Intraseptal Procaine Abolishes Hypothalamic Stimulation-Induced Wheel-Running and Hippocampal Theta Field Activity in Rats

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Rats were implanted chronically with hippocampal recording electrodes, a microinfusion guide cannula aimed at the medial septal nucleus, and an electrode for electrical stimulation of the posterior hypothalamic nucleus (PH). PH stimulation elicited running in rats placed in a wheel and simultaneously occurring hippocampal theta field activity (HPC- θ). In the preprocaine (PRE) testing condition, a positive linear relationship was demonstrated among the intensity of electrical stimulation of the PH, wheel-running speed, and the peak frequency of HPC- θ . HPC- θ amplitude reached an asymptote at the lowest levels of electrical stimulation of the PH. Procaine hydrochloride (1.5 μ l, 20% solution), a local anesthetic, was then infused into the medial septal nucleus (MS). Five minutes after the infusion, PH stimulation no longer induced wheel-running behavior or HPC- θ , and the remaining irregular field activity was significantly reduced in amplitude. Fifteen minutes after the procaine infusion, PH stimulation still did not elicit HPC- θ or running behavior in the majority of animals but did evoke large-amplitude sharp-waves. Thirty minutes after the procaine infusion, PH stimulation again elicited HPC- θ and running behavior, but HPC- θ peak frequency and running speeds were both

significantly reduced compared with PRE values. Forty-five minutes after the infusion, HPC- θ amplitude had recovered to PRE values, but HPC- θ frequency and running speeds elicited by PH stimulation were still significantly reduced. By 60 min after procaine administration, the amplitude and frequency of HPC- θ and the running speeds elicited by PH stimulation recovered to PRE values. Multiple regression analysis revealed that the recovery pattern of running behavior reflected the frequency rather than the amplitude of HPC- θ . Neither saline control infusions into the MS nor procaine infusions into the lateral septum and paraventricular thalamic nucleus affected HPC- θ or running behavior. These findings are consistent with the notion that both the locomotor activity and "movement-related" HPC- θ frequency induced by electrically stimulating the PH were attributable to ascending activation of a hypothalamo-septal pathway and not to activation of descending brainstem or peripheral motor systems.

Key words: posterior hypothalamus; medial septum; procaine inactivation; hippocampal theta field activity; wheel-running behavior; ascending brainstem synchronizing pathway

Hippocampal theta field activity (HPC- θ) is a rhythmic field potential that oscillates at a frequency between 3 and 12 Hz and has been suggested to be involved in a variety of theories of hippocampal function including attention and motivation (Green and Arduini, 1954; Grastyan et al., 1959, 1966; Bennett et al., 1973), mechanisms of learning and memory (Elazar and Adey, 1967; O'Keefe and Nadel, 1978; Rose and Dunwiddie, 1986; Greenstein et al., 1988; Pavlides et al., 1988; Buzsaki, 1989; Alonso et al., 1990; Eichenbaum et al., 1992; Huerta and Lisman, 1993; Markowska et al., 1995; Staubli and Xu, 1995), and integration of motor programming (Vanderwolf, 1969; Black, 1975; Thompson et al., 1980; Morris and Hagan, 1983; Lopes Da Silva et al., 1985; Bland, 1986). In behaving animals, HPC- θ is present during "exploratory" behaviors, paradoxical and slow-wave sleep (Gatzert et al., 1994), and accompanies "voluntary" motor behaviors such as walking, running, rearing, and head movements (Vanderwolf, 1969). During nonalert immobility and when animals are engaged in "automatic" motor activities such as chewing, licking, grooming, or shivering, large-amplitude irregular activity

is recorded in the hippocampal formation (Vanderwolf, 1969; Whishaw and Vanderwolf, 1973).

The medial septal nucleus (MS) is the primary extrinsic regulator of HPC- θ (Stewart and Fox, 1990). In unanesthetized animals, lesions of the MS abolish HPC- θ (Green and Arduini, 1954; Petsche and Stumpf, 1960; Petsche et al., 1962; Donovan, 1968; Gray, 1971; Myhrer, 1975; Kolb and Whishaw, 1977; Winson, 1978; Andersen et al., 1979; Monmaur et al., 1979; Rawlins et al., 1979; Sainsbury and Bland, 1981). Transection of the fimbria/fornix, which connects the MS to the hippocampal formation, also abolishes HPC- θ (Green and Arduini, 1954; Ranck, 1973; Buzsaki et al., 1983). Electrical stimulation of the MS, when delivered in theta-patterned bursts, elicits theta-like activity in the hippocampal formation; however, the behavior of the animals during MS stimulation is uncorrelated with the induced HPC- θ (Kramis and Routtenberg, 1977). Conversely, septal inactivation, achieved via the microinfusion of a local anesthetic directly into the MS, temporarily abolishes HPC- θ and reduces the occurrence of locomotor activity (Brücke et al., 1959; Mizumori et al., 1989; Lawson and Bland, 1993).

