The septal EEG suggests a distributed organization of the pacemaker of hippocampal theta in the rat

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Abstract
Individual neurons in the medial septum and diagonal band fire in phase with, and appear to act as a ‘pacemaker’ of, the hippocampal theta rhythm. We investigated the relationships of periodic EEG both among various parts of the septum and with dorsal hippocampal theta recorded concurrently in freely moving rats. Most septal sites showed theta rhythm concurrent with hippocampal theta during locomotion. However, periods with theta at hippocampal but not septal sites were more frequent than the reverse. Theta waves in different parts of the septum were synchronized with each other but medial septal sites showed less frequent theta than other sites. The phase delays between medial and lateral septal sites were < 10 ms, suggesting that the hippocampus does not act as a simple relay between the two. Spectral analysis revealed periods (> 5 s) of theta at hippocampal sites coinciding with rhythms at multiple septal sites that were slower than theta. Even slower were the ‘slow septal waves’ (mean 2.7 Hz), which were present in the absence of locomotion and did not ‘drive’ the hippocampus. Our data suggest that the pacemaker of hippocampal theta may best be thought of as a set of functionally differentiated components rather than as a single homogenous unit.

Introduction
Septal input to the hippocampus, via the fornix, is essential for normal hippocampal function. Destruction of either the fornix or the septum eliminates the slow rhythmic electroencephalogram (EEG), ‘theta rhythm’, in the hippocampus (Green & Arduini, 1954). The relationship between the hippocampal EEG and unit activity of septal neurons was first studied by Petsche et al. (1962) in curarized rabbits. They found that some cells fired bursts of action potentials in phase with concurrent hippocampal theta. These cells maintained their rhythmic bursting activity in the absence of hippocampal theta (after a hippocampal seizure or after pharmacological treatment). They concluded that hippocampal theta is the consequence rather than cause of the bursting unit activity in the septum.

This ‘pacemaker’ theory of theta (Tombol & Petsche, 1969) answered ‘what drives what’ but has left open how (or indeed whether) the septum functions as a simple, clock-like, pacemaker of the hippocampal theta (O’Keefe & Nadel, 1978; Stewart & Fox, 1990; Buzsaki, 2002). While individual septal units fire bursts phase-locked to hippocampal theta, they do so at different phases of theta (Gogolak et al., 1968). A burst of one septal cell, for example, occurred at the peak of hippocampal theta while the burst of another cell occurred near its trough. The population distribution of the activity of individual neurons approximates the accompanying hippocampal theta waves (Gogolak et al., 1968). However, overall, such septal activity in anaesthetized or curarized rats does not seem generally to produce an oscillating field potential (Petsche et al., 1962; King et al., 1998). Indeed, strong theta was reported to be rarely recorded from the septum except in the vicinity of the fornix (Green & Arduini, 1954) and this may be a reason why there is almost no literature on septal EEG.

In the experiment described in the present paper, we were able to record movement-related (Type 1) theta from all parts of the septum in freely moving rats. On the simplest pacemaker hypothesis, rhythmic septal unit activity (that could manifest as septal theta) drives hippocampal theta. This would predict that: (i) the septal pacemaker should not be ‘silent’ when hippocampal theta is present (whereas the opposite could occur); (ii) if any differences and/or similarities in theta are to be found between the medial septum (MS), the hippocampus, and lateral septum (LS), they should reflect the order in which theta waves travel among these structures (Fig. 1A); (iii) the conduction delay of ~ 5–15 ms from the septum to the hippocampus (Gogolak et al., 1968; McNaughton & Miller, 1984) and substantial phase shifts within the hippocampus (Petsche & Stumpf, 1960; Buzsaki et al., 1986) should work to separate MS and LS theta in time.

