Hippocampal θ Field Activity and θ -ON/ θ -OFF Cell Discharges Are Controlled by an Ascending Hypothalamo-Septal Pathway

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The nature of the control of hippocampal formation field activity [theta (θ) and large-amplitude irregular activity (LIA)] and θ -on/ θ -off cell discharges by an ascending hypothalamo-septal pathway was investigated in urethane-anesthetized rats.

Electrical stimulation of the dorsomedial-posterior hypothalamus in the range of 0.1–1.0 mA in 0.1-mA steps produced θ in the hippocampal formation, with a linear positive relation between stimulus intensity, θ frequency, and θ amplitude. Reversible blockade of the medial septal (MS)/vertical limb of the diagonal band of Broca (vDBB) region by microinjection of procaine hydrochloride abolished spontaneous and hypothalamically elicited θ , resulting in a field activity at 1-min post-procaine that had a lower power than pre-procaine LIA levels. The efficacy and recovery of the MS/vDBB suppression was tested at 1, 10, 20, 30, 40, 50, and 60 min post-procaine using 0.5, 0.8, and 1.0 mA of hypothalamic stimulation. All three of the dependent measures of hippocampal field activity (frequency, amplitude, and power) showed a progressive recovery during the 60-min post-procaine period. Frequency exhibited a rapid recovery with a shallow slope between 20 and 60 min post-procaine. In contrast, the amplitude and power of θ exhibited a gradual recovery with a steeper slope between 20 and 60 min post-procaine. During the time of maximal MS/vDBB suppression, θ -on cell discharges were reduced to 0 in most cases. The initial recovery of θ -on cells in the post-procaine condition was correlated with the first appearance of θ and was characterized by low discharge rates. The gradual recovery to pre-procaine discharge levels (along with discharge pattern) paralleled the recovery of θ amplitude and power, not the recovery of θ frequency. In contrast to the suppression of θ -on cells during the maximal suppression of the MS/vDBB, θ -off cells continued to discharge at rates that were not significantly different from the pre-procaine levels accompanying LIA. The inhibition of θ -off cells normally produced by hypothalamic stimulation was also abolished during the time of maximal suppression of the MS/vDBB. The hypothesis

that hippocampal formation θ -on cells received excitatory cholinergic projections and θ -off cells received inhibitory GABAergic projections was supported.

The existence of an ascending brainstem pathway that produces synchronous theta (θ) field activity in the hippocampal formation when electrically stimulated has been well documented (Bland, 1986; Vertes, 1986). This pathway originates in the rostral pons, ascends through the medial hypothalamus to the medial septal (MS)/vertical limb of the diagonal band of Broca (vDBB) region, and from there projects to the hippocampal formation. Electrical stimulation of the pathway at the level of the dorsomedial-posterior hypothalamic nuclei has been shown to activate the discharges of θ -on cells and suppress those of θ -off cells, in both the hippocampal formation and MS/vDBB (Colom et al., 1987; Bland et al., 1990). Pharmacological studies utilizing urethane-anesthetized rats demonstrated that θ -on cells were sensitive to cholinergic agonists and antagonists while θ -off cells were not (Bland and Colom, 1988, 1989). Based on these observations and recent immunohistochemical work (Nyakas et al., 1987; Freund and Antal, 1988), it was suggested that hippocampal θ -off cells may be inhibitory interneurons, receiving inhibitory GABAergic inputs from the MS/vDBB.

The present research had three main objectives: (1) to characterize the effects of electrically stimulating the dorsomedial-posterior hypothalamic nuclei on hippocampal formation field activity, specifically on θ frequency, amplitude, and power; (2) to determine if the effects of hypothalamic stimulation on hippocampal field activity were mediated by the MS/vDBB region, by reversibly suppressing the latter with procaine HCl; and (3) to determine if the effects of hypothalamic stimulation on hippocampal θ -on and θ -off cells were mediated by the MS/vDBB region, again by reversibly suppressing the latter by procaine HCl. Our guiding hypothesis for the third objective, based on our previous research, was that θ -on cells in the hippocampus received excitatory cholinergic inputs, and θ -off cells received inhibitory GABAergic inputs, from the MS/vDBB. Temporary blockade of the MS/vDBB should therefore have opposite effects on the discharge properties of θ -on and θ -off cells. The studies were carried out using acute urethane-anesthetized rats and reversible blockade of the MS/vDBB by procaine hydrochloride (Malpeli and Schiller, 1979).

