Research report

Hippocampal theta activity and facilitated locomotor stepping produced by GABA injections in the midbrain raphe region

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Abstract

Inactivation of neurons in the midbrain raphe region produces increases in locomotor activity, and it appears that they function to suppress locomotion. Inactivation of neurons there also produces hippocampal slow wave (theta) activity and it appears that they also function to inhibit rhythmic activity in the hippocampus. We determined whether the degree of association between the two effects was consistent with the operation of a single mechanism. Stimulation electrodes were implanted into locomotor sites of the hypothalamus of 34 urethane-anesthetized rats. Hindlimb stepping was elicited by 5.12-s trains of perifornical electrical stimulation presented once per minute. Hippocampal theta activity was recorded across the CA1 layer of the dorsal hippocampus. GABA injections were used to locate raphe sites at which neuronal inactivation influenced stepping and hippocampal activity. A glass pipette (80-μm tip) was inserted into the midbrain, and injections of GABA (50–100 mg/0.1–0.2 μl) were made in 70 sites in the midbrain. Injections at 34 sites facilitated stimulation-elicited stepping, and at 17 sites, they also produced intertrial stepping. Facilitating injections, but not ineffective or suppressive injections, increased the mean peak frequency of hippocampal activity, and increased power in the 4–5 Hz band during the period that preceded the stimulation trains, but did not change the 5–6 Hz activity produced during the stimulation trains. Priming locomotor stimulation which also facilitated stepping produced generally similar increases in pre-stimulation peak frequency and 4-Hz power. The magnitudes of the increases in stepping and 4-Hz power were uncorrelated. The increase in 4-Hz power appeared earlier than the increase in stepping in 18 of 34 cases, and later in 11 cases; no increases in 4-Hz power were apparent in five cases. The results indicate that pre-locomotor 4-Hz hippocampal activity in the urethane-anesthetized rat is loosely coupled with facilitated locomotor initiation. Neurons in the midbrain raphe region appear to suppress both processes, but the low degree of association between the magnitudes and onset times of increases in stepping and hippocampal 4-Hz power indicate the operation of multiple mechanisms. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Locomotion; Hippocampal theta activity; Median raphe; GABA

1. Introduction

One of the clearest behavioral correlations with hippocampal rhythmic slow wave activity (theta) is locomotor behavior [27]. Stimulation in sites throughout the hypothalamus elicits hippocampal theta, and locomotion is commonly associated [3,30,31]. This association appears to reflect an important linkage between theta activity and locomotor control. With stimulation of the posterior hypothalamus, the frequency of the elicited theta activity and the speed of locomotion covary as the intensity of stimulation is changed [3]. Locomotion and theta activity elicited by posterior hypothalamic stimulation also show a parallel reduction when the septum is inactivated by procaine injections [11]. The frequency of theta activity progressively increases prior to a jumping response [10,30]. Stimulation of the posterior hypothalamus increases the force and decreases the latency of jumping [3]. Sites throughout the lateral hypothalamus, including the perifornical...
area, also produce locomotion associated with theta activity [31], and hypothalamic sites that support stimulation-elicited stepping show slow wave activity at theta frequencies during locomotion [25].

Locomotor stepping can be elicited in the anesthetized rat by stimulation of the hypothalamus [21], and the use of this preparation offers advantages in the study of locomotor initiation and control. Anesthesia allows repeatable and standardized stepping, and minimizes the uncontrolled sensory consequences of extraneous behaviors. At the high doses typically 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 activity. The frequency of hippocampal activity under anesthesia appears to be lower than the frequency in the unanesthetized condition. One important property of stimulation-elicited locomotion that is preserved under anesthesia is the priming effect [24,26]. 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. The priming paradigm provides a means of repeatedly modulating the excitability of locomotor initiation. To show priming effects, a pair of stimulation trains, Control and Test, separated by ≈10 s are presented on trials that occur at 1-min intervals.