Materials and methods
Experimental subjects
Subjects were five male, experimentally naïve, Sprague–Dawley rats (weighing between 515 and 775 g, aged 5–7 months), obtained from the University of Otago Department of Laboratory Animal Sciences, Dunedin, New Zealand. They were housed individually in plastic box cages in a temperature-controlled room (21 °C) with incident daylight supplemented by fluorescent lighting between 06.00 and 18.00 h. The experiment was approved by the University of Otago Animal Ethics Committee following guidelines that are in accordance with the European Communities Council Directive (86/609/EEC).
Surgery
Atropine sulphate (25 mg/kg) was administered prior to surgery in order to reduce respiratory congestion. Electrodes were stereotaxically implanted in a Kopf stereotaxic machine, with the skull horizontal, under anaesthesia with sodium pentobarbital (60 mg/kg, i.p.). All coordinates were with reference to the skull surface with the skull horizontal between lambda and bregma and were based on the stereotaxic atlas of Paxinos & Watson (1982).

The chronically implanted electrodes were monopolar and aimed at a number of placements in the septum and dorsal hippocampus for simultaneous recording of EEG activity. All septal electrodes were mounted in a single multielectrode headstage. It consisted of three guiding needles (0.3, 0.45 and 0.3 mm in diameter) soldered onto a horizontal bar. The spacing between the needles was 0.5 mm. The electrodes were made of a 0.0762-mm stainless steel Teflon-coated wire (A-M Systems, Inc., Everett, USA). The wires were pulled through the needles, bent at the headstage end and glued with epoxy. The bending was necessary to prevent the later sliding of the electrodes in the epoxy. The final length of each electrode was adjusted during gluing under the microscope as measured from the horizontal bar. The target coordinates of septal electrodes were (dorsoventral distances measured in mm from dura): AP +1.2, ML 0.0, DV 6.3; AP +0.7, ML 0.0, DV 6.4; AP +0.2, ML 0.0, DV 5.85; AP +0.7, ML 0.2, DV 7.3; and AP +0.7, ML −0.35, DV 4.8. In one animal (rat LN05), the septal coordinates were AP +0.8, ML 0, DV 6.9; AP +0.5, ML 0, DV 6.9; and AP +0.2, ML 0, DV 6.9. The hippocampal electrodes were aimed at dentate gyrus (with the exception of LN05) and the CA1 region (in all animals) of the dorsal hippocampus. Two electrodes (0.0762 mm stainless steel Teflon-coated wires) were pulled through a 26-gauge needle (0.45 mm in diameter). Their target coordinates were AP-3.8, ML 2.5, DV 2.8 and AP-3.8, ML 2.5, DV 3.5.

The skull was exposed and small holes were drilled for four anchor screws, one for the ground screw (overlying the right cerebellum), one for the reference ‘electrode’ (a skull screw above the left cerebellum) and one for the hippocampal electrodes, and a narrow slit anterior to bregma was drilled for the array of septal electrodes 2.3 mm laterally from the midline. The array of septal electrodes was implanted at an angle of 20° to the vertical. The electrodes were fixed in place with dental acrylic and the space between the headstage and skull was filled...
with dental acrylic as well. The skin was then sutured and animals were given 10 days to recover.

EEG recordings were carried out in an open-field arena (~ 4000 cm² area) during a food-foraging task. A rat was connected to the recording apparatus via a cable with a multichannel source follower at the headplug end. The signal was amplified and band-pass filtered (Grass P511 series preamplifiers, 3–30 Hz) and digitized (CED 1400 Plus A/D converter, Cambridge Electronic Design, Cambridge, UK; sampling rate, 100 Hz). CED’s Spike 2 program was used for data acquisition. Eight EEG channels were recorded simultaneously (including the common reference electrode) plus a marker channel. There were thus seven EEG channels stored resulting from the differential activity between each monopolar electrode and the common reference. The marker channel contained characters that the experimenter typed on the computer keyboard during recording while watching the rat. Only two marks were used: one for the initiation of locomotion and other for the end of it.

Upon completion of the experiment, the animals were exposed to a supra-anaesthetic level of halothane. After stopping breathing, an anodic current of 20 µA lasting 20 s was passed through each electrode to mark the electrode tips with iron ions. The rats were then perfused with a standard formalin solution with 1% potassium ferrocyanide to stain the iron deposits (Prussian Blue). The brains were removed and stored in the same solution with 20% sucrose. The brains of the rats were then cut into sections of 50 µm and the sections were mounted on microscope slides and stained with thionine. Location of the electrode tips as marked by Prussian Blue could be easily detected on the background of violet thionine staining. The stereotaxic atlas of Paxinos & Watson (1982) was used to localize structures in the brain and reconstruct electrode positions.