Portions of this research have been presented previously (Christie and Bland, 1989; Smythe et al., 1990a,b).

Materials and Methods

The data were obtained from 40 male black-hooded rats (0.250–0.400 kg) supplied by the Animal Care Services at the University of Calgary.

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Figure 1. Diagrammatic representation of the recording, stimulation, and microinfusion arrangement. A tungsten microelectrode was placed into the stratum moleculare (upper blade) of the dentate gyrus as a reference to monitor hippocampal formation field activity. Cells were recorded in the opposite hippocampal formation (HIP) using a glass microelectrode. A bipolar, twisted-stainless-steel stimulating electrode was placed in the dorsomedial-posterior nuclear region of the medial hypothalamus. A guide tube was implanted above the septum to facilitate the placement of the microinfusion cannula in the MS/vDBB.

The rats were initially anesthetized with halothane while tracheal and jugular cannulas were inserted. Halothane was then discontinued, and urethane (0.8 gm/ml) was administered via the jugular cannula to maintain an appropriate level of anesthesia during the remaining surgical and experimental procedures.

The rats were placed in the stereotaxic instrument with the plane between bregma and lambda leveled to horizontal. An uninsulated tungsten wire placed in the cortex, anterior to bregma, served as an indifferent electrode, and the stereotaxic frame was connected to ground. A tungsten microelectrode (0.2–0.5 M Ω) for recording hippocampal field activity was placed in the right dorsal hippocampal formation in the dentate molecular layer (3.0–3.3 mm posterior to bregma, 2.0–2.5 mm lateral to the midline, and 2.4–2.8 mm ventral to the dural surface). Cell recordings in the left hippocampal formation were made at the same posterior and lateral coordinates, starting at the alvear surface and continuing ventral to the lower (endal) blade of the dentate granule cells. Cells were recorded with glass microelectrodes (4–6 M Ω) filled with 0.5 M sodium acetate, mixed with 2% Pontamine sky blue. The hypothalamic stimulating electrode was bipolar, constructed of 250- μ m stainless-steel wire, insulated to the tip, and soldered to male subminiature connectors. The coordinates were 3.3 mm posterior to bregma, 0.0 mm lateral to the midline, and 6.5–7.0 mm ventral from dural surface. The hypothalamic coordinates resulted in electrodes positioned in the region of the pars dorsalis portion of the dorsomedial nucleus and the posterior hypothalamic nucleus. The infusion cannula consisted of a 30-gauge hypodermic needle fastened to a length of PE50 intramedic tubing. The infusion cannula was inserted into a 23-gauge hypodermic guide tube and extended beyond it 2 mm (Rowntree and Bland, 1986). The guide tube served as a holder to facilitate stereotaxic placement of the infusion cannula. The infusion cannula was placed in the MS/vDBB region, 0.5 mm anterior to bregma, 0.0 mm lateral to the midline, and 5.5–6.0 mm ventral from the dural surface. A diagrammatic representation of the recording, stimulating, and infusion placements in the brain is shown in Figure 1. Infusion pressure was generated and maintained by a microinfusion pump. Procaine hydrochloride (10% by weight, pH 6.6) was infused at a rate of 1 μ l/min. Tip locations of the microelectrodes were marked by passing 50 μ A of current for 10 min (5 min cathodal, 5 min anodal). Frozen brain sections (40 μ m) were taken serially and mounted on glass slides for subsequent thionine staining. Brain signals were amplified and displayed in the manner we have described previously (Co-

lom and Bland, 1987). All signals were stored on an FM tape recorder for subsequent computer analysis.

In the first series of experiments, the effects of hypothalamic stimulation on hippocampal formation field activity were determined with the MS/vDBB intact, during procaine hydrochloride blockade of the MS/vDBB, and finally, during the time up until the MS/vDBB recovered. In the second series of experiments, the same procedure was followed, with the addition of recording from hippocampal formation θ -on and θ -off cells. The hypothalamus was stimulated with square-wave pulses, 0.1 msec in duration, at a frequency of 100 Hz for 5 sec. The durations of the stimulation periods were recorded on tape by means of a synchronizing pulse from the stimulator. Current amplitudes used were 100–1000 μ A, in 100- μ A steps. Each current level was administered three times, and all current levels were presented in random order. Due to the 100-Hz artifact, we could only visually and auditorily analyze the cell data during the 5-sec stimulation period. Quantitative analysis began immediately after the stimulation was terminated. For linear regression analyses, only the first 1 sec of the poststimulation period was analyzed.

