PRIMING PATTERN DETERMINES THE CORRELATION BETWEEN HIPPOCAMPAL THETA ACTIVITY AND LOCOMOTOR STEPPING ELICITED BY STIMULATION IN ANESTHETIZED RATS

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Abstract—The after-effects of locomotor stimulation are a transient facilitation of locomotor initiation (the priming effect), and a transient increase in hippocampal rhythmic slow activity in the 3–6 Hz band of the theta range. The similar time course of the two effects suggests that hippocampal 3–6 Hz activity may be linked to the excitability of locomotor initiation. This study tested the hypothesis that power in the 3–6 Hz band that is present prior to stimulation would predict the magnitude of elicited stepping. Stimulation electrodes were implanted in 15 locomotor sites of 10 anesthetized rats (urethane, 800 mg/kg). Hindlimb stepping was elicited by a single control train of electrical stimulation presented once every 62 s. On test trials, a test train at the same intensity followed the control train at varying control/test intervals (15–36 s) to assess the priming effect on stepping. The priming pattern determined whether hippocampal 3–6 Hz power predicted the amount of stepping to be elicited by a stimulation train. Positive correlation (0.47 > r > 0.22) was found for seven out of eight sites showing positive priming effects. Correlation was absent for three other sites that showed non-significant priming effects and were mixed for four sites that showed negative effects. Sites with positive priming patterns, compared to sites with inconsistent or negative priming patterns, had similar trends in post-stimulation 3–6 Hz power, smaller increases in 6–8 Hz power during the control train and lower 1–3 Hz power during the periods immediately before the control stimulation. For six of 15 sites, regardless of the priming pattern, 1–3 Hz power was inversely related to subsequent stepping, and in three cases provided an independent predictor of stepping. Stimulation at two sites produced discrete episodes of post-stimulation stepping. In one of these cases, a 0.5-Hz increase in peak frequency of hippocampal activity preceded stepping.

The results show that the association between hippocampal 3–6 Hz activity and the excitability of locomotor initiation is sufficiently specific to allow prediction of the magnitude of stepping by the prior levels of 3–6 Hz power. However, the occurrence of negative priming effects during prominent 3–6 Hz activity indicates that other factors determine the actual stepping and they can suppress the correlation between theta activity and subsequent locomotion. © 2000 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: hypothalamus, movement initiation.

Since the seminal description of the co-occurrence of locomotion and one prominent electroencephalographic (EEG) pattern in the hippocampus, rhythmic slow-wave activity (theta),25 a range of evidence has supported the existence of a linkage between hippocampal activity and locomotor control. The hypothalamus appears to contain circuitry that activates both locomotion and theta activity. In the awake rat, stimulation in sites throughout the lateral hypothalamus produces locomotion associated with theta activity,3,21,29,30 and hypothalamic sites that support stimulation-elicited stepping show slow-wave activity at theta frequencies during locomotion.22 The speed of locomotion and the frequency of the hypothalaminically elicited theta activity vary with the intensity of stimulation.3 The frequency of theta activity also correlates with the speed of naturally elicited locomotion,14,21 and it progressively increases prior to a jumping response.9,30

At the high doses often used for anesthesia, hippocampal rhythmic activity is abolished,7 but in locomotor studies, surgical anesthesia is maintained at lower doses and hypothalamic locomotor stimulation continues to produce hippocampal slow-wave activity, although at lower frequencies than in the awake rat.19

In the midbrain median raphe region, the relationship between locomotion and theta is the converse of the pattern for the hypothalamus. Inactivation of neurons in the midbrain raphe region leads to increases in locomotor activity,15,31,32 and produces theta activity,17,27 In a study that determined whether the two effects covaried,19 GABA injections into the raphe region increased both stepping elicited by stimulation of the hypothalamus and the amount of theta activity that occurred in the periods. Injections that did not increase stepping did not increase theta and injections that blocked stepping also reduced the pre-stimulation rise in theta activity. These findings indicated that increases in theta activity were associated with facilitation of locomotor initiation. However, the magnitudes of increased theta and increased stepping could not be shown to correlate. This lack of correlation raised a question of the specificity of the relationship between theta activity and locomotor initiation.

