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SIGNIFICANCE OF CA3 REGION OF HIPPOCAMPUS IN MEMORY FUNCTION

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Abstract: In mobile unanaesthetised adult rabbits, spontaneous single unit activities of CA3 neurons of Hippocampus showed a specific change in firing pattern in response to conditioned stimuli (CS+). This pattern could be correlated with changes in the general behaviour of these animals. Major deafferntation of the CA3 region did not alter the conditioned response, though neuronal firing pattern and behavioural response changed to that of the pre-conditioned state after antidromic stimulation of CA3 region through fimbria. The importance of CA3 region in memory retention process has been indicated in these experiments.

Keywords : CA3 conditioning hippocampus memory single unit activity

INTRODUCTION

The key role played by hippocampus in memory functions has been well documented in the past (1, 2, 3, 4, 5, 6, 7). The organised, widespread afferent and efferent connections of hippocampus with other association areas of cortex, limbic systems and subcortical regions (8, 9, 10) probably make it a neuronal computer with sensitive memory functions. Behavioural correlation with hippocampal unit activities have been worked out earlier by giving different memory tasks by many workers (7, 11, 12, 13, 14, 15, 16, 17, 18, 19). However, the importance of the sub-regions within the hippocampal formation have not been worked out in detail. Most of the emphasis on hippocampal memory retention have been attributed to the granule cells of dentate region and the CA1 region (13, 15, 16). On the other hand, in vitro studies of CA3 neurons showed post tetanic potentiation and long-lasting after-discharge (20, 21). Also, in earlier studies in mobile rabbits, these neurons demonstrated "habituation" property (17, 22, 23). Any neuron possessing these properties should be highly suitable for memory functions (6, 22, 23). So the present study was undertaken to assess the role of CA3 neurons by de-afferentation and antidromic stimulation. The CS+ was considered as the memory task.

METHODS

Twenty four adult rabbits of either sex were used. The experiments were done in two phases. In phase A, the animals' behaviour and the single unit activities from CA3 region were studied in pre-conditioned state. In phase B, the same animals were conditioned by a tone of 600 Hz for 6 seconds followed by food. Under local anaesthesia (3 ml of 2.5% Procaine Hydrochloride) the scalp was removed and the skull bones were cleaned. A burr hole of 3 mm diameter was made 5 mm lateral and 3.5 mm posterior to the bregma, by a hand-driven drill gently. After applying procaine hydrochloride locally, the dura was removed gently by a sharp-edged lancet without any pull. A nut with 3 mm internal diameter was placed guiding the hole, which was fixed in situ by a composite dental resin. A small inverted screw (to hold a small sized operational amplifier) was also fixed in the frontal region by the same resin. An indigenously prepared small microdrive was fixed

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vertically over the nut. The microdrive also acted as an electrode carrier. Glass capillary microelectrodes with a tip diameter of 0.5 μ were filled with 3 molar K-acetate, and a DC resistance of 40-60 MΩ were used to record single unit activities. By manipulating the microdrive vertically, the electrode could be guided to a depth of 6-6.5 mm (CA3 region). In phase A, spontaneous single unit activities were recorded on an oscilloscope through an amplifier. The spikes were simultaneously fed into an FM-tape recorder. A tone of 600 Hz for 6 seconds was presented by an audiometer, which at this stage was meaningless tone to these animals. In phase B, the same animals were food deprived for 24 hours and conditioned with the same tone followed by food, repeatedly. The "meaningless tone" became a conditioned stimulus (CS+) now. These conditioned animals were divided into four groups. Group I consisted of controlled conditioned animals. In Group II, under sodium pentothal anaesthesia (40 mg/kg iv), large bilateral lesions of dentate region were made by electronic lesion-maker. Two concentric needle electrodes (insulated by epoxilite except at the tip) with a fine nicrhome wire inside them, kept bare and floating 500 μ below the needle tip, were placed bilaterally in the fimbrial region. They were implanted chronically and a stimulus (5 V, 10 μ amp, pulse width 0.05 msec, frequency 200 Hz) was applied through them for 6 seconds prior to the CS+. Fig. 1 shows the position of lesions and the fimbrial electrodes in group II and group III, animals. Group IV consisted of sham operated animals, where the electrodes were implanted but no lesion or stimulation was given.

