Priming of Locomotor Initiation by Electrical Stimulation in the Hypothalamus and Preoptic Region in the Anesthetized Rat

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Received 7 April 1994

TRESCH, M. C., C. L. MILLER, AND H. M. SINNAMON. Priming of locomotor initiation by electrical stimulation in the hypothalamus and preoptic region in the anesthetized rat. PHYSIOL BEHAV 57(4) 641–648, 1995.—Electrical stimulation at a locomotor site can prime (i.e., shorten the latency to initiate) stepping elicited by subsequent stimulation of the same or a different site. We tested for the priming effect in representative sites along the medial forebrain bundle, and determined if its magnitude showed regional differences. Rats (n = 20) were anesthetized with Nembutal and held in a stereotaxic apparatus over a wheel. Stepping was detected by accelerometers attached to the hindlimbs. Priming and test trains of stimulation (0.5-ms cathodal pulses, 50 Hz, 25–75 μA, 7–9-s train duration) separated by 20 s were delivered every 90 s. When the priming and test stimulations were applied to the same site, the priming effects were similar along the entire extent of the medial forebrain bundle. When the priming and test sites were different, the priming effect depended on their relative positions. Anterior stimulation primed posterior sites at magnitudes comparable to those produced by stimulating the same posterior site. Posterior stimulation primed anterior sites at a level half of that produced by stimulation of the same anterior site. This pattern was found for priming and test sites that were ipsilateral and contralateral. Priming is a general and robust phenomenon with properties that may be useful for studying locomotor initiation pathways.

Method

Subjects and Surgery

Male Sprague–Dawley rats (n = 20), weighing between 260 and 550 g (median 390 g) were initially anesthetized with 2% halothane in oxygen, and then injected intraperitoneally with 25 mg/kg of Nembutal. The rat was placed in a stereotaxic apparatus

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and four holes were drilled in the skull to receive stainless steel screws. An uninsulated wire was wrapped around the screws to provide the return line to the stimulator. Additional holes were drilled to receive the stimulation electrodes. Supplemental anesthesia was provided by intraperitoneal injections of Nembutal of 3.5 mg/kg, typically at 25–30-min intervals. Nembutal injections were given as needed whenever the rat showed increased respiration or limb movements in the absence of stimulation. Lidocaine injections were made into the incision every 2.5–3 h. Rectal temperature was maintained throughout the experiment between 37.0 and 38.0°C by means of a heat lamp.

**Apparatus**

The rat was held in the stereotaxic apparatus and suspended over a wheel with a diameter of 30 cm and a surface width of 10 cm. The head of the rat was held by earbars and a bitebar; the thorax, abdomen, and pelvis were supported by a platform. When the rat was at rest, the hindlimbs hung in passive extension, usually with their anterior aspects in contact with the surface of the wheel. The forelimbs also extended with the flexed digits touching the wheel.

Brain stimulation was provided by a constant-current stimulator that delivered 0.5-mA cathodal pulses at 50 Hz. Stimulation was delivered through 125-μm diameter tungsten wires insulated with Teflon except at the tip. The leads connecting the electrodes to the stimulator were unshielded.

The train lengths were equal for sites tested together; it was set for a duration that was sufficient to reliably produce locomotion for the least effective site. Between subjects the durations ranged from 5–10 s; for a typical subject the duration varied from 7 to 9 s over the course of testing. Currents ranged between 25 and 75 μA. It was usually necessary to increase the current over the course of the test; for the typical subject the increase was 10 μA. Most sites were tested with currents between 40 and 50 μA.

When stimulation was applied to one electrode, the other electrode was disconnected from the circuit.

**Measure of Locomotor Stepping**

Stepping latency was determined from the output of a pair of accelerometers, one attached to the dorsum of each metatarsus. The accelerometers (Etran Devices, Model EGAX 10) had a range of ±10 g, a sensitivity of 8 mV/g, and could follow frequencies greater than 200 Hz. Each weighed about 0.5 g and, with dimensions of less than $7 \times 4 \times 4$ mm, appeared to present no impediment to the stepping behavior. They were secured to the limb with masking tape. The differential signals of the accelerometers were led to a standard transducer amplifier that in turn was led to an analog/digital converter sampling at 100 Hz and displayed on a computer monitor. Displayed along with the accelerometer traces was a stimulus marker and a manually operated stepping onset marker. This redundant marker helped to locate the first step on the accelerometer record in the rare ambiguous cases. The latency of the first extension was measured using a cursor to an accuracy of 0.1 s.