Locomotor stimulation has a residual excitatory effect, a priming effect, such that the latency to step is shorter and stepping is more robust on a subsequent stimulation train.30,24 Associated with the priming effect are increases in
Subjects and surgery

The goal of this study was to determine the degree to which hippocampal theta activity was related to the initiation of stepping in the anesthetized rat. As in the previous work, the priming paradigm was used in order to vary the excitability of locomotor initiation, but the sensitivity of the analysis was improved. Rather than correlating theta and stepping values averaged for a site, each site was tested separately correlating over individual trials. In addition, the study used a range of intervals between the priming and the test stimuli, and provided a continuous spectral analysis of hippocampal activity throughout the trial. The original plan was to focus on periformal stimulation sites, and based on prior studies, it was anticipated that priming effects would monotonically decline over time. However, over the course of the study, it became clear that more complex priming effects could provide important information, and additional sites were added to increase the range of the priming patterns.

EXPERIMENTAL PROCEDURES

Subjects and surgery

Male Sprague-Dawley rats (n = 10), weighing an average of 356 g, were initially anesthetized with 2% halothane in oxygen for 5–10 min, then given an initial intraperitoneal injection of 600 mg/kg urethane. After an additional 5–10 min of halothane, the anesthetized rat was placed in a stereotaxic apparatus. A scalp incision was made, and lidocaine was injected around its margins. Holes were drilled in the skull for the stimulation and recording electrodes, and for screws, which provided a recording ground and a stimulation return. The stimulation and recording electrodes were 125-μm diameter tungsten wires insulated with Teflon except at the tip. An uninsulated wire wrapped around two screws in the skull completed the circuit to the amplifier. When the rat stepped, the extensor phase of the hindlimb movement caused the digits to engage the mesh surface of the wheel and rotate it. When the rat stepped, the extensor phase of the hindlimb movement caused the digits to engage the mesh surface of the wheel and rotate it. Hindlimb stepping movements were transduced by a pair of accelerometers, one attached to each metatarsus. Forelimbs are infrequently involved in the stepping elicited by this preparation and they were not monitored. The accelerometers (Etan Devices, Model EGAX 10) have a range of ±10 g, a sensitivity of 8 mV/g, and a linear frequency response from 0 to 150 Hz. Each weighed about 0.5 g, and with dimensions of less than 7 × 4 × 4 mm², did not impede stepping. The amplified output of the accelerometers was led to an analog/digital converter sampling at 500 Hz and displayed on a computer monitor.

To quantify the stepping, a customized program computed a step index (SI) from the accelerometer values. It was defined as a single number over the overall amplitude and frequency of stepping by both hindlimbs. It was computed for each 5.12 s epoch (256 samples) throughout the trial even though stepping generally occurred during and shortly after the stimulation. The computation involved the following steps: the original sampling rate of 50 Hz was reduced to an effective sampling rate of 5 Hz by averaging every 10 samples. The typical frequencies of the flexion–extension cycles of stepping are less than 5 Hz and to minimize the extraneous higher frequencies related to acceleration and foot contacts with the wheel, the records were low-pass filtered by twice applying a four-sample moving average, correcting for lag each time. The records of the left leg were inverted and summed with the records of the right, a fast Fourier transform was computed, the root-mean-square of the spectrogram was multiplied by the square root of the peak frequency. The resulting SI had a wide dynamic range that was monotonic with respect to the latency, amplitude and frequency of stepping. Because the SI used the differential accelerometer values of the right and left hindlimbs, it was optimized for alternating gaits; galloping gaits (in which both hindlimbs flex and extend together) were avoided by using moderate current levels.

Recording of hippocampal activity

The recording electrode was lowered into the hippocampus in 0.1 mm steps to a depth at which a tail pinch elicited a clear theta pattern. After amplification at a gain of 1000, with a band pass of 1–500 Hz, the activity was sampled at 500 Hz. The original sampling frequency was reduced to an effective sampling frequency of 50 Hz by averaging every 10 samples, and fast Fourier transforms were computed using Hanning windowing for 5.12 s (256-sample) epochs continuously throughout each trial. Because of limitations in the sequencer control equipment, the interval between the end of one trial and the start of the next was 62 s, and therefore with a trial duration of 61.44 s, there was a gap of 0.56 s during which data were not recorded. Otherwise, the 12 epochs accounted exhaustively for the time from 10.24 s before the onset of one control trial to the next. The spectral analysis of the power values was characterized by power values at seven frequency bands. The mean values for five consecutive frequency intervals were used to characterize the power values at seven frequency bands. Expressed in terms of lower and upper limits, they were 1–2, 2–3, 3–4, 4–5, 5–6, 6–7 and 7–8 Hz. For the power values of these 1-Hz bands, the lower limit of the band is used to identify the band. To simplify the presentation of results, the 1-Hz band power values were summed for 1 and 2 Hz, for 3, 4 and 5 Hz, and for 6 and 7 Hz. The wider bands are identified by their upper and lower limits: 1–3 Hz, 3–6 Hz and 6–8 Hz. In addition, the peak frequency, defined as the frequency with maximum power, was determined for each epoch with a resolution of 0.2 Hz.

