

Extrinsic Modulation of Theta Field Activity in the Entorhinal Cortex of the Anesthetized Rat

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ABSTRACT

Field recordings of the entorhinal cortex (EC) were studied and compared to those recorded concomitantly in the dentate region of the hippocampal formation (HPC) in the urethane anesthetized rat. The EC, like the HPC, showed two main variations of spontaneous field activity: a desynchronized, large amplitude irregular activity and a synchronized, rhythmic, slow frequency field activity (RSA or theta). Corroborating previous research, a phase reversal was seen across layer II of the EC and when recorded superficial to this layer, EC theta was phase-locked to that recorded from the HPC (dentate). Entorhinal cortex (and HPC) theta could be evoked by the application of moderate tail pinches (sensory stimulation), by pharmacological treatments enhancing cholinergic transmission, and by electrical stimulation of the posterior hypothalamus. Spectral analysis revealed that in all cases, theta was produced coherently across the two limbic structures. Entorhinal cortex (and HPC) production of theta could be abolished by pharmacological treatments disrupting cholinergic transmission, and by reversible procaine inactivation of the medial septal region. Therefore, it was concluded that limbic theta is modulated spontaneously, and with sensory and hypothalamic stimulation through the activity of cells in the medial septal region via muscarinic neurotransmission. It was also hypothesized that the activation of cells in the posterior hypothalamus linearly codes the frequency, and to a lesser extent the power, of EC and HPC theta. Given these findings and the coincidence and coherence of the occurrence of theta across the EC and HPC, it was postulated that it occurs via a parallel mechanism in the two areas.

Key words: ascending synchronizing system, acetylcholine, oscillatory activity, limbic cortex

The study of rhythmical brain activity at the intracellular and extracellular level has recently generated a number of hypotheses relating to mechanisms and functions (Steriade et al., 1990; Bland and Colom, 1993). An example of synchronous field activity is the theta rhythm generated in various limbic cortices. The study of theta is of interest both because it is the largest amplitude rhythmical slow wave in the mammalian brain (a fact that facilitates its study) and because it has strict behavioral correlates (Vanderwolf, 1969; Bland, 1986). Therefore, its study will undoubtedly lead to insight in brain-behavior relationships.

In the rat, theta has traditionally been studied primarily within the hippocampal formation (HPC), the cornu ammonis, and dentate regions (Bland, 1986). However, other limbic

areas are capable of independently generating theta rhythm. One such region is the entorhinal cortex (EC) (Mitchell and Ranck, 1980), a periarchicortical region bordered by the rhinal fissure laterally and dorsally, the parasubiculum medially, and the paraamygdaloid and prepyriform regions ventrally. The EC is a nodal point in the processing of limbic information for three reasons: it is the source of the most extensive HPC afferent (the perforant path); it receives the bulk of the HPC efferent system through the subiculum; and it makes reciprocal projections with the entire cortical mantle (for anatomical reviews see Swanson et al., 1987, Lopes da Silva et al., 1990).

This study addressed some basic questions related to the expression of EC theta in the urethane anesthetized rat. First, is the coexpression of theta coincident and coherent between the HPC and the EC and can it be elicited by the same sensory stimulation (e.g., in response to tail pinch)? Second, is it mediated primarily by cholinergic neurotransmission similar to the HPC? (Kramis, et al., 1975). Third, is the EC subject to the same septally mediated influences of the ascending brainstem

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synchronizing system as the HPC? (Smythe et al., 1991, 1992). Portions of this research have been presented in abstracts (Dickson and Bland, 1991, 1992).

MATERIALS AND METHODS

Twenty male Long-Evans rats weighing between 300 and 500 g and supplied by the Animal Care Services at the University of Calgary were used in this study. Each rat was housed along with up to four littermates in clear Nalgene cages with ad libitum access to water and food. The day and night cycle was 12 hours on and off with lights off at 8:00 PM.

Rats were anesthetized with halothane and implanted with a jugular catheter. Halothane was discontinued and ethyl carbamate (urethane) was administered intravenously in a concentration of 0.8 g/mL. The animals were then placed in a standard stereotaxic apparatus, their skulls exposed and leveled to horizontal. Core temperature was maintained at $37^{\circ} \pm 1^{\circ}\text{C}$ with a servo-driven heating blanket connected to a rectal probe.

An indifferent electrode consisting of an uninsulated tungsten wire was implanted in the frontal cortex and fixed to the skull using dental acrylic. A tungsten microelectrode (0.2–1.0 M Ω ; measured at 100 Hz) was placed in layer I or occasionally in layer III or deeper in the right medial entorhinal cortex (8.8 mm posterior, 5.3 mm lateral to bregma, and 4.5–5.5 mm ventral to dural surface). Another tungsten microelectrode was positioned in the dentate molecular layer of the right dorsal hippocampus (3.3–3.5 mm posterior, 2.0–2.2 mm lateral to bregma, and 2.1–2.6 mm ventral from dural surface). Recordings were made with the stereotaxic frame connected to ground. Electrical activity from both areas was amplified differentially with respect to the indifferent and to each other at a gain of 200 and filtered at a bandpass of 1–35 Hz. Signals were displayed on both an oscilloscope and polygraph in addition to being stored on VHS tape for off-line analysis.

Ten rats were prepared as previously described but in addition to entorhinal and hippocampal recording electrodes, a bipolar stimulating electrode made of twisted 250- μm diameter insulated stainless-steel wire was implanted in the posterior hypothalamus (PH) (3.3 mm posterior, 0.1 mm lateral to bregma, and 7.5–8.3 mm ventral to dural surface). Five of these animals were implanted with a cannula in the dorsal aspect of the medial septum (MS) (0.5 mm anterior, 0.0 mm lateral to bregma, and 5.5 mm ventral to dural surface). The cannula consisted of a section of 30 gauge tubing fastened to a length of PE50 intramedic tubing. The cannula was fixed into a length of guide tubing (23 gauge) and extended beyond it 5.0–6.0 mm. The guide tube facilitated stereotaxic placement of the cannula. Infusion pressure was generated by a Harvard Instruments infusion pump using a Hamilton 50 μL syringe. A diagrammatic representation of this experimental preparation is shown in Figure 1.

After an experiment was concluded, small electrolytic lesions were made at recording and stimulating sites and the animal was perfused through the heart with formal saline (10%). The brain was removed and stored overnight in formal sucrose (30%). The brain was frozen sectioned and 40- μm slices showing electrode or cannula placements were mounted on gelatin-coated glass slides and stained with either cresyl violet or thionin.

Testing procedure

Spontaneous and sensory-evoked theta

Baseline samples of large amplitude irregular activity (LIA), and spontaneous, or tail pinch-evoked theta were obtained prior to all other experimental manipulations.

Cholinergic manipulations

Five rats were tested. After baseline recording, the level of anesthesia was increased to limit the generation of spontaneous theta. Physostigmine salicylate (1.0 mg/kg) was then administered slowly via the jugular catheter over a period of 2 minutes. Its effect on the field activity of the limbic cortex was recorded for a period of 5–20 minutes postinfusion. Atropine SO_4 (25–50 mg/kg) was then administered to three animals in the same manner and its effects on the physostigmine-evoked field activity were recorded after a 15-minute interval.

Manipulations of the ascending synchronizing system

Ten rats were tested. After spontaneous recordings were obtained, a stimulation protocol (baseline) consisting of 3 randomized sets of six different levels of intensity of hypothalamic (PH) stimulation from 0.1–1.0 mA was conducted and recorded. Individual stimulation trials lasted for a duration of 10 seconds at a frequency of 100 Hz. Following completion of this protocol, 2.0 μL of procaine hydrochloride (20% by weight) was infused at a rate of .5 $\mu\text{L}/\text{min}$ into the MS in five rats. PH stimulation and tail pinches were administered immediately, and at 15, 30, and 60 minutes postinfusion.

At the last time point (50–60 minutes postinfusion) the ability of PH stimulation to evoke theta had fully recovered and data from this stimulation protocol was used as a comparison for the next manipulation. Atropine SO_4 was administered intravenously (25 mg/kg) and in one animal, intraseptally (100 μg in 1.0 μL). Fifteen minutes following this last manipulation another stimulation protocol was given.