Data were analyzed off line in the manner we have described previously (Bland and Bland, 1986; Colom and Bland, 1987). The simultaneously occurring hippocampal field activity accompanying every sample of cell activity was led into a dual-channel signal analyzer yielding frequency spectographs with the amplitude expressed as power (dB). Field activity was classified as either θ or LIA by the following criteria: (1) θ was defined as a sinusoidal-like activity with a peak frequency of 2–8 Hz and a small bandwidth, and (2) LIA was defined as a large-amplitude, irregular activity with a broad frequency band (0.5–25 Hz; Leung et al., 1982). Power was determined at the peak frequencies of both spontaneous and hypothalamically elicited θ . In the cases where no peak θ frequency occurred (LIA and post-procaine conditions), power was measured where the peak frequency of spontaneous θ occurred for that animal.

Results

Histology revealed that in all 40 rats the reference electrode for monitoring hippocampal formation field activity was accurately positioned close to the stratum moleculare of the dentate gyrus. The tips of the stimulating electrodes were in the dorsomedial-posterior hypothalamic nuclei in 36 out of the 40 rats. In three rats, the tips were close to the border region of these nuclei, and the remaining tip was located in the ventromedial nucleus. All the infusion sites that produced an effective block of hypothalamically induced θ (30 out of 40 rats) were located in the MS/vDBB. Of the 30 cells recorded, 11 were located in the CA1 layer, 11 were located in the dentate granule layer (upper blade), and eight were located in the hilar region.

Hypothalamic stimulation and hippocampal field activity

Electrical stimulation of the dorsomedial-posterior hypothalamic nuclei produced θ field activity in the hippocampal formation, as did stimulation of the three sites close to these nuclei. In addition to producing θ field activity, stimulation of the dorsomedial-posterior nuclei with increasing intensity resulted in a corresponding increase in θ frequency and amplitude. This relationship was evident over the stimulation range from 0.3 to 1.0 mA. Figure 2 presents a graphic presentation of these results. The relationship between stimulation intensity and θ frequency was linear ($y = 3.8223 + 3.8045x$; $R^2 = 0.977$; Fig. 2A). The relationship between stimulation intensity and θ amplitude was also linear ($y = 0.882 + 0.378x$; $R^2 = 0.22$; Fig. 2B).

Effects of procaine suppression of MS/vDBB on hippocampal field activity

Infusions of 2–3 μ l of 10% (1 μ l/min) procaine hydrochloride into the MS/vDBB of 30 animals blocked electrically induced hippocampal θ . Using the failure of 1.0-mA electrical stimulation of the dorsomedial-posterior hypothalamus to elicit θ as a

Figure 2. Graphs showing the relationship between the intensity (mA) of stimulation of the dorsomedial-posterior hypothalamus and the frequency (Hz; *A*) and the amplitude (mV; *B*) of the first 1 sec of elicited θ . The data represent the means for 10 experiments, each point being the grand mean of three 1-sec samples for each animal. Vertical bars are the SEs. Note the linear positive relationships.

criterion of the effectiveness of the neural blockade of the MS/vDBB, a mean blockade duration of 27.14 min was found. A 10- μ l control infusion of physiological saline (pH, 7.07) failed to block the effects of hypothalamic stimulation. The effects of the procaine hydrochloride suppression of hippocampal θ was unlikely to be due to direct spread of the procaine to the hippocampal formation because in a number of experiments an infusion 1 mm above the MS/vDBB failed to block the stimulation effects. Figure 3*A* presents a graphic summary of the relationship between the frequency of hippocampal formation field activity and the various pre- and post-procaine MS/vDBB suppression conditions (group data for 14 experiments). θ was totally abolished at the 1-min and 10-min post-procaine times. Note the rapid recovery of θ frequency between 20 and 60 min post-procaine suppression of the MS/vDBB, as measured in response to 1.0-mA stimulation of the hypothalamus. By 40–60 min post-procaine, spontaneous θ had also recovered with frequencies equivalent to pre-procaine levels. Figure 3*B* presents a graphic summary of the relationship between the amplitude of hippocampal formation field activity and the various pre- and post-procaine MS/vDBB suppression conditions (group data for 14 experiments). There was a gradual recovery of θ amplitude between 1 and 60 min post-procaine suppression of the MS/vDBB as measured in response to 1.0-mA stimulation of the hypothalamus. Note, however, that by 60 min post-procaine, the amplitude of spontaneous θ and the θ induced by 1.0-mA

