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Original Article

Unilateral degeneration in hippocampus of female rat

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ABSTRACT

Study demonstrates occurrence of unilateral type neurodegeneration in hippocampal sub region (CA2 and CA4) of female rats that given four hours swimming stress and unique rotational behavior. Stressed rats' brain sections, stained with cresyl violet, passing through bregma -1.8 to -3.8 levels, demonstrated degenerative changes in the cell bodies of CA2 and CA4 hippocampal sub regions of right hemisphere. These changes were observed in 50% of rats. Reason lying behind the unilateral degeneration after exposure to swimming stress could not be explained. To our knowledge, it is the first report demonstrating unilateral hippocampal degeneration. © **Neuroanatomy. 2008; 7: 1–5.**

Key words [hippocampus] [swimming stress] [CA2] [unilateral degeneration] [rotational behavior]

Introduction

A number of investigations have shown that hippocampal sub regions are particularly vulnerable during stressful conditions [1-5]. Cell loss in hippocampus during normal ageing and also in age-related disorders has also been reported [6]. During the exposure to acute or chronic stress and ischemia, cell loss in hippocampus has been demonstrated [7,8]. Exposure to external stressors like heat can damage hippocampus [9,10]. It has been exhibited that glucocorticoids, which are secreted during the stress, play an important role in stress-related pathological consequences [11]. Excess of glucocorticoids or prolonged exposure to them may enhance neurotoxic insults in brain [12,13] and increase age-related loss of pyramidal cells in hippocampi of rats [14]. Degeneration is depicted by enlarged intracellular spaces, condensation of chromatin, pyknotic neurons in CA1, CA4 and Dg [15]. Cerebral ischemia as a model for analysis of mechanism of neuronal cell death or damage in ischemia is studied by Matsuka et al [16].

CA1 sub region of hippocampus is relatively vulnerable to various stress conditions such as hypoxia and ischemia [1,17]. Cerebral ischemia induces various degrees of brain damage depending on intensity and duration. CA1 pyramidal cells in Mongolian gerbil are selectively damaged even after 3-5 minutes exposure to ischemia [1,18].

In the present study, we report specific neurodegeneration observed in hippocampal sub regions of young adult rats after exposure to swimming stress for a specific period.

Materials and Methods

Young adult female Swiss albino rats (body weight 100±5 grams, aged one month) were procured from veterinary college, Mhow, India. After receiving the animals from the supplier, rats were separated in groups and housed in polyurethane cages, for 10 days to acclimatize them with laboratory conditions. Animals were kept under controlled temperature (25±2 ºC) and illumination (14 hours light and 10 hours dark) conditions. They were provided food and water ad-libitum. All the experiments were cleared by institutional ethical committee (*Registration No. 973/AC/06/CPCSEA*).

Animals were divided into control and experimental groups, all the groups constituted of 6 animals each. Control group (n=6) rats were housed in complete non-stress condition. Prior to decapitation, animal room was locked for 24 hours to avoid any stressful situation even due to handling. Room was opened next day morning and animals were decapitated immediately for collection of brain tissues.

Experimental group (n=24) animals were subdivided into four subgroups (n=6) and subjected to cold-water swimming stress for 7, 14, 21, and 28 days, for 4 hours a day. Animals were forced to swim in a plastic bucket

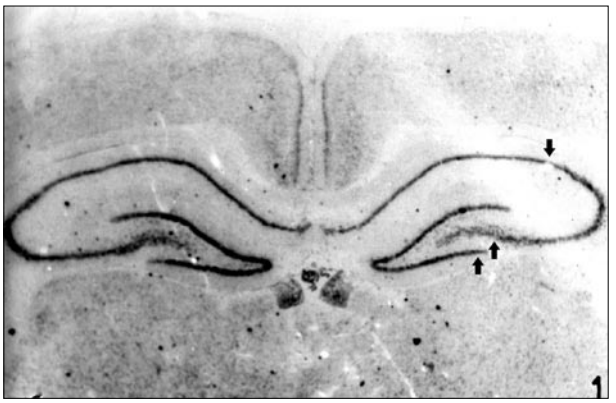

Figure 1. Section through hippocampal sub region of stressed female rat. Arrows demonstrating the unilateral degeneration in CA2 and CA4 sub region in the right hemisphere. No degeneration was observed in left side. ***Arrows*** *(Degeneration and thinning of layers) (Cresyl violet staining, 40X).*

filled with cold water (18±2 ºC) under observation. On completion of experiment, rats were decapitated.