Data analysis

Data were analysed off-line using non-commercial EEG software powered with Intel Signal Processing Library 4.5. Raw signals were cleared of artefacts (defined as high-amplitude irregular events occurring abruptly and spanning all channels) and saved as new files. On average, 3.0 ± 3.3% of data with artefacts were removed from each file. For the calculation of power spectral density (PSD) using fast Fourier transform, the EEG amplitude in all channels was normalized to a pure sine oscillation of 10 Hz to match the standard deviation (SD) of its amplitude, making the values of PSD in all channels mutually comparable. Mathematically, the value of each signal sample was multiplied by the ratio of the SD of the sine and the SD of the signal calculated over the whole length of the recording. Without amplitude normalization, the differences among PSD values in individual channels would be due to differences in signal amplitude rather than (or in addition to) reflecting the amount of spectral component.

If waves of a particular frequency f were searched for in the record, a Morlet wavelet was cross-correlated with the signal. The maxima of the cross-correlogram (above the threshold set arbitrarily to 15 and used with all our data) were used as marks for the found waves. The wavelet was defined by the following function:

\[ M(i) = e^{-\frac{(i-N/2)^2}{w^2}} \cos(2\pi F(i - N/2)) \]

where i represents the sample number (index), N is the number of samples in the searched section of EEG, w is the ‘width’ of the wavelet (250 in our case) and F = f/sampling (where sampling is the sampling rate of 100 Hz).

For a symbolic representation of the superimposition of epochs with theta in different channels (Fig. 7), a logical OR operation on marked epochs was used. For instance, if theta was present in MS between 4 and 11 s (time measured from the beginning of the recording) and in VDB between 7 and 12 s, the superimposition of theta in these structures would mark a period between 4 and 12 s. Note the superimposition was performed with both hippocampal and separately with all septal channels, for each rat and each recording. As a first step, theta frequency was determined from the power spectra of the hippocampal and the septal channel with the highest amount of theta and used in the wave-searching routine (in each channel), as described in the previous paragraph. The found peaks of theta waves were marked with ‘1’ in a newly created binary channel, the rest of the samples being assigned 0 (refer to Fig. 7B for an example). Contiguous sequences of marks (with gaps not wider than 350 ms, thus allowing for one or two missing theta waves) in each binary channel were ‘merged’, i.e. the space between marks was filled with 1s. All septal binary channels were ORed to form a new channel called A, and both hippocampal binary channels (with the exception of rat LN05 in which only one hippocampal channel was used) were ORed to form a new channel B. Hence, whenever theta was present in the septum, the amplitude in A was 1; if no septal channel displayed theta,
the value in A was 0. The same held for the hippocampal binary channel (B). In order to assess the time for which theta was present in the septum only, B (hippocampus) was subtracted from A and any −1 turned to 0. In order to assess the time for which theta was present in the hippocampus only, A (septum) was subtracted from B and any −1 turned to 0. This operation was equal to A and (NOT B) and B AND (NOT A), respectively, where AND and NOT are logical operators. Finally, in order to assess the time theta was present overall, A and B were added (ored). In all three cases, however, any isolated segment of continuous 1s < 500 ms (three or four theta waves) was removed to make the procedure more robust. The percentage of time theta was present in the septum alone was calculated as 100 × (A AND (NOT B))/(A OR B). The percentage of time theta was present in the hippocampus only was calculated as 100 × (B AND (NOT A))/(A OR B). For each animal, the means for these two values were calculated from all available recording sessions and plotted in a horizontal bar graph.