Electrical stimulation at various levels of the brainstem reticular formation and caudal diencephalon elicits HPC- θ . Increasing the intensity of stimulation in these areas elicits increasing frequencies of HPC- θ (Torii, 1961; Yokota and Fujimori, 1964; Grastyan et al., 1966; Bland and Vanderwolf, 1972a; Klemm,

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1972a,b; Macadar et al., 1974; Paiva et al., 1976; Robinson and Vanderwolf, 1978; Vertes, 1980, 1981). The synchronizing effect these regions have on hippocampal field activity may be mediated via the MS and originate in the rostral pons region (PO) (Macadar et al., 1974; Vertes, 1980, 1981). Fibers from the PO reach the MS via nuclei in the caudal diencephalon, namely the supramammillary nucleus and the posterior hypothalamic nucleus (Vertes, 1982, 1984, 1988); both nuclei project to the MS (Vertes, 1992; Vertes et al., 1995). The discharge patterns of cells in these nuclei show distinct relationships to hippocampal field activity (Kirk and McNaughton, 1991; Nunez et al., 1991; Kocsis and Vertes, 1994; Bland et al., 1995), and the infusion of a local anesthetic in this area blocks the induction of HPC- θ with PO stimulation (Kirk and McNaughton, 1993; Oddie et al., 1994).

Electrical stimulation of the posterior hypothalamic nucleus (PH) not only elicits HPC- θ , but also induces locomotor activity; however, the motor behavior is dependent on the environmental situation (Gerben, 1968, 1969; Bland and Vanderwolf, 1972a; Whishaw et al., 1972). The same intensity of PH stimulation can induce a rat to run in a wheel, jump out of a box, dig in bedding, or explore a novel environment. Increasing the intensity of PH stimulation not only increases the frequency of HPC- θ , but also increases the intensity with which motor programs are initiated and maintained in each situation (e.g., increasing running speeds are effected by increasing intensities of stimulation). In freely moving rats, PH lesions decrease the frequency of HPC- θ and the occurrence of voluntary motor behavior while leaving "automatic" movements intact (Robinson and Whishaw, 1974).

Taken collectively, these studies indicate that the PH may exert its influence on both HPC- θ and concomitant locomotor activity via a hypothalamo-septal pathway. Alternatively, PH stimulation may induce movement by activating descending motor systems. Locomotor activity elicited in this manner would then cause "movement-related" HPC- θ . In this experiment, rats were implanted with hippocampal recording electrodes and induced to run in a wheel with electrical stimulation of the PH. Procaine, a local anesthetic, was then infused into the medial septum, and its effect on the previously elicited behavior and field activity was examined. In the event that MS procaine abolished HPC- θ , and had no influence on the PH elicited motor behavior, the alternative hypothesis would be confirmed. Conversely, if MS procaine abolished both HPC- θ and the elicited wheel-running behavior, then the aforementioned hypothesis would be confirmed; that is, PH-elicited behavior and the accompanying HPC- θ are elicited by activating an ascending hypothalamo-septal pathway that synchronizes the hippocampal field activity and plays a role in sensorimotor integration (see also Bland and Colom, 1993).

MATERIALS AND METHODS

Animals. Male Long-Evans rats (300–550 gm) were used. The animals were supplied by the University of Calgary Animal Vivarium, housed individually, and maintained on a 12 hr light/dark cycle. Food and water were provided *ad libitum*.

Chronic implant procedure. Animals were implanted chronically with bilateral hippocampal recording electrodes, a guide tube aimed at the medial septum, and a bipolar electrode for electrical stimulation of the PH. Each animal was given atropine methyl nitrate (10 mg/kg) and then anesthetized with sodium pentobarbital (65 mg/kg). The animal was placed in a stereotaxic instrument, and bregma and lambda were leveled to horizontal. An uninsulated tungsten wire implanted into the cortex anterior to bregma served as an indifferent electrode. Tungsten microelectrodes (0.2–0.8 M Ω) were placed bilaterally into the stratum moleculare of the fascia dentata (3.3 mm posterior to bregma, 2.4 mm lateral to the midline, and 2.7 mm ventral to the dural surface). A guide tube, constructed of 14.6-mm-long 23-gauge stainless steel tubing, was im-

planted 2.7 mm ventral to the dural surface. The guide tube positioned a microinfusion cannula, constructed of 17.6-mm-long 30-gauge stainless steel tubing, in the medial septum. The tip of the microinfusion cannula extended 3.0 mm beyond the guide tube to 5.7 mm ventral to the dural surface. A bipolar stimulation electrode (MS 303/1, Plastics One, Roanoke, VA), ~250 μ m in diameter, was implanted into the PH 3.5 mm posterior to bregma, 0.0–0.2 mm lateral the midline, and 7.6 mm ventral to the dural surface. A subminiature connector soldered to a panhead screw and placed in the skull adjacent to lambda served as a ground electrode. The electrodes and guide tube were held in place using dental acrylic fixed to panhead screws placed in the skull. The recording, ground, and indifferent electrodes were then connected to a female 4-pin Amphenol plug that was fixed to the skull using dental acrylic. The animals were given a minimum 1 week recovery period before testing began. Thirteen animals that had stable chronic recording and stimulation arrangements were involved in the behavioral testing described below.