Figure 3. Graphs showing the relationship between the frequency (Hz; *A*) and the amplitude (mV; *B*) of hippocampal formation field activity and the various pre- and post-procaine MS/vDBB suppression conditions. *A*, Each bar is the mean of 14 experiments with a sample duration of 8 sec. Post-procaine values for conditions of 1, 10, 20, 30, 40, 50, and 60 min were obtained during 1.0-mA hypothalamic stimulation. Vertical lines are SEs. *B*, Each bar is the mean of 14 experiments, with the amplitude from each experiment taken as the mean of the amplitudes averaged over three 5-sec samples. Post-procaine values for conditions of 1, 10, 20, 30, 40, 50, and 60 min were obtained during 1.0-mA hypothalamic stimulation. Vertical lines are SEs.

hypothalamic stimulation had not recovered to pre-procaine levels.

Effects of procaine suppression of MS/vDBB on cell discharges θ -on cells

A total of 17 phasic linear θ -on and two tonic non-linear θ -on cells were tested during the pre-procaine and post-procaine conditions of the MS/vDBB. All θ -on cells were equally affected by procaine suppression of the MS/vDBB, regardless of their location in the hippocampal formation. One phasic linear θ -on cell was lost prior to the 60-min recovery condition. The data are summarized in Table 1. All 17 phasic linear cells had a positive linear slope ($b = 3.54$).

Analysis of the recovery of θ -on cell discharges through the post-procaine times of 1–60 min revealed some interesting findings (Fig. 4). The first significant increase in discharge rate was

Figure 4. Graph showing the discharge rates of a phasic θ -on cell in response to stimulation of the dorso-medial-posterior hypothalamus, in the pre- and post-procaine MS/vDBB suppression conditions. Each bar is the mean of three 1-sec periods. Stimulus intensities of 0.5, 0.8, and 1.0 mA produced a linear increase in discharge rates in the pre-procaine condition. Stimulation of 1.0 mA was used in all the post-procaine conditions. The cell was totally silent at the 1-min and 10-min post-procaine times. The cell discharge rates gradually recovered to near pre-procaine levels, between 30 and 60 min post-procaine, with rhythmicity not appearing until 40 min post-procaine. Vertical lines are SDs.

correlated with the appearance of discernible low-amplitude θ (usually in response to 1.0-mA stimulation of the hypothalamus). The frequency of this θ was almost equal to that of the 1.0 mA-stimulation induced pre-procaine θ . Although a θ -on cell's first significant increase in discharge rate was correlated with this low-amplitude θ , the discharge rate was still much reduced compared to the equivalent pre-procaine condition. In addition, if the cell was a phasic θ -on cell, rhythmicity was not readily observable at this time. In other words, the recovery of discharge rate and pattern paralleled the recovery of θ amplitude and power, rather than the recovery of θ frequency.

The detailed analysis of a phasic linear θ -on cell during pre-procaine, 1 min post-procaine suppression of the MS/vDBB, and 60 min post-procaine recovery conditions is given in Figure 5. The upper panels in Figure 5A show 2-sec samples of the θ (left) and LIA (right) field activities and the simultaneously occurring cell discharges during the pre-procaine condition. The short vertical lines under the field activity are the digitally converted cell discharges. The middle panels show the cross correlations between the θ field activity and cell discharges (left) and the LIA field activity and cell discharges (right). The bottom panels in Figure 5A show the autocorrelations of the cell discharges during θ (left) and LIA (right). Note that during θ the cell discharged rhythmically just preceding the positive peak of each wave while the discharge pattern was nonrhythmic with no preferred phase during LIA. Figure 5B shows two 2-sec samples of raw data at 1 min post-procaine suppression of the MS/

vDBB. The upper panel is an example of the spontaneous activity, and the lower panel was taken during 1.0-mA stimulation of the dorsomedial-posterior hypothalamus. Note the suppressed field activity and lack of cellular discharges in both conditions. Figure 5C shows that by 60 min post-procaine the field activity and cellular discharge patterns have recovered to resemble the pre-procaine condition.