Data analysis

Analysis was conducted by comparing the phase of the signals and the individual power, cross power, and coherence spectra between states and for each of the pre- and post-conditions described. Analogue data segments 8 seconds in length were fed into a Bruel and Kjaer Dual Channel Signal Analyzer (Type 2032). Field activity from both areas was sampled simultaneously at a rate of 256 Hz. The signal analyzer performed a Fourier transform of the data and displayed the results as instantaneous power spectra. A log ratio (db) scale was used with $\text{db} = 20 \log (V \times 200/1\text{mV RMS})$. Typically, an ensemble average of five or more samples was taken and auto, cross, and coherence spectra were all computed and plotted. Limbic wave states (i.e., theta and LIA) were compared by computing the auto and cross power at the frequency corresponding to the theta peak in both and conducting dependent *t*-tests. Coherence was averaged across the theta peak half amplitude bandwidth and its significance assessed by using tabled values for Pearson's rho at $P < .05$ ($df = 25$). This method of statistical testing is analogous to that used by Lopes da Silva et al. (1973) who converted coherence values to Fisher-z scores.

The relative phase of the theta signals was computed using

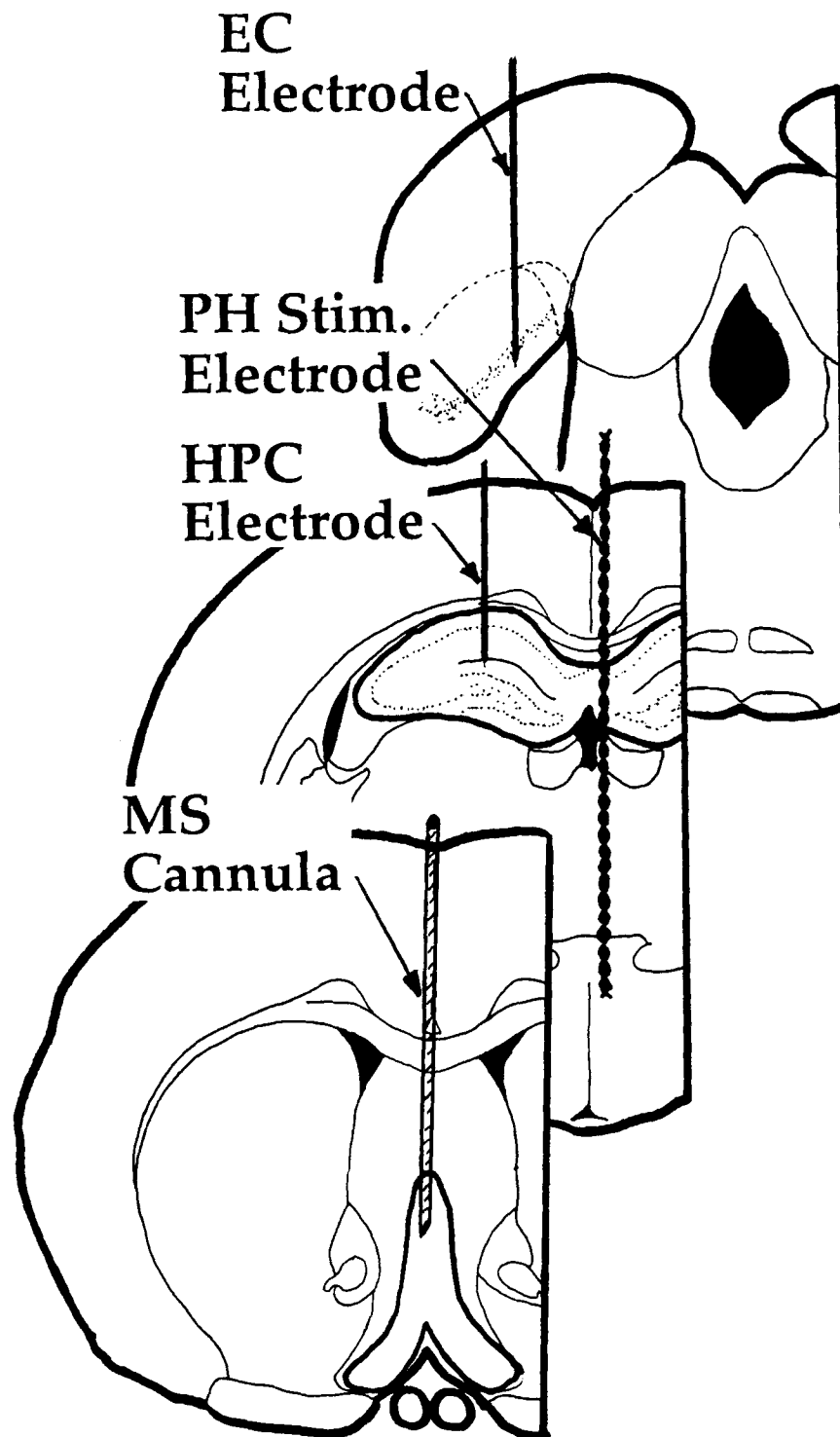


Fig. 1. Diagrammatic representation of the recording, stimulating, and microinfusion arrangement used to assess the extrinsic modulation of entorhinal cortex (EC) field activity. Experiments conducted to assess the spontaneous, sensory, and pharmacological manipulations were conducted with two tungsten microelectrode implants, one in the medial EC and the other in the dorsal hippocampal formation. Experiments conducted to assess the effects of manipulations of the ascending synchronizing system had two additional implants: a twisted, bipolar stainless-steel stimulating electrode was placed in the posterior hypothalamus and a microinfusion cannula was placed in the medial septal region.

a cross phase spectrum. This method of measuring the phase relationship was chosen due to its correspondence with the exact frequency of the theta peak. Previous measurements of HPC/EC theta phase relations have been determined by the time lag and lead method using the first peak of the cross-correlation function and its distance from the origin (Mitchell and Ranck, 1980; Alonso and Garcia-Austt, 1987a). This latter method represents all frequencies present in the signal and may therefore lead to an inaccurate measure of the exact phase relationship of theta.

Analysis of the electrical stimulation trials was made by computing instantaneous spectra for each level of stimulation and measuring the peak frequency and power for both the EC and HPC. If no peak was observed in the conditions following the two manipulations, a power reading was taken at the average frequency of the appropriate baseline condition and the frequency recorded as zero for that trial. All values were then averaged for each protocol and difference values computed by subtracting measures postmanipulation from the appropriate premanipulation measures. These results were then plotted and subjected to statistical evaluation (regression analysis and dependent *t*-tests, one-tailed).

RESULTS

Histological analysis revealed that all HPC recording electrodes were located in the molecular layer of the dentate, near the hippocampal fissure. In 17 animals, the tip of the EC electrode was located close but superficial to layer II and in the other three the tip was located deeper to layer II but with a large degree of variance in distance from this layer. The locations of the microinfusion sites were confirmed to be in or immediately adjacent to the medial septal nucleus and hypothalamic stimulating electrodes were localized in the region of the dorsomedial and posterior hypothalamic nuclei.

Spontaneous and sensory-evoked theta

In all animals, theta recorded from electrodes placed superficially to layer II in EC was at an average of $-0.9^\circ \pm 8.9^\circ$ phase lag to that recorded from the dentate electrode. This value was found to be not significantly different from 0° , therefore, superficial EC theta was said to be in phase with dentate theta. The phase lead computed for EC theta recorded from layers deeper than II was at an average of $197.6^\circ \pm 34.9^\circ$ compared to that of the dentate. The latter value was found to be not significantly different from 180° , and thus, was referred to as being phase reversed with respect to dentate theta. The variance for the phase average for deeper EC electrode sites was greater due to the low number of conditions in addition to variation in electrode placements.

