NeuroReport 6, 2166–2170 (1995)

Medial septal control of theta-correlated unit firing in the entorhinal cortex of awake rats

Kathryn J. Jeffery,[CA] James G. Donnett and John O'Keefe

Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK

[CA]Corresponding Author

THE present study investigated whether the medial septal nucleus controls theta-correlated unit activity in the entorhinal cortex (EC), as it does in the hippocampus. Single neurones were recorded from the medial EC of rats as they ran on a linear track or chased food pellets on a small platform. The most prominent pattern of cell activity observed was burst firing occurring near the peaks of the ongoing dentate gyrus theta rhythm. This rhythmic unit activity was abolished by microinjections of lignocaine into the medial septal nucleus, but was resistant to cholinergic blockade.

Key words: Entorhinal cortex; Theta cells; Medial septum

Introduction

A prominent feature of the physiology of the rat hippocampus (HC) is the occurrence of a sinusoidal electroencephalographic (EEG) rhythm, the theta rhythm, which appears during certain types of movement. The function of theta forms an integral part of most theories of the hippocampus and its neuronal substrate is now well characterized. In particular, it appears that although the potential itself is generated by two zones within the hippocampus (the dentate gyrus and CA1 subfields), its occurrence and frequency depend on an intact input from the brainstem via the medial septum (MS).[1] Neocortical information reaches the HC via the entorhinal cortex (EC), and both theta rhythm and theta-correlated unit activity also occur in this structure.[2] HC and EC theta share many characteristics: for example, they are phase-locked[2] and both are abolished by MS lesions.[3] However, normal HC theta also depends on an intact EC input[4,5] because it is of lower frequency following EC lesions and is abolished by cholinergic blockade,[6,7] and the power peak usually seen at the hippocampal fissure is absent.[7] One hypothesis is that the MS input to the EC causes oscillations in EC cells, which are then the source of the EC contribution to HC theta. Evidence for this includes the finding by Dickson *et al*[8,9] that urethane-related theta and theta-correlated unit activity are both suppressed by MS inactivation. However, theta activity seen under urethane anaesthesia differs in several respects from that seen in freely moving animals: it is slower, sensitive to cholinergic blockade, and units show different firing patterns and phase relationships. Mizumori *et al*[10] found that MS inactivation in awake rats reduced the firing of EC cells, but it is not known whether these were theta-related cells. The present study therefore investigated whether the theta-related firing of EC cells in freely moving animals was abolished by MS inactivation. The effect of systemic atropine administration on theta-related activity was also assessed, to determine whether this activity is dependent upon a cholinergic input in the awake animal, as it is under anaesthesia.[9]

Materials and Methods

Male Lister hooded rats (350–400 g) were chronically implanted under isoflurane/N_2O anaesthesia with an EEG electrode pair, medial septal cannula (where required) and lightweight microdrive holding two tetrodes, from which single unit data could be obtained as previously described.[11] The EEG electrodes comprised a twisted pair of 75 μm Teflon-coated tungsten wires (California Fine Wire, Grover Beach), with the tips separated vertically by 1.0 mm. The electrodes were positioned so that the longer of the two wires was situated in the dorsal dentate gyrus (coordinates: bregma −3.8 mm AP, 2.2 mm ML, 3.0 mm deep) and the shorter wire in CA1. The 25 gauge medial septal guide cannula was implanted to a depth of 5.0 mm, 0.2 mm anterior to bregma in the midline. Each tetrode consisted of four twisted strands of 25 μm Teflon-coated platinum or HM-L-coated stainless-steel wire (California Fine Wire) cut flat at the tips. The two tetrodes were separated vertically by approximately 0.5 mm to allow one set of four wires to detect unit activity while the other acted as a reference. The tetrodes were mounted in a cannula attached to the microdrive, enabling them to be advanced through the brain in 25–100 μm steps.

© Rapid Science Publishers

They were implanted near the EC contralateral to the EEG electrodes by tilting the tetrodes in the sagittal plane 30° from vertical, and lowering them from the brain surface (bregma −60 mm AP, 4.0 mm ML) along this trajectory until 4.0 mm of the deeper tetrode were embedded in the brain. A 19-gauge sleeve was then pulled down over the remaining exposed wire and the assembly cemented to the skull. Buprenorphine (45 μg) was given i.m. for postoperative analgesia.

