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Original Research

Unilateral lesion of fastigial nucleus in Wistar albino rats and its effect on motor coordination - A preliminary study

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ABSTRACT

Aim: The objective of this present study was to investigate whether unilateral lesion in the fastigial nucleus (deep cerebellar nuclei) which is believed to influences posture and locomotion, could alter motor coordination in a rat model. **Materials and Methods:** In order to understand motor activity a combination of three motor tasks, which include rotarod, grid walk, and narrow beam test were performed. Rats were divided into three groups namely control, sham, and lesion groups. **Results:** The lesion group on 10th day showed increased hind limbs, forelimbs slips, and time spent on grid runway and beam walk along with decrease performance in the rotarod test, which is an indicative of altered motor behavior. However, no significant change was observed on the 15th day study in the entire test. **Conclusion:** This transient effect displayed on the 15th day, suggest that it might have been due to the ability of the animal to rehabilitate/neuronal plasticity on the lesioned side or it may have been due the compensatory changes on the non-damaged brain areas.

KEY WORDS: Fastigial nucleus, grid runway, motor coordination, neuronal plasticity, narrow beam, rotarod

INTRODUCTION

The cerebellum or little brain is located posterior to the brain hemispheres covering the dorsal part of the brainstem. It is believed that cerebellum has long been called as the silent area of the brain because electrical excitation does not cause any conscious sensation and rarely produces any motor movement, however muscle movement especially coordination and smooth motions, are product of feedback loop involving the cerebellum and frontal cortex. The cerebellum has been considered to contribute to motor control and coordination, [1] control of upright stance and balance [2-4] and since then, many other functions have been attributed to the cerebellum, such as motor learning, [5] language, [6] planning, prediction [7], and perceptual ability [8]. Afferent connections via the corticopontine-cerebellar tract with the premotor and motor cortex carry a "copy" of motor demands to the cerebellum. The cerebellum then compares feedback from the muscle spindles, joints, and tendons via the cerebellar peduncles to modify motor behavior, maintain coordination, and perform skilled movements [9]. Because of the prominence of sensory input to some parts of the cerebellum, some physiologists have believed the cerebellum functions more as a sensory analyzer than as a movement controller [10]. Based on afferent and efferent connectivity cerebellum is divided into distinct functional zones [11,12]. All zones can influence locomotion though in different ways. The most medial zone of the cerebellum (vermis) receives input from the primary vestibular afferents and vestibular nuclei, [13] reticular [14,15], and pontine nuclei, as well as from the spinal cord [16], via dorsal and ventral spinocerebellar tracts. As suggested by Chambers and Sprague [17,18] it is likely that the vermis and the fastigial nucleus (nuclei responsible for cerebellar output; which efferent fibers from the vermis project) are concerned with maintaining posture and with elaborating associated body and neck movements. This is well-supported by Armstrong [19] who proposed that fastigial nucleus influences posture and locomotion by coordinating the activities of axial muscle groups via the reticulospinal and vestibulospinal tracts.

Cerebellar Ablation Studies

Animal studies have helped to elucidate the role of different cerebellar regions in the motor control and coordination.

Literature studies suggested the role of deep cerebellar nuclei in motor functioning. During cat locomotion, there is rhythmic neural activity in the fastigial nucleus that modifies descending brain stem pathways that influence spinal cord locomotor generators [20,21]. Yu and Eidelberg [22] also demonstrated that cats with fastigial lesions bilaterally, produced ataxic locomotor movements. Thus, taking account on the role of fastigial neuleus the present study aims to evaluate whether lesioned in the fastigial nucleus unilaterally can influence motor coordination in Wistar albino rats. The combination of three motor tasks which includes rotarod test, grid walk test, and narrow beam test was performed to understand the motor activity and motor learning behavior in experimental rats.

MATERIALS AND METHODS

Ethical Approval

The study was approved by the Institute's Animal Ethical Committee (IAEC No 08/034/07) and the Committee for the Purpose of Control and Supervision of Experiments on Animals. Healthy adult male Wistar albino rats weighing about 200-220 g have been used for this study and allowed to have food and water ad libitum.

Experimental Groups

Animals were divided into three groups with six animals in each group. The control animals formed the Group I. As surgical procedures are involved it is essential to have sham animals, which are treated similarly to the lesioned animal including the surgical procedures, however no current was passed through the electrodes while lesioning the fastigial nucleus this forms the Group II. The Group III animal includes the unilateral fastigial nucleus lesioned electrically on the left side.

