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A study on effect of hippocampal dentate gyrus lesion on acquisition and retention of working memory assessed using radial arm maze in wistar albino rats

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Abstract:

Hippocampal lesion can impair internally cued discrimination in rats. This may be due to either impairment of the perception of the internal cue (subjects do not experience hunger) or its use (the subject experiences hunger but cannot solve some other aspect of the problem). There is strong evidence that hippocampal lesions also impair use of spatial cues by rats. The aim of this study was to investigate the allocentric spatial learning affected by damage to hippocampal Dentate gyrus studied using all arm baited procedure in eight arm radial arm maze, a task involving spatial learning and memory retention. A total of 24 male wistar albino rats were were randomly assigned into four groups with 6 rats in each group. Dentate gyrus lesion was carried out by placing the electrodes stereotaxically into the brain area through the respective holes in the skull. All the behavioral experiments were carried out in three phase's viz. orientation and training session, learning performance test (retention test) by using radial arm maze. Dentate gyrus lesion by electrolyte & thermo coagulatory method caused significant impairment in the acquisition of spatial task, but not the retention. We conclude that Dentate gyrus is found to be an important area for spatial information coding and processing during the early stage of spatial learning in the rats.

Key words: Dentate gyrus lesion; Radial arm maze; Spatial learning; Stereotaxi; Working memory.

Introduction

Understanding the physiological basis of behavior is one of the major goals in the contemporary neuroscience. Behavior is considered as the outcome of an interaction between inborn (Nature) and environmental factors (Nurture). The most important environmental factor in shaping the brain and behavior is learning. Learning can be defined as the process of modification in the organization of the brain. Learning is not a unitary process; it has multiple processes and is defined as 'an enduring change in the mechanism of behavior that results from experience with environmental events'. While learning is concerned with acquiring new information, memory refers to the persistence of a change in behavior over time, in a state that can be retrieved at a later time. Learning and memory form an important tool for an organism to interact with the environment, resulting in the modification of the nervous system. Accordingly, unraveling the physiological basis of learning and memory may lead to the understanding of the neural basis of behavior.

The hippocampus is one of a group of structures within the limbic system called the hippocampal formation, which includes the dentate gyrus, hippocampus, subiculum, and pre subiculum and para subiculum and entorhinal cortex [1]. The existence of relatively direct connections between hypothalamic nuclei and ventral hippocampus suggests that the ventral hippocampus may be involved in acquisition of information regarding internal cues (hunger). Dorsal hippocampus lesion impairs the formation of spatial memory [2]. Working memory is defined as retention of information that is useful within a session of an experiment, and reference memory is defined as retention of information that can be used across sessions. The allocentric place discrimination task (APDT) is simple and useful radial arm maze test for spatial working memory in rats [3,4].

Animals with lesions of the hippocampal system (hippocampus proper, fornix, anterior thalamus) are severely impaired in spatial task such as place learning in the water maze, searching for food in the radial maze, T-maze alternation and object - place association [5,6,7]. These findings together with the discovery of place cells in the hippocampus gave rise to the theory that the hippocampus functions to construct a map like representations of the world, where in places are defined allocentrically, in terms of their relationships to landmarks [8,9]. It has been widely accepted that the integrity of the hippocampus formation is essential for the efficient performance of conditional discriminations by the rats [10,11] Hippocampal lesion can impair internally cued discrimination in rats. This may be due to either impairment of the perception of the internal cue (subjects do not experience hunger) or its use (the subject experiences hunger but cannot solve some other aspect of the problem). There is strong evidence that hippocampal lesions also impair use of spatial cues by rats [8].

The aim of this study was to investigate the allocentric spatial learning affected by damage to hippocampal Dentate Gyrus studied using all arm baited procedure in eight arm radial arm maze, a task involving spatial learning and memory retention.

Materials and Methods

The present study was an experimental study conducted at Little Flower Medical Research Centre. Angamaly, Kerala, India.

Ethical approval: The study protocol was approved by Institutional Ethics Committee (**LFMRC-344N-2012**) of Little Flower Medical Research Centre, Angamaly. **Subjects:** A total of 24 male wistar albino rats were used for this study. They were housed in groups, in propylene cages in an acclimatized (25-27^{o}C) room and were maintained on a 12hr light / dark cycle. Food and water was given *ad libitum* until they aged 60 days at the beginning of the experiment.

