ORIGINAL RESEARCH PAPER

Volume-7 | Issue-7 | July-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ROLE OF HIPPOCAMPUS IN NAVIGATIONAL ABILITIES IN RATS WITH SELECTIVE LESIONS.

Physiology

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ABSTRACT

The role of the hippocampus in memory storage in the mammalian brain has been examined in depth. Previous studies show a differential effect of dorsal and ventral hippocampus lesions on various some operant tasks. Which predicts the non homogeneous structure of hippocampus and it can be predicted that hippocampus may have differential role in storing memory. So to examine the effects of discrete hippocampal lesions present study was carried out on navigational ability of rats by using water maze paradigm.

The detailed analysis of the behavioral performance of each lesioned group in present study provided some insight into the nature and the magnitude of the deficits after hippocampal lesions.

KEYWORDS

Dorsal and ventral hippocampus, memory, navigational ability.

Introduction

Despite large number of studies on all aspects of hippocampus the functions of this structure more studies are needed (Kosaki et al., 2014). Considerable advance in functional anatomical knowledge through tracer techniques and electrophysiological studies is made; but still it is not yet known what processes work on incoming signals or what functions are served by hippocampal output signals (Kosaki et al., 2014, Chen et al., 2013, Stig et al., 2001).

Although the hippocampus is involved in a broad range of memory types, it appears to have a particular function in spatial navigation and memory (Burgess et al., 2002, Hartley et al., 2000, Hughes KR. 1965). In spite of this, almost everyone would agree that the hippocampal system is involved in higher cognitive functions including memory. Hippocampal damage has been traditionally held as a cause of amnesia. The severity of memory deficit shows appositive correlation with the extent of damage to the hippocampus, It is also noted that lesions limited to hippocampal formation have been associated with almost negligible deficit in some studies and moderately deficit in others (Huxter et al., 2003., Kosaki et al., 2014, Lorens et al., 1975, Myhrer T. 1975).

Some studies have compared the effect of lesion in dorsal and ventral hippocampus show a differential effect of these lesions on some operant tasks (Hughes KR. 1965, Stig et al., 2001, Kosaki et al., 2014). It is known that hippocampal formation is not homogeneous structure. Based on this it is possible to think that dorsal and ventral hippocampus may have a differential role in storing memory. Therefore, it was proposed to examine the effects of discrete hippocampal lesions on navigational ability of rats by using water maze paradigm.

2. Material and Methods

2.1 Animals- 35 adult male wistar rats of 120 to 150 days (weighing 180 to 200 gms) were selected for the study. The surgical animals were housed in separate polyvinyl cages under 12 hour light and dark regime [light on at 600 hours and off at 1800 hours] and were fed with standard diet and water ad libitum. A prior permission was taken from the Institutional Ethics Committee of Rajarambapu College of Pharmacy, Kasegaon (RCPK) for use of rodents for the present research work. RCPK is registered under CPCSEA [Committee for the Purpose of Control and Supervision of Experiment on Animals]. The protocol of the present study was approved by the CPCSEA approved committee of RCPK.

2.2 Chemicals- Anaesthetics used for operative procedures are sodium pentobarbitone and ether. Sodium pentobarbitone is used to maintain a steady level of anaesthesia .Ether was used for induction of anaesthesia.The chemicals used for perfusion of animal at the end of experimental session are formal saline and potassium ferrocyanide. The chemicals used for histological procedure are absolute alcohol and alcohol in various concentration, chloroform and eosin.

2.3 Stereotaxy- The stereotaxic instrument (INCO, Ambala, India) was used for the present study. Lesion Maker-_S.S 44 square wave stimulator was used for making anodal lesions in predetermined sites. While the suction apparatus was used to suck the brain tissue by applying precise negative pressure. Intermittent section of the brain tissue was carried. The methods for preparation of microelectrodes their calibration and marking of lesions is described in detail in our previous studies.

2.4 Stereotaxic operative procedure for sham, ventral and dorsal suction of hippocampal lesion (Somade et al., 2015)**-**

The anesthetized rats were fixed in a stereotaxic apparatus through the ear bars. To achieve this first the ear clips were fixed into the ear of the animal. Then the ear bars were inserted in these clips and adjusted so that their distance on either side is equal and the head is fixed .The incision of 1.5 cm length was made with razor blades on the skin of the skull starting at level behind eyes, the membranous fascia covering the skull was cut away and the skull surface was made clear ,so that external landmark of sutures become visible. The bregma was taken as reference point and with the use of stereotaxic coordinates the site to be implanted where marked with ink using a dummy electrode holder. The holes where drilled at the marked points through the skull. The unipolar electrode varnished except at the tip was lowered sterotaxically in predetermined sites using coordinates from Paxinos and Watson. The other electrode was fixed to the ear of the animal. By using research stimulator S.S 44, a D.C anodal current of 1.5 mA intensity was passed for 20 seconds in lesions groups. The lesions where made bilaterally.