For the region of the median raphe, the relation between locomotion and theta is the converse of the pattern for the hypothalamus. In the awake rat, electrical stimulation of the median raphe suppresses locomotion [5,14]. In the anesthetized rat, stimulation there blocks stepping elicited by prior stimulation of the hypothalamus [23], and produces post-stimulation stepping which suggests a post-inhibitory rebound [20]. Various pharmacological methods that decrease neuronal activity in the median raphe increase locomotor activity [15,19,32,33]. The effects of electrical stimulation in the median raphe on hippocampal theta are complex and controversial [5,14,28] which may reflect the heterogeneity of the region as well as activation of fibers of passage. Pharmacological approaches using agents that presumably decrease the activity of neurons in the median raphe produce hippocampal theta activity [17,29]. Some of these agents also reduce serotonin activity in the hippocampus [19,32], and it has been proposed that serotonergic neurons in the median raphe function to antagonize theta activity [28,29].

The purpose of this study was to determine if activation of the midbrain raphe would increase stepping and theta activity under anesthesia, and if so, to determine the type and degree of association between the two effects. High positive covariance would be consistent with the idea that neurons in the region of the median raphe modulate theta activity and locomotion via a common mechanism. The approach used anesthetized rats that stepped in response to locomotor stimulation of the perifornical hypothalamus and GABA injections to block neuronal activity in the raphe region. GABA injections are useful because their effects are brief, and multiple sites can be tested in a single acute experiment. This method has been used in an earlier study to identify sites in the anterior midbrain required for hypothalamic stepping [22]. Theta activity patterns were compared for GABA injection sites at which stepping was increased, reduced, or unaffected. The use of the priming paradigm allowed the comparison of the effects of GABA injections to another manipulation that also increases stepping and theta activity. The focus was on theta activity that occurred prior to the locomotor stimulation because activity during that time would more clearly relate to locomotor initiation as opposed to locomotor execution.

The specific questions about the increases in stepping and in theta activity produced by GABA injections were: (1) whether increased theta activity was selectively associated with sites at which GABA injections increased stepping; (2) whether the magnitudes of the increased stepping and theta activity correlated; (3) whether the increases in stepping and theta activity had the same time of onset.

2. Material and methods

2.1. Subjects and surgery

Male Sprague Dawley rats (N = 34), weighing an average of 360 g were initially anesthetized with 2% Halothane in oxygen for 5–10 min, then given an initial intraperitoneal injection of 500 mg kg⁻¹ 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, for the drug injection pipette, and for screws which provided a recording ground and a stimulation return. The stimulation electrode was a 1 25-μm diameter Tungsten wire insulated with Teflon except at the tip. The target was the perifornical nucleus of the hypothalamus, 3.6 posterior to Bregma, and 1.2 mm lateral to the midline. An uninsulated wire wrapped around four screws in the skull completed the circuit to the stimulator. The recording electrode was a twisted pair of 125 μm stainless steel wires with a vertical separation at the tip of 1 mm. It was aimed to straddle the CA1 layer of the dorsal hippocampus at 4 mm posterior to Bregma and 3.0 mm lateral to the midline. The appropriate anesthetic level was achieved by additional intraperitoneal
injections of 50–100 mg kg\(^{-1}\) urethane over approximately the first hour of the experiment. Injections were given whenever the rat showed increases in respiration, vibrissae movement, mouth movement, or limb movement in the absence of brain stimulation. When the appropriate urethane level was reached, the rat would maintain an anesthetized state for several hours without a supplemental injection. Almost all testing was performed after the rat had reached this stable level. The mean cumulative dose of urethane administered was 806 mg kg\(^{-1}\) (SEM = 13.2). Body temperature was maintained by means of a heat lamp. Procedures were approved by the Wesleyan University Animal Care and Use Committee.

2.2. Stimulation

Brain stimulation, provided by a constant current stimulator, was composed of biphasic pulses, each phase 0.5 ms, delivered in trains of 5.12 s, with interpulse intervals of 20 ms. The priming paradigm was used. A trial consisted of two trains. The first was designated as the Control train; it was followed in 10.88 s by a second train, the Test. The inter-trial interval was 1-min. Stepping in response to the Test train typically showed a priming effect. For a test at an injection site, the current was fixed (mean 44 \(\mu A\), range 27–60 \(\mu A\)) to produce minimal stepping during the Control train and a priming effect on the Test train.