Apparatus:

Radial arm Maze: Radial arm maze is made of Plexiglas; consist of eight equally spaced arms radiating from an octagonal central platform. Each arm was having a length of 56.2cm, width of 7.9 cm and height of 10 cm. The entire maze is elevated 80 cm above the floor for easy locating of spatial cues by rats.

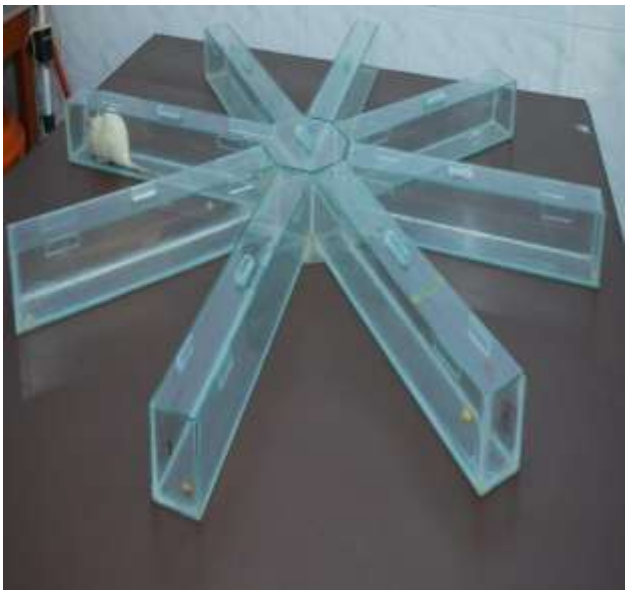
Experimental design:

The rats were randomly assigned into four groups with 6 rats in each group.

(a) Control group
(b) Sham group
(c) Lesion before acquisition
(d) Lesion after acquisition

Weight of the rats in lesion group was noted. Then they were anaesthetized with Ketamine hydrochloride.

Figure 1: Radial arm maze



The experimental animal's hair on the skull from neck to eye level was shaved cleaned and 2% Lignocaine (local anesthesia) was injected subcutaneously. The anaesthetized rat was fixed on the stereotaxic instrument. The surface of the skull was cleaned with cotton soaked in physiological saline (0.9%). The head of the rat was fixed horizontally with the head holder of the stereotaxic apparatus. The bregma was taken as the reference point. After the skull was dried the position of the area to be lesioned were marked stereotaxically and small holes were made on the skull by drilling.

Lesion was carried out by placing the electrodes stereotaxically into the brain area through the respective holes in the skull. The ear bar served as cathode and electrode as anode. A steady flow of Direct Current of 20mA was passed for 5 sec in order to lesion the dentate gyrus. The electrode was thereafter withdrawn from the brain.

The wound was covered with zinc cement. After covering the wound, using zinc cement the antibiotic powder was applied. At least for 12 hours of postoperative period, the animals were not given food and water. The operated animals were kept in separate cage with food and water.

All the behavioral experiments were carried out in three phase's viz. orientation and training session, learning performance test (retention test). The rats were semi starved for 48 hrs before the start of behavioral experiments. The body weight was maintained at 85% of the original body weight, throughout one session of behavioral experiments.

Figure 2: Stereotaxic instrument with the anesthetized rat

Behavioral experiments were conducted in the same room with the same allocentric cues, such as doors, windows, posters and experimenter. Experimenter always maintained same position though out the whole of the experiments.

Radial arm Maze task:

Orientation phase was for three days, where the starved rats were allowed to familiarize themselves with the radial arm maze. To avoid olfactory cues, the maze was wiped with 70% ethanol prior to each session. In this protocol 1, all the eight arms were baited with food pellets (cornflakes). The rat was placed in the center of the maze and allowed to freely explore the maze for 15 minutes on the first day. The rats were required to take the food pellets from each arm without making a reentry into the arm already visited. The trial was terminated when the animal takes the food reward from all the eight arms or after 10 minutes if all the eight arms were not visited. A correct score was given when the rat visits an arm and collects the food reward, and a maximum score of '8' can be attained per trial.

When a rat reenters an already visited arm or doesn't enter an arm, it was taken as a working memory error. The acquisition test was continue until the rats attained learning criteria of obtaining a correct score >= 7, and an error <= 1, for three consecutive trials. Four trials / day was given with an inter trial interval of one hour. Ten days after the last day of acquisition of the task, the rats were subjected

retention test. It was continued until the learning criteria were attained. The memory score was calculated by taking the difference between the number of trials required for acquisition test and number of trials for retention test.