Procedure

After the hippocampal electrode was fixed in place, a stimulation electrode was placed in the hypothalamus to find sites that would elicit theta activity that persist until the subsequent stimulation train. Like the increases in theta produced by GABA injections into the raphe region, the average increase in theta activity produced by priming stimulation was found to be not correlated with the average increase in stepping. It appears that hippocampal theta is increased under conditions in which locomotor initiation is facilitated, but the strength of the association remains uncertain. One possibility is that the use of average levels of theta and stepping for the correlation analysis was insensitive because of variation in average stepping levels produced at different stimulation sites. Alternatively, the lack of correlation between theta activity and locomotor initiation could indicate that the two phenomena were not actually related.
reliable stepping. The minimal current that would produce reliable stepping was selected and held constant for the testing sequence. A typical testing sequence involved approximately 70 trials, each of 62 s in duration. In a standard trial, a single control train was presented. In a test trial, a second train (the test train) was presented at 15, 26, 31 or 36 s (on epochs 3, 5, 6 or 7) after the onset of the control train. Typically, every fourth trial was a test trial. In an ideal sequence, an ascending/descending series was presented twice to yield four tests at each control/test (C/T) interval. On the trial immediately after a test trial, stepping on the control train was usually enhanced (the priming effect of the previous test train), so this trial was not used as a standard trial. When sites showed variability in the stepping, additional standard trials were given between test trials. In cases of variable stepping to the test train, additional test trials were given. For some sites, the entire sequence could not be completed because of threshold changes. In two experiments, an additional site was tested by moving the stimulation electrode to a more ventral location (at least 0.4 mm), and in two experiments an additional site was tested in a different electrode track. The electrodes were fixed at the deepest point in the tracks.

**Results**

The results were based on 15 stimulation sites in 10 rats. They ranged from the perifornical area (PEF) to the ventral tegmental area (VTA). Eleven of the 15 sites supported bilateral hindlimb stepping, and the others supported stepping that was primarily by the contralateral hindlimb. In agreement with earlier study, most sites showed positive priming effects at the shortest Control/Test interval. However, the use of multiple C/T intervals revealed variability in the time course of the priming effects. The magnitudes of Test stepping expressed as a percentage of the Control stepping for the different sites are shown in Fig. 1 along with the locations of the stimulation sites.

Of the eight sites that showed positive priming, three showed a clear linear decline (Group A), and five showed no decline within the tested range (Group B). Three sites classed as Group C, Inconsistent Priming, were characterized by occasionally increased but variable Test stepping. Of the sites that showed negative priming at one or more C/T intervals, Group D, two showed increased Test stepping at the 15-s C/T interval, and two showed priming at later C/T intervals. There were no obvious regional patterns related to the different priming patterns. The mean control stepping for the sites that showed consistent positive priming (Groups A and B, mean = 394) and those that did not (Groups C and D, mean = 522) did not differ \( t(13) = 0.51, P > 0.10 \). Nor did the groups differ in current used \( A \text{ and } B, 47 \mu A; C \text{ and } D, 44 \mu A; t(13) = 0.62, P > 0.10 \) or in the cumulative dose of urethane at the time of testing \( A \text{ and } B, 794 \text{ mg/kg}; C \text{ and } D, 843 \text{ mg/kg}; r(13) = 1.34, P > 0.10 \).

Two sites (10A, 10D) also supported post-stimulation stepping episodes that were sufficiently consistent to be tested for a relationship with prior hippocampal activity. Two other sites showed occasional post-stimulation leg movements but they did not show the step-like flexion–extension sequences that characterize stepping. The recording sites are illustrated in Fig. 2. All but one was in close proximity to the hippocampal fissure. The one exception was site 10 in the dorsal granule cell layer.

