Research Note

Generation of Theta Activity (RSA) in the Cingulate Cortex of the Rat

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Summary. Unit activity recorded from the cingulate cortex during theta rhythm shows periodic trains of spikes which are phase-locked to the local theta field potential waves. These cortical theta units were also shown to be correlated with hippocampal theta units. These findings, along with the fact that theta field potentials show a phase reversal within the cingulate cortex, lead to the conclusion that this cortical area is a source of theta activity.

Key words: Theta rhythm – Spike trains – Field potential – Cingulate cortex – Hippocampus

Theta field potentials, or rhythmic slow activity (RSA) in the frequency band 4–10 Hz in the rat, recorded in parts of the cortex overlying the hippocampal complex, have been noted by several authors. Although without specifying the cortical areas from which it was recorded, Artemenko (1972), Winson (1974), Bland and Whishaw (1976), and Gerbrandt et al. (1978) described cortical theta to be isomorphic with theta in the neighboring part of the hippocampus, the amplitude decreasing toward the cortical surface. Cortical theta would thus be accounted for in terms of volume conduction theory, since a stationary neuronal source in a purely resistive medium causes a field potential, the amplitude of which decreases with increasing distance from this source without phase shift (Holsheimer and Feenstra 1977).

However, Petsche and Stumpf (1960) found a “loose phase coupling” between cortical and hippocampal theta field potentials in the rabbit; the theta waves in these two regions were never more than a fraction of a period apart. During arousal they found regular theta rhythm in the hippocampus, but irregular theta activity in the cortex of the awake rabbit. Winson (1974) reported that both intermittent theta or absence of theta in the cortex could be found

during regular hippocampal theta rhythm in the awake rat. These findings suggest that theta field potentials in parts of the cortex overlying the hippocampal complex may not be considered solely the result of volume conduction from a hippocampal source, i.e., there is a possibility that hippocampal and cortical theta have different neuronal sources.

We made a quantitative analysis of the relationship between theta field potentials in the laminae of the dorsal hippocampus and overlying cortex of the urethane anesthetized rat, which indeed revealed phase differences of approximately 180 deg not only in the hippocampus but also in the overlying cortex (Feenstra and Holsheimer 1979; Holsheimer et al. 1979). These theta phase reversals, on tracks normal to the hippocampal and to the cortical surface (laminar profiles), were considered as indicating that neuronal sources of theta activity exist in each of these structures; each source causing a dipole-like potential field. The cortical areas concerned were those parts lying near the midline, namely the cingulate cortex (Fig. 1). The corresponding neuronal source might be the population of large pyramidal cells having their somata in layer V, since the theta phase reversal was found in this layer. We also found that the coherence function between theta field potentials recorded in the dorsal hippocampus and in the cingulate cortex had a value > 0.8 at theta peak frequency (4–5 Hz), indicating a strong linear relationship between the theta components of the field potentials in these regions.

Beside this evidence for a cortical theta source, based on spatio-temporal field potential properties, a second type of evidence has been found. Spike activity, recorded from cortex overlying the medial part of the hippocampus, appeared to have a periodicity which is strongly related to theta field potentials in this region. The results from the analysis of these *theta units* are presented here.

Fig. 1. **A** Simultaneous field potential recordings in layer V of cingulate cortex (1) and in CA1 pyramidal layer of dorsal hippocampus (2); electrodes at 3.4 mm anterior. **B** Recording in layer V of cortex (3) at 2.6 mm anterior. Registrations divided into slow wave component (0.5–100 Hz) and spikes (300–3,000 Hz); calibration: abscissa 1 s, ordinate 0.3 mV (slow waves) or 0.1 mV (spikes), positivity upward; c: cingulate cortex

Spontaneous theta activity was recorded simultaneously by way of two electrodes from four female Wistar rats (200–220 g) under light urethane anesthesia (1.1–1.4 g/kg). For details see Feenstra and Holsheimer (1979) and Holsheimer et al. (1979). Platinum electrodes with a tip diameter of 2–3 μm (impedance 2–3 M Ω at 1 kHz) were used for recording both spike activity and slow waves; the recording system had a bandwidth of 0.5–5,000 Hz. After high-pass filtering at 300 Hz, unit activity was selected by amplitude discrimination. The criteria used for this selection were the amplitude of the spikes (at least twice the background activity), the mean discharge frequency (at least 3/s) and the stationarity of amplitude and discharge pattern. Slow wave activity was selected by using a low-pass filter at 100 Hz to minimize the resulting phase shift of the theta waves. Both signal components are shown in Fig. 1. Signal analysis was performed on a PDP-11 computer (Holsheimer et al. in prep.). The electrode tracks were verified histologically from serial sections.