In eleven animals, the field activity of both the entorhinal cortex and hippocampus spontaneously cycled between LIA and theta. In the other 8 animals theta was elicited mainly in response to mild tail pinch stimulation. Examples of these are shown in the upper panels of Figure 2. Spontaneous theta appears concomitantly in both structures, as does that evoked by tail pinch. The occurrence of LIA is also coincident in both locales.

Spectra for theta and LIA are plotted in Figure 3. As can be seen, during the occurrence of theta, both autospectra and

the cross spectrum show prominent peaks centered at 4.25 Hz. For the same field samples, the frequency of these peaks were always aligned across the EC and HPC (average value for spontaneous-tail pinch theta = 4.15 ± 0.11 Hz). This is reflected in the presence of a peak in the cross spectrum that is a weighted cross-product of the two individual autospectra and reflects the correspondence of phase and power across the two areas. The average theta peak power readings for the EC and the HPC were 34.9 ± 1.0 and 41.8 ± 1.0 db, respectively. The average theta peak power reading for the cross spectrum was 38.3 ± 1.0 db. Another observation to note is that the bandwidth corresponding to the ($1/2$ amplitude) cross spectral peak in Figure 3A (during theta) shows a large and systematic degree of coherence. The average coherence for the theta peak measures 0.94 ± 0.01 ($P < .01$).

As can be seen in the power spectra for LIA, theta peaks are no longer apparent. The decrease in power at the corresponding peak theta frequency (in this example 4.25 Hz) is, on average, 10.5 ± 1.0 , 13.7 ± 1.0 , and 14.3 ± 1.0 db for the EC, HPC, and cross spectra, respectively, when the two states are compared. In addition, the level of coherence in the same theta bandwidth is not as high nor is it as systematic. At the frequency corresponding to the peak itself, the average coherence value is 0.36 ± 0.05 (nonsignificant). Although some seemingly significant levels of coherence exist in the bandwidth from 10–35 Hz this is due to the small sample size. With larger samples, only low (< 0.3) levels of coherence exist in the range from 1–35 Hz. This is true also of theta except in the bandwidth corresponding to the cross spectral theta peak where the coherence values are consistently above 0.9.

Cholinergic manipulations

In all five animals, both the EC and the HPC began to show continuous trains of theta 3–5 minutes after the infusion of physostigmine (bottom left panel, Fig. 2). The phase relationship between the signals was found to be consistent to that seen prephysostigmine. Spectra for the physostigmine-evoked theta (Fig. 4A) are similar to those presented for spontaneous theta in Figure 3A. Two noticeable exceptions are the presence of a weak harmonic peak in all three power spectra (present in all experiments) and the slightly lower frequency at which the peak manifests itself (averaging 4.09 ± 0.25 Hz across all animals). Peak power at the theta peaks averaged 38.7 ± 0.7 , 46.7 ± 1.4 , and 42.7 ± 0.8 db for the EC, HPC, and cross spectra, respectively. These peaks reflected an average increase in power of 10.3 ± 0.9 , 15.2 ± 2.9 , and 13.0 ± 2.3 db for the EC, HPC, and cross spectra, respectively, comparing the pre- and postphysostigmine conditions at the same frequency. Also prominent is the high degree of coherence shown in the bandwidth of the cross spectral peak. The average coherence for the peak frequency during physostigmine theta was 0.98 ± 0.01 ($P < .01$) compared to its prephysostigmine value of 0.56 ± 0.07 (NS). Note that this level of coherence was not observed for the harmonic peak.

In some cases, following an initial insufficient injection, theta activity became decremental and was eventually abolished (confirmed by spectral analysis) in either the EC or HPC uniquely. This was the only example of theta occurring in one

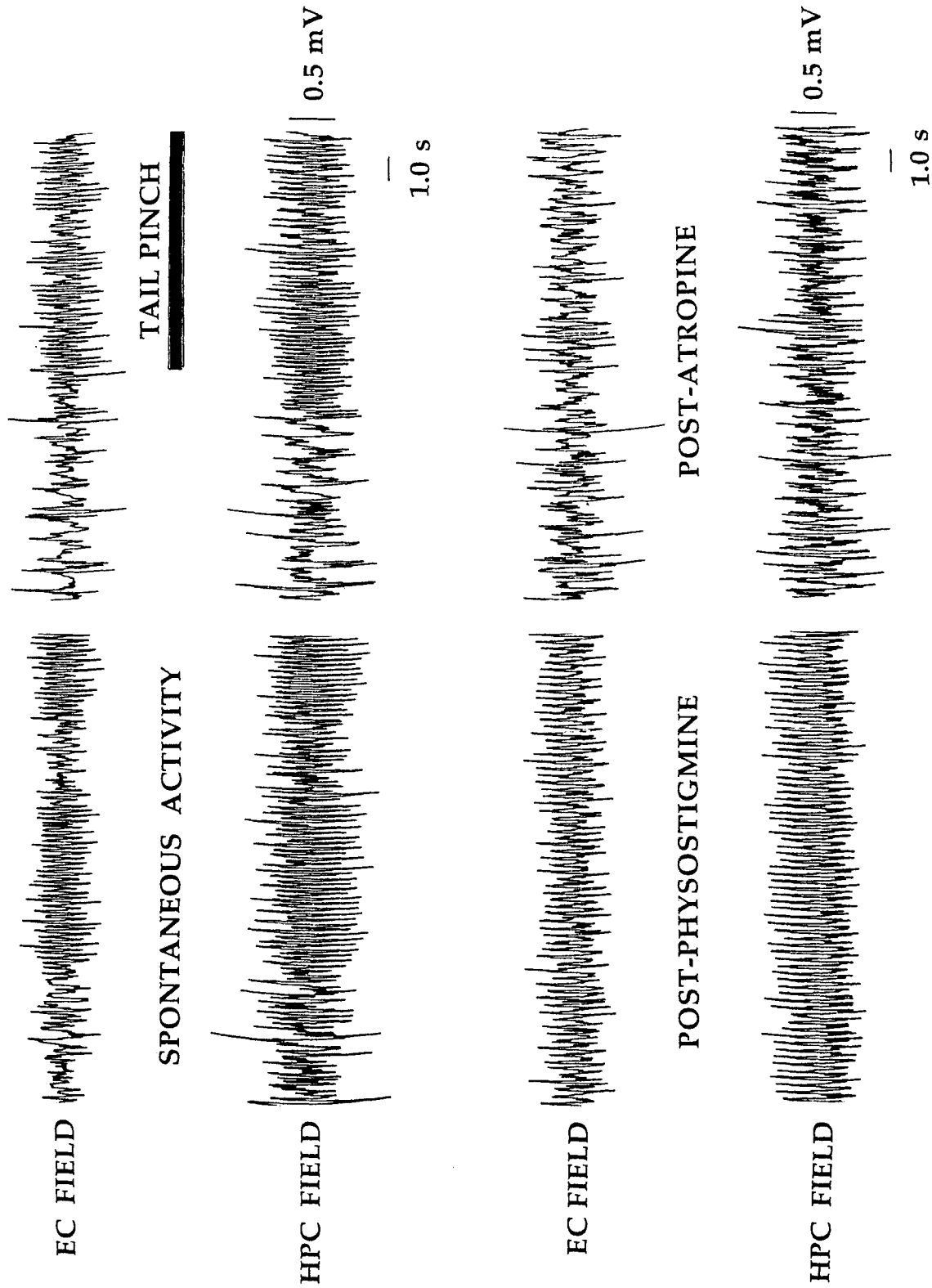


Fig. 2. Analogue recordings showing the field activity of the entorhinal cortex (EC) and hippocampal formation (HPC) occurring spontaneously, in response to tail pinch, and after treatments with both physostigmine and atropine. Theta is present concomitantly in both structures during spontaneous cycling, during a moderate tail pinch, and after an i.v. injection of physostigmine. Following an injection of atropine, theta is abolished and replaced by an LIA-like activity.

