Hippocampal Stimulation of Fornical-lesioned Rats Improves Working Memory

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Abstract: Intrinsic rhythmic electrical activity in the brain, such as the hippocampal theta rhythm, might serve important roles in normal cognition. Lesions to the medial septal nuclei, or to the fimbria/fornix, disrupt the hippocampal theta rhythm and lead to memory impairment. We have superimposed an artifical stimulating rhythm to the hippocampus of rats with prior lesion of the fornix, during testing in the Morris water maze. This intervention improves performance in a test of working memory, and lends support to the view that intrinsic rhythmic activity may play an important role in normal physiology, and in certain disease states.

Résumé: La stimulation de l'hippocampe de rats porteurs d'une lésion du fornix améliore la mémoire. Il est possible que des rhythmes électriques intrinsèques au cerveau jouent un role important dans la mémoire et dans d'autres processus physiologiques. Des lésions aux noyaux septaux ou au fornix perturbent les rhythmes thêta et résultent en un déficit de memoire. Nous avons stimulé l'hippocampe de rats dont le fornix a été sectionné, et les avons soumis à des tests de mémoire. L'intervention produit une amélioration importante de la mémoire et renforce l'idée que les rhythmes électriques intrinsèques au cerveau jouent un rôle important dans les processus physiologiques et pathologiques.

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It now seems likely that most types of memory entail changes to synapses distributed over a large number of neurons, such that the discharge pattern of a network of neurons is changed.[1] The anatomical localization of these putative networks of neurons is not known, but the integrity of the hippocampus and related structures appears essential for certain types of learning.[2,3] If appropriate temporal-spatial relationships are to be preserved, it also seems likely that some form of temporal synchronization of the neural activity over the neural net in question must exist. It has recently been suggested that spatially separate neurons in the cat visual cortex can display synchronous activity and that this synchrony provides a temporal code allowing for the reintegration of the distributed activities and unambiguous representation of visual objects.[4]

Synchronous activity at the theta frequency can be recorded in the rat hippocampus under diverse conditions, including those when learning might be occurring (reviewed in O'Keefe and Nadel[5]). It is likely that the hippocampal theta rhythm in rats is not generated in the hippocampus per se, but involves an external pace-maker in the basal forebrain (medial septal region/vertical limb of the diagonal band of Broca).[6-8] Complete lesions to this area result in loss of hippocampal theta rhythm,[9] and memory loss in rats[10] while incomplete lesions, which do not result in loss of theta activity, do not result in memory impairment.[10] Septal anaesthesia can impair short term spatial memory in rats.[11] Long term potentiation (LTP), a model of learning and memory, is best accomplished in rats when triggering stimuli are time-locked to the theta rhythm,[12] and Valjakka et al.[13] have demonstrated that dentate LTP is impaired in fimbria-fornix lesioned rats. Finally, the basal forebrain in man is affected in certain diseases producing memory loss, such as Alzheimer's disease where it appears as an early, marked, and consistent finding.[14] On the basis of these observations, we postulate that some form of synchronizing activity is essential for human memory, that some of the memory disturbance in certain amnestic states may arise from a lack of synchronising activity, and that some of the amnestic deficit in these conditions might therefore be reversed by artificially imposing a synchronizing rhythm to the hippocampus. The following experiment was designed to provide an initial test of this hypothesis.

Methods

We used a fine encannulated wire knife[15] to produce fimbria/fornix cuts in 12 male Long-Evans hooded rats weighing 380-450 g. In this procedure, a cannula with an angled tip is lowered to a point above the intended site of transection. The wire is then pushed out of the cannula for several millimeters (depending upon the intended width of the cut). The cannula is lowered for the intended depth of the cut, after which the wire

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and the cannula are withdrawn. The coordinates were selected from the atlas of Paxinos and Watson,[16] and adjusted according to a pilot experiment to produce complete fimbria/fornix lesions. Six control animals were treated identically but the final knife-cut to the fornix was not made. One month after the initial surgery all 18 animals underwent a second procedure to place electrodes in the dentate gyrus and the perforant path of the right hemisphere. Electrodes were implanted using electrophysiological monitoring procedures, as previously described.[17] A permanent acrylic skull cap secured the electrodes. Eight weeks after the second surgery the animals were tested in a water maze test[18] using standard tests of acquisition and reversal. As expected, the lesioned animals demonstrated impaired performance compared to controls. These results do not bear on the present experiment, and will be described in a separate paper.