2.3. Measure of hindlimb stepping

The body of the rat was supported by an acrylic platform attached to the side rails of the stereotaxic apparatus. Cutouts in the platform allowed stepping movements of the limbs. Below the platform was a wheel with a diameter of 30 cm and a surface width of 10 cm. At rest, the rat’s hindlimbs passively extended to make contact with the wheel. 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 (Etran Devices, Model EGAX 10) have a range of \(\pm\) 109, a sensitivity of 8 mV g\(^{-1}\), and follow frequencies > 200 Hz. Each weighed about 0.5 g, and with dimensions of \(< 7 \times 4 \times 4 \text{ mm}\), did not impede stepping. The amplified output of the accelerometers was lead to an analog/digital converter sampling at 1 KHz and displayed on a computer monitor.

To quantify the stepping occurring during stimulation, a customized program computed a stepping index which reflected in a single number the overall amplitude and frequency of stepping by both hindlimbs. The computation involved the following steps: the sampling rate was reduced by averaging to 50 Hz; the records of the left leg were inverted and summed with the records of the right, a Fast Fourier Transform was computed for the 256 samples during stimulation, the root-mean-square of the spectrogram was multiplied by square root of the peak frequency. The resulting index was nonlinear but monotonic with respect to both frequency and amplitude of stepping. An example is shown in Fig. 1.

2.4. Recording of hippocampal activity

The recording electrode was lowered into the hippocampus in 0.1 mm steps to a depth at which locomotor stimulation elicited a clear theta pattern. After amplification at a gain of 1000, with a band pass of 0.1–500 Hz, the activity was sampled at 1 kHz. The sampling frequency was reduced to 50 Hz by averaging and Fast Fourier Transforms were computed using Hanning windowing for 5.12 s (256-sample) epochs around and during the Control and Test stimulation trains. The epochs were termed PreControl, Control, PostControl, PreTest, Test, and PostTest. Fig. 1 shows power values for a representative trial. Because of limitations in the sequencing control equipment, the interval between the end of the Control and start of Test stimulation was constrained at 10.88 s, and therefore there was a gap of 0.64 s between the PostControl and PreTest epochs. Otherwise, the epochs exhaustively accounted for the time from 5.12 s before the start of the Control train to 5.12 s after the offset of the Test stimulation train. The power values at the frequencies of 4–5, 5–6, and 6–7 Hz, and the frequency band with the maximum power were determined for each epoch. The lower limit of the band is used to identify the band.

2.5. Procedure

When the stimulation electrode was fixed in the hypothalamus, the site was stimulated on a 1 trial min\(^{-1}\) schedule that was maintained for the duration of the experiment. GABA (Sigma) was injected after the baseline for stepping had been recorded for at least 7 min. GABA was delivered in saline at a concentration of 250 \(\mu g \text{ ml}^{-1}\). The GABA was injected through a glass pipette (80–100 \(\mu m\) tip diameter) which was connected by PE 20 tubing to a 1-\(\mu l\) syringe. The injection was made at a rate of 0.5 \(\mu l\) min\(^{-1}\). Flow through the system was confirmed by observing the movement of an air bubble in the tubing. For 65% of the sites, the standard volume injected was 0.1 \(\mu l\). For the other sites, to achieve the equivalent bubble movement, the syringe plunger was additionally displaced by a nominal 0.05 \(\mu l\) (18% of sites) or 0.1 \(\mu l\) (16% of sites). If there was no
Fig. 1. A representative trial with the associated measures of hippocampal theta activity. Each trial was composed of a Control phase and a Test phase in which a 5.12 s locomotor stimulation train was applied to the hypothalamus. Each phase was divided into three 5.12 s epochs, before, during and after stimulation. An interval of 16 s separated the onsets of the Control and Test stimulation trains. The middle vertical dashed line indicates a gap of 0.64 s in which no samples were taken. The top trace shows hippocampal slow wave activity sampled at 100 Hz; voltage calibration, 800 mV. Below it are the accelerometer records for the right and left hindlimbs (RHL, LHL); flexion produced an upward deflection and extension produced a downward deflection. The next three traces represent power of hippocampal activity during the six epochs. Calibrations: 123 µV^-2 for the 4 and 5-Hz bands, and 246 µV^-2 for the 6-Hz band. The lowest trace shows the Stepping Index computed for each epoch. The arrows indicate the 4 Hz power in the PreControl and PreTest epochs which are the principal focus of the analysis. Note that the enhanced stepping during the Test train was preceded by an increase in PreTest 4 Hz power.