The first step in a locomotor sequence was determined in accord with classifications that have become standardized over the course of a series of studies (22). The standard requirement for a hindlimb movement elicited by stimulation to be considered a locomotor step was that it had to involve a flexion–extension sequence that was of sufficient force to rotate the wheel. The requirement that the extension produce wheel movement provides an objective basis for declaring the occurrence of stepping and avoids the difficulty of classifying small tremors or flexion–extension sequences that can precede the bout of stepping.

**Procedure**

In the preliminary phase of the experiment, electrodes were placed into two or more locomotor regions in the preoptic area, hypothalamus, or midbrain. When sites were found that supported locomotion with trains of less than 10-s duration and currents of 50 μA or less, the electrodes were secured to the skull with dental cement. Most rats received two electrodes, two received three, one received four, and one received five.

Figure 1 schematically illustrates the testing procedures. The sequencing of the stimulations differed for the cases when the priming and test stimulation was applied to the same site and to different sites, but they had the following common characteristics. Pairs of stimulation trains were presented with 90 s between the onsets of the first members of the pairs. A 20-s interval separated the onsets of the members of a pair. The 90-s intertrial interval and the 20-s priming interval were invariant for every subject. The train duration could change during the course of a test series, but data based on different train lengths were not combined. A generally successful attempt was made to maintain the presentation of trials without interruption to allow any long-term priming effects to stabilize.

In the condition in which the same site was stimulated twice in a trial, two sites (A and B) were tested in a sequence with the form of AA, BB, AA, . . . , BB. The latency of stepping to the first train in the pair provided the control (unprimed) value and the latency to the second provided the test (primed) value. The

![FIG. 1. Schematic representation of electrode arrangements and procedures. (A) Four possible positions for electrodes (most subjects had two). Pairs of stimulation trains were applied to the same site (same-site condition) and to separate sites (different-site condition). In the different-site condition, priming stimulation could be applied to any of the three other locations. We determined whether there was a difference in the capacity of stimulation to produce priming effects depending on whether the sites were contralateral or ipsilateral to each other or whether they were posterior or anterior to each other. (B) Illustration of the testing procedures. The onsets of the two trains were separated by 20 s. Pairs of stimulation trains occurred every 90 s. In the different-site condition, the order of the stimulation sites alternated between pairs of stimulation. A priming effect occurred when the first stimulation train reduced the latency to initiate locomotion to the second stimulation train.](image)
The priming effect was defined as the difference between the control and test latencies. The reliability of the priming effect was determined for each site by a one-tailed t-test for correlated observations with $p < 0.05$. If the rat did not locomote to the control stimulation, the trial was not used. The mean number of trials in this condition was 13 (SD 6.1). In general, the less consistent the priming effect appeared to be, the more trials were given. The same-site condition was tested first and the same pair of electrodes was tested in the condition in which the priming and test trains were applied to different sites.

In the different-site condition, each site alternately acted as the priming and test site. The sequence was AB, BA, AB, . . . BA. The second site in each pair was the test site, and to determine if it showed priming, its test latency was compared to its latencies in the prior and subsequent trials in which that site was stimulated first. If both values were available, the mean was used; if only one was available, then it was used alone. If the rat failed to locomote to the adjacent control stimulations, then the test latency was not used. The mean number of trials in this condition was 19 (SD = 8.3).

The level of the maintenance anesthesia affects the latency of locomotor initiation. Stepping latencies are longer early in the Nembutal cycle, and they become progressively shorter as the anesthetic level wanes. Locomotion at some sites is sensitive to the depressant effects to the point of being blocked for 5–10 min after the injection of Nembutal (27,28). Because we did not know if the priming effect would depend on anesthetic levels, we adapted the test procedure to assure that each site was tested throughout the Nembutal cycle. In general, a test series began with a Nembutal injection and continued at least until the next injection. In the same-site condition, a sufficient number of trials was usually obtained in one Nembutal cycle. The median duration of a same-site test condition was 24 min. In the different-site condition, which did not produce as consistent priming effects, the test series spanned multiple Nembutal cycles. The median duration of these test series was 44 min.