Histograms were plotted by an Epson MX-80F/T plotter after analysing the inter-spike intervals (in mesc by logarithmic scale) and the percentage of the number of spikes in each interval time was calculated

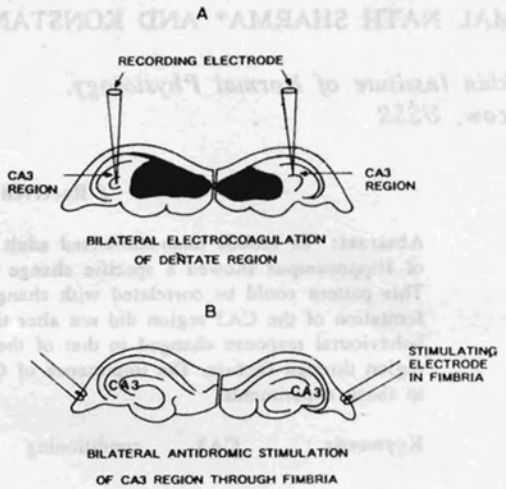

Fig. 1- A: Shows the site of bilateral lesions of dentate region in group II animals. B - Shows the placement of stimulating electrodes in fimbrial regions.

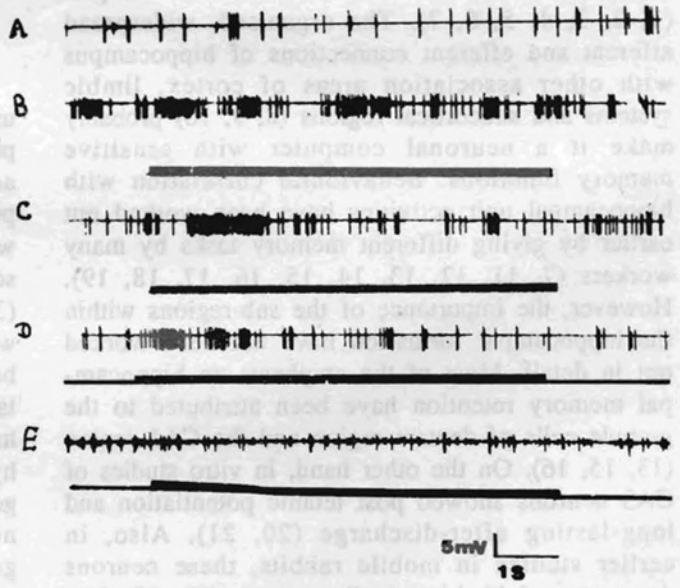

Fig. 2: Single unit activities recorded from CA3 region. A - Spontaneous unit activities; B - Increased burst activities in response to tone in unconditional animal (Phase A). C - Typical spike discharge in response to CS+ in conditional animals (Group I). D - Spike discharge in response to CS+ in Group II animals (after bilateral dentate lesion). E - Spike activities in response to CS+ after fimbrial stimulation (Group III). The thick black lines under the spikes indicate the duration of tone or CS+ (in B, C, D, E).

by using an Apple II computer. Histology was done at the end of the experiments in each group to confirm the electrode positions. In group II, the exact extent of damage of dentate region was noted. The general behaviour and single neuronal activities from CA3 region were compared in phase A and phase B of all the four groups of animals. The histograms of corresponding inter-spike interval patterns were also compared. Histology was done in all the groups of animals to confirm the position of electrodes and the site of lesions and stimulation.

RESULTS

In phase A, the animals were familiarized with experimental cage. In response to the tone (600 Hz) they demonstrated apprehensive and puzzled behaviour and retreated to the corner of the cage. During this pre-conditioned state, the normal spontaneous irregular spike activity (<2 - 40 per sec) from CA3 region (Fig. 2 A) changed to the burst activities throughout the duration of the tone (Fig. 2 B). Histograms of inter-spike intervals showed a pattern which was common to all the animals in phase A (Fig. 3 A & B). In phase B, after conditioning, specific changes in general behaviour of the animals were observed in response to CS+, in all the groups of animals except in group III. In group I, II and IV, animals approached the food plate which opened synchronously following the CS+. In group I animals, CA3 unit activities showed typical bursts only during initial 2-3 seconds of the duration of CS+. In group II (after bilateral lesions of dentate region) and in group IV animals (sham operated), identical pattern of discharge was observed from CA3 neurons in response to CS+ (Fig. 2 C&D). The histograms of inter-spike intervals also demonstrated a comparable pattern in groups I, II and IV (Fig. 3 B, C&D).