Fig. 1 shows hippocampal activity and stepping activity in a typical baseline trial and provides an overview of the measures. The time base shows the six epochs (each 5.12 s) that were used in the spectral analysis and markers for the Control and Test stimulation trains. With an intertrial interval of 1 min, and a Control-Test interval of 16 s, the priming effect on stepping occurred on most trials. It is seen in the accelerometer tracings for the right and left hindlimbs (RHL, LHL) as a faster onset and greater amplitude of stepping during the Test train, and in the higher stepping index value for the Test epoch in the bottom trace. The upper trace shows the hippocampal activity from which the power values in the 4, 5 and 6-Hz bands are graphed in the lower part of Fig. 1. During the baseline before GABA injections, the PreControl period was dominated by large amplitude irregular activity. Control stimulation sharply increased power in the 6-Hz band and slightly decreased power in the 4-Hz band. In the PostControl period, 6-Hz activity returned to its baseline level, but 4-Hz power increased. The post-stimulation increase in 4-Hz power carried over into the PreTest period, such that its level consistently exceeded the PreControl level (arrows). The Test train produced a pattern of hippocampal activity that was similar to that produced by the Control train. This example suggests that increased 4-Hz power in the behaviorally inactive period between the trains predicted the facilitated stepping seen on the Test train. In the analyses that follow, the principal focus is how GABA injections that facilitated stepping like priming stimulation also affected 4-Hz power during the periods that preceded the two stimulation trains.

2.6. Histology

At the end of the experiment, the rat was given a lethal dose of Nembutal and perfused through the heart with 10% Formalin. The fixed brain was sectioned transversely every 100 µm with a vibratome. Unstained sections were viewed with a microscope at 40 × magnification. Stimulation, recording and injection sites were localized with reference to the atlas of Paxinos and Watson [13].

3. Results

3.1. The parameters studied

Injections sites were classified as Step-Increased, Step-Decreased, or No-Effect. An increase or decrease in the stepping index was declared when the values differed from the mean pre-injection baseline by three standard errors on four consecutive trials, the first of which had to occur within 5 min of the injection. Recovery was declared when the values did not exceed the criterion for two consecutive trials.

Fig. 2. Representative trials from a case of increased stepping produced by an injection of GABA into the raphe region. Each of three trials shows tracings of hippocampal activity (voltage calibration, 800 μV) and stepping during the Control and Test phases. Trials are shown at 2 min before, 2 min after and 19 min after an injection of GABA. Same general format as Fig. 1. Note the increased stepping that occurred during the stimulation trains and the additional stepping that appeared during the PreControl and PostControl periods on the +2 min trial.

3.2. Increased stepping and theta produced by GABA injections

At 34 sites, GABA injections increased stepping. The effects were often large: the median increases in stepping between 2 and 7 min post injection were 540% for the Control trains and 343% for the Test trains. Fig. 2 shows an example of a stepping increase and the associated effects on hippocampal activity. Trials are shown at 2 min before, 2 min after and 19 min after an injection of GABA. In the +2-min trial, the injection produced an increase in stepping during both trains. The latency to increased stepping for the 34 sites ranged from 1 to 5 min with a median of 1 min. In Fig. 2, stepping returned to baseline levels at 19 min after injection. For the group, there was wide variation in the durations of the facilitation. For half of the sites, stepping returned to baseline levels in <20 min, but the rest had longer durations, and in nine cases, stepping had not returned to baseline levels after 50 min when testing ended.