HPC recording, PH electrical stimulation, and MS infusion procedure. Signals from the brain were amplified by an operational amplifier set at 10 $\times$ gain (LF 444 CM, Linear Technology, Milpitas, CA) located in a 4-pin male Amphenol plug that was connected to the animal. Signals passed through a cable into a commutator that then fed to band-pass (1–35 Hz) Grass P511 AC preamplifiers. The signals were displayed on a storage oscilloscope and stored on an FM tape recorder for subsequent analysis. The experimenter's vocal description of ongoing behavior during testing was also recorded on the FM tape. In the preprocaine (PRE) condition, electrical stimulation of the PH was delivered in a block containing two sets of three stimulus intensities (0.1 msec duration, 100 Hz square wave). Stimulus intensities varied for each animal and were chosen using the following criteria: (1) the lowest intensity that elicited wheel-running behavior, and (2) two higher intensities at 0.1–0.2 mA increments from the lowest intensity. Stimulus intensities ranged from 0.1 to 1.0 mA and were delivered to the animal via the commutator using a Grass S88 stimulator equipped with a stimulus isolation unit. Any given stimulus intensity within a block lasted ~10 sec and was followed by a 10–30 sec delay. Thus, a stimulation block contained six 10 sec stimulus bouts delivered to the PH, two at each of the three intensities. In the drug testing condition, a solution of procaine hydrochloride in physiological saline (20%) was infused into the MS. The microinfusion cannula was connected to a 10 μ l Hamilton Gas-Tight syringe (Reno, NV) via a length of PE 50 intramedic tubing that contained the procaine solution. The syringe was mounted on a Harvard Apparatus (Boston, MA) infusion pump that delivered 0.5 μ l/min for 3 min. The microinfusion cannula was not removed until 3 min after the termination of the MS infusion. Control infusions used only physiological saline. After drug or control infusions, a stimulation block was delivered to the PH nucleus for each of the postprocaine experimental conditions as in the PRE testing condition.

Apparatus and behavioral testing procedure. The experiments were conducted in a running-wheel (43 cm diameter) placed in a Faraday cage (122 $\times$ 65 $\times$ 65 cm³). The running wheel was equipped with a speedometer (Cateye Mity Cyclocomputer, Osaka, Japan, model CC-MT100), which converted wheel revolutions to km/hr based on the wheel diameter. The speedometer recorded the maximum speed achieved during PH stimulation. A testing session began by connecting the PH stimulation and hippocampal recording leads to the animal and then placing the animal in the running-wheel. The lowest level of PH stimulation that induced wheel-running was determined, and then two higher levels were chosen. The stimulus levels were input into a PC equipped with a BASIC program that generated six blocks of randomly ordered stimulus intensities: one block for each of the PRE, 5 min postprocaine (P5), 15 min postprocaine (P15), 30 min postprocaine (P30), 45 min postprocaine (P45), and 60 min postprocaine (P60) experimental conditions. In the PRE condition, a stimulus block was delivered to the animal. For each intensity in the block, the maximum running speed elicited by the PH stimulation was recorded on the FM tape along with the simultaneously occurring hippocampal field activity. The speedometer was then zeroed, and the subsequent stimulus intensities were administered. After the PRE condition, the procaine microinfusion cannula was inserted into the guide tube. Procaine hydrochloride or saline was infused into the MS, and 5 min after the termination of the infusion the P5 stimulation block was delivered to the PH and the running speeds and simultaneously occurring hippocampal field activity were recorded. The same procedure followed for the P15–P60 experimental conditions. Behavior and hippocampal field activity were also recorded using a video recorder and a split-screen technique. One camera recorded the oscilloscope display of the hippocampal field activity while a second camera recorded ongoing behavior.

Figure 1. Procaine microinfusions in the MS. Diagrammatic reconstructions illustrating the location of the medial septal microinfusion cannula tips. The infusions were localized to three coronal planes: 0.45 mm anterior to bregma (A), 0.10 mm anterior to bregma (B), and 0.0 mm anterior to bregma (C). All stimulation electrodes were in the PH or within close proximity; an example is provided in D. The diagram in the top left panel shows the location of the coronal sections in a mid-sagittal view. *ac*, Anterior commissure; *cc*, corpus callosum; *CM*, central medial nucleus of the thalamus; *DMH*, dorsal medial hypothalamic nucleus; *HDB*, horizontal limb of the diagonal band; *LS*, lateral septal nucleus; *MS*, medial septal nucleus; *oc*, optic chiasm; *VDB*, vertical limb of the diagonal band.

The two video inputs were recorded simultaneously and viewed by the experimenter during testing. Thirteen animals were tested with procaine microinfusions. Three of these animals also served as controls and, in addition to procaine, received physiological saline infusions. After perfusion and fixation of the brain, frozen sections (40 μ m) were taken serially and mounted on glass slides and stained with cresyl violet for subsequent verification of hippocampal field electrode, microinfusion cannula, and PH stimulation electrode tip placements.

Data analysis. Analysis of the data segments was accomplished off-line using a PC and a software acquisition and analysis package (DataWave Technologies, Longmont, CO). A data segment was collected during each level of PH stimulation (6–8 sec of data were selected from within the 10 sec stimulus train). Hippocampal field activity was sampled at a frequency of 133 Hz. Each data segment was subjected to a real-time fast Fourier (FFT) analysis, and the peak frequency and amplitude values were recorded. Theta was defined as a sinusoidal-like waveform with a peak frequency of 3–12 Hz and a small bandwidth. If there was no prominent peak in the spectrum, the largest power value in the 3–12 Hz range was recorded. The maximum running speed achieved during the 10 sec of PH stimulation was also recorded. The peak frequency, amplitude, and running speed values elicited by PH stimulation at the same intensity within a block were averaged. A one-way repeated-measures ANOVA was used to analyze each of the peak frequency, amplitude, and running speed measures. Multiple regression and Dunnett's *t* test for pairwise comparisons were used for post hoc analyses. Histological illustrations were generated using the computer graphics files that accompany *Brain Maps* (Swanson, 1992).