The duration of the testing procedure depended on the number of electrodes, on the consistency of the elicited locomotion at a particular test site, and on the consistency of the priming effect (smaller effects required more trials to access reliability). For rats with two electrodes ($n = 15$), the procedure required a median duration of 164 min (range 39–247 min). One rat with three electrodes required 492 min, and one rat with five electrodes required 372 min. When time permitted, the test series was repeated. Of the 15 rats with two electrodes, 11 had multiple series of same-site or different-site tests, or both. In rats with more than two electrodes, each site was generally tested with one same-site series and one different-site series.

Histology

When the testing was completed, the rat was administered a lethal dose of Nembutal and, when insensate, was perfused transcardially with 0.9% saline followed by formalin. Following 1 or more days of further fixation, the brain was sectioned transversely at 100 µm with a vibrotome. The sections were viewed with a projection microscope at 23 magnifications. The stimulation sites were plotted onto drawings of the brain adapted from the atlas of Paxinos and Watson (18).

RESULTS

Overview

The 20 rats had a total of 44 sites. Priming was tested by applying the priming and test stimulation to the same site in 41 cases. Priming and test stimulation was applied to different sites in 45 cases (three sites were tested twice with multiple priming sites). There were 16 cases in which the test and priming sites were on the same side (ipsilateral) and 29 cases in which they were on opposite sides (contralateral). Most ($n = 13$) subjects had two electrode sites, each of which was used in both test conditions.

Control Latencies

The mean latency to step during the control stimulation train was 4.95 s (SE 0.19). The control latencies observed in the same-site and in different-site conditions did not differ, $t(39) = 1.26$, $p > 0.20$. We found a significant correlation between a site's mean control latency and its anterior–posterior location, $r(38) = -0.48$, $p < 0.01$. Anterior sites in and near the preoptic area had longer control latencies whereas posterior sites near the ventral tegmental area had shorter control latencies. This relationship was not due to larger currents being used in the more posterior sites.

Priming Effect of Prior Stimulation at the Same Site

Prior stimulation at the same site reduced the latency to locomotor to test stimulation by a mean of 1.64 s (SE 0.15), about one-third of the control value. All but 5 of the 41 cases tested with the same-site stimulation showed significant ($p < 0.05$) priming effects. The locations of the sites are illustrated in Fig. 2. The positive sites (filled symbols) ranged from the preoptic area through the longitudinal extent of the hypothalamus to the ventral tegmental area. The five negative sites (unfilled symbols) showed no obvious common anatomical characteristic. No site showed an indication of a suppressive effect (i.e., one in which the test latency was consistently greater than the control latency). These data indicate that the facilitation of locomotor initiation by prior stimulation at the same site is a widespread phenomenon.

The size of the priming effect correlated with the control latency: sites with larger mean control latencies showed larger priming effects, $r(39) = 0.62$, $p < 0.001$. This relationship might appear trivial because the priming effect is defined as a difference, and larger reductions simply reflect larger initial values. However, as will be seen below, the relationship is not inevitable. Although the priming effect positively correlated with control latency, and control latency depended on anterior–posterior level, the magnitude of the same-site priming effect did not correlate with anterior–posterior level. Although the simple correlation approached significance, $r(39) = -0.28$, $p < 0.10$, the correlation fell to virtually zero when the contribution of the control latency was removed [partial $r(38) = -0.02$]. On the other hand, the correlation between control latency and anterior–posterior level was maintained after the contribution of the priming effect was removed [partial $r(38) = -0.33$, $p < 0.05$]. It is concluded that, unlike the control latency, the magnitude of the same-site priming effect is similar among sites along the entire anterior–posterior extent of the medial forebrain bundle.

Priming and Test Stimulation Applied to Different Sites

The different-site condition generally was not as effective as the same-site condition in producing priming effects, but, nevertheless, sites that were reliably primed in this condition were common and widespread. The locations of the sites are illustrated in Fig. 3. Similar proportions of reliable priming were seen among sites that were ipsilateral to one another (11 of 16) and among sites that were contralateral (19 of 29). The magnitudes of the mean priming effects for the ipsilateral sites ($1.08 \pm 0.24$
s) and the contralateral sites (1.11 ± 0.20 s) were similar. Positive sites were located at all levels. Negative sites appeared to be more common in the anterior levels. A more refined analysis of this issue, one that accounts for magnitude of the priming effect and for the location of the priming site, is provided below. Two sites, both in the ventral tegmental area (VTA), produced suppressive effects on each other that approached significance (p < 0.10).