In the case shown in Fig. 2, the GABA injection also produced brief episodes of stepping during some intertrial and inter-train periods. Intertrial stepping occurred in 17 of 34 cases in which GABA injections increased stepping during the trains, and it never occurred in the absence of injections. Intertrial stepping also occurred in 2 of the 14 cases in which stepping during stimulation trains was depressed. Intertrial stepping episodes generally occurred in the first 5 min of the GABA effect.

As seen in the +2-min trial after the injection, hippocampal activity was characterized by rhythmic activity in the PreControl period when GABA increased stepping. The increases in PreControl hippocampal rhythmic activity did not require the occurrence of intertrial-stepping episodes, which were brief and sporadic. By 19 min after injection, hippocampal activity and stepping returned to baseline patterns.

At 22 sites, GABA injections produced no significant effects on stepping. At 14 sites, GABA injections blocked stepping. The depressions resembled the effects described in an earlier study of GABA injections into the anterior midbrain [22]. The depressions were characterized by a complete block of stepping occurring at a latency of 1–3 min (median = 1 min). The durations of the blocks ranged widely with most under 20 min but some lasting over 50 min. The recovery was more gradual than the onset, usually appearing first as a return of stepping on the test train.

3.3. Injection, stimulation and recording sites

Fig. 3 illustrates the locations of the injection sites. Sites in the Step-Increased group were generally, but not exclusively, located in and around the median (MnR) and paramedian (PMR) raphe [13]. The densest collection of facilitating injections were at anterior–posterior level 7.8 mm. Most of the sites at which GABA produced intertrial stepping were located within 0.5 mm of the midline. The sites in the Step-Depressed group were mostly located dorsal to the facilitating sites, scattered around the superior cerebellar peduncle (scp). Sites in the No-Effect group at which GABA produced no detectable effect on stepping were scattered.

The locomotor stimulation sites were in the perifornical hypothalamus at anterior posterior levels between 3.3 and 4.1 mm posterior to Bregma; they were similar to the sites used in previous studies [8,22,24]. The hippocampal recording sites were in the dorsal hippocampus between 3.8 and 4.8 mm posterior to Bregma. Typically, the deep pole of the electrode was close to the hippocampal sulcus and the superficial pole was near the CA1 layer.
3.4. Analysis of theta patterns in the three GABA groups

The theta patterns for the three groups of sites, Step-Increased, Step-Depressed and No-Effect, were examined for relations to the effects on stepping. The increases in the maximum frequency of hippocampal activity produced by the stimulation trains are shown in Fig. 4. This value is the frequency band that had the maximal power for each epoch. For each group, we compared the means of the pre-injection baseline trials to the means of post-injection trials 2–7 when the effects of GABA on stepping were most pronounced. A repeated measures analysis of variance for two factors (Epoch, Injection Condition) was followed by post hoc t-tests using the Fisher procedure. In the pre-injection baseline period, the three groups of sites showed similar patterns of maximum frequencies in the six epochs. The low frequency (2–3 Hz) in the PreControl epochs increased to 5–6 Hz during the Control stimulation and remained higher than PreControl levels in all subsequent epochs (P < 0.01). Note in particular that the 3–4 Hz maximum frequency in the PreTest epoch (arrowheads) distinguished this epoch from the Pre-Control epoch. The maximum frequencies during the Control and Test trains were 5–6 Hz and did not differ. GABA injections changed these patterns only slightly. For the Step-Increased group (upper panel), the maximum frequency in the PreControl epoch increased from a mean of 2.7 Hz in the pre-injection baseline to a mean of 3.0 Hz after the GABA injection (P < 0.01). The
GABA injections did not change the maximum frequency in any other epoch in any of the three groups. At least with the 1-band width resolution used, the maximum frequency measure did not strongly correlate with the effects on GABA injections on stepping. We focused therefore on the power values in specific frequency bands to increase the sensitivity of the analysis.