The lesioned animals were then randomly divided into two groups of six animals, and together with the controls were subjected to testing in a water maze using a slight modification, described below, to the "working memory" protocol[19] known to be sensitive to hippocampal dysfunction. The water maze was a circular white fibreglass tank, 185 cm in diameter, and filled to a depth of 22 cm. The animals were tested daily. At the beginning of each day the platform was placed in one of three quadrantic locations, in a pseudo-randomized fashion, while the starting location in the fourth quadrant was constant. A total of four trials were given per day. The first trial served to familiarize the animal with the new location, and the principal outcome measure was the time taken to reach the platform on the three subsequent trials. If the platform was not reached within 60 seconds the trial was stopped and the animal placed on the platform for 20 seconds. Each trial was separated by 20 seconds. Since several animals with fornical lesions were removed at 60 seconds, the outcome measures were not normally distributed. We therefore used non-parametric methods (Kendall rank order; Friedman ANOVA by rank) to compare the three groups, with conservative assumptions for multiple comparisons.

On selected test days lesioned animals received a stimulation train through the electrode in the perforant path. The stimulation was begun 5 minutes prior to the first trial of the day and continued until all four trials were completed (about 10 minutes). It consisted of a train of pulse-pairs, with an interpulse interval of 10 ms, and an intensity just sufficient to produce a population spike in the dentate electrode on the first stimulus. The pulse-pairs were repeated at a frequency of 5 Hz. The choice of stimulation parameters was arbitrary, and represented a compromise between stimulation intense enough to induce synchronicity and low enough to prevent spontaneous discharges and seizure. On the first day, no stimulation was given to either lesion group. For the subsequent five days, one of the lesioned groups was stimulated during testing. After a rest period of two weeks, all three groups were tested again without stimulation, and for the subsequent five days those lesioned animals receiving stimulation during the first week were not stimulated, while those not receiving stimulation the first week were stimulated. Finally, all animals were tested in the water maze with the platform visible, to control for psychomotor differences not related to memory deficits. No animal was stimulated during this phase, and a total of 8 trials were run sequentially for each animal with the platform placed randomly in a different quadrant on each trial. At the end of this experiment, all animals were sacrificed to verify the completeness of the lesion to the fimbria/fornix, and the position of the electrodes.

Results

The primary results are presented in Figure 1. This figure shows the mean time to escape to the platform for trials 2-4 of each daily block of 4 trials. Twelve blocks were run as explained above. On blocks 1 and 7 no synchronizing stimulation was given to any group and since there were no significant differences between the two blocks, for simplicity the results were averaged and shown as block 0, representing the unstimulated control condition. During blocks 2-6 one group of lesioned animals (open circles) received stimulation to the perforant path. During blocks 8-12 the other group (open squares) was stimulated. Data from the stimulated animals are identified on the graph by an asterix. The unlesioned animals (closed circles) received no stimulation throughout. For convenience, the average time taken to escape to a visible platform is included in the same figure, and is shown arbitrarily as block 14.

It is possible to draw three comparisons for each block (control vs. stimulated, control vs. unstimulated, stimulated vs. unstimulated). It is possible to draw similar comparisons for the combined results of blocks 2-6; and of blocks 8-12. Lastly it is possible to compare blocks 2-6 with 8-12 for the same animals (that is same group stimulated vs. same group unstimulated). There is no ideal measure since individual comparisons lack power and cumulative comparisons assume independence and further ignore such factors as learning between blocks. For these reasons we show the calculated p values for all comparisons, in Table 1. Three general conclusions are possible. First, the two groups of unstimulated lesioned animals each differ significantly from unlesioned controls but not from each other. This is expected. Second, stimulated animals perform better than

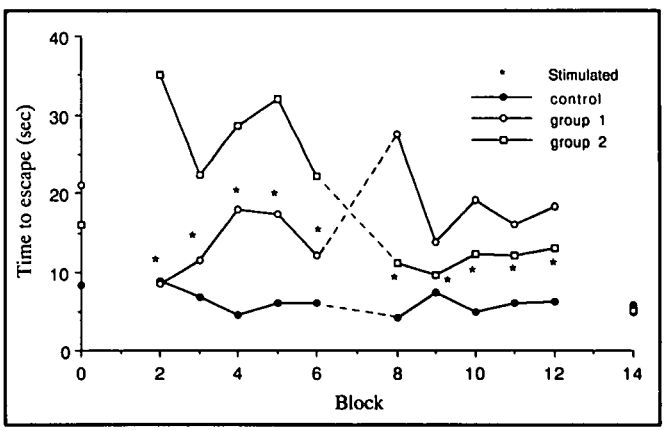

Figure 1: *The average time to escape to the platform for trials 2-4 of each block is shown. As indicated one group of lesioned animals was stimulated during blocks 2-6, and the other group stimulated during blocks 8-12. For convenience two controls points are included in the same figure. No animals were stimulated in block 0, and the time to escape to a visible platform is shown in block 14.*

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Table 1. P Values for Comparisons of Escape Times.