θ -off cells

A total of 11 θ -off cells were recorded. All θ -off cells were equally affected by procaine suppression of the MS/vDBB, regardless of their location in the hippocampal formation. Of these, five were classified as phasic linear θ -off cells. All five were recorded in the pre-procaine condition, three of five were recorded at 1 min post-procaine suppression of the MS/vDBB, and two of five survived to the post-procaine recovery time. Six cells were classified as tonic nonlinear θ -off cells. All six were recorded in the pre-procaine condition, five of six were recorded at 1 min post-procaine suppression of the MS/vDBB, and two of six survived to be recorded at the 60 min post-procaine recovery time. The data are summarized in Table 2. All five phasic linear cells had a linear negative slope ($b = -1.41$).

The detailed analysis of a phasic linear θ -off cell during pre-procaine, 1 min post-procaine suppression of the MS/vDBB, and 16-min post-procaine recovery conditions is given in Figure 6. The upper panels in Figure 6A show 2-sec samples of the θ (left) and LIA (right) field activities and the simultaneously oc-

Table 1. Summary of the effects of microinfusions of procaine HCl in the MS/vDBB on the discharge rates of hippocampal θ -on cells

θ -on cell	Pre-procaine	Post-procaine	
		1 min (θ abolished)	60 min (θ recovering)
Phasic linear ($n = 17$)			
θ	11.89 ± 3.3	0.36 ± 0.45	10.13 ± 3.35
LIA	4.67 ± 2.79		4.19 ± 2.63
Tonic nonlinear ($n = 2$)			
θ	8.99 ± 2.27	0.52 ± 0.85	4.88 ± 1.81
LIA	4.21 ± 2.6		3.10 ± 1.41

Data are presented as mean discharge rate (in spikes/sec) $\pm$ SE.

PHASIC THETA-ON CELL

Figure 5. Detailed analysis of a phasic linear θ -on cell during pre-procaine, 1 min post-procaine suppression of the MS/vDBB, and 60 min post-procaine recovery conditions. The upper panels in *A* show 2-sec samples of spontaneous θ and LIA field activities and the simultaneously occurring cell discharges (short vertical lines) during the pre-procaine condition. The middle panels show the cross correlations between the θ field activity and cell discharges (left) and the LIA field activity and cell discharges (right). The bottom panels show the corresponding auto correlations of the cell discharges during θ (left) and LIA (right). *B* shows examples of 2-sec samples of raw data at 1 min post-procaine suppression of the MS/vDBB. The upper panel shows the spontaneous activity, and the lower panel was made during 1.0-mA stimulation of the dorsomedial-posterior hypothalamus. *C* is the equivalent of *A*, taken during the 60-min post-procaine recovery condition.

curing cell discharges, during the pre-procaine condition. The middle panels show the cross correlations between the θ field activity and cell discharges (left) and the LIA field activity and cell discharges (right). The bottom panels of Figure 6*A* show the autocorrelations of the cell discharges during θ (left) and LIA (right). Note that during lower-frequency θ the cell discharged rhythmically, midway between the positive and negative peaks of each θ wave. At higher θ frequencies, this and all the other phasic linear θ -off cells were totally silent. The discharge pattern was nonrhythmic, with no preferred phase during LIA. Figure 6*B* shows two 2-sec samples of raw data at 1 min post-procaine suppression of the MS/vDBB. The upper panel is an example of the spontaneous activity, and the lower panel was taken during 1-mA stimulation of the dorsomedial-posterior hypothalamus. Note that, in contrast to the θ -on cells, the θ -off cell continued to discharge. Also note that the inhibition of discharges that normally occurred during θ in the pre-procaine condition fails to occur with the MS/vDBB suppressed. Figure 6*C* shows that by 16 min post-procaine the field activity and

cellular discharge patterns have recovered to resemble the pre-procaine condition.