Electrophysiological recording: The tetrode technique is an extension of the stereotrode[12] and has been described previously.[13] Briefly, the close apposition of the four electrode tips allows simultaneous recording and optimal separation of several neighbouring single units by a clustering algorithm that compares the size and shape of the signal seen on each of the wires. Recording began at least one week after surgery. The potentials recorded on each electrode were passed through field effect transistors mounted on the rat's head, and led to custom-built equipment where the signal was amplified and filtered. Each of the four wires of one tetrode was recorded differentially with respect to one of the wires of the other, the signal tetrode being that on which units were appearing. For unit recording, the signal was amplified 30 000–50 000 times, bandpass filtered (800 Hz to 6 kHz) and sampled at 20 μs intervals. Whenever the signal on any of the four channels exceeded a threshold of 3 V (a presumptive spike), 50 points per channel were stored (including the preceding 200 μs). For EEG, the output of one of the signal tetrode wires or one of the fixed hippocampal electrodes was filtered (1–125 Hz), amplified 12 000–40 000 times and sampled continuously at 250 Hz.

Unit activity was initially assessed while the rat was on a small 40 × 40 cm square wooden platform. When cells had been isolated the room lights were switched off, a low intensity desk lamp switched on and the rat induced to run around the platform chasing food pellets held in the experimenter's hand. Long runs of large amplitude theta were elicited by inducing the rat to run on a linear track 2 m long by 5 cm wide. The position of the rat was monitored by a video camera mounted directly above the platform and converted into x–y coordinates by a TV tracking system (SP111, HVS Image Analysing, Kingston, UK) which detected a small DC light mounted on the rat's head. Each spike event was stamped with the location of the animal and the time since the start of the recording. The data were stored on a hard disk and later transferred to a Sun 4 workstation for analysis.

Data analysis: An autocorrelation function was computed to ±500 ms for each cell.

The phase of the theta rhythm was determined by setting the positive-to-negative zero crossings to 0°/360°. The mean firing phase of a given cell was calculated by attributing to each spike a unit vector whose angle equalled its firing phase, and finding the resultant vector for the recording period. All values presented are given with respect to dentate theta, so 180° was added to the phase for cells whose phase relationship had been recorded relative to CA1 or EC.

Drug administration: A 2% solution of lignocaine hydrochloride solution (Phoenix) was injected in 1 μl volumes via the medial septal cannula until hippocampal theta was abolished (2–6 μl). Atropine sulphate powder (Sigma) was dissolved in water to a concentration of 10 mg ml^{-1}, and a dose of 50 mg kg^{-1} was injected i.p.

Histology and cell localization: After completion of recording each rat was perfused transcardially with paraformaldehyde and the brain extracted and stored in formalin for later sectioning either parasagittally (EC) or coronally (HC). The 40 μm sections were Nissl stained to allow visualization of the electrode tracks. Cells were localized by two methods. First, the theta activity recorded by the tetrode was compared with hippocampal theta in order to determine whether the cell was in deep EC (in phase with CA1 theta), at the border between layers III and II (null zone, or theta minimum) or in superficial EC (in phase with dentate theta[2]). Second, the depth of the electrode at implantation plus the distance through which the microdrive had been advanced was superimposed on the electrode track obtained histologically.

Results

Classification of entorhinal neurones: A general survey of entorhinal neurones was carried out in three animals. Cells for which <400 spikes in total had been collected were discarded because of the difficulty in analysing their firing pattern. The firing of the remaining cells (n = 88) was tested for rate, place specificity, rhythmicity and phase relationship to theta. The cells fell broadly into two classes, those that showed clear periodic firing and those that did not. The first class comprised periodic cells. Of these, simple cells (n = 30) showed a characteristic firing of single spikes or short bursts, at the frequency of the ongoing theta rhythm. Rhythmicity was not always evident on the spike trace but the autocorrelation function showed a characteristic pattern of peaks which decreased and troughs which increased smoothly with increasing temporal distance (see Fig. 2). These cells increased their firing rates when the animal moved and showed a relatively high average rate, with a mean (±s.e.m.) of 8.48 (±1.8) Hz. They did not possess place fields, firing uniformly over the platform. The phase relationship was determined for

20 cells and found to be approximately normally distributed with a mean of 218 (±14)° relative to dentate theta. A frequency phase histogram of a theta cell, obtained by dividing the theta cycle into 12 bins and counting the spike occurrences in each bin, is shown in Figure 1. A further 11 theta-correlated cells also had place fields (spatial theta cells), preferring to fire when the theta peaks coincided with the rat's location on a restricted part of the platform or track. Five place cells from one rat showed a biperiodic theta rhythmicity, preferring to fire on alternate theta peaks.

