Widespread functional connectivity of rat hippocampal CA1 neurons revealed in spontaneous complex-spike activity

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The lamella hypothesis of hippocampal organization was tested by simultaneous recording of CA1 complex-spike activity from pairs of pyramidal cells in both anesthetized and awake rats. The complex-spike activity of the CA1 pyramidal neurons separated in the medio-lateral direction for distances of up to 2.0 mm was correlated. The normalized cross-correlation coefficient showed an uniform distribution along this axis in both anesthetized and awake animals. A similarly uniform distribution of correlated activity was also observed between neurons separated in the rostro-caudal direction for distances up to 1.6 mm. These results suggest that the spontaneous activity of CA1 neurons is shaped by the presence of weak but widely distributed functional connections between the neurons of this region, and thus does not strictly follow the pattern of activity predicted by the lamella hypothesis.

Key words: Lamella hypothesis, unit activity, cross-correlation, hippocampus, rats

INTRODUCTION

The neuronal circuits of the hippocampal formation form a closed loop in the plane perpendicular to the septo-temporal axis. This loop consists of the perforant path, which connects the entorhinal cortex to the dentate gyrus, the mossy fibers, which connect the dentate gyrus to area CA3, the Schaffer collaterals, which connect CA3 area to CA1 area, and lastly the subiculum through which CA1 area is connected to the entorhinal cortex. At each level, the axons forming this loop remain spatially localized (approximately 800 μm) in septo-temporal direction, and therefore form narrow bands or lamella, which have been proposed to form the functional units of the hippocampal formation. Consistent with this anatomical organization, spread of evoked potentials are limited in the septo-temporal axis to a narrow band-like distribution in rabbit hippocampus. Moreover, Buzsaki et al. showed that gamma oscillations generated by intra-circuits of GABA in rat hippocampus have a similar restricted distribution of about 600 μm. However, recent anatomical data have demonstrated extensive neuronal connections in the septo-temporal axis, which presumably also contribute to patterns of activity across the hippocampus. The Schaffer collaterals, for example, connect not only within a sagittal band area, but also more widely in the septo-temporal direction. In the present study we tested the lamella hypothesis for the hippocampal CA1 region by simultaneously recording of complex-spike activity from pairs of cell in anesthe-
tized and non-anesthetized rats.

**MATERIALS AND METHODS**

Fourteen albino Wistar rats (body weight, 250-350g) were used in acute experiments where hippocampal activity was recorded under urethane anesthesia (1.6g/kg, i.p.). Following anesthetization the animal’s head was fixed in a stereotaxic frame and the bone overlying the hippocampus was removed. The dura mater was then cut to expose the cortex overlying the hippocampus. An electrode retainer was fixed to the skull with dental acrylic over the recording site (A3.5-5.0 mm, L2.0-4.5mm from bregma) as described in previous papers. The retainer was made of a molybdenum electron microscope grid (100-mesh, 250 μm spacing, Nishin EM Co., Tokyo) and two tungsten rods for reinforcement (200 μm in diameter). The grid had a 200 μm thick silicon rubber (Syrigard, #3140RTV, Dow Corning) lining.

Three male albino Wistar rats (250-350g) were used in chronic experiments where recordings were obtained from awake animals. Initially, each rat was anesthetized with pentobarbital sodium (40mg/kg, i.p.) and restrained in a stereotaxic apparatus in order to attach a restrainer to the rat’s skull for subsequent painless immobilization of the head in the correct stereotaxic planes. The skull was exposed and six small screws were implanted to act as anchors. A receptacle for four modified ear bars was then formed using dental cement built up around the working region. After completing the surgery, the wound was cleaned, and the scalp was sutured. The day before beginning the recording sessions the rats were anesthetized with ketamine (30 mg/kg, i.m.) and a 2-mm hole was drilled in the skull over the recording site. The opening in the receptacle was filled with a steroid paste, covered with a thin layer of dental cement, and the animal was returned to its home cage. Just before unit recording, the dura mater was incised with a fine needle for electrode insertion under local anesthesia (1% lidocaine).

In the acute experiments, two extracellular action potentials were recorded separately with glass-coated tungsten electrodes. In the chronic experiments, simultaneous recordings from two sites were obtained using pairs of bipolar glass-coated tungsten electrodes whose tips were separated by 30-50 μm in the vertical axis for elimination of the EMG artifact. Electrodes were independently fixed using micromanipulators. Signals were amplified with conventional preamplifiers (band width, 150-10kHz; AB-610J, Nihon Kohden). All signals were recorded with a data tape recorder for later offline analysis (band width, DC-5kHz; RD-125T, TEAC). For off-line analysis, signals for 60 sec periods were fed through a data acquisition device (5 kHz sampling rate; 1401plus, CED) to a PC-compatible computer. Spikes were detected using a window discriminator software ( Spike2, CED). A normalized cross-correlation function was calculated for time lags of 128 msec to investigate the relationship between the activity of two given neurons. The peak correlation value over this time span was defined as the normalized cross-correlation coefficient (NCC) from one spike train to another. To test the statistical significance of the NCC, the interspike intervals of all spike trains were randomly shuffled and the NCC was determined for the shuffled data. The degree of coherence (dNCC) between two neurons was defined as the difference in the NCC values of the true spike train and the shuffled data. To quantify further the shape of the cross-correlogram, we defined the peak to baseline ratio (RPB) as the peak value (after smoothing by 5 bins) to the average bin height of the correlogram (excluding zero bins).

The results were expressed as means±SEM. For statistical analysis, one-way ANOVA and t-tests were used as indicated. This experiment was permitted by animal committee of the university (2001-32).