Electrical Lesioning of Fastigial Nucleus

Rats were anesthetized with pentathol sodium (40 mg/kg b.w). The hair on the scalp was removed, and the animal was fixed to the stereotaxic apparatus frame (INCO, AMBALA). The coordinates used for fastigial nucleus lesion was minus 10 mm from bregma, lateral from the midline was 1.10 mm, and 4.80 mm from dura (depth) as per the Pellegrino *et al.* [23]. Appropriate holes were made using a dental drill. Stainless steel electrode of 0.22 mm diameter was lowered to the required depth, and anodal electric lesions of the fastigial nucleus were also made using direct current of 2 mA at 100 volts for 10 s. The day of the lesion was considered as "0" day. At the end of experiments, the control and lesioned animals were sacrificed with the overdose of pentothal sodium and perfused. The cerebellum was taken for histology studies, and the lesion site was confirmed [Figure 1a and d]. The data from the properly lesioned animals only considered for the study.

Behavorial Parameter

Narrow beam-walk [24]: Animals were trained to cross an elevated wooden beam rapidly. The beam was 2.5 cm wide and was elevated to a height of 1 m above the floor. Each test session consisted of three trials in which latency to cross the beam, and the number of foot faults was observed. Rats will normally walk with their feet flat on the surface of the beam. Therefore, a fault was defined as any use of the forelimb or hind limb on the side of the beam or if either foot slipped off the top surface of the beam. The results of the three trials were averaged to give a mean latency and foot fault score.

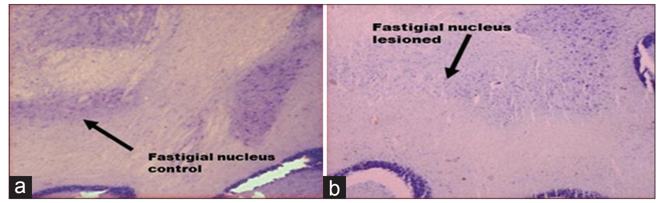

Figure 1: (a) Fastigial nucleus in control group ×40, (b) lesioned fastigial nucleus ×40

Grid walk [25]: Sensorimotor coordination was tested on the 10th and 15th day after the unilateral fastigial nucleus lesion. The apparatus consisted of an elevated 30 cm wire grid bridge that measured 120 cm × 30 cm and contained a dark escape box at one end. The rat's progress across the bridge and counted the number of the foot faults were observed; the numbers were then averaged. Total time to cross the bridge was also recorded. Foot faults per min were used to estimate performance.

Rotarod [26]: Motor coordination was tested with rotarod on the 10th and 15th day after the unilateral fastigial nuclei lesion. The apparatus consists of a horizontal iron rod, 2.5 cm in diameter and 15 cm long with a roughened surface, moving on its axis at 10 rpm. The duration for which the animal is able to balance on the moving rod (until it falls) is noted as the coordination time.

Histology

Animals were deeply anesthetized with ketamine hydrochloride. Rats were then perfused transcardially with phosphate-buffered saline, followed by buffered 10% formalin. The brain was removed, and preserved in formalin until processed for histology. Then kept on running water to remove formalin pigments and dehydrated with ascending grades of alcohol. After impregnation with paraffin wax, the paraffin blocks were made. They were processed and sections were cut with 6 μm in thickness using Spencer Lens, rotatory microtome (no 820, Newyork, USA) and then, stained with hematoxylin and eosin stain.

Statistical Analysis

Data are expressed as mean ± standard deviation. All data were analyzed with the SPSS for windows statistical package (version 19.0, SPSS Institute Inc., Cary, North Carolina). Statistical significance between the different groups was determined by one-way Analysis of Variance. When the groups showed a significant difference, then Tukey's multiple comparison test were followed, and the significance level was fixed at $P < 0.05$.

RESULT

All the animals in this study appeared healthy without signs of weight loss. The motor coordination was assessed on the 10th as well as on the 15th day after lesion. For the evaluation of lesion effect in this study, the sham animals are compared and considered as strict controls.

Narrow Beam

The data from various groups are tabulated in Table 1. Unilateral lesioned rats showed marked increase in the time to cross the beam, forelimb slips, and hind limb when compared to the controls, as well as sham animals on the 10th day after lesion procedure. However, none of the groups studied showed any variation among themselves on the 15th day.

Grid Runway

The data from various groups are tabulated in Table 2. Unilateral lesioned rats showed marked increase in the time to cross the grid runway, forelimb slips and hind limb when compared to the controls, as well as sham animals on the 10th day after lesion procedure. However, none of the groups studied showed any variation among themselves on the 15th day.