After the completion of the experiment, the animal is weighed and the amount of fixative required is 2-3 times the weight of the animal. The animal is deeply anaesthetized with chloroform. The thorax was cut opened and heart was exposed; a needle connected to the tubing from the fixative bottle, is inserted into left ventricle. The right atrium was cut open to drain out the blood and fixative. First, 20-30 ml of saline is passed transcardially to flush out the blood then per fused with formalin. After perfusion the animal is decapitated, the brain is shelled out and kept in 10% formalin for minimum 2-3 days for proper fixation. Commonly used fixatives are 10% formalin, 4% paraformaldehyde, 1% paraformaldehyde + 1.25 glutarldehyde etc.

The fixed tissue has to be processed further to get good sections. Tissues are either freezed or embedded in paraffin wax or hard material like celloidin, low viscosity nitrocellulose, araldite, epoxy resin, etc for sectioning. L-shaped metal pieces are used to form paraffin blocks. Wax is poured in the block and air bubbles are removed. The tissue is quickly placed with the help of a warm forceps and oriented as desired (In our study the brain tissue is placed with the spinal end facing down). The block is trimmed and a wooden block is fitted to it and kept ready for sectioning. The paraffin block sections are obtained using rotary microtome. The microtome is adjusted for suitable thickness of 5-10 micrometer. The level of knife and block is approximately adjusted and sections were taken. The sections are collected in water. The sections are mounted on albumin or gelatin coated slides and the slides are kept on the slide warmer. The paraffin will melt slowly and it helps in spreading of the sections and also the sections will adhere to the surface of the slide. Sections are taken on egg albumin coated slides. This gets coagulated and once water evaporates sections adhere firmly to the slide.

The slides are dipped in xylene and the sections are pressed using series of alcohol grades (down grading-100%, 90%, 80%, and 70%). Then the sections are treated with Haematoxyline and Eosin. The slides are mounted encased in a medium of suitable refractory index for microscopic observation and also for preservation.

The site of lesion was confirmed after completion of all the behavioral experiments. The rats were sacrificed and brain shelled out, processed, sectioned, stained and then observed for lesion site. The histology revealed the exact location of lesion (Fig. 3,4).

Figure 3: Section of a sham group rat brain showing dorsal hippocampus

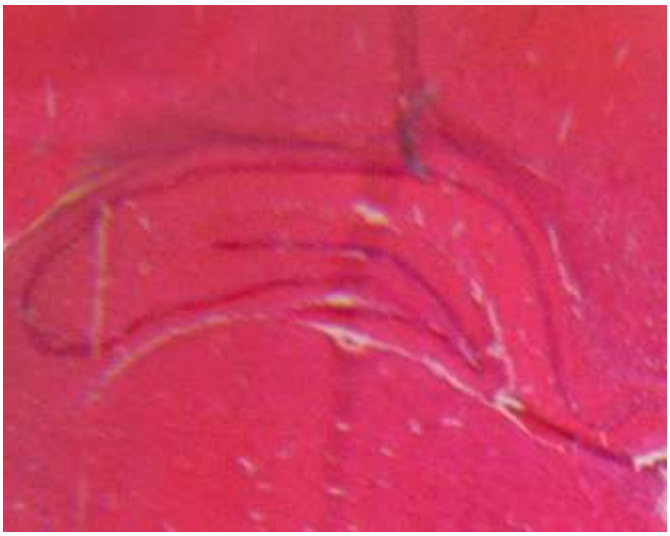
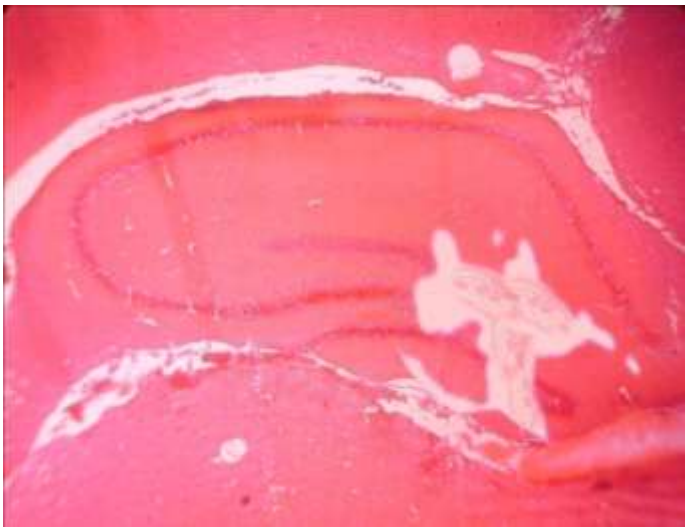


Figure 4: Section of a rat brain from DG lesioned group showing dorsal hippocampus with lesion at DG



Data analysis:

Statistical comparison between groups for behavioral studies was analyzed by one-way analysis of variance (ANOVA) and followed by Tukey–Kramer multiple comparisons test. To compare means of acquisition to retention within the same group paired t test was used. Analysis was performed using GraphPad InStat version 3.06 for Windows, GraphPad Software, San Diego California USA.