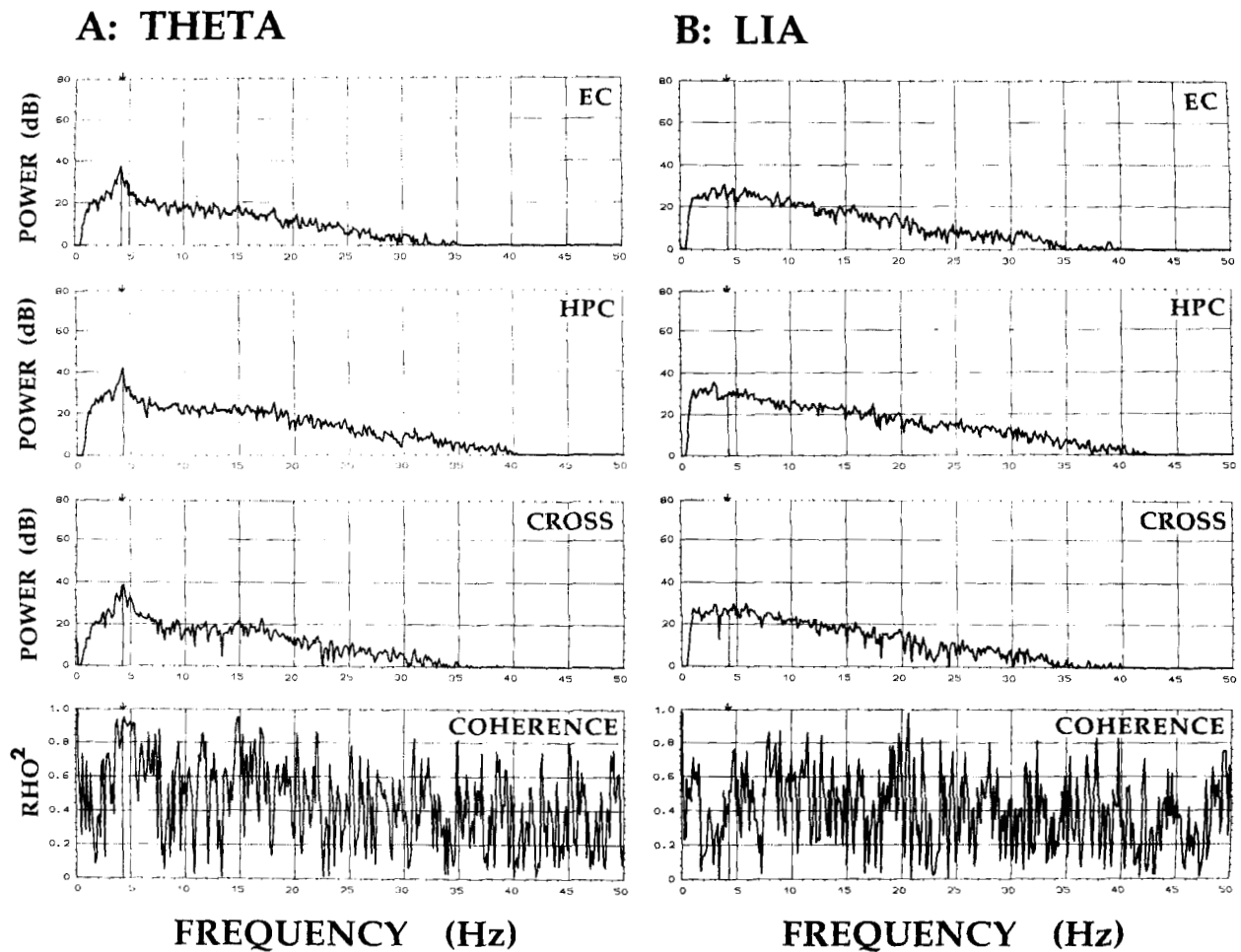


Fig. 3. Individual power, cross power, and coherence spectra of entorhinal cortex (EC) and hippocampal formation (HPC) (A) theta and (B) LIA field activity (from signals shown in Fig. 2). Theta activity is dominated by the presence of a peak in the autospectra for the EC, HPC, and the cross spectrum. This peak is centered at a frequency of 4.25 Hz (bandwidth 3.5–5.375) and is not present in the spectra for LIA. It represents a 14.0, 12.4, and 13.9 db increase in power at that frequency for the EC, HPC, and cross spectra, respectively, when comparing the two states. The coherence spectrum during theta demonstrates a large and systematic degree of correlation between the signals in the bandwidth of 3.5–5.375 Hz. Such a degree of coherence is not seen in the spectrum for LIA. The coherence value at 4.25 Hz during theta is .926 compared to the value of .387 for the same frequency during LIA.