Behavioral Studies

During the course of stress period, animals were assessed for any behavioral dysfunction occurrence. Amazingly, rotational behavior was observed by the third day of stress treatment. Considering this unusual non-hippocampus dependent behavior, extra care was taken. Immediately after stress exposure, the paws of fore and hind limbs of the rats were soaked in ink and then animals were placed on plain white paper sheet to assess the rotational behavior. Impressions of fore and hind limbs during the circulating behavior formed a circle and impressions were considered as the circumference of the circle. Diameter of the circle was measured using compass.

Histological Studies

Brains were dissected out from each animal and were fixed in 10% chilled neutral formalin for 14 hours at 4ºC. Paraffin embedded sections (bregma -1.8 mm to -3.8 mm) of 10 μm thickness were cut on rotary microtome and stained with cresyl violet for demonstration of nerve cell bodies. Quantitative analysis of neuron cell bodies in the pyramidal cell layer of Ammon's horn and granule cell layer dentate gyrus of hippocampus was performed, using calibrated ocular micrometer. Sections passing through bregma level -2.8 to -3.3 mm were used to count the neuron cells. The cell count was corrected by using the Abercrombie's formula and thus the absolute cell count was obtained. Round, clear and medium or large neurons with distinct nucleus were counted. Cells with darkly stained shrunken cells and cells with fragmented nuclei were considered and excluded from the count. The differences in cell count data were analyzed using one tail paired *t* test.

Results

Recent investigations in our laboratory led us to demonstrate stress induced cell loss in specific sub regions of hippocampus and cerebral cortex. But all these observations were related with the degeneration in both the side of brain.