Interestingly, in group III, after fimbrial stimulation the animals showed apprehensive behaviour in response to CS+. It seemed as if these animals had no memory of the CS+ and behaved like the animals of phase A (pre-conditioned state). The CA3 unit activities, did not however show any type of burst activity (Fig. 2E). The inter-spike interval patterns were also different from the other groups, which were more like phase A (Fig. 3 A&E).

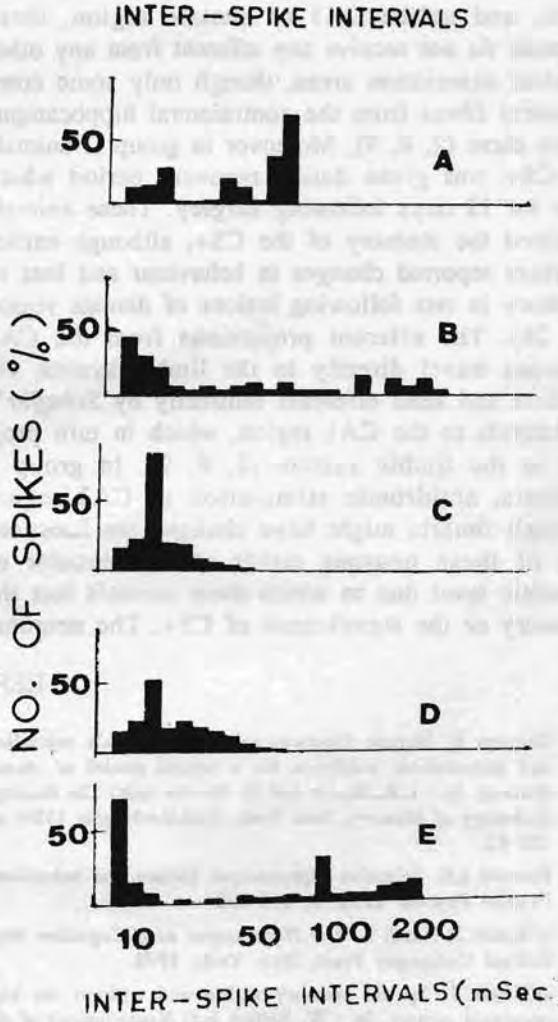

Fig. 3: Inter-spike interval pattern histograms plotted from the unit activities of CA3 region. The abscissa shows inter-spike intervals in milliseconds (logarithmic scale) and the ordinate, the number of spikes in percentage. A - Pattern of spontaneous spikes from CA3 region. B - The pattern obtained during the application of tone in unconditioned animals (Phase A). C - Typical similarity of pattern in response to CS+ in Group II (animals with bilateral lesions in the dentate region). E - Shows changes of pattern in response to CS+ after fimbrial stimulation (Group III).

DISCUSSION

The results obtained from these experiments indicate that the CA3 neurons retain the memory of CS+, which remains intact even after major de-afferentation by bilateral electro-coagulation of dentate region. It is known that afferents reach the CA3 neurons mainly through the dentate granule cells, and unlike CA1 or dentate region, these neurons do not receive any afferent from any other cortical association areas, though only some commissural fibres from the contralateral hippocampus reach there (2, 8, 9). Moreover in group II animals no CS+ was given during recovery period which was for 12 days following surgery. These animals retained the memory of the CS+, although earlier workers reported changes in behaviour and loss of memory in rats following lesions of dentate region (2, 24). The efferent projections from the CA3 neurons travel directly to the limbic system via fimbria and send efferents indirectly by *Schaffer's* collaterals to the CA1 region, which in turn project to the limbic system (2, 8, 9). In group 3 animals, antidromic stimulation of CA3 region through fimbria might have changed the functioning of these neurons either at a molecular or synaptic level due to which these animals lost the memory or the significance of CS+. The neuronal connections of CA1 region as well as the dentate granule cells were normal in these animals, still the memory of CS+ could not be retained by them.

In a recent study, it was shown that after complete transection of fimbria/fornix in rats, new learning and retention of earlier learnt memory was abolished completely (7). The results of those experiments can be explained further in view of the present observations that, after complete transection of fimbria/fornix, only the CA3 region remains completely isolated whereas CA1 region still receives inputs directly from medial entorhinal cortex. Thus it seems that within the hippocampal formation the CA3 neurons play the key role in memory retention or memory indexing. In the absence of proper functioning of these neurons, other regions of hippocampus fail to retain the memory, at least temporarily, as observed in the present experiments.

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