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Latency to Initiate Locomotion Elicited by Stimulation of the Diencephalon Positively Correlates in Awake and Anesthetized Rats[1]

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SINNAMON, H. M. AND B. SKLOW. *Latency to initiate locomotion elicited by stimulation of the diencephalon positively correlates in awake and anesthetized rats.* PHARMACOL BIOCHEM BEHAV 36(4) 725–728, 1990.—Locomotor stepping can be elicited by brain stimulation at various diencephalic sites under moderate levels of Nembutal. This study determined if locomotor initiation measured under anesthesia provides a valid measure of the intersite factors which determine initiation in the awake condition. We compared the latencies to inititate locomotor stepping elicited by electrical stimulation (50 μA, 0.5-msec pulses, 10 to 160 Hz) by rats tested while awake and unrestrained in a rotary runway or anesthetized and held in a stereotaxic apparatus. In the latter tests, initial anesthesia was provided by Nembutal (25 mg/kg) and 2% halothane and maintenance anesthesia was provided by 7 mg/kg as needed and local injections of lidocaine. For 30 sites in 16 rats, average locomotor initiation latency in the awake condition and the shortest latencies in the anesthetized condition were positively correlated ($r = .78$). Locomotion at sites with long latencies in the awake condition was frequently blocked in the anesthetized condition, but sites with short latencies were rarely blocked. The results indicate that the shortest locomotor latencies in the anesthetized condition approximate the latencies measured in the awake condition. It is concluded that the anesthetized condition can provide valid initiation measures, but sites with long latencies in the awake condition are prone to depression under anesthesia.

Locomotion Nembutal Electrical stimulation Rats

THE earliest report that locomotor stepping can be elicited under anesthesia was the work of Brown in 1913 (1). Mel'nikova (4) reported that locomotor stepping could be elicited by electrical stimulation of the brain of the anesthetized rat. This preparation has advantages in terms of efficiency and behavioral control and has been useful to identify locomotor areas in the brainstem (5,9), diencephalon (3,8) and basal forebrain (6,7) and the connections between them (2, 10, 11). In these studies, the method has involved anesthesia primarily by means of Nembutal and the rat has been restrained in a stereotaxic apparatus. Testing usually begins 1 hour after initial anesthesia with the rat maintained in a anesthetized state by injections of Nembutal at 7 mg/kg at 30–60-min intervals supplemented with local injections of lidocaine.

Sites at which electrical stimulation elicits locomotor stepping are usually found after anesthesia is induced. With this procedure it is uncertain whether a site that might have supported locomotion under the awake state would continue to do so under anesthesia. Although there is a correspondence between the distributions of locomotor sites in awake and anesthetized rats [see (3, 6, 9)], there is evidence that Nembutal may not affect all locomotor sites in the same way. In studies which required that locomotion be repeatedly elicited over several hours (2, 10, 11), it was noted that sites in the lateral hypothalamus were relatively stable, but other diencephalic sites seemed to be more depressed by the maintenance injections of Nembutal. In a test of this possibility, Sklow and Sinnamon (12) determined the effects of 7, 14, and 28 mg/kg Nembutal on the locomotion of awake rats with chronically implanted electrodes in various diencephalic sites. The low dose had few effects and the high dose blocked locomotor initiation at almost all sites. At the intermediate dose of 14 mg/kg Nembutal, qualitative differences between sites in the lateral hypothalamus and more dorsal and medial regions emerged. These findings suggest that testing locomotion under anesthesia might be well suited for some types of locomotor sites but inappropriate for others.

The purpose of this study was to directly compare the locomotor initiation latencies for the same sites in the anesthetized condition and in the awake condition. In the anesthetized test condition the rat was held in a stereotaxic apparatus with postural support provided by a sling; in the awake test condition the rat was unrestrained and locomoted in a rotary runway. Previous work

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(12) has demonstrated large differences among diencephalic sites in initiation latencies in the awake condition. Given the differences between the postural states of the rat and the methods of detecting stepping in awake and anesthetized test conditions, there would be little a priori reason to expect the intersite differences to be preserved under anesthesia. Therefore, a positive correlation between the locomotor initiation measures in the two conditions would indicate that the anesthetized test condition can provide valid measures of certain factors that determine locomotor initiation in the awake rat.