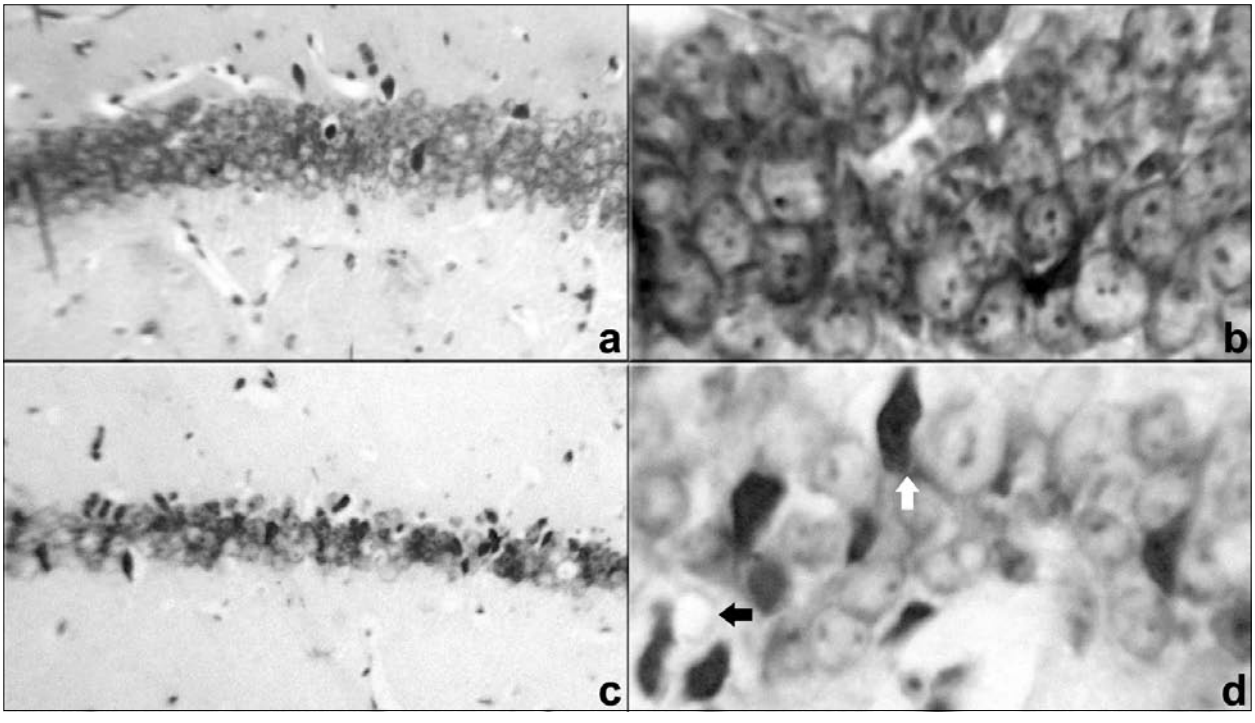

Figure 2. Section through CA2 hippocampal sub region of stressed female rat. Figures a and b demonstrating left side of the hemisphere. Note normal neuron cell bodies with distinct nucleus and nucleoli. Cytoplasm is evenly filled with Nissl substance. Figures c and d are from the right hemisphere demonstrating degenerating nerve cell bodies. Degenerating cell bodies are marked with pyknotic nuclei and vacuolar spaces. ***Arrows:*** *(Black: Hydropic cell) (White: Dark Cell) (Cresyl violet staining, 2a and 2c 100X and 2b and 2d 400X).*

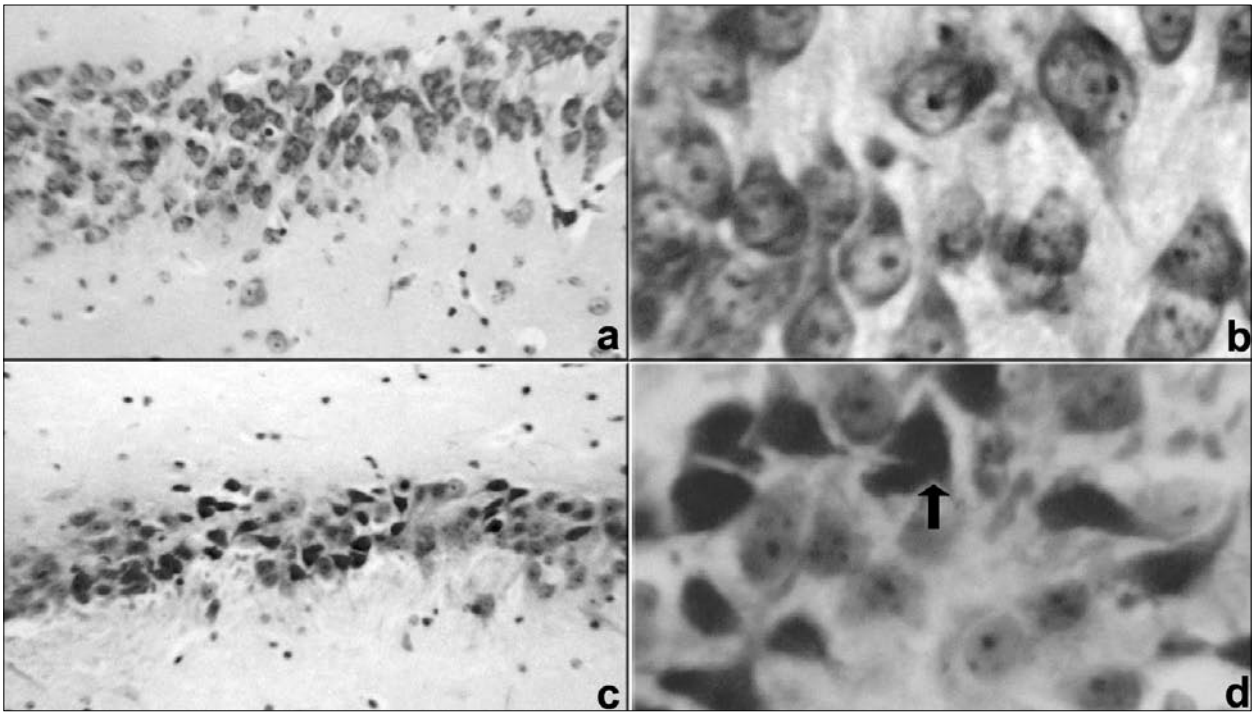

Figure 3. Section through CA4 hippocampal sub region of stressed female rat. Figures a and b demonstrating left side of the hemisphere. Note normal neuron cell bodies with distinct nucleus and nucleoli. Cytoplasm is evenly filled with Nissl substance. Figures c and d are from the right hemisphere demonstrating degenerating nerve cell bodies. Degenerating cell bodies are marked with pyknotic nuclei and dark cells. ***Arrows:*** *(Dark Cell) (Cresyl violet staining, 3a and 3c 100X and 3b and 3d 400X).*