Figure 7 provides a diagrammatic summary of the effects of MS/vDBB suppression on hippocampal field activity and θ -on and θ -off cell discharges. When the MS/vDBB was intact, phasic θ -on cells in the hippocampal formation discharged in an arrhythmic manner with lower discharge rates during LIA, compared to the rhythmic discharge pattern and higher discharge rates associated with θ . Tonic θ -on cells had similar rates of discharge in relation to LIA and θ , but discharged in either a regular or an irregular nonrhythmic pattern. Procaine suppression of the MS/vDBB resulted in suppressed field activity, even when 1.0-mA hypothalamic stimulation was applied. The discharges of θ -on cells were correspondingly suppressed. By 60 min post-procaine, both the field activity and the θ -on cell discharge patterns and rates returned to pre-procaine characteristics. θ -off cells proved to have opposite discharge relations, during both the pre-procaine condition and the post-procaine suppression of the MS/vDBB. With the septum intact, phasic

Table 2. Summary of the effects of microinfusions of procaine HCl in the MS/vDBB on the discharge rates of hippocampal θ -off cells

θ -off cell	Pre-procaine	Post-procaine	
		1 min (θ abolished)	60 min (θ recovering)
Phasic linear ($n = 5$)			
θ	2.09 ± 2.16	6.04 ± 2.50	1.80 ± 2.49
LIA	5.94 ± 2.20		5.65 ± 1.39
Tonic nonlinear ($n = 6$)			
θ	0.0 ± 0.0	7.53 ± 1.23	0.0 ± 0.0
LIA	6.80 ± 1.54		9.08 ± 5.50

Data are presented as mean discharge rate (in spikes/sec) $\pm$ SE.

PHASIC THETA-OFF CELL

Figure 6. Detailed analysis of a phasic linear θ -off cell during pre-procaine, 1 min post-procaine suppression of the MS/vDBB, and 16 min post-procaine recovery conditions. The upper panels in *A* show 2-sec samples of spontaneous θ and LIA field activities and the simultaneously occurring cell discharges (short vertical lines) during the pre-procaine condition. The middle panels show the cross correlations between the θ field activity and cell discharges (left) and the LIA field activity and cell discharges (right). The bottom panels show the corresponding auto correlations of the cell discharges during θ (left) and LIA (right). *B* shows examples of 2 sec of raw data at 1 min post-procaine suppression of the MS/vDBB. The upper panel shows the spontaneous activity, and the lower panel was made during 1.0-mA stimulation of the dorsomedial-posterior hypothalamus. *C* is the equivalent of *A*, taken during the 60-min post-procaine recovery condition.

θ -off cells discharged in an irregular manner and at higher rates during LIA, compared to those associated with θ . At higher θ frequencies, phasic θ -off cell discharges ceased completely, as indicated during hypothalamic stimulation. In contrast to θ -on cells, θ -off cells continued to discharge during the procaine suppression of the MS/vDBB, and 1.0-mA hypothalamic stimulation (or tail pinches) was no longer effective in suppressing the discharges. By 16 min post-procaine, the pre-procaine characteristics of the field activity and θ -off cell discharge patterns and rates had returned.

Discussion

The present study confirmed and extended previous observations that electrical stimulation of the dorsomedial-posterior hypothalamic region exerted a potent synchronizing influence

on the field activity recorded from the hippocampal formation (cf. Bland, 1986). An obvious conclusion of the present study was that the synchronizing influence brought about through activation of the dorsomedial-posterior hypothalamus was mediated by the MS/vDBB, either synaptically or through axons of passage. The data also support the notion that LIA was a result of an active input mediated by the MS/vDBB, because the power of the suppressed field activity was below that of spontaneous LIA (see Leung et al., 1982).

All three of the dependent measures of hippocampal field activity (frequency, amplitude, and power) showed a progressive recovery during the 60-min post-procaine period. There was, however, a marked difference in the time course of recovery between these three measures. θ was recorded at 20 min post-procaine (in response to 1.0-mA hypothalamic stimulation) with

Figure 7. Diagrammatic summary of the effects of MS/vDBB suppression on hippocampal field activity and θ -on and θ -off cell discharges.

a frequency that was nearly equal to that of its equivalent pre-procaine condition. Frequency therefore exhibited a rapid recovery with a shallow slope between 20 and 60 min post-procaine. In contrast, the amplitude and power of θ exhibited a gradual recovery with a steeper slope, between 20 and 60 min post-procaine. In addition, there was a rank order of recovery in terms of θ production: hypothalamically induced θ produced by 1.0-mA stimulation recovered first, followed by that produced by 0.8-mA and 0.5-mA stimulation, and finally by spontaneous θ .