RESULTS

Histology

In all animals tested, hippocampal recording electrodes were localized to the molecular layer of the dentate gyrus. Figure 1 illustrates the location of the microinfusion cannula in 11 of 13 animals tested with procaine hydrochloride (A–C). The microinfusion cannula tips were localized to the MS in three coronal planes: 0.45 mm anterior to bregma (A), 0.10 mm anterior to bregma (B), and 0.00 mm anterior to bregma (C). Figure 1 (top left panel) shows the location of the coronal sections in a mid-sagittal view. Figure 1D illustrates the placement of a PH stimulation electrode. All PH stimulation electrode tips were located in the PH (3.4–3.7 mm posterior to bregma and 0.0–0.9 mm lateral to midline). In 2 of 13 animals, the microinfusion cannula was not

localized to the MS, and the data from these animals served as histological controls (see below).

PH electrical stimulation and procaine microinfusion in the medial septum

Figure 2 provides a representative example from one animal illustrating the effect of procaine microinfusion into the MS on HPC- θ and wheel-running elicited by 0.15 mA PH stimulation. Data segment analogs of the hippocampal field activity at the onset of PH stimulation (solid bar) are shown. Figure 2 (middle graph) also provides the corresponding spectra from FFT analysis for each data segment in the PRE–P60 experimental conditions (spectra plot linear values). Before the microinfusion of procaine in the MS (PRE), electrical stimulation in the PH elicited HPC- θ with an amplitude of 55.8 dB and a peak frequency of 10.0 Hz and induced a maximum running speed of 17.0 km/hr. At P5, the same intensity of PH stimulation no longer elicited HPC- θ (absence of peak in the P5 spectrum) or induced wheel-running behavior (running speed = 0.0). At P15, PH stimulation still did not elicit HPC- θ or wheel-running behavior (absence of peak in the P15 spectrum) but, rather, evoked large amplitude sharp waves in the hippocampal field. At P30, PH stimulation again elicited HPC- θ , but at a reduced amplitude and frequency, and induced wheel-running at speeds that were also reduced compared with PRE values. In the P30 condition, 0.15 mA PH stimulation elicited HPC- θ with an amplitude of 35.1 dB, a peak frequency of 7.8 Hz, and induced a maximum running speed of 13 km/hr. Although the amplitude of HPC- θ had nearly recovered to PRE values at MS P45 (53.5 dB at P45), the peak frequency of HPC- θ and running speed elicited by PH stimulation were still reduced (8.3 Hz and 14 km/hr, respectively). By MS P60, the amplitude of HPC- θ had recovered to PRE values (55 dB), and in this animal the peak frequency HPC- θ and running speed had nearly recovered to PRE values (9.1 Hz and 14 km/hr, respectively).

Behaviorally, the microinfusion of procaine into the MS resulted in animals that appeared catatonic, but when handled they moved voluntarily without overt postural abnormalities. Figure 3

Figure 2. The effect of medial septal procaine on hippocampal field activity and running speeds induced by electrical stimulation of the posterior hypothalamus (0.15 mA): representative recordings and graphic summary (stimulation onset indicated by solid bars). The middle graph provides the spectra caused by FFT analyses of the data segments (only partial data segments are shown) for each of the experimental conditions (graph plots linear values). Before the infusion of procaine into the medial septum (PRE), PH stimulation induced a running speed of 17 km/hr and simultaneously occurring HPC- θ with a peak frequency of 10 Hz and an amplitude of 55.8 dB (see the corresponding data analog and FFT spectrum for the PRE condition). Five minutes after the infusion of procaine into the medial septum (P5), the same intensity of PH stimulation no longer induced running or elicited hippocampal theta (no peak in spectrum at P5), and the remaining field activity was reduced to 26 dB. The frequency and amplitude of HPC- θ gradually recovered to PRE values, and by 60 min after procaine administration (P60), 0.15 mA PH stimulation induced running at 14 km/hr and simultaneously occurring theta with a peak frequency of 9.1 Hz and an amplitude of 55 dB.

shows the group data obtained from 11 animals that received procaine microinfusion in the MS at each of the three PH stimulation levels used. The PH stimulation values shown in the legend of Figure 3 are the averages for each of the low (mean 0.28 mA), medium (mean 0.41 mA), and high (mean 0.54 mA) intensity levels chosen for each animal.