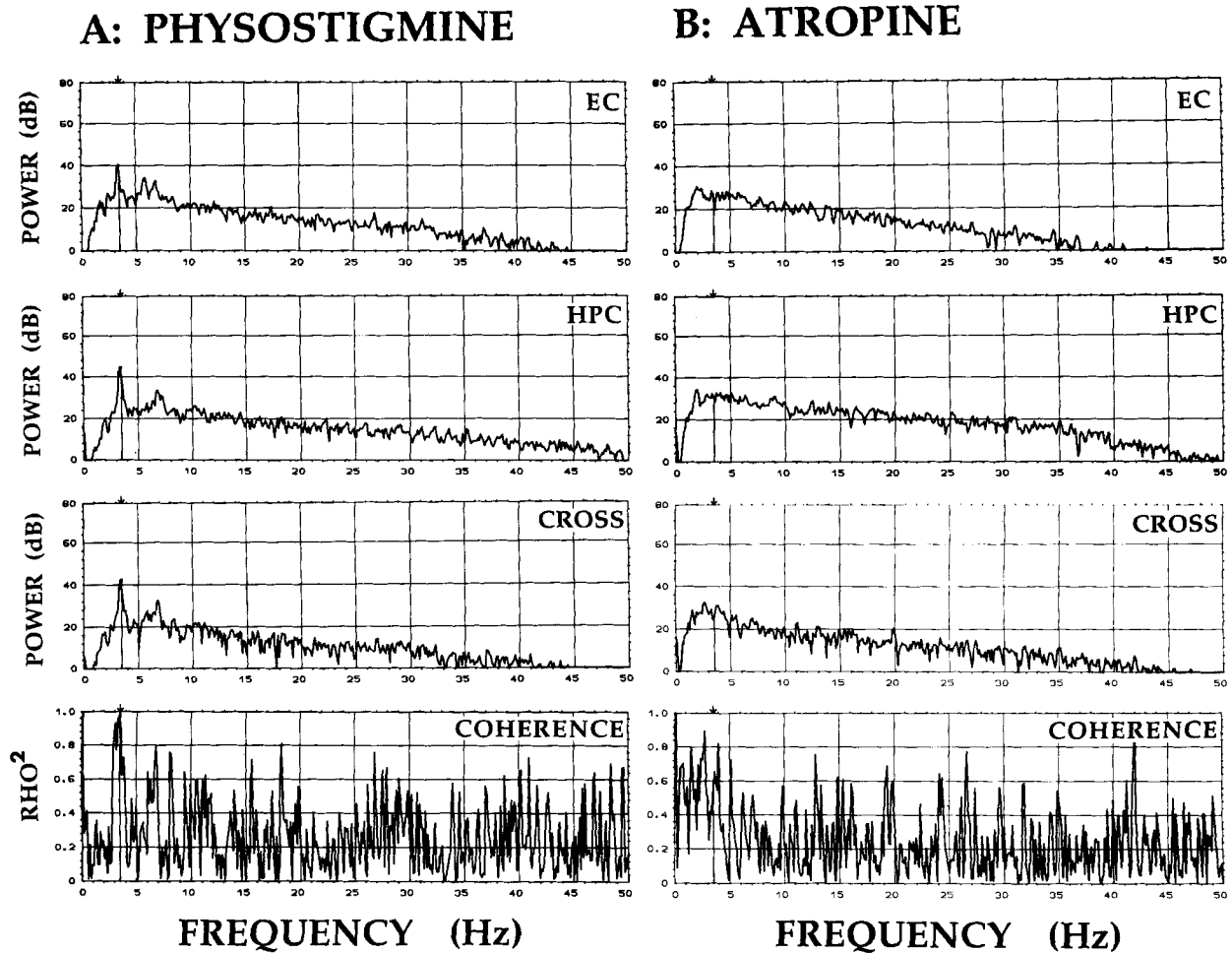


Fig. 4. Individual power, cross power, and coherence spectra of entorhinal cortex (EC) and hippocampal formation (HPC) (A) physostigmine- and (B) atropine-evoked field activity (from signals shown in Fig. 2). The presence of a theta peak centered at 3.5 Hz can clearly be seen in the auto and cross power spectra after physostigmine treatment. This peak reflects a 14.6, 14.0, and 11.4 db increase in power compared to the preadministration (LIA) condition (not shown) for the EC, HPC, and cross spectra, respectively. In addition to the power peak for theta, a harmonic peak (at double the theta peak frequency, 7 Hz) can also be seen in all three power spectra. Note that as for spontaneous and tail pinch theta, the cross spectral peak shows a high level of coherence (0.990, an increase from its pretreatment level of 0.313). Following treatment with atropine, theta peaks are no longer observed in any of the three power spectra that resemble those taken during LIA (Fig. 3B). The decrease in power at 3.5 Hz is 13.3, 14.2, and 18.4 db for the EC, HPC, and cross spectra, respectively, when comparing the physostigmine and atropine conditions. This is comparable to the differences found between the pretreatment and physostigmine conditions. There is no systematically large degree of coherence seen in the bandwidth of interest; there is a loss of coherence at 3.5 Hz to a level of 0.539.

structure and not the other. Following a subsequent top-up injection, theta could again be restored in both structures for long periods of time (up to 45 minutes).

In the three animals administered atropine the physostigmine-induced theta was abolished and could not be reevoked by subsequent injections of physostigmine. This is shown in analogue form in the bottom right panel of Figure 2. Note the similarity of the waveform seen during LIA and that induced by atropine. This similarity can also be seen in the frequency domain by comparing spectra for the field activity following treatment with atropine (Fig. 4B), to that seen for LIA (Fig. 3B). No theta peaks are seen and there is a decrease in power and coherence at the frequency corresponding to the physostigmine theta peak. The average decrease in power at the physostigmine peak frequency was 9.8 ± 1.8 , 14.3 ± 0.3 , and 15.0 ± 0.9 db for the EC, HPC, and cross spectra, respectively. There is a decrease in the coherence for the same frequency to a value of 0.45 ± 0.15 (NS).

Manipulations of the ascending synchronizing system

Moderate to high levels of PH stimulation were a potent activator of EC and HPC theta activity. This is illustrated in the top left panel of Figure 5. Theta was again evoked coincidentally in both structures and maintained a relative phase consistent with that expressed during spontaneous theta. Threshold stimulus intensities (0.3–0.4 mA) were similar for both areas.

Theta evoked by 1.0 mA stimulation of the PH is shown in spectral plots in Figure 6A. Again, theta peaks are seen in all three power spectra centered this time at a frequency of 6.875 Hz. The average frequency generated by this level of stimulation was 6.18 ± 0.36 Hz, increased from that seen during either spontaneous, tail pinch, or physostigmine theta. The average power at peak was 29.58 ± 0.5 , 39.0 ± 2.3 , and 34.24 ± 1.0 db for the EC, HPC, and cross spectra, respectively. On average, the power peaks represented an increase of 7.9 ± 1.8 , 13.6 ± 2.8 , and 14.4 ± 2.9 db for the EC, HPC, and cross spectra, respectively, compared to spectral readings for LIA. Coherence for the cross spectral peak is again high, being on average 0.97 ± 0.01 ($P < .01$) as compared to 0.33 ± 0.13 (NS) for LIA. Again, as with physostigmine theta, the presence of harmonic peaks were observed in the power spectra of every animal at this level of stimulation intensity. However, the harmonic peaks do not show the same degree of coherence as the fundamental.

There was a positive linear relationship between the intensity of stimulation and peak frequency of EC theta evoked as measured by spectral analysis (Fig. 7A). This relationship is identical to that seen in the HPC (which is not surprising given that the frequency of the power peaks in both regions were consistently locked). A positive linear relationship also existed between the intensity of stimulation and peak power of both EC theta (Fig. 7B) and HPC theta (not shown).

Immediately following septal inactivation, the ability of all levels of PH stimulation as well as tail pinch to elicit EC theta was abolished in all five animals tested. The top right panel of Figure 5 illustrates this effect. The degree of depression was decreased at 15 minutes, and full recovery was achieved at both 30 and 60 minutes postinfusion (see the bottom left

panel of Fig. 5). Spectra showing the abolition of stimulation-induced EC theta following intraseptal microinfusion of procaine are shown in Figure 6B. As is plainly seen, peaks are no longer visible and there is a decrease in power and coherence at the frequency corresponding to the PH stimulation theta peak. The average decrease in power at the peak frequency was 15.7 ± 3.6 , 17.2 ± 3.5 , and 16.3 ± 2.3 db for the EC, HPC, and cross spectra, respectively. There is a decrease in the coherence for the same frequency to a value of 0.43 ± 0.17 (NS).

Fifteen minutes following either a systemic or an intraseptal infusion of atropine the ability of PH stimulation to evoke EC theta was also severely attenuated (bottom right panel of Fig. 5). These data are represented graphically in Figure 8. Although there was a consistent decrease in the power at the frequency corresponding to the theta peak evoked during stimulation (Fig. 8B) an analogous depression was not observed in the frequency plot (Fig. 8A). At low stimulation intensities, the ability of PH stimulation to induce theta was abolished along with spontaneous theta. However, at higher levels, although significantly depressed, some theta can still be observed both spectrally and by observations of the analog records. The ability of tail-pinch to evoke theta, however, was completely abolished.

DISCUSSION

Studies of hippocampal formation field activity of urethane-anesthetized rats have demonstrated the spontaneous occurrence of two dominant patterns: theta, a near-sinusoidal waveform with peak frequencies in a narrow band between 2–8 Hz, and large amplitude irregular activity (LIA) with a broad spectral band from 0.5–25 Hz (Bland, 1986). The present study has demonstrated not only that the same holds true for the EC, but that the maintenance of the two types of field states is instantaneously coincident across both areas. This corroborates the findings of Mitchell and Ranck (1980).

Although lower in amplitude than theta recorded from the dentate region, (likely due to differences in such variables as cellular morphologies, density of cellular packing, and alignment of cellular dipoles) EC theta was produced coherently with that of the HPC. Individual values ranged from 0.85 to 0.99 which overlaps with the range of intrahippocampal coherence values for theta (Bland et al., 1975; Leung et al., 1982). This denotes a high degree of phase correspondence of theta between the two limbic areas.

The intracortical site specificity described for phase locking and reversal of the theta signal found in this study is in agreement with previous results (Mitchell and Ranck, 1980; Alonso and Garcia-Austt, 1987a). These researchers demonstrated a phase reversal of theta across layer II of the EC compared to HPC theta recorded from either the stratum oriens of CA1 or the dentate molecular layer. In agreement with Alonso and Garcia-Austt (1987a), no systematic phase lag and leads were found between the HPC and EC in the present study. The phase lag and leads reported by Mitchell and Ranck (1980) may be due to the fact that they studied freely moving rats. Both researchers in addition to others (Adey, 1958; Alonso and Garcia-Austt, 1987b; Dickson and Bland, 1991, 1992; Stewart et al. 1992) have also described rhythmical bursting cells in this region, phase locked to the local and hippocampal