**RESULTS**

Simultaneous recordings of complex-spike activity were obtained from pairs of electrodes inserted into the hippocampal CA1 pyramidal layer. The first (reference) electrode was fixed in place upon isolating a pyramidal layer unit displaying clear complex-spike activity. The second (target) electrode was mobile and was used to record units at different locations with respect to the reference electrode. Signal to noise ratios of the records ranged from 3:1 to 4:1, and signals displayed typical complex-spike bursts consisting of 3-5 spikes. The mean intraburst interspike interval, for an example, was 7.7±2.4 msec (mean±SD, n=93 interspike intervals from a record). Examples of cross-correlograms are shown in Figs. 1A a and b and correspond to a sample of points a and b in Fig. 1B. The locations a and b were separated from the reference electrode at the lateral distance of 250 μm and 2.0 mm, respectively. The cross-correlogram shows a broad increase in activity about 0 ms, with NCC values of 0.046 (a) and 0.024 (b). The dNCCs of both coupled neurons were
0.045 (a) and 0.012 (b). Figure 1B plots the dNCC as a function of medio-lateral distance (250 μm steps) from the reference electrode for recordings made in two medio-lateral planes. At each position complex-spike activity was isolated at a similar depth and recorded 2-5 times. The mean firing rate of the reference electrode was 5.62 ± 1.62 spikes/sec (n=42 records) during the approximately 4 hr recording session and that of the target electrode was 4.20 ± 2.03 spikes/sec. The mean NCC over all records was 0.030 ± 0.012, which was higher than that for the shuffled spike trains (0.013 ± 0.004, p < 0.001, t-tests). The dNCC was almost uniform along the medio-lateral axis for distances of up to 2.0 mm, with a mean value of 0.017 ± 0.013 (n=42 records). The mean RPB was 1.93 ± 0.39.

Figure 1C plots the dNCC as a function of rostro-caudal distance from the reference position for recordings in two parasagittal planes. The cross-correlograms between the reference and target electrodes displayed clear peaks at all separation distances. As an example, the cross-correlogram between the reference electrode and the activity recorded at position c (Fig. 1C) is shown in Fig. 1Ac (NCC=0.092, dNCC=0.072). The mean NCC over all records was 0.045 ± 0.021, and the NCC (shuffled) was 0.014 ± 0.005 (n=28 records). The dNCC was relatively uniform along the rostro-caudal axis for at least 1.6 mm, with a mean value of 0.034 ± 0.023 (n=28 records) and a mean RPB of 2.35 ± 0.51. The mean firing rate of activity recorded by the reference and target electrodes was 3.34 ± 1.41 spikes/sec, and 3.50 ± 1.66 spikes/sec (n=28 records), respectively.

To determine whether these results reflected the effects of the anesthesia, similar recordings were performed in a quiet condition without any apparent
movements in awake animals. Complex-spike activity, similar to that in the anesthetized state, was recorded following insertion of the electrode into hippocampal CA1 pyramidal layer. The target electrode was moved along medio-lateral axis with steps of 200 µm. An example of the correlation between activity recorded by the reference electrode and a unit 500 µm posterior and 1.1 mm lateral to it is shown in Fig. 1 Ad. The activity recorded on the two electrodes was correlated with the peak of the correlogram occurring approximately at a time lag of 0 ms and having an NCC of 0.063. The dNCC was 0.031. The NCC over all records was 0.033 ± 0.016 and the NCC (shuffled) was 0.015 ± 0.004 (n=23 records). Figure 1D shows lateral distribution of the dNCC. The values were uniformly distributed along the medio-lateral direction (dNCC=0.019 ± 0.017, n=23 records). For the estimation of degree of peak shape, the mean RPB was 1.68 ± 0.73. The mean frequencies of reference and target electrodes for 60 sec recording periods were 2.59 ± 1.45 spikes/sec and 2.38 ± 0.78 spikes/sec (n=23 records), respectively.

**Discussions**

Our results indicate that the functional connectivity of neuronal activity is uniform at least over 2.0 mm along the medio-lateral direction and over 1.6 mm along the rostro-caudal direction of rat hippocampal CA1 in both anesthetized and non-anesthetized (awake) states, although the distance was limited by the size of retainer. Also, the dNCC values in the anesthetized state were similar those found in the awake state. These results suggest that the connectivity was not affected so much by the anesthetized drug in the rat hippocampus.

Anderson et al. provided the first electrophysiological evidence in support of lamella organization of the hippocampus by recording the distribution of evoked potentials following electrical stimulation. Stimuli only evoked hippocampal responses within a limited extent in the septo-temporal direction. A similar narrow banding distribution was found by Burzaki et al. for spontaneous gamma oscillations in hippocampus. These results support the lamella hypothesis of the hippocampus. However, recent anatomical evidence has demonstrated the existence of widespread connections in the septo-temporal direction in the entorhinal cortex-dentate gyrus-CA1-CA3-entorhinal axis. Our data are consistent with these results and suggest that spontaneous complex-spike activity displays a uniform functional connectivity along the medio-lateral axis. Buszaki et al. have suggested that the sharp wave is dominant in rat hippocampus and is distributed over a wide region that extends for over 2 mm laterally. They also showed that complex-spike activity is often synchronized with a sharp wave. These results suggest that complex-spike activity could also be correlated over a wide region; however, under their recording conditions the correlation distribution displays a clear banding organization. Optical recording experiments in the anesthetized rat also suggest that stimulation of the perforant path fibers may evoke correlated activity over a widespread region of CA1. One possible explanation of these differing results is that the broad functional connectivity is a result of the urethane anesthesia. However, our results indicate that such activity patterns occur in the awake state as well. A likely explanation is that the pattern of connectivity during spontaneous activity may be different from that during information processing of stimuli. In this sense, the lamella hypothesis might reflect the dynamic organization of the hippocampal loop during information processing.

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