For the power analysis, we examined the two epochs that preceded the stimulation trains, the PreControl and the PreTest. The pre-stimulation epochs were more relevant to initiation processes, and as shown above, GABA injections increased the maximum frequency during the PreControl epoch in the Step-Increased group, and the priming stimulation increased the maximum frequency during the PreTest epoch for all groups. Each group was analyzed separately using a repeated measures analysis of variance for three factors (PreControl versus PreTest, Baseline versus GABA, and Band) that was followed by post hoc tests. The main finding was that GABA injections produced small but significant increases in 4-Hz power during the PreControl and PreTest periods for the Step-Increased group but not for the other groups. The results are summarized in Fig. 5. The two traces in each panel represent 4- and 6-Hz activity. The 5-Hz activity is not represented because it showed fewer differences, and they were similar to the 4-Hz effects. The left side of each panel shows theta activity during the PreControl epoch before and after the GABA injection. The right side shows theta activity during the PreTest epoch, before and after the GABA injection. The arrows indicate significant GABA effects. For the Step-Increased group, GABA injections enhanced 4-Hz power by a median of 37% during the PreControl epoch and by a median of 5% during the PreTest epoch ($P < 0.01$). GABA injections also decreased 6-Hz activity in the PreControl ($P < 0.01$) but not the PreTest period. GABA injections that produced no effects on stepping did not affect theta activity. In the Step-Decreased group, GABA injections decreased 4-Hz power during the PreTest epoch ($P < 0.05$).

It was expected that the magnitude of effects of the GABA injections on 4-Hz power and on stepping would correlate since they both increased during the same time periods. However, this was not the case. Table 1 shows that the magnitude of the principal effect on GABA injections which was to increase 4-Hz power in the PreControl epoch failed to significantly correlate with the GABA-induced increases in stepping during either the Control train ($r = 0.08$) or during the Test train ($r = 0.15$). Normalizing the data by expressing them in terms of percentage of baseline did not increase the levels of the correlations. This paradoxical lack of association is attributable to the variability in both the power and stepping measures, combined with the relatively small effects of the GABA injections on 4-Hz power. GABA injections, at Step-Increased sites, increased Control stepping by 540%, and Test stepping by 343%, but increased 4-Hz power in the PreControl epoch by 37% and in PreTest epoch by 5%. By contrast, priming stimulation had effects that were more similar in magnitude; it increased Test stepping by 116% and increased PreTest 4-Hz power by 89%.

3.5. Time course of GABA effects on stepping and theta

In another approach to the question of the relation between the increases in PreControl 4 Hz power and stepping, we examined the times of onset for the two effects. Fig. 6 shows 4-Hz power in the PreControl epoch relative to the time of onset of the first effect on stepping. At Trial 0 (arrow) changes in theta activity in the PreControl period occurred before the start of stimulation and therefore preceded effects on stepping. There was a significant increase in 4-Hz power for the Step-Increased group on this trial relative to the three trials prior to the injection.

![Fig. 5. Effects of GABA injections on Hippocampal activity during the PreControl and PreTest epochs for sites with different effects on stepping. The power scale is constant for all panels. The baseline values are the means of all trials prior to the injection. The GABA values are the means of trials 2–7 after the injection. Where no standard errors are shown, they are smaller than the data symbol. The arrows indicate significant effects of GABA injections relative to the corresponding baseline value.](image-url)
Table 1
PreControl 4-Hz power and stepping to control and test trains and their relation during pre-injection baseline and after GABA injection*

<table>
<thead>
<tr>
<th></th>
<th>PreControl epoch</th>
<th>Stepping Index during Control Train</th>
<th>Stepping Index during Test Train</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Hz power (mV²)</td>
<td>Base-line</td>
<td>GABA Diff</td>
<td>Base-line</td>
</tr>
<tr>
<td>Mean (SEM)</td>
<td>4993 (726)</td>
<td>6390 (804)</td>
<td>1396 (657)</td>
</tr>
<tr>
<td>Correlation with 4-Hz</td>
<td>r = 0.04</td>
<td>r = 0.04</td>
<td>r = 0.08</td>
</tr>
<tr>
<td>power</td>
<td></td>
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</tbody>
</table>