The effect of MS procaine on the amplitude of hippocampal theta elicited by PH electrical stimulation

Figure 3 (top graph) shows the effect of MS procaine on the amplitude of hippocampal field activity. After MS inactivation, PH stimulation no longer elicited HPC- θ . A one-way repeated-measures ANOVA revealed that MS procaine had significantly reduced the amplitude of the remaining irregular hippocampal field activity during PH stimulation ($F_{(5,10)} = 54.6, p < 0.0001$). The main effect of PH stimulation intensity on the amplitude of HPC- θ was not significant. The average amplitude of HPC- θ (averaged across all levels of PH stimulation) before the microinfusion of procaine in the MS was 47.7 ± 1.4 dB ($\pm$ SEM). After the microinfusion of procaine into the MS, HPC- θ was abolished and the average amplitude of the remaining irregular hippocampal field activity (when measured as the largest amplitude within the theta bandwidth) was 30.1 ± 1.8 dB. Thus, 5 min after procaine infusion into the MS, the amplitude of the hippocampal field activity during PH stimulation was reduced by ~ 1.0 mV. Figure 3 (top graph) also shows that in the earlier period of MS recovery, the amplitude of HPC- θ was rapidly recovering to PRE values (P15, 35.9 ± 1.8 dB; P30, 38.7 ± 1.7 dB; P45, 42.0 ± 1.8 dB; P60, 44.1 ± 1.6 dB). There was no significant difference in the amplitude of HPC- θ elicited by the highest level of PH stimulation at the PRE and P60 conditions, indicating that the MS had recovered from the procaine microinfusion (48.2 ± 2.5 dB PRE vs 45.2 ± 3.0 dB P60; $t_{(10)} = 2.1, p = 0.06$).

The effect of MS procaine on the peak frequency of hippocampal theta elicited by PH electrical stimulation

Figure 3 (middle graph) shows the group data for the effect of procaine microinfusion into the MS on the peak frequency of HPC- θ . In the PRE condition, increasing the intensity of PH

stimulation significantly increased the peak frequency of HPC- θ ($t_{(32)} = -64.6, p < 0.0001$). PH stimulation at the low intensity (0.28 mA) elicited hippocampal theta with an average peak frequency of 8.4 ± 0.1 Hz, 8.9 ± 0.2 Hz at the medium intensity (0.41 mA), and 9.4 ± 0.3 Hz at the high intensity (0.54 mA). At P5, HPC- θ was completely abolished in 11 of 11 animals at the lowest level of PH stimulation (0.28 mA), abolished in 10 of 11 animals at the medium PH intensity (0.41 mA), and abolished in 9 of 11 animals at the highest intensity of PH stimulation (0.54 mA). Thus, the values plotted in Figure 3 (middle graph) for the P5 condition (right of dashed line) are peak frequency values in only 1 animal at the medium (0.41 mA) intensity of PH stimulation and 2 animals at the highest (0.54 mA) intensity of PH stimulation. The peak frequency of HPC- θ (in the 3 animals in which theta was recorded in the P5 condition during PH stimulation) was reduced compared with PRE values. At P15, PH stimulation elicited HPC- θ in only 2 of 11 animals at the low (0.28 mA) PH intensity, in 9 of 11 animals at the medium (0.41 mA) PH intensity, and in 9 of 11 animals at the high (0.54 mA) intensity. The frequency of HPC- θ in these animals was reduced compared with PRE values. Only large-amplitude hippocampal sharp-waves were recorded at this time. At P30, PH stimulation elicited HPC- θ in 6 of 11 animals at the 0.28 mA intensity, in 11 of 11 animals at the 0.41 mA intensity, and in 11 of 11 animals at the 0.54 mA intensity. The frequency of HPC- θ remained at values that were reduced compared with PRE values. At P45, PH stimulation elicited HPC- θ in 8 of 11 animals at the 0.28 mA intensity, in 11 of 11 animals at the 0.41 mA intensity, and in 11 of 11 animals at the 0.54 mA intensity; the frequency of HPC- θ remained reduced compared with PRE values. At P60, PH stimulation elicited HPC- θ in 9 of 11 animals at the 0.28 mA intensity, in 11 of 11 animals at the 0.41 mA intensity, and in 11 of 11 animals at the 0.54 mA intensity. The frequency of HPC- θ in these animals was still slightly reduced compared with PRE values. In 2 animals in the P60 condition, no HPC- θ was recorded at the lowest PH stimulation level.

Given the nature of the effect of MS procaine on the peak frequency of HPC- θ , the data could not be analyzed using repeated-measures ANOVA because of the large number of

Figure 3. The effect of medial septal procaine infusion on theta and running speed. Group data on the amplitude (*top*) and peak frequency (*middle*) of HPC- θ and running speed (*bottom*) induced by electrical stimulation delivered to the PH. The PH stimulation values shown in this legend are the averages for each of the low ($\bar{x}$ = 0.28 mA; □), medium ($\bar{x}$ = 0.41 mA; ◇), and high ($\bar{x}$ = 0.54 mA; ○) intensity levels chosen for each animal. Before the infusion of procaine into the medial septum, increasing the intensity of posterior hypothalamic stimulation had no significant effect on the amplitude of HPC- θ (*top*, left of dashed line, MS Procaine), significantly increased the peak frequency of HPC- θ (*middle*, left of dashed line), and significantly increased the speed of running (*bottom*, left of dashed line). After MS procaine, the amplitude of hippocampal field activity was significantly reduced (P5), HPC- θ could no longer be elicited by PH stimulation in most animals (see Results), and running could no longer be elicited by PH stimulation. As the medial septum recovered from the procaine infusion the amplitude and frequency of hippocampal field activity gradually recovered to PRE values, as did the speed of running induced by each PH stimulus level. There was no longer any significant difference between the amplitudes and frequencies of HPC- θ or the running speeds induced at the PRE and P60 experimental conditions, indicating that the MS had recovered from the procaine inactivation.