Rotarod

The data from various groups are presented as bar diagrams [Figure 2 a andb]. Unilateral lesioned rats showed a marked decrease in their performance on the rotarod from the controls, as well as from the sham animals on the 10th day after lesion procedure. However, none of the groups studied showed any variation among themselves on the 15th day.

DISCUSSION

In order to avoid variation in behavior induced by handling stress, the animals were carefully handled in the similar way on a regular basis. Earlier reports state that midline cerebellar injuries and atrophies cause instabilities of posture and locomotor movements [27]. It is a known fact that cerebellar hemispheres regulate the ipsilateral side of the body, unlike cerebral cortex, which controls the opposite side of the body [28]. Colombel *et al.* [29] have previously reported that the functional lateralization of the cerebellum concerning motor

Table 1: Effect of unilateral lesion on time taken to cross the narrow beam, number of forelimb, slips fore, and hind limb slips on the 10th day and 15th day

Parameters	Control	Unilateral sham	Unilateral lesion
Time taken to cross in seconds (day-10)	5.33±1.21	5.5±1.04	17.66±2.73*#
Time taken to cross in seconds (day-15)	5.5±1.04	5.83±1.16	9.66±2.65
Number of forelimb slips (day-10)	0.16±0.40	0.5±0.54	3±0.89*#
Number of forelimb slips (day-15)	0±00	0.33±0.51	1.16±0.75
Number of hind limb slips (day-10)	0.16±0.40	0.66±0.51	2.66±0.81*#
Number of hind limb slips (day-15)	0±00	0.16±0.40	1.0±0.89

The data from various groups for the individual test are presented as bar diagram with mean±SD. Significance fixed at $P<0.05$, the lesion group when compared to control significance is marked as* and lesion group when compared to sham groups significance is marked as#, SD: Standard deviation

Table 2: Effect of unilateral lesion on time taken to cross the grid runway, number of forelimb, slips fore, and hind limb slips on the 10th day and 15th day

Parameters	Control	Unilateral sham	Unilateral lesion
Time taken to cross in seconds (day-10)	6.26±0.74	6.2±0.93	9.83±1.15*#
Time taken to cross in seconds (day-15)	6.95±0.93	7.18±1.18	7.35±1.32
Number of forelimb slips (day-10)	0.5±0.54	0.83±0.40	4.16±0.75*#
Number of forelimb slips (day-15)	0.33±0.51	0.66±0.51	1.16±0.75
Number of hind limb slips (day-10)	0.16±0.40	0.5±0.54	4.33±0.51*#
Number of hind limb slips (day-15)	0.16±0.40	0.83±0.75	1.33±0.81

The data from various groups for the individual test are presented as bar diagram with mean±SD. Significance fixed at $P<0.05$, the lesion group when compared to control significance is marked as* and lesion group when compared to sham groups significance is marked as#, SD: Standard deviation

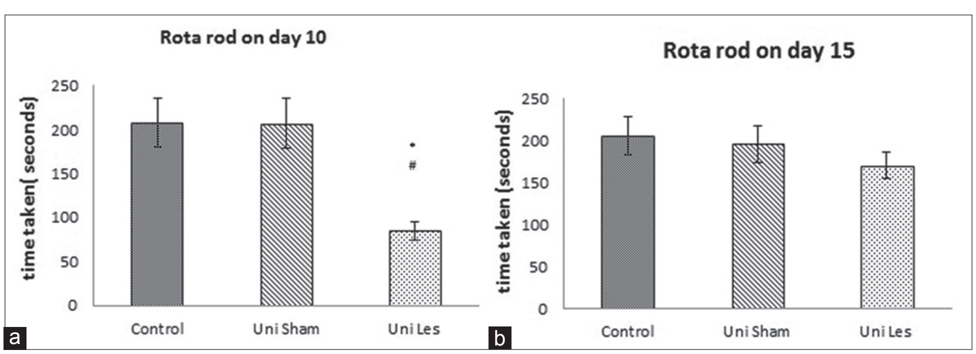

Figure 2: (a and b): Effect of unilateral lesion on rotarod performance on the on the 10th day and 15th day, the data from various groups for the individual test are presented as bar diagram with mean ± STD, significant fixed at $P < 0.05$, the lesion group when compared to control significant is marked as* and lesion group when compared to sham groups significance is marked as#

function, the motor deficits being more pronounced after left side lesion as opposed to right cerebellar damage. Taking account on this report, the left side of the cerebellar fastigial nucleus was unilaterally destroyed by an electrolytic lesion in this study. Unilateral brain damage results in deficits of symmetry, therefore it is useful to rely on tests that have the ability to detect asymmetries [30].