Cytological examination of cresyl violet stained sections of stressed animals, depicted unusual unilateral degeneration in CA2 and CA4 areas of hippocampus. The degeneration was mainly in the right hemisphere. This degeneration is evident by the thinning of hippocampal blade in right side as compared to left side, thinning of the blade was observed in all the sub regions of hippocampus i.e. CA1-CA4 and Dg (Figure 1). It was evident by presence of pyknotic nuclei, darkly stained cells, cells with condensed nuclei, as well as vacuolated spaces. Consequently, cell density was comparatively lesser in these areas compared to the contrlateral side, i.e. left hemisphere and control animals (Figures 2, 3). Degenerative changes were evident in all the regions including CA1, CA3 and Dg but in CA2 and CA4 regions they were maximum in the right blade of hippocampus (Figures 4, 5). When the neuron cell count was subjected to *t* test, significant statistical difference was observed between left and right blade of stressed animals ($P<0.05$). Total number of normal cells in CA2 and CA4 was approximately reduced by 50% on the right side in comparison to left side of hippocampus. Number of cells present in the right blade of hippocampus was comparable to the control animals and no significant statistical difference was observed ($P>0.05$).

Rats demonstrating unilateral degeneration exhibited unique rotational behavior. Interestingly, the rotations

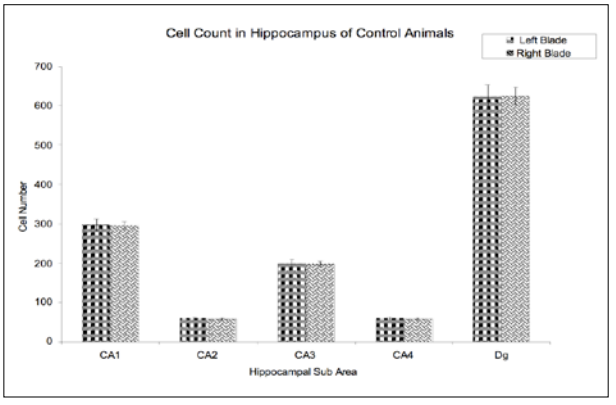

Figure 4. Graph depicting cell count in Left and Right blade of hippocampus of control animals.

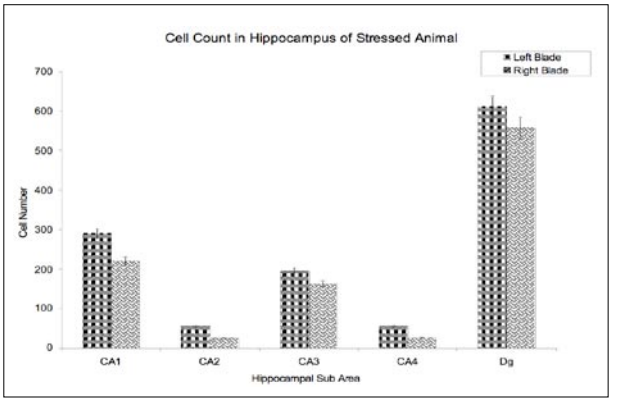

Figure 5. Graph depicting cell count in Left and Right blade of hippocampus of stressed animals.

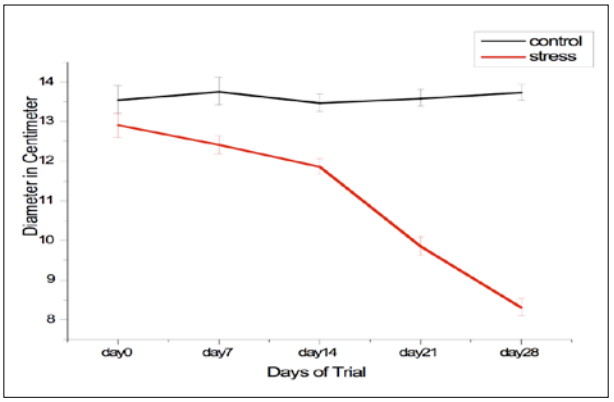

Figure 6. Graph depicting circle diameter after the stress treatment. Color version of figure is available online.