Electrodes were lowered into the cortex at 1.6–3.0 mm lateral and 3.0–3.6 mm anterior, according to the stereotactic coordinates of the Groot (1959). In three rats theta units were found in this cortical area, overlying the medial hippocampus (Fig. 1), at a dorso-ventral level of 3.4–4.1 mm (mean = 3.7 mm) (de Groot 1959). Hippocampal theta units from the CA1 pyramidal layer were observed at 0.5–1.0 mm (mean = 0.8 mm) below the units in the deep cortical layers. Of 12 cortical units 60–80 s recordings were analyzed. The mean spike frequencies of the units varied between 8–58/s (mean value = 25/s). Some units had a regular pattern of spike bursts, which was phase-locked to the theta field potential recorded with the same electrode, while the spike pattern of other units was more irregular (Fig. 1B). These phenomena can be better put in evidence

by estimating the renewal density (often called autocorrelation) (Perkel et al. 1967a) and the (spike-)-triggered correlation with the theta waves of the same record. The estimated renewal density function $V(\tau)$ shows the probability of spike generation within successive intervals of 4 ms during 1 s after the occurrence of a spike at $\tau = 0$ s (Fig. 2). Eleven cortical units had renewal densities with a periodicity which corresponded with the theta waves. The triggered correlations $W(\tau)$ are the average (in mV) of 1 s epochs of the slow wave activity triggered at $\tau = 0$ s by a spike (Fig. 2A–B). All plots presented are smoothed: each one of 64 values plotted is the average of four successive 4-ms intervals.

The triggered correlation W in Fig. 2A, resulting from signal 3 in Fig. 1B, shows that the greater part of the spikes are near the negative peak of the theta field potential recorded with the same electrode. It is also shown that spikes are correlated only with the theta component of the slow wave, because all other components are lost by averaging. W in Fig. 2B shows that the mean moment of firing of this cortical theta unit is past the positive peak of the theta wave (approximately 52 deg).

As mentioned above, we found a linear relationship between theta field potentials in dorsal hippocampus and cingulate cortex. This relationship is also found by estimating the cross-correlation function of cortical and hippocampal theta units (Perkel et al. 1967b). The estimated cross-correlation function $C(\tau)$ shows the probability of spike generation by one unit conditional upon the occurrence of a

Fig. 2. A V (estimated renewal density of a spikeseries) of cortical unit from Fig. 1B (3), calculated from 829 spikes; W (triggered correlation of slow wave field potential and a spikeseries) from the same unit (in mV): mean wave of 809 triggered waves; note that the mean position of the spikes (at $\tau = 0$) is near the negative peak of the theta wave. B V of cingulate unit (ant. 3.0, lat. 2.0 mm) calculated from 1,276 spikes; W from the same unit: mean wave of 1,253 triggered waves (in mV); note that the mean position of the spikes is past the positive peak of the theta wave. C V of cingulate unit (1) and hippocampal CA1 unit (2) from Fig. 1A: V1 calculated from 2,294 and V2 from 247 spikes in simultaneous recordings; C (estimated cross-correlation of two spikeseries) of these units: maximum at ~ 0 s (synchronous firing patterns of the two units). D V of cortical unit (1) and hippocampal CA1 unit (2) at 3.0 mm anterior: V1 calculated from 806 and V2 from 332 spikes in simultaneous recordings; C of these units: maximum at ~ -0.1 s. All functions V, W and C presented, are obtained from 60.4 s recordings. Note that from each recording the functions presented have identical periodicities: ~ 250 , 260 , 240 , and 260 ms in A, B, C, and D, respectively

spike at $\tau = 0$ s by the other unit (Fig. 2C–D). In Fig. 2C the renewal densities V of the cingulate unit (1) and of the simultaneously recorded hippocampal CA1 unit (2) from Fig. 1A are shown, as well as the cross-correlation C of these theta units. The two units are firing almost synchronously, because the max-

imum of C is at about 0 s. In Fig. 2D V and C of another pair of (cortical and hippocampal) theta units are shown. These units have alternating spike trains, because the maximum of C is at about -0.1 s or 147° . The degree of theta modulation of cortical unit activity did not differ in a substantial way from theta modulation of unit activity in dorsal hippocampal areas of the rat (Holsheimer et al., in prep.).

From these results, regarding theta spike activity in the cingulate cortex (and possibly from areas somewhat more lateral), as well as from our previous results concerning theta field potential profiles in the cingulate cortex, it can be concluded that this cortical area is a source of theta activity. There is anatomical evidence that the medial septal-diagonal band complex, which functions as the main generator of hippocampal theta, also projects to the cingulate cortex. Swanson and Cowan (1979) have described fibres which originate in the nucleus of the diagonal band and pass over the genu of the corpus callosum to terminate in layer I and in the deeper layers of area 24 in the rat. We must point out, however, that we found intracortical reversals of the theta field potential and recorded theta units at sites lying more caudally than the septal projections to the cingulate cortex found by Swanson and Cowan. Nevertheless, the simplest explanation for the linear relationship found between theta field potentials in the hippocampus and the cingulate cortex, as well as between unit spike activities in these two regions, is that they have a common neuronal generator being the diagonal band of the medial septum.

Acknowledgements. I thank C.J. Stok for preparing the signal analysis program, Dr. F.C. Kuipers and his staff of the Laboratory for Pathology (Enschede) for preparing the brain serial sections, Prof. Dr. F.H. Lopes da Silva and Prof. Dr. A.H.M. Lohman for their useful criticisms and suggestions.

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Received January 6, 1982 / Accepted March 29, 1982