Block	Control vs group 1	Control vs group 2	Group 1 vs group 2
0	.01	.01	.76
2	.70	.04	.02
3	.01	.01	.08
4	.01	.01	.30
5	.03	.01	.05
6	.21	.02	.23
8	.01	.01	.01
9	.12	.68	.18
10	.01	.01	.94
11	.07	.24	.60
12	.02	.01	.89
Blocks 2-6	.01	.01	.01
Blocks 8-12	.01	.01	.04
			Stimulated vs unstimulated
Blocks 2-12			.01

Significance values are shown for comparisons between the unlesioned (control) animals, and the two otherwise identical groups of lesioned animals (group 1 and group 2). Three comparisons are possible for each block (control vs. group 1, control vs. group 2, group 1 vs. group 2). These are shown for blocks 0 (no stimulation to any group), blocks 2-6 (group 1 stimulated) and blocks 8-12 (group 2 stimulated). Significance data are also shown for the pooled data from blocks 2-6 (group 1 stimulated), and blocks 8-12 (group 2 stimulated). Also shown is the comparison of group 1 stimulated compared with group 1 unstimulated, combined with similar data for group 2 stimulated and group 2 unstimulated.

unstimulated animals. This is true in all 10 individual blocks, and reaches customary statistical significance in 3, (2 of which are less than $p = 0.02$). When the results are combined to give greater power, the results are highly significant in favour of stimulation, especially in blocks 2-6 (before cross-over). This conclusion holds using either combined Kendall rank order or a Friedman non-parametric analysis of variance. Third, neither stimulated lesioned group performed as well as the control group. This was true for 19 of 20 possible individual comparisons, 14 of which reached customary statistical significance. There was no difference in the time taken to reach a visible platform. All animals performed well and any effect is not due to differences in psychomotor function resulting from the surgery. In addition all trials were videotaped and it is clear that stimulated animals were neither better coordinated nor faster swimmers than unstimulated animals.

Discussion

We have demonstrated that the artificial imposition of synchronizing activity at the theta frequency to the hippocampus of fornical-lesioned animals improved performance in a working memory variation of the Morris water maze. The results were significant, and were likely due to the intervention and not to chance occurrence, (since the results changed following cross-over of the stimulated and unstimulated groups). The difference was most pronounced before cross-over, suggesting that some learning had occurred in the stimulated animals. Even though the phenomenology appears robust, it is not yet clear that the explanation for the improvement in memory is due to restitution of synchronizing activity in the hippocampus as we postulated, and several alternative explanations are possible. For example, the improved performance in the stimulated conditions may reflect a general facilitation that is not specific to the train frequency. Lower or higher frequencies might work as well. In subsequent experiments it will therefore be important to verify that the effect is specific to the driving of the hippocampus at the theta frequency. Even if the theta frequency proves optimal, it remains to be seen whether this simply reflects the best frequency for a facilitation effect, or if the theta pattern is providing a missing temporal or "clocking" cue. Whatever the final conclusion, any intervention which improves memory deficit to this extent has intrinsic interest.

Similarly, it will be important to understand if this effect can be reproduced in aged animals, either by stimulating the hippocampus or the medial septum. If so, then considerable light could be shed on the mechanism of memory deficit in senescence, and in certain disease states such as Alzheimer's disease. Positive results might also explain why cholinergic supplementation does so little to improve the amnestic deficit in Alzheimer's disease because, even though there are severe deficits in the cholinergic septal system, the synchronizing input would still be missing.

Last, it should be noted that brain stimulation did not restore memory in this test to normal, and stimulated animals still performed less well than unstimulated controls. Our stimulation was unilateral and it is conceivable that bilateral hippocampal stimulation would improve the results. (However, since unilateral hippocampal lesions are generally well tolerated, this seems unlikely.) Alternatively, different stimulation sites or parameters might yield improved results. We did not observe adverse effects from the stimulation regimen we employed. There was no evidence of long-term potentiation, kindling of seizures, or sleep disturbance. Nonetheless the risk of these effects could be increased by alternative (longer, higher intensity) stimulation protocols.

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