In sham group the electrode was lowered to dorsal and ventral hippocampus but no current was passed. The coordinates for dorsal hippocampal lesion were as under- Bregma - 3.8 mm, Lateral - 1.7 mm, Deep - 3.5 mm, Coordinates for ventral hippocampus were, Bregma - 5.8 mm, Lateral - 4.5 mm, Deep - 7.0 mm. After the procedure was over the electrodes were removed from the skull and skin was sutured and the site was cleaned with spirit. Nebasulf powder was applied and injection benzathene penicillin 10000 I.U. was given intramuscularly and animal was transferred to its cages.

2.5 Suction of hippocampus group- The animals assigned for suction group were anaesthetized and fixed in stereotaxic frame as above. Midline incision of 1.5 cm was taken on the skull and fascia and muscles were retracted. Bone surface was cleaned until its sutural landmarks were exposed. After adjusting the stereotaxic coordinates to dorsal hippocampus bilaterally points were marked on the skull surface and holes were drilled through it. A polythene tube of 48 gauge was attached to suction apparatus. The pressure of suction apparatus was adjusted to 40 mm Hg. The tip length of polythene tube was then passed through drilled hole and hippocampus was performed on the other side. Brain matter in the tube indicated that hippocampus is sucked. The animals allowed to recover from the operation and a week after the surgery, escape latencies were again determined for different group of rat as earlier.

2.6 Morris Water Maze (Morris 1981**) -** Morris water maze is a circular tank of 1.33 meters in diameter. 3/4th of the tank is filled with water and a wooden platform of 15 x 10 cm dimensions is placed 1cm above the water level in the tank at a fixed point of 0.33 meter from periphery. The water temperature was adjusted to 27°c ± 1°c during test. All the tests were conducted at 4 p.m. in evening.

2.7 Method to determine escape latencies- Rats were trained to locate the platform in Morris Water Maze in shortest possible time. First the animals were released in water facing the will from a fixed point opposite to the platform placed 1 cm above water level at a distance of 0.3 meters from the periphery. The rats learned to orient themselves correctly and swim towards and platform in shortest possible time. The time taken by rat to reach the platform after being placed in water is called as escape latency. The rats were given 3 trials at an interval of 10. The critical level for these escape latencies was less than 10 seconds. Once the score stable all the animals were blinded by enucleation under aseptic precaution by giving sodium pentobarbitone anesthesia intra peritoneal in a dose of 35 mg/kg body weight. They were allowed to recover from the operation and again tested for escape latencies after seven days. It was observed that all the animals regain preoperative score of escape latencies within 5-9 days after recovery from ennucleation.

The rats were assigned to 4 different groups randomly; group I; Sham group n=8, group II; Ventral hippocampal lesion n=8, group III; Dorsal hippocampal lesion n=8; group IV- Hippocampal suction n=13.

2.8 Statistical analysis- The mean of all trials for each set was calculated and analysis of variance was applied to see if the differences observed in individual groups were significant. This was followed by Tukeys multiple comparison test to test the level of significance of observed differences in individual groups.

3 Results

If rats are place in morris water learn to escape by they will quickly learn to escape by finding and climbing on to the small platform placed at affixed location over series of trials. That is the rat learns not only to recognize vicinity of safe place when they reach it, but also learn to swim towards it when they are released at a distant place in the water maze. By comparing the performance of control and lesioned groups we have examined the role of hippocampus in simple navigation.

3.1 Escapes latencies before and after enucleation- All rats swam effectively using the characteristic adult swimming posture. The time taken to escape from water by all rats before enucleation was 9.23 ± 3.53 seconds. After enucleation the rats did find slight difficulty in locating the platform during first three days. However the escape latencies were normal after 4th day. The mean escape latencies of this group were 11.92 ± 4.57 seconds.