missing cells in the P5 and P15 conditions. That is, the failure to observe a peak in the FFT analysis of a data segment during PH stimulation (indicating that theta had been abolished) caused a missing data cell. Thus, to examine the repeated-measure effect of MS procaine on the frequency of HPC- θ , the following data analysis was performed. If HPC- θ was elicited by the highest intensity of PH stimulation in any of the postprocaine experimental conditions, the peak frequency values were plotted for each of the remaining experimental conditions and the slope of those values (which represent recovery of the MS from the procaine microinfusion) for each animal was determined. A one-sample t test revealed that the values were significantly different from zero,

indicating that the peak frequency of HPC- θ changed significantly as the MS recovered from procaine inactivation ($t_{(10)} = 4.9$, $p < 0.0006$; mean slope = 0.7). The peak frequency of HPC- θ was not significantly different between the PRE and P60 conditions at the highest level of PH stimulation (9.4 ± 0.3 Hz PRE vs 8.9 ± 0.4 Hz P60; $t_{(10)} = 0.97$, $p = 0.35$), indicating that the MS had recovered from the procaine microinfusion.

The effect of MS procaine on the speed of wheel-running induced by PH electrical stimulation

Figure 3 (*bottom graph*) illustrates the group data for the effect of MS procaine on wheel-running induced by PH stimulation. Before the microinfusion of procaine into the MS, increasing intensities of PH stimulation induced significantly increasing wheel-running speeds in the animals ($F_{(2,30)} = 14.4$, $p < 0.0001$). PH stimulation at the low intensity (0.28 mA) caused a wheel-running speed of 5.8 ± 0.7 km/hr, 10.9 ± 1.2 km/hr at the medium intensity (0.41 mA), and 15.7 ± 1.9 km/hr at the high intensity (0.54 mA). After the microinfusion of procaine into the MS (P5), wheel-running was totally abolished in all animals (11/11) at the low intensity, in 8 of 11 animals at the medium intensity, and in 7 of 11 animals at the highest intensity (*bottom graph*, right of dashed line). A repeated-measures ANOVA revealed that running speeds were significantly reduced ($F_{(5,150)} = 41.2$, $p < 0.0001$), causing a running speed of zero at the lowest intensity (0.28 mA), a mean running speed of 1.6 ± 0.9 km/hr at the medium intensity (0.41 mA), and 3.6 ± 1.6 km/hr at the highest intensity (0.54 mA) of PH stimulation in the P5 condition. Figure 3 also shows that in the P15–P60 conditions, running speeds gradually recovered to PRE values. PH stimulation at the highest intensity induced running speeds of 9.4 ± 1.8 km/hr at P15, 12.5 ± 1.9 km/hr at P30, 13.2 ± 1.9 km/hr at P45, and 13.9 ± 1.9 km/hr at P60. By the P60 condition, running speeds induced by PH stimulation were no longer significantly different from the PRE condition, indicating that the MS had recovered from the procaine microinfusion (15.7 ± 1.9 km/hr PRE vs 13.9 ± 1.9 km/hr P60 at the highest stimulus level; $t_{(10)} = 1.4$, $p = 0.19$).

Those animals in which HPC- θ was recorded during PH stimulation in the P5 condition were the same animals that showed wheel-running behavior, with one exception. Two additional animals in which no prominent theta peaks appeared in the FFT analyses did show wheel-running behavior at reduced speeds compared with PRE values.

Saline and histological controls

Three animals received injections of physiological saline (NaCl) into the MS before the microinfusion of procaine. Table 1 presents the results of these microinfusions. The microinfusion of NaCl into the MS had no effect on the amplitude and peak frequency of HPC- θ or on wheel-running speeds induced during low-, medium-, or high-intensity PH stimulation. Figure 4 presents the results of two animals that received microinfusions of procaine in the lateral septal nucleus (LS) and the paraventricular thalamic nucleus (PVT). The top illustrations reconstruct coronal sections that show the location of the procaine microinfusion cannula tips in these animals. The bottom graphs present the results of a $2.5 \mu\text{l}$ infusion in the LS and $5.0 \mu\text{l}$ infusion in the PVT on the amplitude and peak frequency of HPC- θ and the running speed induced at the highest level of PH stimulation. The graphs show no significant difference between the pre- and postprocaine values for these measures, with the exception of the LS microinfusion, which revealed a significant reduction in the amplitude of HPC- θ (51.2

Table 1. The result of physiological saline microinfusion into the MS on the amplitude, frequency, and speed of wheel-running

	PH stimulus intensity	Pre-NaCl	Post-NaCl	<i>t</i> -value (df = 2)	<i>p</i>
Amplitude of theta (dB)	low	40.3 ± 6.3	38.0 ± 4.9	0.73	0.54
	medium	40.7 ± 5.9	43.3 ± 4.3	−0.78	0.52
	high	46.3 ± 6.7	41.7 ± 5.7	3.6	0.07
Peak frequency of theta (Hz)	low	8.1 ± 0.3	8.3 ± 0.2	−0.56	0.63
	medium	8.8 ± 0.3	8.6 ± 0.4	4.0	0.06
	high	9.1 ± 0.3	9.1 ± 0.3	0.189	0.87
Wheel-running speed (km/hr)	low	7.1 ± 1.3	8.5 ± 4.3	−0.39	0.73
	medium	16.8 ± 1.9	15.5 ± 3.9	0.54	0.64
	high	19.4 ± 3.3	18.4 ± 4.0	0.44	0.70

± 0.2 dB PRE vs 43.8 ± 1.5 dB postprocaine). However, after the LS procaine microinfusion, the frequency of HPC- θ and the running speed elicited by PH stimulation remained unchanged.