The second class comprised aperiodic cells. No rhymicity was evident in 47 cells. Five cells showed a broad place field and of these, four showed a rather striking pattern of steeply and smoothly decreasing autocorrelation function. The remaining 42 cells showed neither place fields nor rhythmic firing, and though many tended to increase their activity when the rat moved, their firing was not tightly coupled to the occurrence of theta. They may, however, correspond to the 'tonic theta-on' cells seen by Dickson *et al*[9] in anaesthetised rats. No cells were found that decreased their firing during theta ("theta-off" cells).[9]

Effect of MS inactivation on theta cell firing: The effect of MS inactivation on theta-related firing was investigated in 12 simple theta cells from an additional three rats. MS inactivation was accomplished by pressure injecting 1 μl volumes of 2% lignocaine hydrochloride via the MS cannula until movement-related theta in the dentate gyrus was abolished (2–6 μl). Seven injections were made in total, with a period of several days or weeks separating successive injections in the same animal. MS inactivation was

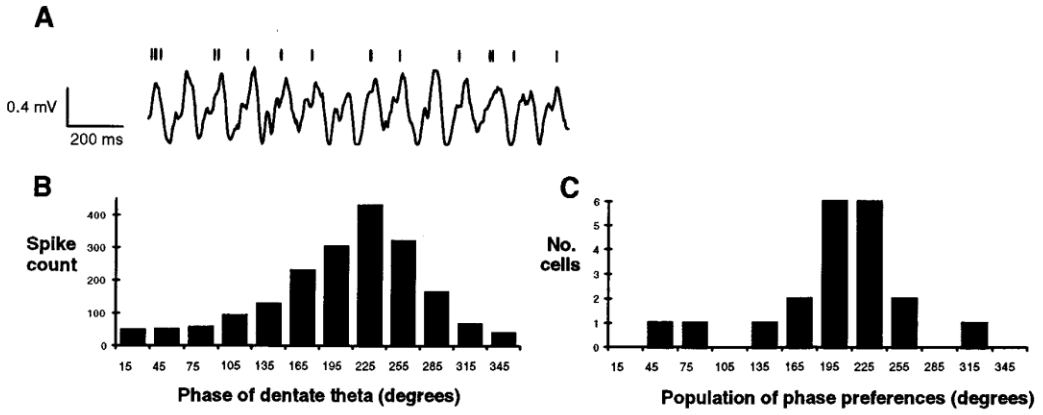

FIG. 1. (**A**) Relationship between firing of an entorhinal cell (tick marks) and dentate theta rhythm. (**B** and **C**) Frequency phase histograms for a single EC theta cell (B) and the population of 20 cells (C).

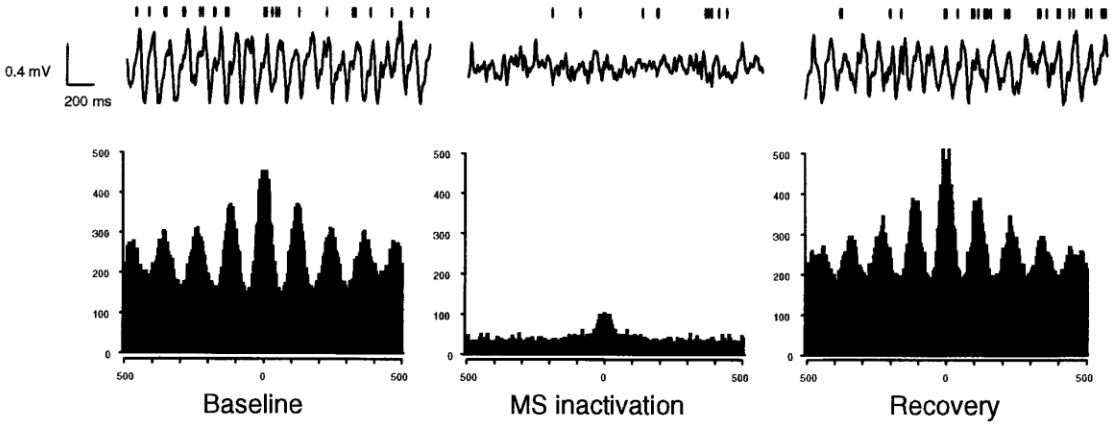

FIG. 2. Effect of medial septal inactivation on entorhinal theta cell firing. Upper trace: relationship of unit activity (tick marks) to dentate EEG. Lower panels: autocorrelation functions from −500 ms to +500 ms showing the loss of rhythmicity and marked decrease in firing rate of the entorhinal unit during medial septal inactivation.