3.2 Escapes latencies in sham, ventral, dorsal and hippocampal suction groups- In the sham group the mean escape latencies were 9.78 ± 6.90 seconds which is comparable to the preoperative score i.e. before and after enucleation. Similarly the animals in whom the ventral hippocampus was lesioned the mean escape latencies were 10.53 ± 2.03 seconds therefore same as sham group. In dorsal hippocampus lesioned group the mean escape latencies were 18.30 ±12.29 seconds. Although there is an apparent difference in the mean escape latencies this is the statistically not significant. However in suction group the mean escape latencies were 67 ± 30.22 seconds by applying ANOVA followed by Tukeys multiple comparison test it was seen that escape latency in this group was significantly high than other group.

4. Discussion

Converging evidence suggests that the hippocampus is essential for goal-directed spatial navigation. Successful navigation requires not only the ability to compute an appropriate path toward the target but is also guided by recognition of places along the trajectory between start and goal (Stig et al., 2001). Rats with hippocampal lesions were able to learn a cued version of the task, which implies that the failure to slow down was not attributable to motor inflexibility (Myhrer 1975). Thus, hippocampal lesions caused a severe but selective deficit in the identification of a location, suggesting that the hippocampus may be essential for image recognition during spatial navigation.

It is suggested that an intact hippocampus is necessary for the formation of stimulus-goal associations that permit successful passive spatial leaning (Burgess et al., 2002, Stig et al., 2001). It is further suggested that an intact hippocampus is not necessary for the formation of stimulus-response associations, except when they involve information about length or distance (Hartley et al., 2000, Kosaki et al., 2014).

The detailed analysis of the behavioral performance of each lessoned group of present study provided some insight into the nature and the magnitude of the deficits after hippocampal lesions. In the present study, the rats learned to locate the platform in few trials by using visual clues for navigation. They must have also used environmental cues close to platform. When faced with a problem such as escaping from water to a platform, the rat's usually do not act randomly but generate and test hypothesis about the solution. The hippocampal cognitive map theory states that the hippocampus calculates the animals location in an environment and also the locations of objects such as reword and threats.

The hippocampus is the brain structure that is necessary for normal memory functions in humans and in animals as seen in patients following medial temporal lobe removal or in patients with lesions limited to hippocampus (Huxter et al., 2003). It has been shown that lesions of hippocampus and extrinsic connections to the hippocampus cause profound deficits in learning and memory, as assessed in rats by performance in water maze tasks (Sinnamon et al., 1978, Kosaki et al., 2014). These finding have stressed the importance of hippocampus in memory storage as our study of hippocampus lesions showed a variable effect on escape latencies.

The present study also evaluated the role of dorsal hippocampus is spatial memory. For perceptual processing in neocortex to persist as a long term memory, information from neocortex must reach the medial temporal lobe. The projections from neocortex arrive initially in para hippocampal cortex and parrahinal cortex. Further processing then occurs that next stage,the entorhinal cortex and in the several stages of the hippocampul formation. This connectivity provides the hippocampus and related structures with access to ongoing cortical activity at wide spread sites through the neocorterx.

In suction groups the escape latencies were much higher ($P < 0.01$) than the sham and other lessoned group. These animals took a longer time to reach the goal as they were deprived of guidance and place hypothesis. The impairment displayed was also peculiar. They stopped short of the platform before reaching it and they frequently circled the area of platform before they found it. Curiously they also seemed to brush against the platform without orienting to it as in the control animals. Occasionally, they seemed to become lost and if they missed the platform on their search they displayed circling behavior. These behaviors are the main cause of their elevated latencies. These results indicate that the hippocampal lesioned rats can learn some sort of escape strategy to reach the platform. But despite extensive preoperative training they are substantially poor at locating the platform.

In present study, all the rats of hippocampus suction group were found to have a total or near total destruction of dorsal and ventral hippocampus, small amount of neocortex overlying the hippocampus with minimal damage to adjacent structures depicted in figure 3. These structures could be a part of the neural system involved in memory storage. The interaction between memory storage site and the structures damaged in the suction group may be necessary for retrieval of declarative memory which includes memory for spatial location.

Perhaps it is asking for too much to expect that a simple, one function theory will in time emerge. The hippocampus has enormous number of direct and indirect connections and Mormons cognitive function are notoriously difficult to pinpoint .therefore it should not come as a surprise to learn that there is at present little agreement on to the true function of the system .however pharmacological and electrophysiological studies could provide useful clues towards the understanding of information storage mechanism in hippocampus/

Conclusion

To conclude ventral as well as dorsal lesions of hippocampus were ineffective in producing impairment of escape latencies in rats. While the in the suction group, where nearly whole of the hippocampus with overlying cortex was removed the rats showed a marked impairment in their ability to reach the platform, this implies that nearly whole of the hippocampus has to be damage before the memory deficits are observed.