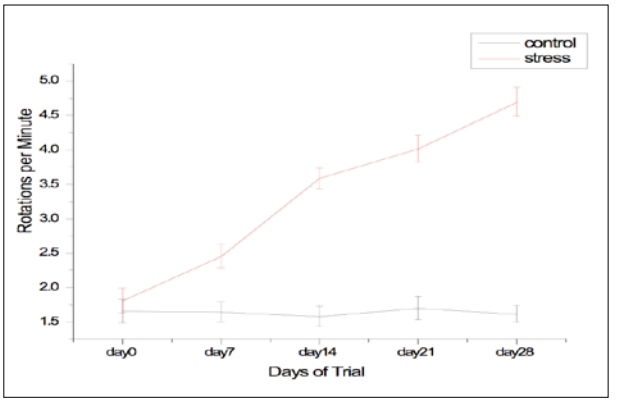

Figure 7. Graph depicting revolution per minute after the stress treatment. Color version of figure is available online.

per minute were increased and the diameter of rotation was decreased, as the stress was continued for longer period of time (Figures 6, 7).

Discussion

Thus far, no direct evidence of such a unilateral degeneration in hippocampus was observed, possibly this is the first report.

Different aspects of unilateral degeneration and behavioral asymmetries have been evaluated earlier by unilateral injection of 6-hydroxy dopamine (6-OHDA) into substantia nigra [19]. Unilateral injections of 6-OHDA into the substantia nigra destroy ipsilateral nigrostrital dopamine projections and cause the animal to rotate toward the side of lesion [20]. Ziegler and Szechtman demonstrated the relation between motor asymmetry and direction of rotational behavior under amphetamine and apomorphine, in rats with unilateral degeneration of the nigrostriatal dopamine system [21].

The reason of neuron loss in one side of the brain cannot be explained. But several evidences suggest that neuron cell loss in hippocampal sub regions are due to various types of stress exposure [9,10,12,13]. Neurons are especially vulnerable to free radical attacks. Free radicals contribute to neuronal loss in cerebral ischemia, hemorrhage and may be involved in damage during epilepsy, schizophrenia, neuronal ageing, Alzheimer's and Parkinson's diseases [22-24].

The hippocampus, a structural part of the limbic system, receives its primary afferent inputs from the polymodal association areas of the entorhinal cortex. The hippocampus, in turn, sends outputs to the nucleus accumbens [25], dorsal striatum [25], and neocortical areas [26-28], which have been implicated in the production and modulation of locomotor activity, rotational movement, and stereotyped behavior induced by the indirect dopamine agonists, methamphetamine and amphetamine [29-33].

In the present study a unique right side rotational behavioral pattern that correlates with the cell degeneration in right blade of hippocampus was observed. Possibly dopamine might have played a pivotal role in this pattern. The hippocampal formation receives projections from the midbrain's dopaminergic cell groups and contains mRNA for all five dopamine receptors [34]. Earlier reports suggest that cholinergic and catecholaminergic systems are involved in the rotational behavior. As a strong correlation between catecholaminergic and cholinergic systems have been demonstrated in vivo: treatment with a D(2)R agonist increases hippocampal ACh release while treatment with a D(2)R antagonist attenuates this effect [35]. We have also observed decrease in dopamine staining after the stress condition (unpublished data).

Another explanation could be the decreased blood flow to hippocampus after the stressful conditions. Hypoperfusion of the frontal cortex has been reported with pathological temporal limbic activation in unilateral epilepsy [36] and reciprocal inhibition between frontal and limbic areas has been hypothesized in theories on the etiology of schizophrenia [37]. This behavior weakens with time, but only reappear when animal is stressed again after a time interval or injected with drugs affecting the dopaminergic system [20,38].

Present study suggests that unilateral degeneration of hippocampus is in concert with the rotational behavior as other areas like basal ganglia and nigrostrital region have; and this behavior can be induced by forced cold-water swimming stress.

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