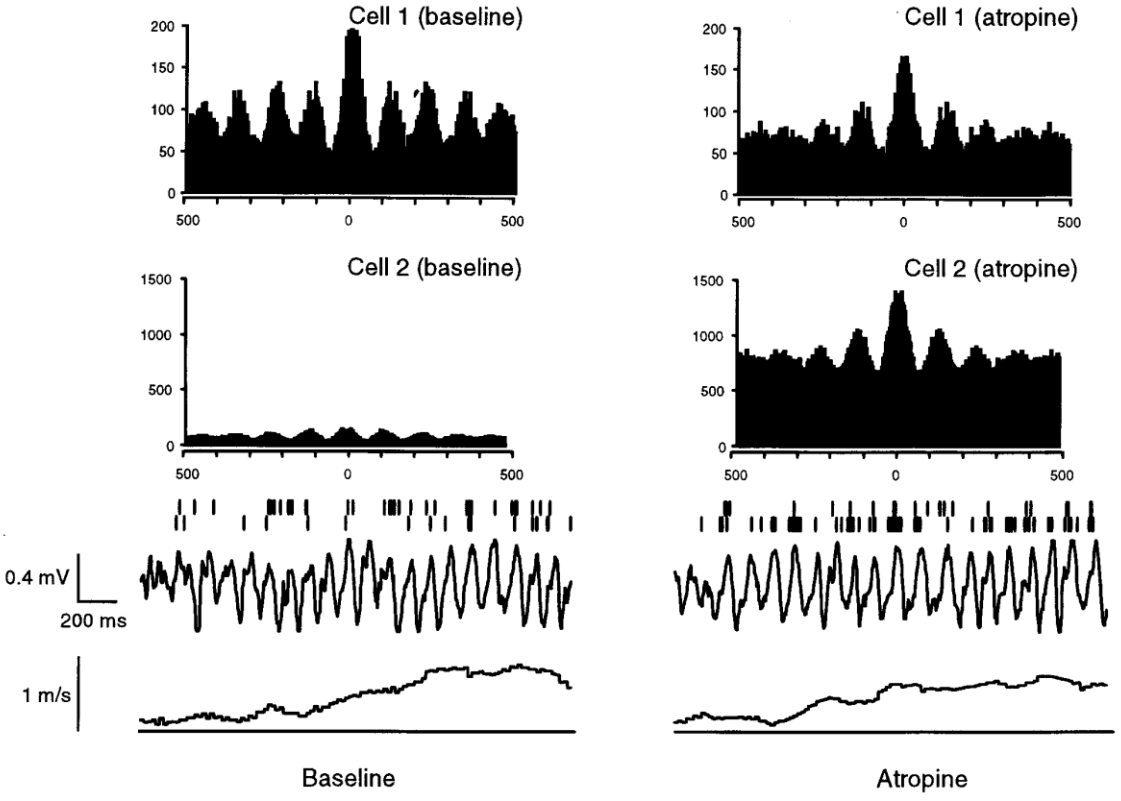

FIG. 3. Differential effects of 50 mg kg^{-1} atropine i.p. on firing of two simultaneously recorded theta cells. Autocorrelation functions and spike traces showed marked increase in firing of cell 2 but no change in cell 1. The spike traces were obtained during two periods of comparable running on the linear track, as shown by the matching velocity profiles (bottom traces).

accompanied by a marked decrease in the firing rate of theta cells from 13.84 (±2.92) to 6.25 (±2.49) Hz (t = 7.82, $p < 0.0001$; paired t-test), and a loss of rhythmicity (Fig. 2). One cell, with an unusual firing phase preference (70°), lost the rhythmic component of its firing but maintained a relatively high basal rate. Another cell developed a biperiodic autocorrelation pattern during recovery, showing a tendency to fire on alternate theta cycles.

Five cells, which did not appear to fire rhythmically either on the spike trace or autocorrelogram, nevertheless showed a mean firing phase preference close to the dentate theta peak (242 ± 4.14°). These cells also showed a decrease in firing rate, from 9.22 (±0.73) Hz to 4.77 (±0.55) Hz. The sixth aperiodic cell, with no firing phase preference, showed no decrease in activity.