Figure 1 Escape latencies in groups before nucleation and after ennucleation.

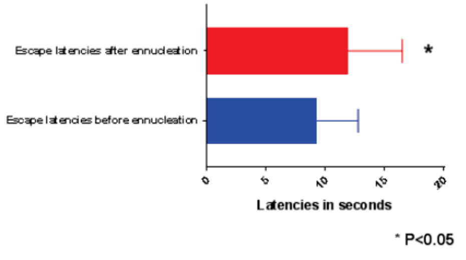


Figure 2A-Mean escape latencies in all groups

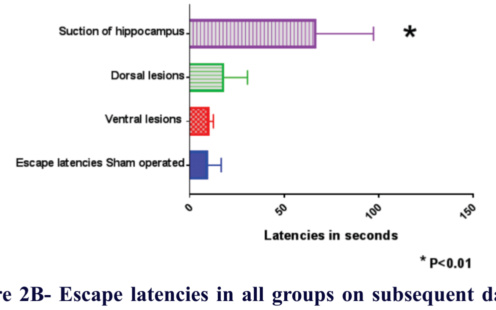


Figure 2B- Escape latencies in all groups on subsequent day of trials

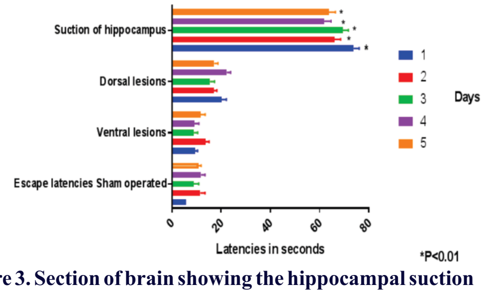
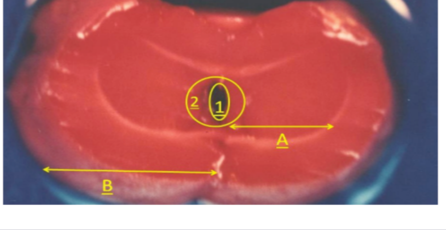


Figure 3. Section of brain showing the hippocampal suction



References

1. Burgess N, Maguire EA, O'Keefe J. 2002. The human hippocampus and spatial and episodic memory. Neuron, 35, 625–641.
2. Chen G, King JA, Burgess N, O'Keefe J. 2013. How vision and movement combine in the hippocampal place code. Proc Natl Acad Sci U S A, 110, 378–383
3. Hartley T, Burgess N, Lever C, Cacucci F, O'Keefe J. 2000. Modeling place fields in terms of the cortical inputs to the hippocampus. Hippocampus, 10, 369–379.
4. Hughes KR. 1965. Dorsal and ventral hippocampus lesions and maze learning: influence of preoperative environment. Can J Psychol., 19(4), 325-332
5. Huxter J, Burgess N, O'Keefe J. 2003. Independent rate and temporal coding in hippocampal pyramidal cells. Nature, 425, 828–832.
6. Kosaki Y, Lin TC, Horne MR, Pearce JM, Gilroy KE. 2014. The role of the hippocampus in passive and active spatial learning. Hippocampus. 24(12), 1633-1652.
7. Lorens SA, Köhler C, Guldberg HC. 1975. Lesions in Guddesn's tegmental nuclei produce behavioral and 5-HT effects similar to those after raphe lesions. Pharmacol Biochem Behav., 3(4), 653-6599.
8. Morris RGM. 1981. Spatial localisation does not depend on the presence of local cues. Learning and Motivation 12, 239–260.
9. Myhrer T. 1975. Locomotor, avoidance,and maze behavior in rats with selective disruption of hippocampal output. J Comp Physiol Psychol. 89(7), 759-777.
10. O'Keefe J. 1976. Place units in the hippocampus of the freely moving rat. Exp Neurol. 51, 78–109.
11. Sinnamon HM, Freniere S, Kootz J. 1978. Rat hippocampus and memory for places of changing significance. J Comp Physiol Psychol. 92(1), 142-55.
12. Somade PM, Chopade AR, Brid SV. 2015. Modulation of Pain by Naloxone and a Possible Role of Neurotransmitters with Selective Lesion of Septal Nuclei. J Spine 4: 263.
13. Stig A. Hollup, Kirsten G. Kjelstrup, Joakim Hoff, May-Britt Moser, and Edvard I. Moser, 2001. Impaired Recognition of the Goal Location during Spatial Navigation in Rats with Hippocampal Lesions. The Journal of Neuroscience, 21(12), 4505–4513.