To determine if the size of the priming effect depended on the relative anterior–posterior location of stimulation sites, we divided the cases into two broad classes: one class in which the priming site was anterior to the test site and the other class in which the priming site was posterior to the test site. Separate classes were formed for the cases in which the sites were ipsilateral and contralateral to one another. Cases in which the two

FIG. 2. Schematic representation of the location of the sites in the condition in which the priming and test stimulation were applied to the same site. Sites are represented on the side of their actual location in the brain. To reduce the number of drawings in the figure, several sites are illustrated on adjacent levels; for the statistical analyses, the actual anterior–posterior level was retained. The number below each drawing is the distance (mm) posterior to bregma. Drawings adapted from the atlas of Paxinos and Watson (18). Abbreviations: AHA, anterior hypothalamic area; DM, dorsomedial hypothalamic nucleus; F, fornix; LH, lateral hypothalamus; LPO, lateral preoptic area; MFB, medial forebrain bundle; MPA, medial preoptic area; MT, mammillothalamic tract; PEF, perifornical nucleus; PH, posterior nucleus of the hypothalamus; R, red nucleus; SNR, substantia nigra reticulata; SUM, supramammillary nucleus; VTA, ventral tegmental nucleus; ZI, Zona incerta.
contralateral sites were placed at the same anterior–posterior level were excluded from this analysis. The matching between corresponding ipsilateral and contralateral groups was adequate: the mean anterior–posterior levels of two anterior groups were within 0.6 mm, and the levels of the two posterior groups were within 0.25 mm.

In Fig. 4, the horizontal band represents the average priming effect for all sites when the priming and test trains were applied to the same site. This priming effect is represented as a single population (mean ± SE) because, as shown above, it did not differ for the anterior and posterior sites. The vertical bars represent the priming effects when the priming and test sites differed. Priming effects were large with anterior priming sites (posterior test sites). Priming effects were small with posterior prime sites (anterior test sites). A two-factor analysis of variance showed an overall effect of anterior/posterior position, $F(1, 29) = 6.95, p < 0.02$, no difference between side of stimulation, and no interaction. The same pattern was seen when the priming effects in the different-site condition were subtracted from the corresponding priming effect in the same-site condition. Thus, the priming effects for anterior sites primed by posterior sites were also low when adjusted for possible differences in their capacity to manifest priming effects. Posterior sites did not show a general weakness in producing priming effects—when the priming and test trains were both applied to the same posterior site, it showed priming effects equivalent to those of anterior sites.
As shown in Fig. 4, the mean magnitudes of the priming effects produced by anterior stimulation on posterior sites approximated the level of the priming effects produced by stimulating the same site twice. The mean magnitude of the priming effect produced by posterior stimulation on anterior sites was about half of that level. The size of the priming effect produced by posterior stimulation did not differ significantly between the ipsilateral group and the contralateral group.

DISCUSSION

Prior stimulation at a locomotor site along the preoptic–hypothalamic course of the medial forebrain bundle usually facilitates the initiation of locomotion in response to subsequent stimulation either in the same site, in an ipsilateral site, or in a contralateral site. This priming effect is a widespread and robust characteristic of hypothalamic locomotor sites. It may therefore provide a useful property by which to study structural and clinically relevant aspects of locomotor initiation circuitry. This exploratory study has described some basic characteristics of the phenomenon. First, priming magnitude produced by stimulation of the same site depended on the characteristic latency of the site, but not on the anterior–posterior location of that site. Second, priming produced by stimulation in different sites depended on the anterior–posterior locations of the priming and test sites. The different-site priming magnitudes were equivalent to the same-site priming levels when anterior sites primed posterior sites, but they were markedly lower when a posterior site primed an anterior site. The pattern was the same regardless of whether the priming site was ipsilateral or contralateral to the test site.

The findings reveal basic characteristics of the locomotor initiation pathways that are activated by stimulation along the medial forebrain bundle, and they also raise interesting questions as well. An important finding is that the priming effects produced by stimulating the same site twice were similar at widely scattered locations. Indeed, the similarity extended to at least some of the sites primed by stimulation in different sites, even sites on opposite sides of the brain. These similarities indicate that priming is largely due to residual facilitation of systems that receive convergent excitation from locomotor sites in the preoptic area and diencephalon. Priming magnitude did not depend on the anterior–posterior level of the site, and therefore, the facilitation must occur at convergence sites that are remote from the stimulation site or are very diffusely organized.