Finally, to examine the relationship between HPC- θ and wheel-running behavior, multiple-regression analysis was used to determine whether the frequency or amplitude of HPC- θ (independent and uncorrelated variables; frequency vs amplitude of HPC- θ , $F_{(1,9)} = 2.2$, $p = 0.17$) accounted for a significant proportion of variance in the speed of wheel-running (the dependent variable). Regression analysis revealed that the frequency of HPC- θ accounted for a significant proportion of variance in the speed data ($t = 4.0$, $p = 0.0002$), whereas the amplitude of HPC- θ did not ($t = -0.589$, $p = 0.5592$).

DISCUSSION

A new and important finding regarding the behavioral correlates of HPC- θ has emerged from the present study. This experiment clearly demonstrates that the wheel-running behavior induced by electrical stimulation of the PH, and the simultaneously occurring HPC- θ , is caused by activation of the ascending hypothalamo-septal pathway. Thus, the motor behavior induced by PH electrical stimulation appears to depend critically on the ascending activation of neural circuitry in the septo-hippocampal structures underlying the generation of theta field activity—not on descending activation of brainstem and/or peripheral motor systems. The PH has been shown to comprise a critical part of the ascending hippocampal synchronizing system in urethane-anesthetized rats (Colom et al., 1987; Bland et al., 1994; Oddie et al., 1994). Furthermore, the hippocampal activation caused by electrical stimulation of the PH in urethane-anesthetized rats is dependent on the integrity of the medial septum (Smythe et al., 1991). The current findings are the first to demonstrate that the integrity of the MS is necessary for modulating both the locomotor activity and the concomitant HPC- θ induced by electrical stimulation of the PH in the freely moving animal.

Before the infusion of procaine into the MS (MS inactivation), PH stimulation elicited HPC- θ and running behavior in rats placed in a running-wheel. In addition, there was a positive linear relationship among the intensity of electrical stimulation of the PH, running speeds, and peak theta frequencies, confirming the previous results of Bland and Vanderwolf (1972a). MS inactivation then abolished PH stimulation-induced wheel-running behavior and the simultaneously occurring HPC- θ . Immediately after MS inactivation, the animals initially appeared catatonic (less likely to initiate motor behavior). Mizumori et al. (1989) and Lawson and Bland (1993) previously demonstrated that MS inactivation in freely moving rats abolished HPC- θ and reduced the amount of spontaneously occurring locomotor activity. The ani-

mals in this experiment showed normal motor activity when handled, but electrical stimulation of the PH was no longer capable of inducing wheel-running behavior after procaine inactivation of the MS. However, PH stimulation did elicit hippocampal sharp-wave activity with no overt behavior changes during this time period. Hippocampal sharp-wave activity and HPC- θ have been shown previously to be mutually antagonistic (Lerma et al., 1984; Suzuki and Smith, 1987). As the MS recovered from the procaine microinfusion, PH stimulation again elicited wheel-running behavior and HPC- θ , although at significantly reduced running speeds and peak theta frequencies, respectively. The amplitude of HPC- θ was also significantly decreased but recovered to PRE values before the recovery of peak theta frequencies. Sixty minutes after the procaine infusion in MS, the amplitude, frequency, and speed of wheel-running elicited by PH stimulation had all returned to PRE values. In addition, a multiple-regression analysis revealed that the frequency, and not the amplitude, of HPC- θ accounted for a significant proportion of variance in the running speed data. This suggested that the frequency of HPC- θ , as opposed to the amplitude, was more critically related to the speed of the elicited wheel-running behavior.

Many studies reinforce the notion that brain regions involved in the production of synchronous field activity play a role in the organization of motor behavior. Lesions of the hippocampal formation consistently produce hyperactivity in animals (for review, see Gray, 1982). MS lesions have been shown to cause hyperactivity that was attributed to the perseveration of body turns (McNaughton and Feldon, 1984). Conversely, MS inactivation initially causes the reduction of voluntary motor behavior (Mizumori et al., 1989; Lawson and Bland, 1993) until animals are forced to move, after which the animals appear to move normally. Moreover, PH lesions reduce the occurrence of voluntary behaviors while leaving “automatic” behaviors intact (Robinson and Whishaw, 1974). These studies are consistent with the notion that lesions at different levels of the ascending brainstem hippocampal synchronizing pathway influence motor behavior.

Numerous studies also demonstrate that manipulations that affect hippocampal synchrony also influence locomotor activity and vice versa. For example, in animals that must jump to avoid foot-shock, the frequency and amplitude of HPC- θ increased as the height that the animal must jump increased (Whishaw and Vanderwolf, 1973; Morris and Hagan, 1983). In this and previous studies noted, PH stimulation that elicits HPC- θ also induces motor behavior. The same stimulation parameters that induce HPC- θ and motor behavior during PH stimulation arrest movement when delivered to the dentate gyrus or MS and desynchronize hippocampal field activity (Bland and Vanderwolf, 1972b;