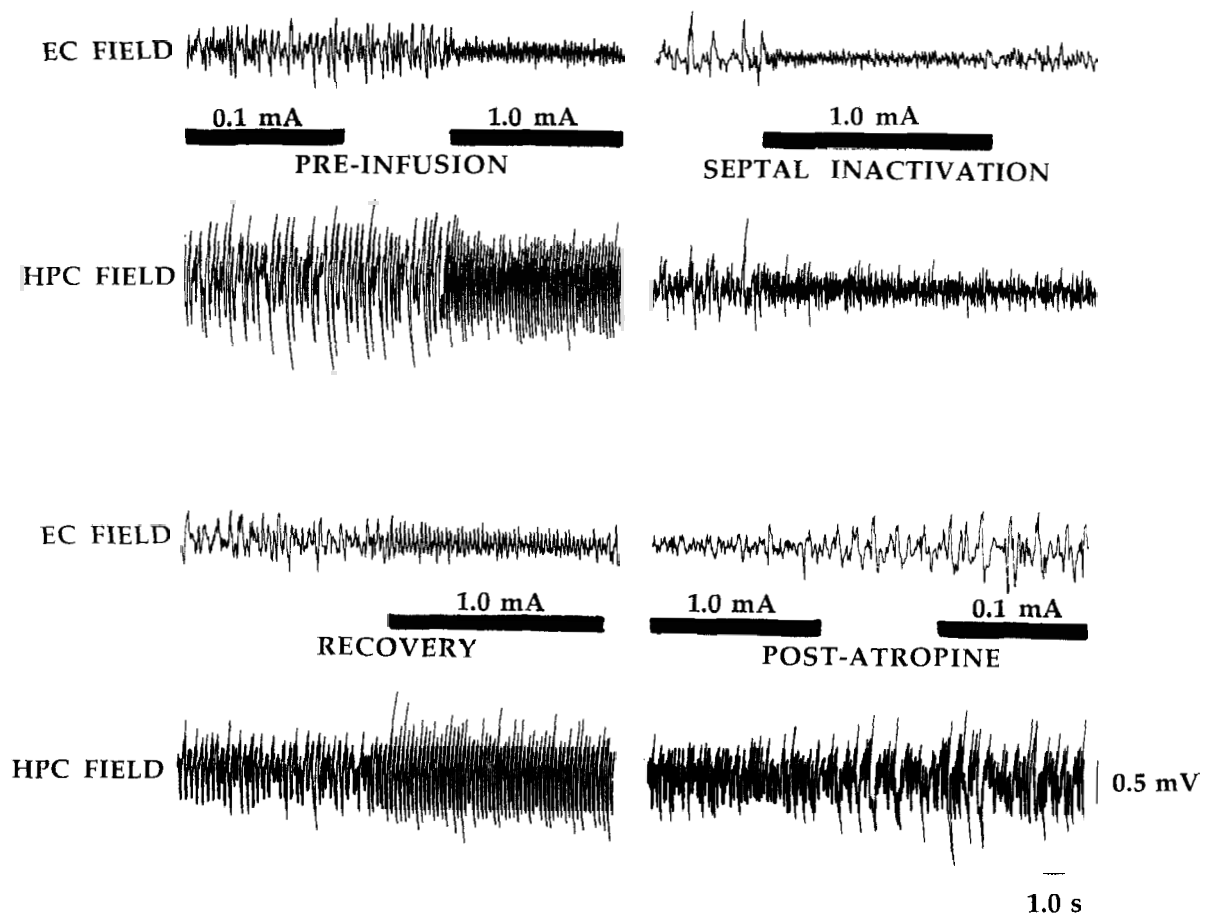


Fig. 5. Analogue traces demonstrating that the theta activating effects of PH stimulation are abolished by septal inactivation and systemic atropine. PH stimulation at an intensity of 1.0 mA, but not 0.1 mA, elicits theta in both entorhinal cortex and hippocampal formation (top left panel). An infusion of procaine in the medial septal region abolished this activation (top right panel). After a 60-minute recovery period, stimulation at 1.0 mA again elicits theta (bottom left panel). An intravenous administration of atropine permanently suppresses the ability of high levels of hypothalamic stimulation to induce theta (bottom right panel).

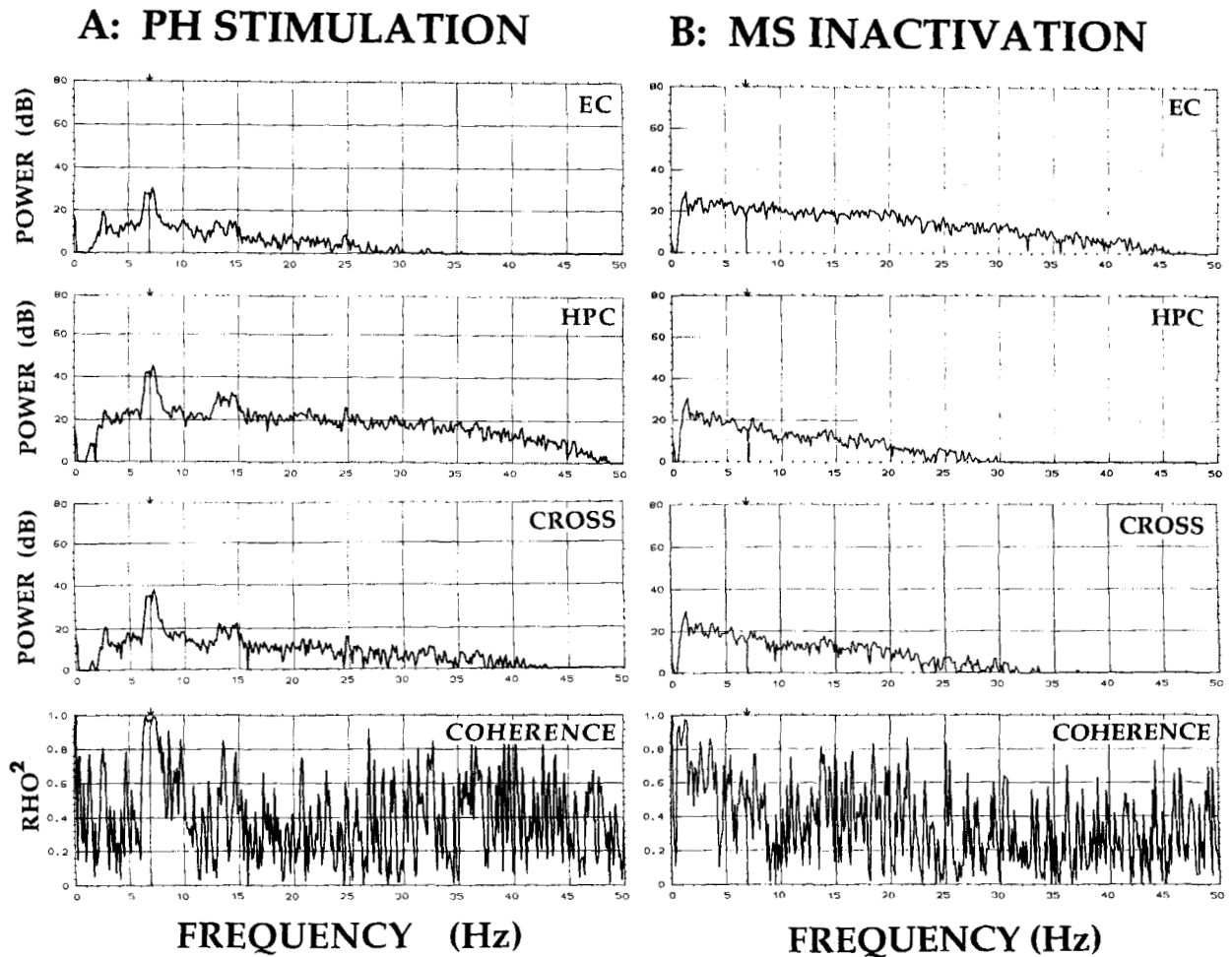


Fig. 6. Individual power, cross power, and coherence spectra of entorhinal cortex and hippocampal formation field activity during 1.0 mA PH stimulation (A) pre- and (B) post-MS inactivation (from signals shown in Fig. 5). Previous to procaine infusion into the medial septal region, stimulation induces a substantial peak centered at 6.875 Hz. This peak corresponds to a 14.1, 20.5, and 24.6 db increase in power for the same frequency during LIA (not shown). In addition to the above peak, there is also a sizable peak at the first harmonic (13.75 Hz) in all three power spectra. The level of coherence for the cross spectral theta peak is very high (0.97) as compared to the prestimulation level (LIA) of 0.04. Subsequent to medial septal inactivation, PH stimulation no longer elicits theta peaks in any of the three power spectra. The decrease in power at 6.875 Hz is 25.8, 12.8, and 21.2 db for the EC, HPC, and cross spectra, respectively, when comparing conditions pre- and postinfusion. Coherence at this frequency has also dropped to a value of 0.42.