Local changes in the excitability of axons of the medial forebrain bundle could be considered a possible explanation for the priming effects. Poststimulation periods of supernormal excitability have been observed after medial forebrain bundle stimulation (31). However, such increases in excitability have a short time course and are followed by a longer-acting subnormal period that, if influential at all, would be expected to reduce priming effects. Further evidence against the possibility of local effects on axonal excitability is that we observed no difference between priming produced at sites contralateral to one another and at sites that were ipsilateral. There was no possibility of cross talk between stimulus channels via current flow through the return electrode (32,44) because nonstimulated electrodes were disconnected. We therefore conclude that the priming effect in this study is not due to changes in local axonal excitability.

Five of 41 locomotor sites did not show same-site priming effects. Characteristics that differentiated these negative sites from the positive sites were not apparent. Their locations were scattered, and they were found in the same general areas as the positive sites. They did not differ from the positive sites in terms of the test currents or the number of trials used for testing. The control latencies for three of the negative sites were relatively low, but five positive sites had control latencies approximately as low. Three of the negative sites were primed by another site, but only one of them primed another site. This pattern might suggest that the lack of same-site priming in these cases was due to a failure of the stimulation to activate the residual excitatory process rather than an insensitivity of the locomotion to reflect the excitation.

The nature of the residual excitation that underlies the priming effect is a significant question. Its long time course, 20 s at least, is compatible with persistent facilitation in locomotor circuitry and/or in autonomic systems, possibly operating on the brain itself, or the cardiovascular and respiratory systems. Only a single priming–test interval was used here because the focus was to test the phenomenon at a large number of sites. More detailed information about the time course of the priming effect and its correlation with autonomic indices would help to restrict the range of possible mediators.

One of the remarkable properties of the residual excitation is that it is independent of the specific location of the locomotor site. In this sense, the underlying process is nonspecific and may correspond to general activation processes proposed to contribute to the diversity of motivated motor patterns elicited by stimulation of the hypothalamus (15) or by mild pressure on the tail (9). Because locomotor stimulation can produce a nonspecific effect manifest as priming, it might be suggested that the locomotor stepping is itself a manifestation of a nonspecific process. By this argument, the stimulation would evoke a generalized state that would produce stepping because it is one of few behavior patterns afforded an anesthetized rat held in a stereotaxic apparatus. This position is consistent with finding that stimulation at diverse sites extending from the preoptic area through the hypothalamus to the midbrain is capable of eliciting apparently similar locomotor patterns (21–23,25,26). The position that both the priming effect and the stepping reflect the same nonspecific process seems incompatible with the findings that stimulation at certain locomotor sites failed to prime their own locomotion, and that stimulation at posterior locomotor sites primes only poorly, if at all, locomotion produced by stimulation at anterior sites. On the other hand, the findings are consistent with the view that electrical stimulation along the medial forebrain bundle activates at least two types of elements: 1) a number of relatively independent...
motivational systems, each with access to specific motor patterns but all with access to locomotor initiation, and 2) a nonspecific system or systems (reflected in priming) that can increase the excitability of the systems underlying multiple motor patterns including locomotion.

The finding that locomotion elicited by electrical stimulation of the medial forebrain bundle in the anesthetized rat shows robust priming effects has implications for the priming phenomenon in ICSS. Hypothalamic stimulation in awake rats produces locomotor excitation (2,20,27,28), and similar to the priming effects shown here, the excitation outlasts the stimulation period by several seconds (20). It has been suggested that the locomotor effects of hypothalamic stimulation and the priming effect in ICSS share a common mechanism (20). Studies of ICSS priming typically use the speed of locomotor approach to index the reinforcing and priming effects (4,6,19). Priming is demonstrated when the speed of locomotor approach is increased by shortened intertrial intervals or by noncontingent stimulation applied shortly before the rat is allowed to initiate the approach locomotion. In the terms of the present study, most of the ICSS studies of priming would be described as using the same-site priming paradigm. Consistent with the findings of this study, there appear to be no reports of regional differences in the magnitude of the ICSS priming effect. Different-site priming, both ipsilateral and contralateral, of ICSS has also been reported (5), but it was not analyzed with respect to anterior—posterior level.