Figure 3 illustrates sample trials for a Group A site and shows key features of the testing procedure. Two consecutive trials are illustrated. Panel A shows a standard trial in which a single control train was given. The hippocampal trace (Hipp) shows the large amplitude irregular activity that was common in the 10.24 s pre-stimulation period. During locomotor stimulation, the hippocampal trace showed high-frequency rhythmic slow activity. After the train, lower frequency rhythmic activity appeared and was clear for approximately 20 s. After that time, the record became mixed and eventually reverted to the large amplitude irregular activity shown in the pre-stimulation period of the Test trial in panel B. The stepping elicited by the stimulation is shown in the accelerometer records for the hindlimbs. The number above the stimulation marker gives the SI during the stimulation period. Panel B shows the effect of delivering a test train 26 s (Epoch 5) after the onset of the control train. The priming effect on stepping is reflected in the shorter latency and higher amplitude of stepping on the test train. Note that during the C/T interval, the hippocampal record continued to show rhythmic activity.

Figure 4 shows the average time courses of the variables for the site illustrated in Fig. 3. The x-axis corresponds to the time
axis in Fig. 3, but is subdivided into the 5.12 s epochs used for the spectral analysis. The bottom panel shows the priming effect on stepping as assessed for four test trials at each of four C/T intervals. Test stepping was high at 15 and 26 s, and appeared to progressively diminish but at the longest C/T interval of 36 s still exceeded the mean control level. The upper two panels show the power values summed between 3–6 Hz, 1–3 Hz and 6–8 Hz. The third panel shows the peak frequency at each epoch. It is important to note that the values in the three upper panels were derived from only 39 standard trials, and not trials in which a test train was delivered, and not the trials that immediately followed a test trial. Up to the point of the test train, the time courses for the test trials were similar to those of the control trials.

During stimulation, the peak frequency increased to a level greater than 6 Hz. Accordingly, power in the 6–8 Hz band showed a dramatic increase while power in the lower bands decreased. At the offset of stimulation, power in the 3–6 Hz band sharply increased in Epoch 1 and then progressively decreased to pre-stimulation levels by Epoch 9 (46 s after the stimulation). Complementing the decline in 4-Hz power, power in the 1–3 Hz and 6–8 Hz bands gradually recovered to pre-stimulation levels. Peak frequencies showed corresponding changes over the epochs but the pattern is complicated by the co-occurrence of sharp waves and lower frequency activity during epochs that were distant from prior stimulation.

This pattern can be seen in Fig. 3. Sharp waves have frequency components in the 6–8 Hz range and during epochs when they were particularly prominent, the peak frequency was in that range even though the hippocampal pattern was also characterized by 1–2 Hz activity. The large variability around the mean of approximately 4 Hz in Epochs 2 and 1, and in the later epochs thus reflected various trials where the patterns were characterized either by lower frequencies (1–2 Hz), prominent sharp waves (6–8 Hz), or intermediate frequencies (3–6 Hz). Most subjects did not show this degree of prominence of sharp waves, and consequently showed lower pre-Control peak frequencies and a progressive decline back to those levels after the Control train.

Figure 4 indicates that stepping is facilitated during periods of increased power in the 3–6 Hz band and decreases in power in other bands. If these average trends reflect specific relationships between hippocampal EEG and stepping, it should be possible to predict, on a site-by-site and trial-by-trial basis, the magnitude of stepping during the control or test trains by the power levels that are present during the 5.12 s epochs that preceded the stimulation. To assess this possibility, correlation was tested using the SIs on the control and test
epochs and EEG values in the preceding epochs of all trials. Figure 5 shows the scatter plots for the site illustrated in Figs 3 and 4. The different symbols denote the three sources of SIs: the open circles indicate control trains of standard and test trials, the filled triangles indicate test trains, and the filled circles indicate the control trains on the trials immediately following the test trials. Note that the filled symbols have generally higher SIs consistent with the priming effect. For this site, the magnitude of stepping was predicted on a trial-by-trial basis by the power values in the 3–6 Hz band ($r = 0.47$), the 1–3 Hz band ($r = -0.32$) and the 6–8 Hz band ($r = -0.55$). Peak frequency in the preceding epoch did not predict the level of stepping when all 87 cases in the sample were tested for a linear correlation ($r = 0.05$). However, when the 13 cases in which the peak frequency was greater than 6 Hz were excluded from the analysis, peak frequency predicted SI ($r = 0.36$). The excluded values are shown to the right of the vertical dotted line in the scatter plot. Not all sites showed as prominent sharp waves as this case, but to maintain consistency and to avoid the use of curvilinear regression, correlation between SI and peak frequency was determined using the restricted range of 1–6 Hz.