METHOD

Subjects and Surgery

Male Sprague-Dawley rats (N = 16) weighing between 268 and 472 g were used. Three stimulation electrodes were implanted stereotaxically in animals anesthetized with intraperitoneal injections of either Chloropent (3.2 ml/kg) or Nembutal (45 mg/kg) supplemented by lidocaine injections into the incision. The electrodes were Teflon-insulated stainless steel wires 125 μm in diameter that were cut to expose the cross section of the tip.

Measurement of Locomotor Initiation

For the three sessions under awake conditions, the rat was tested in a rotary runway. For the session under anesthesia, the rat was tested in a stereotaxic apparatus suspended over a wheel which was rotated by stepping movements.

The rotary runway was made of Plexiglas with an outer diameter of 30 cm and a path width of 8.3 cm. The path length was 45 cm inside and 96 cm outside. Locomotor initiation latency was detected with resolution of 1.0 sec by means of three infrared sensors spaced equidistantly around the circumference. Initiation was defined as the activation of the first sensor; the amount of movement required averaged 15 cm.

In the stereotaxic apparatus, the anesthetized rat was suspended over a wheel with a diameter of 30 cm and a width of 10 cm. The head of the rat was held by earbars and the bitebar, the thoracic and abdominal regions were supported by a sling, and the tail was suspended at its base equidistantly between the two extended side rails of the stereotaxic apparatus. In the stereotaxic apparatus, the hindlimbs of the unstimulated rat hung in passive extension with their anterior aspects contacting the outer surface of the wheel. Usually the forelimbs extended also with the flexed digits touching the wheel. Stepping movements, principally by the hindlimbs, rotated the wheel. A magnetic sensor detected the movement of six magnets equally spaced on the wheel. Locomotor initiation was defined as the activation of the first sensor which reflected wheel rotation produced by the first one or two hindlimbs steps.

Stimulation

A constant current stimulator delivered trains of cathodal rectangular pulses of 0.5-msec duration at 50 μA. The train consisted of a maximum of 50 sec of stimulation in which the pulse frequency doubled every 10 sec, starting at 10 Hz, and progressing to 160 Hz unless locomotion activated two additional sensors. This amount of stepping automatically terminated the stimulation; stimulation also terminated automatically at 50 sec in the absence of stepping.

Procedure

Of the 16 rats, 3 were tested with one electrode, 12 were tested with two electrodes, and 1 was tested with three electrodes. A rotary test session began with a 5–10-min adaptation period in which no stimulation was given. In the test period which was 30–40 min long, a stimulation trial was given every 90 sec. The minimum interval between the end of a train and the onset of the next was 40 sec. If the rat had more than one tested site, each would be tested in turn on successive trials. The data were derived from the final 20 min of the test periods in the three sessions. The median for all of the available trials (typically 7) in the final 20-min period was derived and the mean of these medians was used for the single value of the awake condition of a site. The upper panels of Fig. 1 presents an illustrative case. A minimum of 3 days separated the sessions in the rotary. Immediately following the test periods described here, the rats were further tested in the rotary with injections of Nembutal (7, 14, and 28 mg/kg). These results are reported elsewhere (12).

The test under anesthetized conditions in the stereotaxic apparatus followed the last session in the rotary by a minimum of 3 days. The rat was initially anesthetized by a 5-min exposure to 2% halothane in a small chamber, injected intraperitoneally with 25 mg/kg Nembutal, and returned to the halothane chamber for 10 min. Once mounted in the stereotaxic apparatus, anesthesia was maintained by intraperitoneal injections of 7 mg/kg Nembutal as needed at 30–50-min intervals. Anesthesia level was judged adequate when the rat remained inactive in the absence of stimulation. Lidocaine, 0.5 ml at 2%, was injected into the skin and muscle at the pressure points, and was repeated after 2.5 hours. The temperature of the rat was monitored and maintained with a heat lamp.

Up to 1 hr was required for the initial anesthesia to wane and allow locomotion to be elicited. After that point, the test trials in the stereotaxic condition were conducted similarly to those in the rotary condition. The session always included at least two 7 mg/kg Nembutal maintenance injections and always ended with the anesthetic effects of the last injection waning. The locomotion measures for each site were derived from the final 90 min of the session. A measure of the initiation capacity in the anesthetized condition was provided by the median of the nine shortest latencies. A measure of the depression of locomotion under anesthesia was provided by the percentage of trials in which locomotion failed with 50 sec of stimulation, i.e., percentage of blocked trials.

The test in the anesthetized condition was terminated by giving the rat a lethal dose of Nembutal. Conventional histological methods were used to localize the stimulation sites.