Because locomotion is typically elicited by stimulation at ICSS sites (2,3,20), it is likely that priming of ICSS would also prime locomotor initiation. Locomotor priming effects are robust enough to persist under anesthesia, and it is reasonable to propose that they would importantly contribute to the priming effects in ICSS. In the study of ICSS, considerable attention has been paid to differentiating the priming effect (motivating effect) from the rewarding effect of the stimulation. The available evidence indicates that the priming effect and the rewarding effect show similar quantitative properties (7) but reflect different processes (3,7,30). However, ICSS priming paradigms that use locomotor approach behavior to index the motivating or rewarding value of the stimulation face the problem of disassociating a possible facilitation of motivational or reward processes from the certain facilitation of locomotor initiation. It would seem that effects that appear to be motivational in the awake animal appear to be motonic in the anesthetized animal. Some aspects of motivational processes might persist under anesthesia, but in the absence of evidence to this point, we suggest that ICSS priming reflects a locus of action on locomotor initiation. If this hypothesis is correct, then ICSS sites that do not also support stimulation-elicited locomotion should not show priming, and stimulation in locomotor sites that do not support ICSS should prime ICSS at other sites.

The weak priming produced by posterior sites on anterior sites is open to several interpretations. The finding is consistent with the hypothesis of a rostral—caudal information flow along the medial forebrain bundle (see the Introduction). By this hypothesis, stimulation in anterior sites would activate the anterior sites themselves, posterior sites through synapses (16) and convergent circuitry, but posterior sites would only activate themselves and convergent circuitry. However, the hypothesis is vexed by the unexpected finding that anterior priming sites prime posterior test sites that are contralateral as effectively as it primes the sites that are ipsilateral. This would indicate a greater amount of crossing in the locomotor circuitry at the level of the diencephalon than would be expected from past research (12,16,25).

An alternate (and post hoc) explanation for the weak priming of posterior sites on anterior sites postulates the joint operation of excitation and inhibition. Although there is ample evidence that dopaminergic fibers ascending from the ventral tegmental area and projecting to the nucleus accumbens promote locomotion (14,17), there is evidence that elements located in or passing through the region have an inhibitory effect on locomotion. Stimulation at some sites in the ventral tegmental area suppresses ongoing hypothalamic locomotion and elicits locomotion only as aftereffect (23). GABA injections into the caudal region of the ventral tegmental area increase locomotion (1) and radiofrequency lesions of the ventral tegmental area increase locomotion (11). In the present study, two sites in the ventral tegmental area appeared to show inhibitory interactions. These findings are consistent with the idea that locomotor inhibitory fibers ascending from the ventral tegmental area could be activated by locomotor stimulation in the posterior hypothalamus. Posterior stimulation, by this model, would produce antagonistic effects, a residual excitation on the convergent circuitry and residual inhibition of higher locomotor initiation sites. This explanation raises two complexities: how an inhibitory posterior site could so effectively prime itself, and how a posterior site could inhibit an anterior site that was contralateral to it. It should be possible to design experiments to determine which, if either, of these two explanations is accurate.

The priming phenomenon provides a potentially useful access point for the study of locomotor initiation systems. Priming appears to be one manifestation of the fundamental processes that modulate the excitability of motor systems. These processes appear to be widespread and have characteristics similar to the ascending reticular activating system (20). The cholinergic system associated with the pedunculopontine area of the midbrain, which has been implicated both in general modulatory functions and in specific locomotor functions (8), warrants investigation as a possible mediator for the priming effect. The locomotor initiation deficits associated with Parkinsonism suggest that a dopaminergic mediation of locomotor priming also warrants investigation. Stimulation of the medial forebrain bundle at stimulation parameters effective for producing locomotion causes a transient increase in dopaminergic cell activity and release of dopamine in the striatum (10). The paradigm used to produce priming has potentially useful applications. It provides a robust and reliable means of transiently manipulating the excitability of the initiation system that can be useful for identifying neurons with activity correlated with locomotor initiation. For example, certain midbrain neurons with activity correlated in time to the onset of stepping have been found to show increased activity when priming stimulation facilitates the initiation of locomotion (24).

In conclusion, locomotor priming in the anesthetized rat is a robust phenomenon that can be demonstrated at a wide range of sites. It shows regional variations that may provide insights into the organization of locomotor initiation pathways, and it provides a potentially useful method to systematically manipulate the excitability of locomotor initiation.

ACKNOWLEDGEMENTS

This study was supported by a Wesleyan project grant and the Hughes Medical Foundation.

REFERENCES