As shown in Table 1, increases in power in the 3–6 Hz band similarly predicted stepping for other Group A sites (9 and 12A) and one also showed a positive correlation with peak frequency ($r = 0.66$). The five sites with positive priming but no clear decline (Group B) were less consistent, but they also showed a general association between power in the 3–6 Hz band and subsequent stepping. Two sites (3 and 11A) showed positive correlation with 3–6 Hz power. Two (8 and 12D) that did not have significant correlation with power summed over 3–6 Hz did have small but significant correlation with power in the 3 Hz band ($r > 0.28$). For the remaining Group B site (10D), peak frequency correlated with subsequent stepping ($r = 0.32$). Interpretation of the data for this site is complicated by the presence of post-stimulation stepping which itself was associated with increases in peak frequency in the theta band (see below). For the three sites that showed inconsistent priming (Group C) and the four sites that showed negative priming (Group D), the direction of the correlation with hippocampal activity was inconsistent and depended on the individual characteristics of the priming pattern. Because power in the 3–6 Hz band peaked at the offset of stimulation and gradually declined, Group D sites (5 and 10B) that showed biphasic or flat trends in Test stepping showed no significant correlation. By contrast, a site (7C) that showed negative priming that was more pronounced as the C/T interval increased, showed a positive correlation, and a site (11b) that showed progressively less negative priming at later intervals, showed a negative correlation.

Hippocampal activity patterns in the 3–6–Hz band were similar for the different locomotor sites. Figure 6, upper panel, compares the mean power values for the Standard trials of the sites that showed positive priming (Groups A and B, filled circles) and the sites that showed either non-significant or negative priming (Groups C and D, open squares). The groups were compared using a two-factor (Groups, Epoch), repeated-measures analysis of variance. The groups did not differ in the power levels of the 3–6 Hz band over the various epochs ($F_{1,13} = 0.63, P > 0.05$). However, as seen in the middle two panels of Fig. 6, the groups did differ in the patterns for the other bands. For the 1–3 Hz band, the two types of sites differed at specific epochs ($F_{1,13} = 2.34, P < 0.05$). Tukey tests showed that the sites with consistent positive priming (Groups A and B) had lower ($P < 0.01$) power levels at epochs 1, 2 and 9, the epochs that immediately preceded control trains. The sites also showed an epoch-specific difference in 6–8 Hz power ($F_{1,13} = 4.88, P < 0.01$), which Tukey tests showed to be due to the positive priming sites showing smaller ($P < 0.01$) increases in 6–8 Hz power during the stimulation train. The two groups did not differ in the peak frequencies ($F_{1,13} = 0.06, P > 0.05$).

Table 1 shows that for six of the sites, power in the 1–3 Hz band was negatively correlated with stepping. Power in the 1–3 Hz band was higher immediately prior to the relatively low control stepping, and was lower prior to the relatively greater Test stepping. The typical pattern is shown in Fig. 3. Multiple regression analyses showed that 1–3 Hz power could provide an independent prediction of stepping beyond that provided by 3–6 Hz power. In two cases (9 and 11A), 1–3 Hz power provided additional predictive information, and in one case (10D), 1–3 Hz power correlated with subsequent stepping when 3–6 Hz power did not. For five sites, 6–8 Hz power correlated negatively with subsequent stepping, but these relations are complicated. As described in reference to the case shown in Fig. 3, power in the 6–8 Hz band, in addition to being sensitive to rhythmic activity, reflected the occurrence of sharp waves that are associated with 1–3 Hz activity. The negative correlation of 6–8 Hz power with...
subsequent stepping jointly reflected the occurrence of sharp waves during the initial and terminal periods of the trial, and the decreased synchronous 6–8 Hz activity during the C/T intervals. Only in the case of site 6 did 6–8 Hz power provide additional predictive information over that provided by 3–6 Hz power.