* Data from the Step-Increased group (n = 34). Note: correlations are between stepping index values in the Control and Test train epochs and their corresponding 4-Hz power values in the PreControl Epoch; P > 0.05 in all cases.

preceding trials (Wilcoxon test, P < 0.05). There was considerable variability in the group in that only 18 of 34 sites showed increases above baseline on this trial. In the subsequent trial, after the first effects on stepping had appeared, the increases in 4-Hz power became more common, appearing in seven additional sites. Even with this most sensitive, site-specific analysis, five sites did not show any increase in PreControl 4-Hz power within three trials of the increase in stepping. For the group in which GABA injections depressed stepping, small decreases in PreControl 4-Hz power were detected. They appeared on Trial 1 (P < 0.05), which is the trial after the first stepping decrease.

4. Discussion

Injections of GABA into the midbrain raphe region increased stepping produced by locomotor stimulation of the perifornical hypothalamus. The injections also increased the amount of hippocampal 4-Hz power that appeared prior to stimulation. The large increases in 5–6 Hz activity that appeared during the locomotor stimulation were unaffected by the GABA injections. The GABA effects were similar to increases in stepping and pre-stimulation 4-Hz power produced by priming locomotor stimulation. The findings indicate that hippocampal 4-Hz power in the urethane-anesthetized rat is generally associated with the facilitation of locomotor initiation. They further indicate that neurons in the midbrain raphe region suppress both hippocampal 4-Hz power and locomotion. The possibility that suppression of the two processes is produced by a single mechanism is questioned by several disassociations between the effects on stepping and theta. The disassociations are consistent with evidence that separate elements in the median raphe region modulate stepping independent of effects on hippocampal activity.

The similarity of effects of GABA injections in the midbrain raphe region and of priming stimulation provide evidence for a link between 4-Hz theta and the initiation of stepping. When a GABA injection increased stepping, like priming, it also increased pre-stimulation 4-Hz power. When a GABA injection decreased stepping, it also attenuated the rise in 4-Hz power produced by priming stimulation. The increase in 4-Hz power did not appear to reflect simply a consequence of prior activation of stepping, i.e. feedback or reafference. For half of the sites, increases in 4-Hz power appeared earlier than the first increase in stepping. Intertrial stepping was associated with theta increases, but the episodes rarely occurred in the Pre-Control period sampled in the theta analysis. Moreover, increases in PreControl theta appeared equally often with injections that increased stimulation stepping but not intertrial stepping. However, it is possible that the 4-Hz power increases reflected feedback from unmeasured motor patterns, such as respiration or vibrissae movements. In some cases, early increases in respiration preceded the first increase in stepping, but...
the observations were not systematic. In any case, the increases in 4-Hz power produced by GABA injections occurred in pre-stimulation periods when the rat was inactive, and therefore did not directly relate to execution of stepping.

Bland’s [2,11] model of the functional correlates of theta activity can incorporate the present findings to the extent that processes operating in the unanesthetized rat are similar to those in the stepping rat under urethane. According to this model, low frequency, atropine-sensitive (‘Type II’) theta activity is produced during alert immobility associated with certain motivational stimuli [16] and it ‘provides the motor system with a ‘readiness’ or priming signal’ [11]. There is some direct support for an association between Type II theta and the preparation for locomotion. Type II theta is not present during the immobility that follows inescapable footshock, but it does occur if an escape response is possible [1]. In the present study, the increases in theta activity were in the 4–5 Hz band which appears to correspond to Type II theta for two reasons. First, it occurred during the prestimulation period when there was no stepping and second, the rats were tested under urethane, and Type II theta is relatively resistant to anesthetics [7]. According to the model, once locomotion is triggered, a higher frequency theta pattern appears which reflects the activation and consequences of the locomotor pattern itself. The 6-Hz activity that was elicited during locomotor stimulation here appears to correspond to this locomotor-related, atropine-resistant, theta (‘Type 1’). It is abolished by anesthetics [7] at doses that block the behavior, but apparently not at lower doses sufficient to maintain a surgical level of anesthesia, while permitting the elicitation of locomotor behavior. Type II theta in the awake rat is differentially sensitive to muscarinic antagonists [11]. If the correspondence is valid, then they should block the increases in 4-Hz power produced by priming stimulation and by GABA injections in the raphe, but leave relatively unaffected the higher frequency theta elicited during locomotor stimulation.