RESULTS

The upper panel of Fig. 1 illustrates the results from a representative case of a rat with an electrode in the ventral perifornical area. Each data point represents the initiation latency for a single trial. In sessions 1–3 in the awake condition there was a progressive decrease in the latencies, with the average of the three sessions at 21 sec. In the anesthetized condition, initiation failed on the first three trials reflecting the effect of the initial anesthesia. Thereafter, the latencies waxed and waned with a period determined by the maintenance injections of Nembutal (indicated by diamonds). Note that the two trials in which initiation was blocked in the final 90 min occurred within 5 min of the maintenance injections. The shortest latencies occurred before the maintenance injections and the median of the nine shortest was 27 sec. It is important to note that the anesthetized state is maintained during this period. The two values for this site, 21 sec for the mean awake latency and 27 sec for the median anesthetized latency, are represented by the asterisk in the scatterplot in the lower panel of Fig. 1. This plot shows the relationship between the

shortest latencies under anesthesia and the average latency while awake for the group of 30 sites.

The anesthetized test condition provides valid initiation latencies measures to the extent that the differences between the stimulation sites found in the awake condition are maintained. The scatterplot in the lower part of Fig. 1 shows for the 30 sites that the intersite differences in initiation latencies were preserved under the anesthetized test condition. The shortest latencies in the anesthetized condition correlated significantly ($r = .78$, $p < 0.01$) with the mean locomotor initiation latency in the awake condition. Although it is not essential to the validity question, it is noteworthy that the shortest latencies under anesthesia approximated the latencies in the awake test condition. The dashed line in the scatterplot represents the positions that the values for a site would occupy if the latencies in the stereotaxic apparatus were identical to the corresponding latencies in the rotary. The values for half of the sites were on or below the line, and for these cases anesthesia compromised initiation latencies little if at all. For the other half of the sites, the values were above the equivalence line, and in these cases the shortest latencies under anesthesia were several sec longer than the average latency.

The percentage of blocked trials in the stereotaxic apparatus varied widely between sites. In the example given in Fig. 1, 2 of the 26 trials in the last 90 min were blocked. About one quarter of the sites showed no blocks at all, about one third were blocked on over 50% of the trials, and the majority were blocked on less than 20% of the trials. The percentage of trials blocked correlated with the shortest latency ($r = .80$, $p < 0.01$). Sites with latencies of less than 20 sec rarely showed more than a few blocked trials, and sites with shortest latencies of more than 30 sec were blocked on the majority of trials.

The locations of the stimulation sites ranged from the preoptic area to the caudal hypothalamus and are described in detail in another report (12). They were located in the lateral and medial preoptic area, the lateral hypothalamus, the anterior hypothalamic, dorsomedial, posterior nuclei, and the anterior and posterior zona incerta.

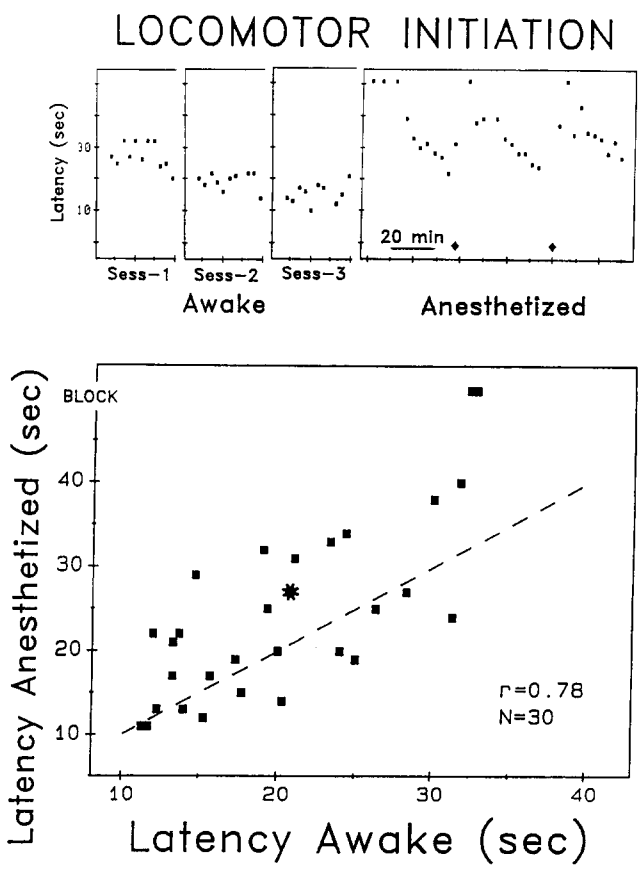

FIG. 1. The relationship between locomotor initiation latencies in the awake and anesthetized conditions with electrical stimulation of the diencephalon. Upper panel: representative results. Each point represents the locomotor latency on a single trial. Three sessions awake in the rotary runway were followed by a single session under anesthesia with the rat held in a stereotaxic apparatus. The five points at the top, corresponding to 50 sec, represent failures to initiate. The time of Nembutal maintenance injections (7 mg/kg) represented by diamonds. Lower panel: the average shortest latency in the anesthetized condition plotted over the average latency in the awake condition. The asterisk represents the data point of the case illustrated in the upper panel.