For the phase relationship of pairs of rhythmic signals (of a known frequency f and number of samples N), one signal was selected as reference and the other was cross-correlated with it (Fig. 2). A window of 64 samples taken from the second signal (starting at the first sample) was cross-correlated with the whole length of N samples of the reference signal. This process was repeated with a window of the same length starting from the next sample, etc. As a result, the cross-correlation was performed N − 64 + 1 times. In each cross-crorrelation, the distance of the second signal’s nearest maximum from 0, if within the set frequency limits (f − 0.5 to f + 0.5 Hz), was used to determine the phase (in degrees) between the two signals. (If all maxima fell within the set frequency limits, we had N − 64 + 1 values to calculate the phase histogram from). The same values were used to
calculate the underlying probability distribution, approximated automatically by a Gaussian curve. This served to estimate the ‘width’ of the phase histogram and the mean value of phase (Fig 2B). The estimated phase histogram width was used as a measure of synchrony between signals, regardless of their phase shift. When the two periodic signals were well synchronized (width < 20 ms), the estimated mean phase was used to calculate their relative lag in ms.

Relative phase shifts among (more than two) channels were calculated in the following way. For each septal channel selected as reference, a mean phase relative to other channels was computed using the procedure described in the previous paragraph. With seven channels we obtained seven values (including the zero phase of the reference channel relative to itself) for each septal channel selected as reference (Fig 2C). In an ideal case, the differences in phase among channels should not be affected by the choice of the reference. In reality, due to the sampling error and arithmetic rounding, they were different. As there was no reason to assume that one set of phase values is better than another, we calculated the mean phase differences among the channels from all septal phase values. The mean phase shifts (relative to LS) were then plotted in a bar graph (such as in Fig. 8B). Although the values of phase for the hippocampus were always calculated and are shown in Fig. 2C, they were never used further. The reason was a low synchronization between hippocampal and septal channels (compared to the synchronization among the septal channels themselves).

For an easy visual assessment of the contribution of various rhythmic components in long epochs of EEG, the signal was transformed into successive power spectra and displayed as shaded stacked spectral arrays viewed from the top. These spectrograms display changes in power simply as changes in darkness of the ascending edge of each peak, not as changes in colour. Power spectra were in this case calculated using a sliding window of 257 samples (zero-padded to the next higher power of 2) with 99% of overlap of successive positions of the window. Therefore, each ‘slice’ of the spectrogram shares 99% of data with the neighbouring slice, making the transition between them appear smooth. The length of 257 samples corresponds to 2.57 s and this defines the maximum inaccuracy with which the time of a particular event in the spectrogram can be determined (Fig. 3).

Results

Histology

All our septal electrodes were located in the septum with the exception of the most anterior electrode in rat LN03, which slid in the guiding needle and ended in the cingulate cortex adjacent to the corpus callosum (Fig. 4A). One electrode was identified in the ventral part of the septofimbrial nucleus and one in the septohippocampal nucleus. In the first case, however, the electrode tip extended into the LS and in the second case into the fornix near the MS. All MS–diagonal band of Broca (DB) complex electrodes were located ≤ 200 μm from the midline.

For convenience, unless noted otherwise, we will hereafter call ‘septal’ all nonhippocampal channels, i.e. all channels anterior to the fornix. Electrode locations in the dorsal hippocampus differed from animal to animal as seen in Fig. 4B.

Septal bursts

Occasionally, the oscillatory EEG activity in the septum occurred in the form of high-amplitude bursts (Fig. 5). This was most likely to happen during fast movements (such as hopping or rearing). The variability among animals was high. The distribution of power at theta frequency across channels during these bursts was not uniform and was different from the distribution of power outside the bursts. It often exceeded the power typical of the hippocampus. A strong burst could occur at one septal site [e.g. MS–ventral DB (VDB); Fig. 5] and be

Fig. 5. A 140-s-long segment of EEG (top) showing ‘bursts’ in the MS-VDB complex. The bursts had an oscillatory character, as seen in the right close-up view. Theta was present both within and outside the bursts. In general, the frequency of the bursts was different from that of hippocampal theta. All our analyses concern recordings cleared of these bursts. CA1, CA1 region of the hippocampus.
absent at another (e.g. LS). This argues against any large-scale volume conduction occurring in the septum.