Figure 4. Procaine infusion controls. The effect of procaine microinfusion into the LS (open circle) and PVT (open square). The illustrations at the top are diagrammatic reconstructions illustrating the location of the microinfusion cannula tips in animals that did not receive procaine in the MS, but received procaine infusions in the lateral septum (top left) and thalamus (top right). The graphs at the bottom present the amplitude (top graph) and peak frequency (middle graph) of HPC- θ as well as the running speed elicited by the highest intensity of electrical stimulation in the PH before the infusion of procaine (left of dashed line, Pre) and 5 min after the infusion (right of dashed line, Post). The procaine infusion in either site had no effect on HPC- θ , with one exception. Procaine in the lateral septum significantly decreased the amplitude of HPC- θ , but did not change the peak frequency of theta or the running speed elicited by PH stimulation. *ac*, Anterior commissure; *cc*, corpus callosum; *fi*, fimbria; *fx*, fornix; *LS*, lateral septal nucleus; *PVT*, paraventricular thalamus; *RE*, nucleus reunions; *vhc*, ventral hippocampal commissure.

Whishaw and Nikkel, 1975). Injections of carbachol, a cholinergic agonist, into the dentate gyrus (Mogenson and Nielson, 1984) or the MS (Monmaur and Breton, 1991) induce HPC- θ and locomotor activity. Interestingly, the locomotor activity elicited by the intrahippocampal infusion of carbachol can be blocked by injections of a glutamate antagonist into the ipsilateral nucleus accumbens (Mogenson and Nielson, 1984). Whishaw and Mittleman (1991) demonstrated that the heightened locomotor activity typical of hippocampectomized rats can be blocked by lesions of the nucleus accumbens. Finally, Lopes da Silva et al. (1985) have suggested that the hippocampus controls movement via a cornu ammonis-subiculum-nucleus accumbens-ventral pallidum-substantia nigra pars compacta pathway. Consistent with these findings is the notion that the hippocampal output may inhibit a motor inhibition system that originates in the nucleus accumbens. The current findings, taken collectively with the studies noted above,

provide support for the notion that synchronous output from the hippocampal formation to motor systems plays a role in the organization of motor behavior.

Evidence has accumulated suggesting that there are two distinct forms of HPC- θ in freely moving animals. HPC- θ that occurs concomitantly with motor behavior has been referred to as type 1 θ (Vanderwolf, 1969), has been postulated to be serotonergic (Vanderwolf et al., 1985; Vanderwolf and Baker, 1986), and is abolished by general anesthetics. Several authors (Bland et al., 1984; Leung, 1984) have provided evidence that a second, cholinergically mediated type of theta (called type 2 θ) occurs coincidentally with type 1 θ . When animals are in a state of "alert" immobility (in a sensory processing mode), type 2 θ can be recorded in isolation from type 1 θ (Bland, 1986). This has been demonstrated in immobile rats preparing to initiate a jump in a jump-avoidance task (Whishaw and Vanderwolf, 1973), in immobile animals placed in close proximity to predators (Sainsbury and Montoya, 1984; Sainsbury et al., 1987), and in immobile rabbits during the processing of relevant sensory stimuli; however, as the animal habituates to a repetitive sensory stimulus, so does the prevalence of HPC- θ (Vinogradova, 1975; Whishaw and Dyck, 1984). Type 2 θ is primarily cholinergic and resistant to general anesthetics (Kramis et al., 1975) (for review, see Bland, 1986). Thus, the type 1 and type 2 θ systems appear to be distinct in their function and pharmacology.

What role does the neural circuitry underlying HPC- θ play in the organization of sensorimotor behavior in freely moving animals? Bland (1986) proposed that the hippocampal formation might serve to integrate sensory and motor behavior. In the proposed model, type 2 θ is initiated by sensory inputs that are processed within the context of being relevant to the subsequent initiation of voluntary movements. Functionally, type 2 θ provides the motor system with a "readiness" or priming signal, for both the preparation of movement per se and the intensity of its initiation. As discussed above, recent work suggests that the anatomical basis of the type 2 θ system is represented in large part by the pathways ascending from PO, through the midline posterior hypothalamus (PH and supramammillary nucleus), to the medial septum and hippocampal formation. The present work, carried out in the freely moving animal, provides very clear and substantive support for this suggestion. Ongoing motor behavior would be reflected in the frequency and amplitude of type 1 HPC- θ , and changes that are required in ongoing motor activity would also be signaled by the coincidentally occurring type 2 system. The inputs for type 1 θ originate as feedback from motor systems to the hippocampal formation. The final result would be a change in the organization or intensity of an ongoing motor program. Thus, the hippocampal formation could serve a functional role in sensorimotor integration.

Although the testing of the sensorimotor integration model based exclusively on PH elicited HPC- θ and locomotor activity must be considered cautiously, the present study clearly indicates that the relationship between HPC- θ and voluntary motor behavior is not a spurious one. That is, the locomotor behavior elicited by PH stimulation is clearly dependent on the activation of ascending projections to the hippocampal formation via the medial septum. The alternative hypothesis, that PH induced motor behavior is attributable to the direct activation of descending projections to the brainstem and spinal motor systems, is not tenable. The findings here are consistent with the notion that locomotor activation and the simultaneously occurring HPC- θ are related and are caused by activation of the ascending brainstem pathway,

which synchronizes hippocampal field activity and directly affects motor behavior. Future studies designed to test the hypotheses generated by sensorimotor integration theory may further clarify the relationship between HPC- θ and voluntary motor behavior.

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