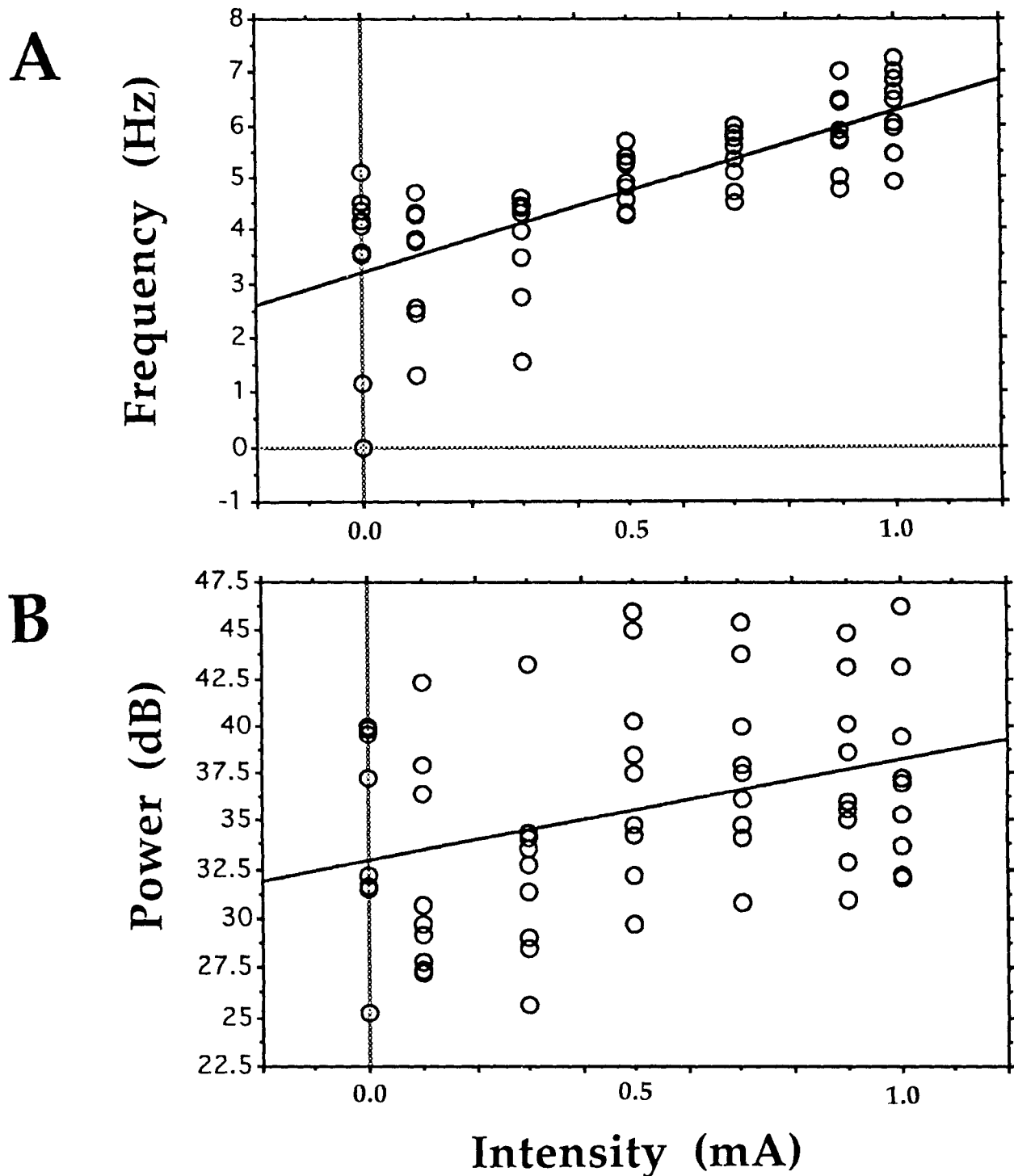


Fig. 7. Regression plots of stimulation intensity versus (A) peak frequency and (B) peak power of EC theta. With increasing intensities, there is a significant increase in the peak frequency ($F(1,61) = 81.535$, $P < .005$) and power ($F(1,61) = 8.906$, $P < .01$) of entorhinal cortex theta. The regression formula for the line of best fit for the scatter plot in (A) is $y = 3.062(x) + 3.214$; $r = 0.756$. The regression formula for the line of best fit for the scatterplot in (B) is $y = 5.274(x) + 32.966$; $r = .357$.

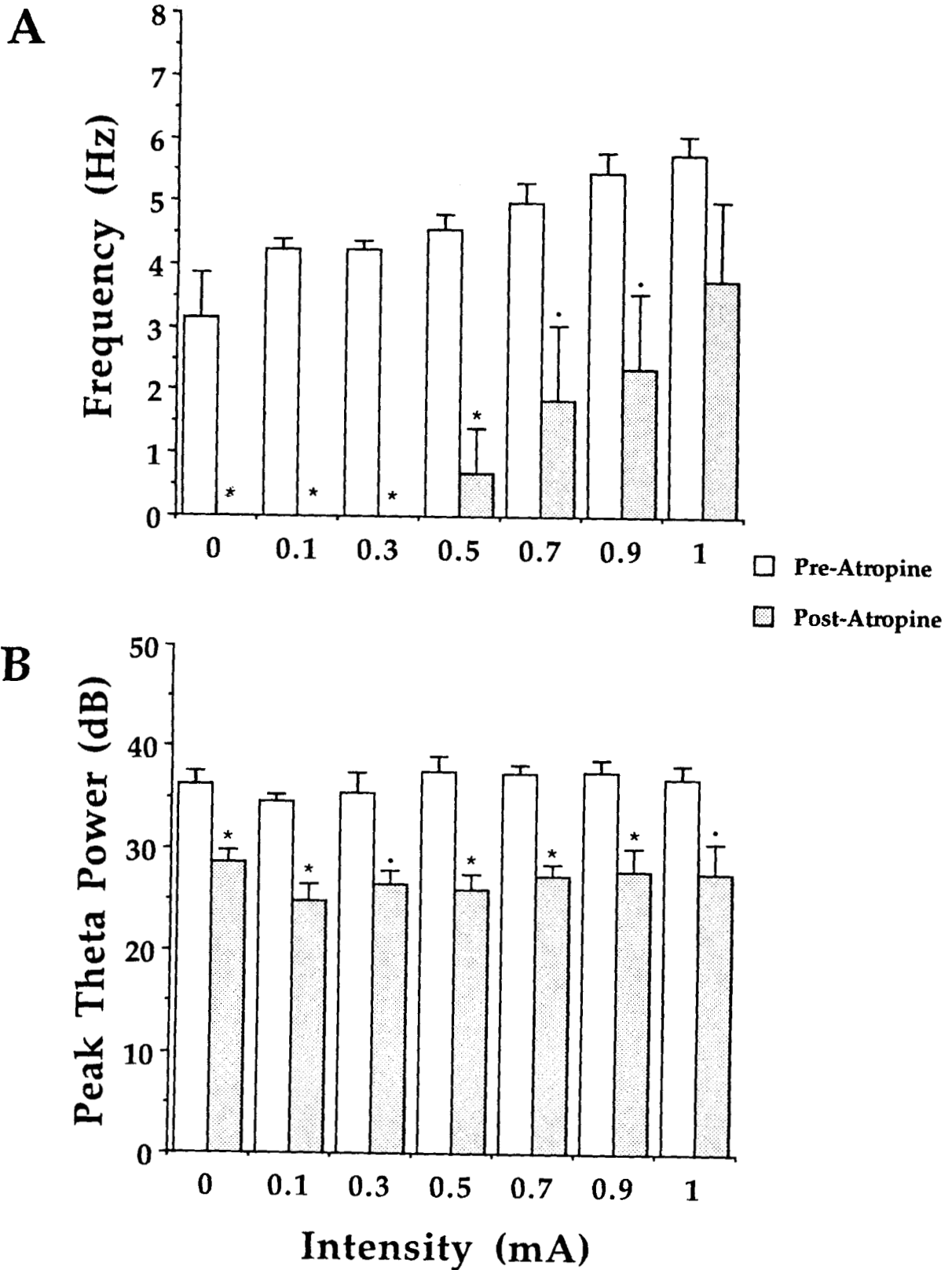


Fig. 8. The effects of atropine on (A) peak frequency and (B) peak power of entorhinal cortex theta as a function of PH stimulation intensity. A small dot refers to a significant difference at $P < .05$. An asterisk refers to a significant difference at $P < .01$. Whereas the theta peak power is depressed equally across all levels of stimulation intensity, the depression of frequency is not as pronounced at the higher levels of intensity.