All our analyses were on data that did not contain these bursts. While deleting the bursts, we adopted a highly conservative approach and cut more rather than less (on average, 38 ± 15% of data were removed, leaving between 5 and 10 min of burst-free EEG). Note that the data were cut from all channels, not only those with the apparent burst.

**Behaviour and theta**

There were notable differences among animals in behaviour, such as the amount of rearing, grooming, acceleration and freezing. Despite these differences, the average percentage of locomotion in an open field was similar among the rats (21–35% of a 10-min recording session) and the locomotion was accompanied by hippocampal theta in all of them. For each animal the peak theta PSD values (found between 6 and 9 Hz) from locomotion-present and locomotion-absent EEG from every recording were entered in an ANOVA with Structure (septum, hippocampus) as a between-subjects factor and Behaviour (locomotion present, locomotion absent) as a within-subject factor. As expected, the effect of locomotion was highly significant in all rats (LN03, \(F_{1,26} = 102.0;\) LN04, \(F_{1,19} = 56.4;\) LN06, \(F_{1,33} = 36.2;\) LN07, \(F_{1,33} = 77.7;\) LN05, \(F_{1,10} = 30.7;\) \(P < 0.001\) in all cases); thus in this study, as in previous studies, theta was much more reliable during locomotion than in its absence. This also suggests that the majority of theta waves that we recorded and analysed are of Type 1 (locomotion-related). Further, in all animals the average hippocampal

![Graphs of averaged PSD](image-url)
theta PSD grew more than the septal theta PSD during locomotion. This interaction effect was significant in all but one rat (LN04, $F_{1,10} = 6.2, \ P < 0.05$; LN06, $F_{1,33} = 5.1, \ P < 0.05$; LN07, $F_{1,33} = 9.6, \ P < 0.01$; LN05, $F_{1,10} = 8.0, \ P < 0.05$).

Spectral content
Unlike the hippocampal EEG, the septal channels contained a large proportion of low-frequency (delta) EEG waves (Fig. 6). Their maximum PSD peak lay at 2.7 Hz and was wider than that of theta. This was most apparent when long periods of EEG (up to 10 min) were analysed; however, on a shorter time scale (≈ 10 s) the frequency of slow septal waves appeared to be more stable and thus PSD peaks were relatively narrow. Sometimes, a distinctive peak in the power spectrum was observed at ≈ 5 Hz (Fig. 6, LN03, left panel).

The occurrence and amount of theta in any structure did not appear to vary systematically across recording sessions for any animal (as is evident from the small SDs obtained below). We therefore treated the values obtained for any particular electrode and animal as independent samples of a central value associated with that electrode. For each bar graph in Fig. 6 (theta part only) one-factor ANOVA was performed with Channel as factor. The effect of Channel was significant in all cases (LN03, $F_{6,119} = 40.2$; LN04, $F_{6,112} = 140.3$; LN06, $F_{6,112} = 334.6$; LN07, $F_{6,98} = 78.8$; LN05, $F_{3,44} = 150.2; \ P < 0.001$ in all cases). Post hoc comparison was made with the largest hippocampal and largest septal value in each graph. In none of the cases was the septal theta power higher than or equal to the theta power in the hippocampus (Scheffé test: $P < 0.05, \ P < 0.001, \ P < 0.001, \ P < 0.001$, respectively, for the five graphs in Fig. 6, filled bars). The number of significant differences among individual septal channels in different animals (as revealed by the post hoc comparisons) varied considerably and the distribution of significant differences was not consistent across animals. No single septal site was therefore found to consistently contain the highest amount of theta in any animal; this result also rules out volume conduction as a source of the general similarities among the different septal electrodes. (The high degree of localization of the septal bursts, see above, also argues against this.)