The association between pre-stimulation 4-Hz power and the facilitation of stepping fits with evidence for an involvement of the hippocampus in motor function (see Section 1). The present findings also showed a number of points of dissociation between hippocampal theta activity and stepping. The magnitudes of increases in 4-Hz power and stepping did not correlate for either priming stimulation or facilitating GABA injections. Priming stimulation produced relatively large effects on 4-Hz power and moderate increases in stepping, but GABA injections produced relatively small increases in 4-Hz power and large increases in stepping. For half of the sites, the increase in theta appeared earlier than the increase in stimulation-elicited stepping, but for others it appeared later, and for a few sites, no increase in 4-Hz was apparent. It appears that hippocampal theta particularly in the 4–5 Hz band is associated with facilitated locomotor initiation under anesthesia, but the coupling is loose.

Multiple factors could contribute to the uncoupling of 4-Hz power and stepping. One is that 4-Hz activity is measured in the pre-stimulation period and the different conditions may be operating when stepping occurs. Another is that hippocampal activity also correlates with nonlocomotor activity, such as bar-pressing [4,18] and sniffing [6,9]. Manipulations such as electrical stimulation and drug injections do not selectively activate only locomotor systems, and extraneous motor systems would contribute to the variability. The loose coupling between 4-Hz activity and stepping is inconsistent with a direct elicitation of motor activity by rhythmically active elements of the hippocampus. It is consistent with a hippocampal modulation of locomotion and other behaviors when complex processing of information is required [12].

A previous study [22] and ongoing mapping work in this laboratory indicate that locomotor blocking effects are the typical effects of GABA injections throughout the upper and lower brainstem. The sites in the present study at which injections increased stepping therefore are distinct. Their general location in the region of the median raphe is consistent with an action of GABA on the neurons in this nucleus. There appears to be no specific information available about the amount of effective spread of GABA at the volumes and concentrations used, but based on a prior study [22], spread of 0.5–1.0 mm is a conservative estimate. Injections on the midline would therefore spread beyond the median raphe neurons. GABA injections in the paramedian raphe also increased stepping and that is a possible site of action. Injections near the midline were likely to produce intertrial stepping in addition to increasing stepping during stimulation. Intertrial stepping could reflect a qualitatively different effect that is specifically associated with the median raphe. Alternatively, midline injections also could spread to suppress paramedian cells on both sides to produce a quantitatively greater facilitation of stepping that is reflected in the occurrence of intertrial stepping.

Inhibiting serotonergic cells in the median raphe may be sufficient to both increase stepping and theta activity, but the pattern of dissociations combined with other evidence make this unlikely to be the sole mechanism. The median raphe contains both serotonergic and nonserotonergic neurons and both types appear to function in locomotor suppression [15,19,32,33]. The increased locomotion produced by a GABA<sub>A</sub> agonist injected into the median raphe is blocked by depletion of forebrain serotonin [15]. Antagonists of excitatory amino acids injected into the median raphe increase locomotor activity while producing correlated decreases
in serotonin activity in the hippocampus [32]. The increased locomotion produced by a GABA<sub>B</sub> agonist injected into the median raphe was not affected by block of serotonin synthesis, and was not associated with a change in hippocampal serotonin [33]. The model of Shim et al. [19] appears to account for these findings and the present results. Adapting this model, it seems likely that the disinhibition of stepping by GABA injections into the median raphe reflected primarily an action on non-serotonergic neurons. On the other hand, the increase in theta activity may primarily reflect a GABA action on the serotonergic cells with rostral projections. An action on two or more types of locomotor suppression neurons combined with a action on hippocampal desynchronizing neurons is consistent with the large increases on stepping, the relatively weak effects on theta, and the low correlation between the two effects.

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References