Significance was accepted at P<0.05. Means ± SD are reported.

Results

Table 1: One way ANOVA table for comparison of acquisition for all the groups

Source of variation	Degrees of freedom	Sum of squares	Mean square (MS)
Treatments (between columns)	3	1753.1	584.38
Residuals (within columns)	20	76.833	3.842
Total	23	1830.0	

Table 2: One way ANOVA table for comparison of retention for all the groups

Source of variation	Degrees of freedom	Sum of squares	Mean square (MS)
Treatments (between columns)	3	2480.8	826.94
Residuals (within columns)	20	55.000	2.750
Total	23	2535.8	829.69

Table 3: Summary of results

Groups	Average no. of trials required for	
	Acquisition	Retention
Control group	$21 \pm 2.280^{***}$	16 ± 1.789
Sham group	$21.833 \pm 1.941^{***}{}_{NS\#}$	$16.33 \pm 1.862^{NS\#}$
Lesion before acquisition group†††	$41 \pm 2.096^{NS*}{}_{\#\#\#}$	$40.83 \pm 1.1690^{\#\#\#}$
Lesion after acquisition group‡‡‡	$21 \pm 1.4142^{NS*}{}_{NS\#}$	$21.17 \pm 1.7224^{\#\#\#}$

Results are mean ± SD

NS*- not significant, *** - p<0.001 average number of trials required for acquisition vs. retention within each group, compared using paired t test.

NS#- not significant, ### - p<0.001 average number of trials required for acquisition or retention in each group compared to respective control group using one way ANOVA and followed by Tukey's post test.

††† - p<0.001 average number of trials required for retention by lesion before acquisition group compared to lesion after acquisition group using one way ANOVA and followed by Tukey's post test.

‡‡‡ - p<0.001 average number of trials required for acquisition by lesion before acquisition group compared to lesion after acquisition group using one way ANOVA and followed by Tukey's post test.

Paired t test is used to compare the acquisition and retention within each group. The number of trials required for acquisition of RAM task by control group was approximately 21 trials where as for retention, control group took only 16 trials, indicating that retention required only 25% less number of trials than acquisition. When number of trials for acquisition and retention of control group was compared using paired t test a very high significant difference at p<0.001 was observed with a t value of 9.682 at 5 degrees of freedom.

Similarly, for sham group also 25% difference in the number of trials taken for acquisition and retention was observed. When the acquisition and retention trials of sham group was compared there was also a very high significance (p<0.001) with a t value of 16.102 at 5 degrees of freedom. The number of trials for acquisition and retention, taken by the sham group and control group were compared by oneway ANOVA followed by Turkey's post test, significant difference was not observed (p>0.05). Since control and sham group took approximately same number of trials for acquisition and retention, and have similar statistical values, these groups were considered as similar. So for further analysis in lesion groups were compared with control group.

The number of trials for acquisition and retention taken by lesion after acquisition group was approximately 20 and 21 trials respectively. When these values were compared using paired t test, there was no significant difference (p>0.05) with a t value of 0.2997 at 5 degrees of freedom. The number of trials for acquisition taken by lesion before acquisition group was more than double that taken by the control group. These values were statistically analyzed using one way ANOVA and followed by

Tukey's post test. A significant difference with $p<0.001$ between the two groups were observed.

The number of trials for retention was also more than double that taken by the control group. The average number of trials were statistically analyzed and found that there was a significant difference with $p<0.001$. The number of trials for acquisition and retention of lesion before acquisition group was compared using paired t test. No significant difference was observed ($p> 0.05$) with a t value of 0.237 at 5 degrees of freedom.

The number of trials for acquisition taken by lesion after acquisition group was approximately 20 trials and for retention they took approximately 21 trials. From that it can be seen that the lesion after acquisition and control group took similar number of trials for acquisition. There is 23% increase in the number of trials for retention taken by lesion after acquisition group than control group. There was no significant difference ($p>0.05$) between the acquisition of lesion after acquisition group and control group, but there exists a significant difference ($p<0.001$) between the retention of lesion after acquisition group and retention of control group. The number of trials for acquisition and retention of lesion after acquisition group was compared using paired t test. No significant difference was observed ($p>0.05$) with a t value of 0.2997 at 5 degrees of freedom.