Grouped septal vs. grouped hippocampal theta
None of the septal channels would thus be able to ‘drive’ hippocampal theta continuously if the simple pacemaker hypothesis were true. It might be, however, that each septal channel displays theta at a different time and their temporal superimposition covers the whole period of theta in the hippocampus. If this were the case, it would be the ‘sum’ of the oscillatory activity of individual parts of the septum that paces the hippocampal theta. To investigate this possibility, we performed a temporal superimposition analysis with all septal and hippocampal channels for each animal. The average

![Fig. 7](https://example.com/fig7.png)

**Fig. 7.** (A) Mean percentage of time (± SD) for which theta was present in the septum only (left bars) and in the hippocampus only (right bars). A dotted line between the two bars in each row represents the percentage of time theta was present simultaneously in both structures. The means were calculated from up to 18 session values. The size of the SD thus reflects the stability of this variable over days. (B) An example of three EEG channels (MS, LS and CA1 region of the hippocampus) recorded simultaneously in rat LN03 and marks as generated by the automated wavelet cross-correlation routine used in A (in A all five septal and both hippocampal channels were used). Note: without normalization, the amplitude of the hippocampal signal would be ≈ 2x larger.
values of the percentage of time theta was found in the hippocampus only and in the septum only are plotted in Fig. 7A. For each rat the two means were compared using a Welsh’s t-test. In all cases the hippocampal theta alone was a significantly more common phenomenon than septal theta alone (LN03, \(t_{20} = 3.09, P < 0.01\); LN04, \(t_{12} = 8.83, P < 0.001\); LN06, \(t_{13} = 15.89, P < 0.001\); LN07, \(t_{12} = 19.70, P < 0.001\); LN05, \(t_{10} = 5.87, P < 0.001\)). The percentage of time theta coincided in the two structures ranged from 57 to 87%. It is noteworthy that the procedure of wave searching inevitably generated some false-positive (‘nonwaves’ identified as waves) and false-negative (waves ignored) hits. As this applies equally to the septum as to the hippocampus, the reported differences would not be affected even if different parameter values were used in the searching routine.

Theta activity in individual septal channels did not appear to be independent. That is, the overall extent to which theta would be seen in one or more channels would not be increased by adding additional septal channels to the one with the highest amount of theta. There were, in that sense, ‘good’ and ‘bad’ septal sites rather than many independent small ‘bad’ sites in the septum.

Phase analysis

Merged sections of EEG in which theta was found in both the septum and the hippocampus (these fall in the dotted space in Fig. 7) were subjected to a phase analysis. As part of this analysis, we obtained estimates of the phase histogram width (see Fig. 2B for an example). Narrow histograms are indicative of good synchronization of rhythms in two channels (regardless of the relative phase of the two rhythms) while broad histograms represent unsynchronized signals (e.g. two rhythms with different or distinctly variable frequencies). The phase analysis was performed on every recording for each rat. The mean values of the phase histogram width for a particular channel (‘desynchronization’) as shown in Fig. 8A are averages over values for that channel relative to each septal channel (the channel itself being

\[A\]

\[B\]

**Fig. 8.** (A) Average width of the phase histogram of theta for four rats with electrodes in septal and hippocampal sites. Each bar is the average over recording sessions and desynchronization values relative to each septal channel (except the analysed channel itself). Note the good synchronization of theta within septum and poor synchronization of hippocampal theta relative to the septal theta. (B) Lag of theta in Cg, SHi, MS, SFi and VDB relative to LS. Negative values represent advancing of theta relative to LS. The error bars are SDs. See Fig. 2 for an example of generating phase lags and legend of Fig. 6 for abbreviations.
excluded from averaging) and then over recording sessions. For example, if values in Fig. 2C were not phases but histogram widths and the table represented a whole session, the mean session desynchronization of MS would be calculated by averaging across values in column LS, excluding the value in row LS. Each bar in Fig. 8A thus represents how well, on average, theta in a given structure and animal is synchronized with theta in the septum (only septal channels are used as reference and all septal channels are used). Septal channels were mutually well synchronized (the differences were not significant as tested by ANOVA).