During the time of maximal procaine suppression of the MS/vDBB, θ -on cell discharges were reduced to 0 in most cases. Analyzing the recovery of θ -on cell discharges, in terms of both rate and pattern, yielded some interesting data related to the above discussion on field activity. The initial recovery of θ -on cell discharges in the post-procaine conditions was correlated with the first appearance of θ . Typically, this occurred at 20 min post-procaine in response to 1.0-mA stimulation of the hypothalamus. At this time, the discharge rate was still greatly reduced compared to pre-procaine levels. In addition, if the cell was a phasic θ -on cell, very little evidence of rhythmicity was evident. As discussed above, the θ at this time was of low amplitude and power, but almost at the same pre-procaine frequency. Thus, the gradual recovery of discharge rate (and pattern of phasic cells) paralleled the recovery of θ amplitude and power, not the recovery of θ frequency.

Taken together, the above data permit some suggestions concerning the nature of the control of hippocampal θ field and cellular activity by the ascending hypothalamo-septal pathway. That is, it may require fewer MS/vDBB cells to translate ascending hypothalamic inputs into specific levels of output for driving the frequency of hippocampal θ field activity, compared to the number of MS/vDBB cells required to produce a maximal amplitude of hippocampal θ field activity. Phasic linear hippocampal θ -on cells code the level of excitation of the ascending hypothalamo-septal pathway by increasing their discharge rates as the frequency of hippocampal θ increases (Colom et al., 1987). As discussed above, θ frequency recovers first after procaine suppression of the MS/vDBB, before θ -on cells recover their discharge rates and patterns. This suggests that the MS/vDBB controls the amplitude of hippocampal θ field activity by recruiting more cells in the MS/vDBB, and in turn, the larger the amplitude of hippocampal θ field activity (i.e., synaptic activity), the greater the probability that phasic hippocampal θ -on cells will increase their discharge rates.

In contrast to the dramatic reduction of θ -on cells during the maximal period of procaine suppression of the MS/vDBB, θ -off cells continued to discharge at rates that were not significantly different from the pre-procaine levels accompanying LIA. The inhibition of θ -off cells normally produced by hypothalamic stimulation was also abolished during the time of maximal suppression of the MS/vDBB.

The results of the present study are in general agreement with those reported recently by Mizumori and colleagues, using freely moving rats. Mizumori et al. (1989a) showed that the microinjection of lidocaine in the MS nucleus greatly reduced the spontaneous firing rates of CA1 θ -on cells. In a second study, Mizumori et al. (1989b) showed that microinjection of tetracaine in the septum abolished θ field activity and reduced the discharge rates of stratum granulosum and CA1 θ -on cells by approximately 50%. These reductions were not as pronounced as observed in the present study. The difference may be due to the

fact that the present study was carried out using urethane-anesthetized rats (where type 2 θ predominates) compared to their work using freely moving rats (where both type 1 and type 2 θ occur). Finally, Mizumori et al. (1990) showed that the microinjection of tetracaine in the MS resulted in the continual discharge of two θ -off cells, irrespective of behavior, with a pronounced 18–20-Hz rhythmic modulation. Interestingly, one phasic θ -off cell in the present study also showed a pronounced rhythmicity during suppression of the MS/vDBB.

In summary, the present study demonstrated that the MS/vDBB nuclei play a major role in the control of spontaneous and hypothalamically elicited hippocampal formation field activity and θ -on and θ -off cell discharges. The findings suggest that it took fewer MS/vDBB cells to control the frequency of hippocampal θ field activity, compared to the control of its amplitude. By extrapolation, the recruitment of MS/vDBB cells controls the increasing discharge rates (and pattern) of phasic hippocampal θ -on cells. Furthermore, the hypothesis that the control of θ -on cell discharges is mediated by excitatory cholinergic projections and the control of θ -off cell discharges is mediated by inhibitory GABA-ergic projections is supported by the results.

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