Lesions before acquisition group took double the number of trials for acquisition and retention when compared with lesion after acquisition group. When they were statistically analyzed using one way ANOVA and followed by Tukey's post test there was a significant difference at $p<0.001$ between lesion after acquisition and lesion before acquisition group for both acquisition and retention.

Discussion

Role of hippocampus in spatial learning was studied in this experiment. The primary finding of the present study was that bilateral lesions of dorsal hippocampus by electrolytic and thermo coagulatory method resulted in spatial learning impairment. Behavioral studies were conducted in RAM/T-maze working memory task. CA3/CA1 lesions resulted in impairment in the acquisition of spatial learning task, since lesion before acquisition group took far more trials to reach the criterion.

Hippocampal structures are linked, one to the next by unique and largely unidirectional projections. From entorhinal cortex information reach the dentate gyrus through perforant pathway and through mossy fiber information will reach the CA3 of hippocampus proper. And CA3 sends information to CA1 of hippocampus proper through Schaffer collaterals. From CA1 the fibers are passing to subiculum and the subiculum has diverse connections with other cortical and subcortical areas [12].

When there is Dentate Gyrus lesion the informational outflow will not be completed. Thus Dentate Gyrus lesion will delink the hippocampus with other structures. This may be responsible for the delay in acquisition of spatial learning by lesion before acquisition group as they took double the number of trials for acquisition than control group. Moreover lesion in rats was unable to improve their performance in postoperative training, while the control rats showed improved performance. Retention of the spatial learning was unaffected by Dentate Gyrus lesion. This might be due to the fact that learnt information is stored in different areas of brain and its recollection may not involve hippocampus. The hippocampal circuitry is found to be involved in computations required to learn about the spatial lay out of the environment[13,14]

The selective lesions of Dentate Gyrus field produce impairment in the acquisition of the complex place and cue tasks in rats. It is suggesting that hippocampus may be required for the conditional learning in rats. Various theories were proposed regarding the hippocampal function. In the spatial mapping theory, hypothesized that the hippocampus is especially involved in establishing a cognitive map of the environment [15]. According to the working memory theory of and the temporary memory buffer hypothesis of Rawlins and Tsaltas, (1983), the spatial or non spatial nature of the information being processed is not important, but rather emphasis is placed on the role of the hippocampus, in processing temporal relation among correct stimuli and representation of recent events [16,17,18]

It was proposed that rats with hippocampal damage show impairment in path integration, i.e. in a process of active monitoring of movements in space, rather than in place learning per say (path integration hypothesis) [19]. But in our study, only spatial learning task was used, hence role of hippocampus in non spatial tasks couldn't be studied. Present study involves the working memory component, since rats with hippocampus lesions showed impairment in the acquisition of rewarded alternation test in T-maze/working memory in RAM. Morris *et al.*, (1990) showed an impairment of memory in maze task in rats after hippocampus lesion [20]

The present study revealed that dorsal Dentate Gyrus lesion by electrolyte & thermo coagulatory method caused impairment in the acquisition of spatial task, but not the retention. It is important to note that the rats with selective lesions of Dentate Gyrus were not able to learn the rewarded alternation task/working memory task in RAM, but was normal in acquiring stored spatial information. Apparently, the normal retention may be possible because animals can use an alternative strategy that involves the learning to approach the correct area of the maze using the neocortical regions, whereas the impairment in acquiring new information may be due to the selective damage in these areas, which may participate in the transferring of processed hippocampal information to the neocortex.

Conclusion

The present study suggests a possible role of hippocampus in spatial learning paradigm, it may be involved in the acquisition of new information rather than retention. The damage to the hippocampal Dentate Gyrus region may cause disruption of information processing. The present study suggests that Dentate Gyrus is found to be an important area for spatial information coding and processing during the early stage of spatial learning in the rats. The result of the present study may indicate the dendritic atrophy of hippocampus neurons following selective Dentate Gyrus lesions. This might be responsible for impairment in spatial memory task of rats. Lesions of Dentate Gyrus area might have also altered the morphology of neurons in regions related to hippocampal formation. The study conclude that pyramidal neurons of the hippocampus play a crucial role in learning and memory and loss of these neurons may cause severe impairments in various behavioral tasks.

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Conflicts of Interest:

The authors hereby declare that there is no conflicts of interest

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