Hippocampal channels were less synchronized with the septal channels than were the septal channels with each other. Note, however, that the synchronization between the two hippocampal channels (i.e. CA1 and dentate; not shown in Fig. 8A) was no better than the synchronization between the hippocampus and septum. Analysis of randomly selected 3-s epochs of theta using interwave periodograms revealed that the relative lack of synchronization between the hippocampus and septum or between the two hippocampal channels is due to wave-to-wave variability in wave length rather than longer-lasting differences in frequency in the two

![Fig. 9. Spectrograms' of a 1.5-min-long segment of EEG from rat LN03. The organization of channels from left to right is arbitrary. Power is represented by the intensity of shadows (rather than colour as in a classical spectrogram) and is optimized relative to the maximum value within each graph. Thick lines on the left denote locomotion as judged and marked by the experimenter. Numbers next to each column are exact frequencies of the strongest peak in power that occurred at that time. If there were two such peaks, their corresponding frequencies are written under each other and separated by a comma. See Fig. 6 legend for abbreviations.](image-url)
channels compared. Thus, on a fairly short time scale, these channels could show leading, lagging and rapid shifts between them. The synchronization tended to be proportional to the amplitude of theta but no systematic inspection of wavelength variations was performed. For reasons explained above, only the septal channels were used in the phase analysis. Figure 8B summarizes the results for four rats. The relative phase shift, expressed as delay in ms relative to LS, was within 10 ms in all cases (i.e. below the level of the maximum accuracy achievable with the sampling frequency of 100 Hz).

**Spectrograms**

Figure 9 demonstrates that theta and slow septal waves generally do not co-occur. The slow septal waves are mostly limited to the septum and behaviourally to the periods of nonlocomotion. Theta, on the other hand, can be seen in both the septum and the hippocampus, although theta in the septum appears to be more vulnerable to disturbance by other (regular or irregular) EEG activity. When periodic signals occur in two or more channels at the same time, their frequencies are not necessarily the same. Indeed, instances of continuous periods with different rhythms in the septum and hippocampus are not difficult to

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**Fig. 10.** Examples of spectrograms of 5-s-long segments of EEG with sustained periodic EEG activity (rats LN03, LN04, LN06 and LN07). (A) Theta in all channels. (B–D) Mixture of hippocampal theta and a septal rhythm of a different frequency. (E) Theta in the septum only. (No examples of this sort could be found in rats LN06 and LN07). (F) Slow septal waves. (G) Slow septal waves ‘leaking’ in the hippocampus. See Fig. 6 legend for abbreviations.
find (Fig. 10). In such cases, the septal rhythm is typically of a lower frequency. Very rarely do the low-frequency rhythms appear in the hippocampus. Within the septum, there are regional differences in the amount of the hippocampal component. Among the septal channels, the MS contains the least amount of theta that is concurrently seen in the hippocampus.

Discussion
We recorded substantial EEG theta activity from the septum of freely moving rats, in contrast to previous studies using drugged rats. The septal theta had a number of properties inconsistent with a simple homogenous pacemaker located in the MS and determining, by itself, hippocampal theta activity.
(i) Septal theta was clear and of substantial amplitude but occurred less frequently than hippocampal theta. Periods with theta in the hippocampus but not in the septum were more frequent than periods with theta in the septum only.

(ii) Theta could be recorded from all tested parts of the septum, but the phase shifts between LS and either MS or VDB were too small for LS activity to be controlled from MS solely via a relay in the hippocampus.
(iii) Different parts of the septum showed different amounts (in terms of power and duration) of theta and these differences were stable over a period of weeks.
(iv) When theta occurred in all channels, it was well synchronized across the septum but less so between the septum and the hippocampus. The septum and hippocampus were able to display rhythms of different frequencies at the same time. When this happened, the septal rhythm did not appear to ‘leak’ into the hippocampus, while some (or no or all) septal channels could contain the hippocampal rhythm to a greater or lesser degree, in addition to their ‘own’ rhythm.
(v) It was hard to find examples in which an oscillation in the septum at theta frequency (~ 7 Hz) appeared in the absence of the hippocampal rhythm of the same frequency. This was not true, however, for septal rhythms of lower frequencies (~< 5Hz). Many existing data are consistent with the simple (homogenous, unidirectional) pacemaker hypothesis, but they have also been consistent with a variety of more complex possibilities (e.g. Fig. 16 in O’Keefe & Nadel, 1978) and the simple pacemaker hypothesis has not been definitively confirmed (Stewart & Fox, 1990). None of the predictions of the simple pacemaker hypothesis advanced in the introduction were supported by our data. A model of the septum as a simple unidirectional source of theta rhythmicity for the hippocampus (Fig. 1A) is not adequate to account for the present results. For locomotion-related (Type I) theta, at least, it could as easily be the hippocampus as the MS that would function as a good pacemaker (Fig. 1B). Its strong and steady theta rhythm would impact on the septal targets. The activity of septal neurons, driven from the hippocampus, would show up as an oscillating field potential at theta frequency. On the EEG evidence the reverse is less likely, or at least less frequent.