EEG. Presumably, cells in layer II of the EC contribute to the generation of EC theta field activity.

Experimental manipulations that are known to affect HPC theta were also shown to affect EC theta coincidentally. Moderate tail pinch elicited EC (and HPC) theta. Systemic administrations of physostigmine (an anticholinesterase) also elicited EC (and HPC) theta, corroborating the results of Alonso and Garcia-Austt (1987a). This type of theta was blocked by systemic injections of atropine, a muscarinic receptor antagonist. Stimulation of the posterior hypothalamus (PH) reliably elicited EC and HPC theta. This type of theta, as well as tail pinch and spontaneous theta, could be blocked by infusions of procaine, a local anesthetic, into the medial septal region (corroborating the electrolytic lesion data for the freely moving rat, Mitchell et al., 1982) as well as with systemic injections of atropine. Therefore, in the urethane anesthetized rat, EC theta, like HPC theta, was modulated by sensory and hypothalamic stimulation through the activity of cells in the medial septal region, and was mediated by muscarinic neurotransmission.

Manipulations enhancing the production of limbic theta do not seem to change the basic properties as previously described. One difference noted, however, was the presence of a harmonic peak in the spectra of robustly elicited theta (i.e., that elicited by physostigmine or PH stimulation). This peak reflects a patterning of the field potentials at twice the given frequency of the fundamental. What this most likely represents is a systematic asymmetry in the extracellular field potentials that stems from the basic asymmetry, in membrane potential, of depolarizing and hyperpolarizing currents in single cells. A finding that supports this contention is that the coherence values for harmonic peaks did not attain significance, reflecting that the processes underlying them were somewhat independent and thus, most likely locally generated.

Experimental manipulations that abolished EC theta seemed to evoke a field activity similar to LIA that suggests that theta requires an active mechanism for elicitation and reflects an active information-processing state of limbic cortex. Another observation that supports this claim is that during deep anesthesia, EC theta cannot be elicited spontaneously.

It is unclear from these experiments at which level muscarinic activation takes place to elicit EC theta. One probable locale is at the level of the medial septal region. In the present study, an intraseptal infusion of atropine abolished EC (and HPC) theta. It has also been demonstrated that intraseptal infusions of carbachol can activate HPC theta (Monmaur and Breton, 1991; Lawson and Bland, 1993). Another possibility is that activation occurs at the level of the EC itself. This is also likely given the cholinergic projection from the septal region to both the HPC and the EC (Lewis and Shute, 1967; Alonso and Kohler, 1984) and the effects of direct intrahippocampal infusions of carbachol and atropine (Rowntree and Bland, 1986). A recent experiment tested this hypothesis directly by perfusing carbachol over an EC slice preparation. This manipulation resulted in the production of theta-like field oscillations (Konopacki et al., 1992). Studies should be conducted to determine if direct intraentorhinal cortex microinfu-

sions of cholinergic agents such as carbachol and atropine can affect the local production of theta *in vivo*.

A linear relationship was demonstrated between the intensity of PH stimulation and both the frequency and power of the elicited EC theta signal. This relationship exists in the HPC as well (Smythe et al., 1991; present study, not shown). This implies that the summed activity of cells in this region linearly codes the frequency and to a lesser extent the power of theta in the entorhinal cortex (see HPC data; Oddie, 1992). The ascending pathway from this region seems to pass through or synapse in the medial septal nucleus since MS inactivation abolished stimulation-induced EC theta. It also appears to be dependent at some level upon muscarinic neurotransmission since atropine severely attenuates it.

It is not clear at this time what neurotransmitter system is involved in the production of theta spared by atropine in the anesthetized rat. Stewart and Fox (1989a) and Colom and Bland (1991) have reported two populations of rhythmically discharging septal cells that show different sensitivities to atropine. Triggering from the impulses of the resistant cells following atropine treatment and averaging the HPC field activity Stewart and Fox (1989b) also demonstrated field rhythmicity at theta frequencies. The identity of these cells remains to be determined but there is a possibility that they are GABAergic septohippocampal cells (Kohler et al., 1984). Smythe et al. (1992) have outlined a possible mechanism for a balance between the two neurotransmitter systems in the production of HPC theta in the anesthetized rat. It is probable that a similar balance controls EC theta as well since the septum projects to this structure in a similar manner (Alonso and Kohler, 1984).

The most striking finding of this report was the almost perfect correspondence of theta field activity in the two areas. There are four possibilities for this: either theta at both sites is driven in a parallel fashion from a common source; the rhythmical wave is transmitted synaptically in a serial fashion from either one to the other; the waveforms seen in the EC are volume conducted from the HPC; or the waveforms seen in the HPC are volume conducted from the EC. The second case (serial conduction) is not likely as the appearance of theta was shown to occur coincidentally and instantaneously in both structures without an appreciable time lag. In addition, a systematic phase shift would also be apparent during ongoing theta. This phase shift would be linearly related to the frequency of theta produced since the synaptic delay would presumably remain constant. Such a phase shift was not observed on average in this study despite the fact that a broad range of frequencies were sampled. The third and fourth possibilities (volume conduction) are also unlikely due to the observed phase reversal of the theta signal across layer II of the EC. This, along with EC cellular correlates of theta suggest that theta is independently generated in this structure (Mitchell and Ranck, 1980; Alonso and Garcia-Austt, 1987a, 1987b; Dickson and Bland, 1991, 1992; present study).

By the process of elimination it would seem that the first possibility is the best explanation. However, in order for parallel driving to occur, a parallel pathway from a common source must exist. As well, the pharmacological modulation of theta at the two sites should be identical. This has been shown to be the case. However, the critical test of independ-

ence would be to demonstrate it directly. This was observed in the present study during subthreshold dosages of physostigmine when either the EC or the HPC produced theta independently of one another. However, it is unclear how this unique activation process takes place and to what extent the recordings represent the activity of the entire limbic structure.

Other ways of inducing independence by perturbing the circuitry of the HPC by a variety of pharmacological and electrophysiological manipulations to abolish the local production of theta are being examined in our laboratory. Preliminary results suggest that when theta is abolished in the HPC, EC theta can still be observed, albeit in a somewhat diminished form (Dickson, Trepel, and Bland, unpublished observations). What these observations reflect is that both the EC and the HPC show intrinsic oscillatory properties that are independent of each other as well as the influence of common extrinsic synchronizing inputs. However, the production and synchronization of physiological limbic theta is undoubtedly a result of a complicated interaction between these various anatomical structures (Bland and Colom, 1993).

To summarize, the field activity of the entorhinal cortex was shown to exhibit theta concomitantly and coherently with hippocampal theta. The spontaneous production of theta and LIA was coincident across both limbic areas. The production of EC (and HPC) theta, was found to be modulated in a similar and coincident fashion by sensory and hypothalamic stimulation through the activity of cells in the medial septal region, and was also found to be mediated by muscarinic neurotransmission (possibly at multiple levels). It was hypothesized that a linear code exists in the posterior hypothalamic region that specifies the frequency, and to a lesser extent, the power of EC theta activity. The theta activity of limbic cortex was postulated to occur through a parallel, yet independent mechanism.

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