As a final approach to the question of the relationship between theta and facilitated stepping, the unanticipated cases of post-stimulation stepping were examined. This post hoc analysis determined whether the increases in the frequency of hippocampal activity preceded the onset of the post-stimulation stepping. Two sites (10A, 10D) showed post-stimulation stepping on a sufficient number of trials for analysis. All trials were used in which at least one step cycle of both hindlimbs occurred more than 10 s after the stimulation train. The hippocampal record was examined for the peak frequency at four 1.28 s epochs before, and four 1.28 s epochs after the onset of stepping. For both sites, there was a sharp

Fig. 3. Representative standard and test trials for Site 6. The trials are consecutive with durations of 61.44 s and onsets separated by 62 s; each began with a 10.24-s pre-stimulation period. Panel A: on standard trials, a single 5.12-s control train of stimulation was given. Stimulation produced stepping movements of the right and left hindlimbs, which were detected by accelerometers (RHL, LHL). Upward deflections in the accelerometer records indicate flexions of the ankles or hips, and downward deflections indicate extensions. In the hippocampal record (Hipp), positive is upward. Panel B: a test trial included a control train of stimulation and an additional test train at varying Control/Test intervals, 26 s in this case. The numbers above each stimulation marker are the SIs. The shorter latency and increased amplitude of the stepping during the test train reflect the priming effect.
increase in the frequency of hippocampal activity on the epoch that began at the onset of stepping. For one site (10D), the epoch prior to the onset of the post-stimulation stepping showed a small but significant increase in the frequency of hippocampal activity. Figure 7, panel A, shows a representative trial for this case. Panel B shows an expanded view of the stepping episode, and the increase in theta frequency can be seen in the Hipp trace before the onset of the bilateral hindlimb flexion which began the stepping episode (vertical line). Panel C summarizes the means for the 39 trials for this case. A repeated measures analysis of variance showed that peak frequency differed over the 1.28 s epochs ($F_{7,266} = 10.67$, $P < 0.01$). The “−1” bin corresponds to the 1.28 epoch prior to the onset of stepping. A post hoc Fisher’s test showed its mean of 4.9 Hz to significantly ($P < 0.05$) exceed the mean of the −2 bin (4.5 Hz) and the −3 bin (4.4 Hz). It is notable that this site was one with positive priming that did not show a general correlation between 3–6 Hz power and stepping. It is likely that the theta frequency changes associated with the post-stimulation stepping episodes were sufficiently large to obscure the correlation with control and test stepping.

**DISCUSSION**

These findings clarify the factors that determine the correlation between hippocampal theta activity and the facilitation of locomotion by brain stimulation. When stimulation produced an immediate increase in locomotor excitability that declined monotonically, power in the 3–6 Hz band closely paralleled it. In these cases, the relationship was sufficiently robust that power in the 3–6 Hz band could predict, on a trial-by-trial basis, the magnitude of the stepping that would be elicited by a stimulation train presented in the next 5-s period. The correlation was not large but it is important in light of the large number of influences that operate when a complex behavioral pattern measured over a 5-s period is predicted on the basis of hippocampal activity occurring in the prior 5-s period. When the priming effects of locomotor stimulation were inconsistent or negative, the correlation with stepping and theta activity was variable. The size and polarity of correlation in these cases were determined by the test stepping as opposed to the time course of theta. Sites with qualitatively different patterns of priming showed similar patterns of 3–6 Hz power during the period when the priming effects were assessed. Therefore it appears that locomotor stimulation of the hypothalamus produces relatively stereotyped after-effects on hippocampal activity--3–6 Hz power and relatively variable after-effects on locomotion. When the facilitating effects on locomotor initiation are simple, and show an immediate rise and monotonic decline, stepping and 3–6 Hz power correlate positively. When the facilitating effects on locomotor initiation compete with suppressive influences, the correlation is unpredictable. These findings indicate that hippocampal activity in the lower theta frequencies is associated with an increased likelihood of the locomotor initiation. However, the ease with which the association can be obscured makes it clear that the linkage between theta activity and locomotor execution is indirect.

For most subjects, the direct effect of locomotor stimulation...
Fig. 5. Prediction of stepping magnitude during control and test trains by hippocampal slow-wave activity in the preceding 5.12-s epoch for Site 6 of Group A. The plots are based on 87 stimulation trains which include 55 control trains from standard and test trials (open circles), 16 test trains (triangles), and 16 control trains from trials, which followed test trials (filled circles). The dashed line indicates the least squares linear regression line. The correlations for 3–6 Hz power, 1–3 Hz power, and 6–8 Hz power were significant \((P < 0.05)\). The correlation for peak frequency was not significant.