DISCUSSION

Locomotor initiation latencies determined under anesthesia were highly correlated with those measured in the awake state. These results show that locomotor initiation determined in the anesthetized rat can provide a valid measure of at least certain factors operating to determine locomotor initiation in the awake condition. The use of the anesthetized preparation is complicated by the finding that sensitivity to locomotor depression by Nembutal varies considerably between sites. For some sites the dose required to maintain anesthesia was only slightly less than the dose which blocked locomotion, and for other sites the locomotor blocking dose greatly exceeded the anesthetic dose. However, for all sites in the present study, there was a Nembutal level sufficient to maintain anesthesia yet compatible with locomotor initiation. The sensitivity to depression was correlated with the locomotor initiation latency. This correlation indicates that variance in the initiation latencies and in the Nembutal sensitivities were both determined by site-specific factors that can be measured in either the awake or anesthetized conditions.

The factors that determine locomotor performance elicited by diencephalic stimulation in the Nembutal-anesthetized rat are becoming better understood. The present findings combined with those of a prior study (12) make a coherent pattern. In the awake condition, sites with relatively short initiation latencies responded to 14 mg/kg Nembutal with shorter latencies. In the anesthetized condition, they had similar short latencies and initiation was rarely blocked by maintenance anesthesia. Sites in the medial forebrain bundle generally had these characteristics. Other sites, generally the medial hypothalamus and zona incerta, in the awake condition had relatively long initiation latencies which were prolonged by 14 mg/kg Nembutal. They had similar long latencies in the anesthetized condition and initiation was likely to be blocked by maintenance anesthesia.

The results indicate that anesthesia does indeed select against certain types of locomotor sites. This bias would be most significant in mapping studies (5, 8, 9) in which a site is tested only briefly. The bias can be minimized by careful control of the anesthesia. In the present procedure, intraperitoneal injections were given at the first signs of spontaneous behavior. This type of delivery results in periods of overdosage which prolong latencies for some sites (e.g., Fig. 1) and block initiation for several trials with other sites. Intravenous delivery on a schedule that would produce an average dose of 5–7 mg/kg/hr (supplemented by a local anesthetic) would allow the maintenance of a stable level of anesthesia compatible with all but the most sensitive locomotor sites.

For certain sites the latencies were similar in the awake and anesthetized states. The exact degree of correspondence should not be overemphasized because it must reflect the details of the specific measurement situations in the two conditions. Nevertheless, it is interesting to consider the factors that might allow the latencies of the anesthetized rat held in a stereotaxic apparatus to approximate those of the awake condition. First, the effort required in the anesthetized situation is minimal; support is provided by the sling and the stepping movements simply rotate the wheel which has low friction and inertia. In the awake condition the rat has to provide its own postural support, and the stepping movements must actually displace the animal. Second, the nonlocomotor behavioral options of the anesthetized rat are fewer, and thus opportunities for competition are lessened. A final factor might be the facilitatory effect of Nembutal on the sites with short latencies (12). More simply, the similarity of the locomotor latencies in the two conditions could reflect the robustness of stepping elicited by lateral hypothalamic stimulation.

In conclusion, it appears that despite the apparent motoric depression produced by anesthesia, measures of locomotor initiation show a consistent relationship to measures in the awake and unrestrained state. Whether this pattern generalizes for locomotor sites in other parts of the brain is not known. The anesthetized preparation has advantages in the study of certain issues in locomotion. First, the locomotor behavior arises from a baseline of no movement and therefore initiation is more clearcut than in the awake animal. Second, the immobile and anesthetized state allows a variety of mechanical and electronic methods to be used to detect the onset and course of stepping. Third, that state also allows a variety of physiological manipulations to be performed that would be difficult in the awake animal. The use of anesthesia is not without costs but for particular types of problems, the benefits of the preparation may mitigate them.

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