Effect of cholinergic blockade on theta cell firing: Administration of atropine sulphate 50 mg kg^{-1}, i.p. resulted in a marked change in the motor behaviour of the animals, producing initial agitation and shivering following by hypokinesis and stereotypy and this complicated analysis of any alterations in theta-correlated unit firing. However, when movements did occur, they were accompanied by apparently normal theta in both HC and EC and normal periodic firing of theta cells (Fig. 3). One theta cell and two aperiodic cells showed a marked increase in activity.

Discussion

The main finding of this study is that theta and theta-correlated unit firing in the entorhinal cortex of freely moving animals were abolished by inactivation of the MS. Both theta and theta-related activity were unchanged or enhanced by cholinergic blockade. We found both periodic and aperiodic EC cells, some of which also showed location-specific firing. Theta-correlated periodic cells showed a marked phase preference, tending to fire ahead of the peaks of the dentate theta rhythm. This agrees with the findings of Mitchell and Ranck[2] and Stewart *et al*[14] who also found that unit activity in the awake animal was phase-locked. Dickson *et al*[9] reported neurones whose tonic firing showed a decrease with the onset of theta, or which fired tonically with the onset of theta ('tonic theta-on' cells). We did not observe

theta-off cells in the present experiment. Some non-rhythmic cells appeared to increase their activity with movement, though their firing was not tightly coupled to theta *per se*, and these possibly correspond to tonic theta-on cells.

Dickson *et al*[9] found that EC theta and theta-correlated unit activity depended upon MS input in the urethane-anaesthetised rat, and were abolished by administration of atropine sulphate. We find that while theta-correlated activity also depends upon the MS in awake rats, it is maintained during cholinergic blockade, suggesting that there is a non-cholinergic component to movement-related EC theta. It remains to be determined whether the non-cholinergic component also originates in the MS.

Conclusion

The present findings demonstrate that the MS is the primary source of rhythmic input to EC cells. Because rhythmic cell firing was maintained during cholinergic blockade, it is likely that in addition to the known cholinergic projection to the EC from the MS, there is also a non-cholinergic component which is able to sustain theta. It is not yet known whether it is the periodic or aperiodic cells, or both, which project to the HC. Both place and theta information are conveyed to the HC from the EC, and cells with either or both of these firing correlates were observed here. However, theta-correlated cells in the EC showed the high firing rates characteristic of interneurones. It is therefore possible that tonic (perhaps place) information carried by perforant path axons contributes to both theta and place cell firing in the HC.

References

1. Fox SE, Wolfson S and Ranck JB. *Exp Brain Res* **62,** 495–508 (1986).
2. Mitchell SJ and Ranck JB. *Brain Res* **189,** 49–66 (1980).
3. Mitchell SJ, Rawlins JNP, Steward O *et al. J Neurosci* **2,** 292–302 (1982).
4. Vanderwolf CA, Leung L-WS and Cooley RK. *Brain Res* **347,** 58–73 (1985).
5. Ylinen A, Soltész I, Bragin A *et al. Hippocampus* **5,** 78–90 (1995).
6. Vanderwolf CA and Leung L-WS. Hippocampal rhythmical slow activity: a brief history and the effects of entorhinal lesions and phencyclidine. In: Seifert W, ed. *The Neurobiology of the Hippocampus,* London: Academic Press, 1983: 275–302.
7. Montoya CP and Sainsbury RS. *Physiol Behav* **35,** 121–126 (1985).
8. Dickson CT, Trepel C and Bland BH. *Hippocampus* **4,** 37–52 (1994).
9. Dickson CT, Kirk IJ, Oddie SD *et al. Hippocampus,* in press (1995).
10. Mizumori SJY, Ward KE and Lavoie AM. *Brain Res* **570,** 188–197 (1992).
11. O'Keefe J and Recce ML. *Hippocampus* **3,** 317–330 (1993).
12. McNaughton BL, O'Keefe J and Barnes CA. *J Neurosci Methods* **8,** 391–397 (1983).
13. Recce ML and O'Keefe J. *Soc Neurosci Abstr* **15,** 1250 (1989).
14. Stewart M, Quirk GJ, Barry M *et al. Exp Brain Res* **90,** 21–28 (1992).

ACKNOWLEDGEMENTS: This work was supported by an MRC programme grant to J.O'K.

Received 10 July 1995; accepted 28 July 1995