Table 1. Correlation* between hippocampal activity in a 5.12-s epoch and subsequent stepping during control and test stimulation for sites classified according to priming effects

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<th>Site</th>
<th>Control stepping</th>
<th>1–3 Hz</th>
<th>Band ‡ 3–6 Hz</th>
<th>6–8 Hz</th>
<th>Peak frequency</th>
<th>Sample size</th>
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<td></td>
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*Pearson correlation coefficient \((P < 0.05)\), positive correlation in bold print.
†Correlation significant only for 3-Hz band.
‡Lower and upper limit of band.
was an increase in 6–7 Hz activity that was followed by a transient increase in the 4–5 Hz activity. These frequencies are population averages under an 800 mg/kg average dose of urethane, and individual differences were evident. A few sites showed mean frequencies that were 1 Hz higher or lower than the group average while maintaining the same pattern. The higher frequency theta activity produced during the locomotor stimulation appears similar to locomotor-related, atropine-resistant, theta (“Type I”).1,2,7,11 It probably has multiple origins that would include the activation of hypothalamic and septal circuitry that produce theta rhythms,27 circuitry that produces locomotor and other somatic and autonomic motor patterns, as well as the sensory consequences of those motor patterns. The post-stimulation theta activity was in the range of 3–6 Hz. This theta pattern should not be assumed to represent simply the declining aftereffects of the 6–8 Hz theta activity activated during the stimulation train. In only four of 15 cases, the initial level of post-stimulation 3–5 Hz power correlated significantly with the level of 6–8 Hz power during the stimulation train. Post-stimulation theta activity appears to correspond to Type II theta that is relatively resistant to anesthetics.7 According to Bland’s model,1,12 this low-frequency, atropine-sensitive activity “provides the motor system with a ‘readiness’ or priming signal”, and is produced during alert immobility associated with motivational stimuli.16 More information is needed regarding the behavioral correlates of this range of theta activity in the non-anesthetized rat. One possible correlate is the suppression of head scanning that occurs when hypothalamic locomotion is primed in the awake rat.20 It would also be of interest to determine whether the post-stimulation theta patterns correlate with the exercise-related vascular and respiratory patterns that show residual activity following a locomotor bout.28

The hippocampal activity pattern in the immediate post-stimulation period was stereotyped compared to the more variable locomotor response to the test trains. Specifically, the post-stimulation hippocampal response in the 3–6 Hz band was indistinguishable in cases in which stepping was facilitated and when it was suppressed. If the correlations between 3–6 Hz power and stepping represent the general case, it implies that facilitation of locomotor initiation is present but not expressed during negative priming. The anatomical complexity of the medial forebrain bundle10 is consistent with the co-activation by electrical stimulation of multiple and antagonistic effects. In a model consistent with this view, activation of locomotor initiation would produce 3–6 Hz activity through ascending projections to the hypothalamo-septal theta driving system,27 and it would produce locomotion through descending projections to the reticulo-spinal stepping execution circuitry.5 Locomotor suppressive processes would operate directly on the locomotor execution system bypassing the initiation circuitry. Postural and locomotor suppressive circuits originating in the anterior pons and posterior midbrain project to the posterior medullary reticulo-spinal system.5,8,22 Thus hippocampal 3–6 Hz activity would reflect the state of locomotor readiness regardless of concurrent locomotor suppression. In the awake rat, the association of slower frequency theta activity with fear-inducing situations16 may be due to the simultaneous elicitation of behavioral suppression (freezing) along with the increased likelihood of escape locomotion. Only the lower theta frequencies associated with the facilitation of initiation, not the higher frequencies associated with execution, would be manifest unless escape locomotion was triggered. It seems unlikely that hippocampal activity would reflect the level of locomotor readiness as well as its execution, but not also the level of locomotor suppression. Therefore, it would be informative to identify the systems responsible for locomotor suppression, and to determine whether their activation is reflected in specific hippocampal activity.

An unexpected finding was that power in the 1–3 Hz band, and to a lesser degree power in the 6–8 Hz band, could provide an independent prediction of the subsequent stepping magnitude. In contrast to the 3–6 Hz band, the correlation between stepping and the 1–3 Hz and 6–8 Hz bands was negative. Power in the 6–8 Hz band partly reflected the number of sharp waves that occurred during slow-frequency periods. In the unanesthetized rat, sharp waves are associated with sleep, immobility and consummatory behaviors,4 all non-locomotor patterns. In this experiment, this type of hippocampal activity, if it appeared, occurred at the end of the trials in the periods that preceded the low stepping on control trains. Slow-frequency activity and sharp waves were minimal prior to the high levels of stepping on test trains. This is the period when 3–6 Hz power was highest. For the site with the best multivariate correlation, power in the three bands combined to account for 38% of the variation in subsequent stepping. It appears that under urethane, hippocampal activity reflects two locomotor states. In the absence of recent locomotor stimulation, it shows an immobility-type
A pattern characterized by slow activity and sharp waves in which the excitability of locomotor initiation is low. In the presence of recent locomotor stimulation, it shows a behaviorally active pattern characterized by the absence of slow waves and the presence of significant power in the 3–6 Hz band. Power in the 1–3 Hz band, even though it was negatively correlated with stepping, is not a locomotor suppression correlate because it was minimal during periods when test stepping was suppressed for the negative priming sites. Rather, high levels of 1–3 Hz power appear to reflect low levels of locomotor facilitation.