However, synchronization and phase relationships did not support a primary role for either structure in the control of their rhythmicity, with evidence of shifts between leading and lagging with only small differences between them. One possibility is that the septum and the hippocampus can each come under the control of other structures (Orban et al., 2001). Certainly, the supramammillary area controls theta frequency in free-moving animals under at least some conditions (McNaughton et al., 1995), and has direct projections to both the septum and hippocampus (Pan & McNaughton, 2004). However, if the control by such a structure were unidirectional then, with fixed conduction times, we would probably not expect the shifts between leading and lagging observed.

Medial septal to hippocampal influences are part of the standard model. However, the reverse is not unreasonable (O’Keefe & Nadel, 1978). There are two classes of projections from the hippocampus to the septum. Pyramidal cells of CA1, CA3 and subiculum send axons to LS (Leranth & Frotscher, 1989; Risold & Swanson, 1997), where they exert an excitatory influence upon local GABA-ergic neurons (Fig. 11A). These in turn send fibres to the MS, synapsing on cholinergic cells (Leranth & Frotscher, 1989); however, they are relatively sparse (Leranth et al., 1992). The second route originates with the hippocampal interneurons and terminates on MS interneurons (McLennan & Miller, 1974; Alonso & Kohler, 1982; Gulyas et al., 2003).

Electrical activity in the hippocampus is thus likely to influence the excitability of MS cells and affect the generation of the local field potential there (Dragoi et al., 1999). The ‘driving’ of one structure by
another is likely to be proportional to the number of impulses arriving in the target structure. This may differ in different behavioural and/or cognitive states resulting in complex relationships between the septal and hippocampal EEG. So, rather than the septum being a simple pacemaker, the appearance of a pacemaker may be an emergent property of septohippocampal interactions. (In this context, ‘pacemaker’ must be understood as the source of global synchrony rather than of the fundamental frequency underlying the synchrony. The latter, at least on some occasions, depends on input to the septohippocampal system from the hypothalamus.)

On this view, each structure would continually influence the other, perhaps to maintain as close to a zero phase relationship as possible. Recent evidence obtained under urethane anaesthesia suggests that this is a general feature of the architecture of the theta system. As we have noted there is good evidence that the supramammillary area can control the frequency of hippocampal theta. However, some cells in the supramammillary area fire with a fixed time delay following hippocampal theta waves (Kocsis, 2006). Blocking of hippocampal theta has also been shown to alter both spikes per burst (Kirk & McNaughton, 1991) and interburst interval (Kirk et al., 1996). There are, then, both ascending and descending interactions between the hypothalamic and hippocampal components of the theta system. As a result, rhythmic activity in the supramammillary area can lead or lag hippocampal theta with leading being most obvious during induced acceleration of frequency and lagging most obvious during deceleration (Kocsis & Kaminski, 2006).

This picture of reciprocal interactions, between several structures, underlying their joint rhythmicity leaves open the question of whether these interactions are fundamentally obligatory or ‘permissive’ (King et al., 1998; Buzsáki, 2002), but is certainly consistent with the fact that both the septum (Vinogradova et al., 1980) and the hippocampus and other limbic structures (Buzsáki, 2002) have an intrinsic oscillatory capacity. It is also consistent with the view that the extrahippocampal and intrahippocampal theta oscillators can be relatively independent.

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Abbreviations
DB, diagonal band of Broca; EEG, electroencephalogram; Hipp., hippocampus; LS, lateral septum; MS, medial septum; PSD, power spectral density; SD, standard deviation; VDB, ventral DB.

References