The grid walking task, often referred to as the foot fault task, is a relatively simple way to assess motor impairments of limb functioning. To begin with the grid walk test was used to analyze sensorimotor coordination and the ability of the animal to perform coordinated forelimb and/or hind limb placement [25]. Animals without brain damage will typically place their paws precisely on the wire frame to hold themselves while moving along the grid. The time-dependent test appears more appropriate for exploring early treatment effects due to spontaneous recovery [31]. Though lesion rats **showed** a significant impairment in sensorimotor coordination as evidenced by more misplaced steps by both hind limb and forelimb along with increased time taken by the animals to cross the grid was observed on the 10th day when compared to control and sham.

The beam walk test has been used extensively to assess vestibulomotor function and fine motor coordination in animal models of traumatic brain injury. An adverse stimulus such as a bright light or white noise was used to motivate the animal to traverse the beam in order to reach a darkened goal box [32]. An increased number of hind limb and forelimb slips along with increased time taken for the animals to cross the beam was also observed on the 10th day in this study, thus suggesting impairment in their ability to integrate appropriate motor commands to balance their posture. Earlier reports showed that lesions of the fastigial nucleus impair the basic gait pattern, with the animals falling to the side of the lesion [33] which support the present finding for the altered motor behavior on the 10th day. However, the lesion group showed a normal response as that of controls and sham in both beam walk and grid test on the 15th day study. This normalization of function after the lesion is likely to have been the ability of the animal to rehabilitate. This is in agreement with Jones and Schallert [34], who reported that in addition to lesion-induced impairments, another striking behavioral effect of the lesions is the development of compensatory behavioral changes.

The rotarod test is another used to assess motor coordination and balance alterations in rodents [35]. Moreover, rotarod performance provides information about muscle tone, balance, and motor coordination by measuring the time/acceleration the animal can cope on the rotating rod [36]. Rodents naturally try to stay on the rotating cylinder and avoid falling to the ground. In this study, the lesion animals showed a marked decrease in the time spent in the rotarod on the 10th day alone. Performance at both mild and moderate injury levels was found to be significantly impaired in the rotarod task, proving the method to be a sensitive index of injury-induced motor dysfunction, whereas other methods (e.g., narrow beam balance) only detect dysfunction at more severe injury levels when comparing rotarod to narrow beam balance at the moderate injury level, rotarod exhibits a slower rate of recovery than the others, which is perhaps indicative of its increased sensitivity to deficits [37]. Animals have been shown to use compensatory behaviors in a variety of situations including the beam walk, staircase test, and the rotarod [38,39] which also supports the present observations.

Unilateral lesioned rats studied for motor coordination, though showed marked changes in this study only on the 10th day from control and sham, however, the entire coordinated motor behavior returned to normalization on the 15th day. Will *et al.*, [40] reported that not only the effects of lesion could be accounted on the basis of lesion-dependent plastic processes in damaged central nervous system (CNS) areas, but also on the other intact CNS areas near the injury. Amrani *et al.*, [41] concluded that the initial, more rapid phase of the recovery after a unilateral cerebellar lesion depends upon intact contralateral cerebellar circuitry, as in unilateral lesion other side nucleus is intact which may be one of the possible causes behind the recovery. Cerebellar networks show long-term synaptic plasticity [42,43] which indicates that experience-dependent adaptive and learning processes are also a salient feature of cerebellar function [5] may compensate for the injury by structural remodeling and plasticity. These reports could justify the normal responses observed on the 15th day lesion group. It has long been apparent that the experiences of an animal could influence behavioral recovery from brain injury. Hence, it is not clear whether the unaffected side nucleus could take over the function entirely to have a normal movement or recovery of behavioral function or may be due compensatory changes by the non-damaged brain areas on the same side. Still elaborate research is still required to understand the molecular mechanisms behind rehabilitation and its motor behavioral outcome.

CONCLUSION

The unilateral fastigial nucleus lesioned animals showed a marked increase in the time taken to transverse across both narrow beam and grid runway. The increased forelimb and hind limb slips on the 10th suggest a significant impairment in their ability to integrate appropriate motor commands to balance their posture. A decrease in time spent on the rotarod on the 10th day alone and a not on the 15th day was observed in the entire motor performance task performed. This study suggests a possible neuronal plasticity or a compensatory behavior on the 15th day. Hence, it is not clear whether the unaffected side nucleus could take over the function entirely to have a normal movement or the recovery may have accounted due to the compensatory changes occurring in the non-damaged brain areas on the same side.

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