The diversity of the priming patterns was not expected. Prior work with single C/T intervals less than 20 s showed that almost all sites from the preoptic area to the ventral tegmental area had positive effects. The priming effects at the 15-s C/T interval in the present study were similarly positive, but the longer C/T intervals revealed complex influences on stepping that occurred between the immediate post-stimulation facilitation and the return to control levels. Yet unknown factors determine whether stimulation produces relatively simple positive priming effects or more complex mixtures of positive and negative effects. A critical factor is

Fig. 7. Case in which post-stimulation stepping was associated with reliable increases in frequency of hippocampal slow-wave activity. Specimen records follow the format of Fig. 3. (A) Representative standard trial in which stimulation produced stepping during the train which was followed in approximately 25 s by a discrete episode of bilateral hindlimb stepping. (B) Time-expanded trace of the post-stimulation stepping episode shown above. In this case, the episode started with a bilateral flexion of the hindlimbs (upward deflections), which was followed by a sharp extension of the right hindlimb, which produced a multiphasic, initially downward pattern. There was considerable variability in the patterns of different episodes. The first clear extension or flexion of either leg was taken as the start of the episode (indicated by vertical line). Voltage calibration 1.5 mV. (C) Mean and S.E.M. of peak frequency in 1.28-s epochs before and after the stepping onset.
probably anatomical, but it may be subtle. Sites in very similar regions showed qualitatively different priming patterns. The groups of positive and negative sites did not differ in the control SIs, or in the current required for reliable stepping. Stimulation at sites with negative priming effects produced higher levels of 6–8 Hz power. Slawinska and Kasicki\(^1\) found that stimulation in the posterior hypothalamus produced higher frequency theta activity than stimulation of the so-called subthalamic locomotor region, but locomotor speeds were equivalent. They suggested that the higher frequency of the posterior hypothalamic stimulation related to its aversiveness. In aversive states there is both facilitation of locomotion (escape) and locomotor suppression (freezing), properties that may have similarities to negative priming. Although they are difficult to study because the behavior of interest is suppressed, negative priming sites may prove useful for understanding locomotor initiation.

Post-stimulation stepping is rare with hypothalamic stimulation. It provides the opportunity to observe an isolated locomotor episode that is temporally separated from the complex events directly produced by the eliciting electrical stimulation. Supporting the main finding of the study, one of the two cases showed a reliable increase in the frequency of hippocampal activity in the 3–6 Hz band that preceded the onset of stepping. As with negative priming, the factors responsible for post-stimulation stepping are not known, but there is some evidence that the two phenomena are related. Stimulation in the area of the median raphe both suppresses locomotion produced by stimulation of the hypothalamus, and produces post-stimulation stepping as an after-effect.\(^8\) Inactivation of the median raphe increases stepping produced by hypothalamic stimulation,\(^1\) and increases theta activity.\(^2\) Inactivation of the median raphe area also produces isolated bouts of stepping\(^9\) that are similar to post-stimulation stepping in not being directly associated with an obvious eliciting stimulus. Post-stimulation locomotion could represent a rebound effect, in which locomotor initiation is relieved from inhibition exerted by the median raphe system. Whether this type of locomotor inhibition is related to negative priming is not known.

**CONCLUSIONS**

This study delineated the boundary conditions within which hippocampal theta activity in the lower frequency range relates to the level of excitability of locomotor initiation in the anesthetized rat. When locomotor stimulation of the hypothalamus produced a relatively simple after-effect of locomotor facilitation characterized by a progressive decay to baseline levels, prior levels of 3–6 Hz power predicted elicited stepping. When locomotor stimulation produced more complex after-effects, power in the 3–6 Hz band was not consistently related to subsequent stepping. Hippocampal activity in the 3–6 Hz band appears to be specifically related to the facilitation of locomotor initiation but additional factors determine whether that facilitation is reflected in the execution of stepping.

**REFERENCES**


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