have been

presumably associated with increased metabolic demands, reduced digestion, and increased adrenal steroid secretion. This observation was in agreement with previously reported studies ((Nagaraja HS and Jeganathan PS 2003, McLaren GW et. al., 2004), but contradicts others Szenasi G et. al., 1988 , Rowland NE and Antelman SM 1976).

Following exposure to swimming stress and immobilization stress, no significant change in the food intake was observed in normal control groups and sham lesioned stress groups though the body weight decreased. Stress is known to trigger CRH secretion which could exert suppressive effect on the food intake in animals(Rowland NE and Antelman SM 1976). However, stress is also known to increase protein catabolism. Normal rats subjected to food deprivation can still maintain the growth and minimize weight loss by increasing the efficiency of utilization of whatever food was taken during the 24-hour period (Rowland NE and Antelman SM 1976). Stress may hamper this mechanism. Therefore, rats subjected to chronic stress continued to lose weight despite of normal food intake (Nagaraja HS and Jeganathan PS 2003) have shown the similar kind of observations. However, in contrast to our results stress induced alterations in the food intake were also observed in some other studies (McLaren GW et. al., 2004, Rowland NE and Antelman SM 1976).The results of the present study showed a significant decrease in water intake during chronic swimming stress. A similar result of decreased water intake during chronic swimming stress was reported by earlier authors (Nagaraja HS and Jeganathan PS 2003, Rowland NE and Antelman SM 1976). In contrast to the present result, chronic stress induced increase in water intake has been reported (Bernatova I et. al., 2002). Therefore, alterations of body weight, food intake and water intake in the different experimental studies probably depend on the types of stressor and its durations.

In present study, following PVN lesion the stress induced alterations in the body weight showed different types of responses. In the PVN lesioned groups, swimming stress decreased the body weight and immobilization stress increased the body weight, compared to its respective sham lesioned groups. This indicates that during the exposure to stressors, PVN plays a role

in stress induced alterations in the body weight which might vary based on the type of the stressor used. Limbic and hypothalamic brain structures integrate emotional, neuroendocrine and autonomic inputs that determine the magnitude and duration of behavior, neural and also hormonal responses to stressful experiences (Herman JP et. al., 2003). Recent studies have shown that inputs from the limbic associated structures are capable of activating the stress responsive CRH neurons of PVN (Beaulieu S et. al., 1989). The 'anticipatory' signals conveyed by the limbic structures to the CRH neurons are integrated with neural pathways subserving "reactive" responses at multiple levels (Beaulieu S et. al., 1989) . In the present study, stress induced changes following PVN lesion might be due to the imbalance of the autonomic inputs that determines the magnitude and duration of stressful experience. Exposure to swimming stress and immobilization stress after PVN lesioning reduced the water intake significantly in the present study. This can be explained by the direct effect of stress on increase in the ADH secretion leading to water reabsorption. This might have caused decreased stimulation of thirst center causing decreased water intake. Based on the present study, it appears that the PVN have a definite role in stress tolerance. After PVN lesion, exposure to both type of stresses showed a significant changes in the food & water intake and bodyweight. Further, the stress induced changes was significantly more during the exposure to swimming stress than the immobilization stress. This shows that PVN nucleus appears to play an important role in minimizing the stress induced changes in these ingestive behaviors during an exposure to the physical type of stress rather than a psychological type of stress like immobilization.

It has been hypothesized that the brain categorizes stressors and utilizes neural response pathways that vary in accordance with the assigned category. If this is true, stressors should elicit patterns of neuronal activation within the brain that are category-specific. 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'. In this direction, the present study provides the supportive evidence that the response of a

physical stress to a greater extent is regulated by paraventricular nucleus of the hypothalamus. There might be the presence of an intricate web of reciprocal independent connections of paraventricular nucleus of the hypothalamus to the brain areas regulating the responses designed for the physical stress. However, the exact role of these nuclei and their interaction among themselves and other brain areas in regulating homeostasis need further indepth study. In brief, the present study emphasizes that paraventricular nucleus of hypothalamus play a definite role in stress mediated body's adaptive mechanisms.

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