Scientific Articles

Long-lasting alterations of postural asymmetry induced by cerebellar lesion and stimulation in rats

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Cerebellar induced spinal fixation was studied in two experiments. In Experiment 1, cerebellar lesions were used to initiate post-spinal-section retention of a postural asymmetry. Bilateral dorsal root rhizotomy performed 45 min after cerebellar lesion failed to disrupt the fixated asymmetry, thus suggesting that muscle feedback to the spinal cord was not necessary for maintenance of the fixation effect. Experiment 2 demonstrated spinal fixation with 50 min of cerebellar stimulation. Effective stimulation sites included anterior cerebellar vermal cortex and the fastigial nucleus.

We recently began an extensive series of studies designed to delineate the neural basis of a nonassociative form of spinal plasticity known as "spinal fixation." Spinal fixation was first described by DiGiorgio (1929). In that study, cerebellar lesions delivered to anesthetized or decerebrate dogs and rabbits caused postural asymmetries that outlasted spinal cord section when 1-2 h elapsed between brain lesion and cord section. Several researchers obtained similar results in anesthetized adult or newborn rats with a variety of cerebellar lesioning techniques (e.g., Chamberlain, Halick, & Gerard, 1963; Mouravieff-Lesuisse & Giurgea, 1968; Palmer & Davenport, 1969). More recently, we developed a standard lesioning preparation for investigation of cerebellar induced spinal fixation in rats (Steinmetz, Cervenka, Robinson, Romano, & Patterson, 1981). Large unilateral lesions of anterior cerebellar cortex or more discrete lesions of underlying nuclear regions were found to effectively produce hindlimb asymmetry. More importantly, consistent postsection persistence of asymmetry was observed when a minimum of 45 min elapsed between the cerebellar lesion and midthoracic spinal section.

Spinal fixation has also been obtained when periods of thigh-skin stimulation, instead of cerebellar

lesions, were used to induce hindlimb flexion. Peripherally induced fixation has been obtained in rats stimulated either before (e.g., Steinmetz, Cervenka, Dobson, Romano, & Patterson, 1982) or after (e.g., Steinmetz, Beggs, Cervenka, Romano, & Patterson, 1982) midthoracic spinal cord section. The peripheral stimulation preparation has proven useful for investigating temporal parameters and peripheral contributions to the fixation process, because the experimenter has precise control over the fixating stimulus. In addition, the experimenter can choose whether or not to include higher brain center contributions to the fixation process by delivering stimulation before or after spinal section.

To this point, our major research efforts have been directed toward investigations of peripherally induced spinal fixation without descending influences from higher brain centers. However, several studies (e.g., Mouravieff-Lesuisse & Giurgea, 1970, 1973; Steinmetz, Cervenka, et al., 1982) have suggested that cerebral or cerebellar activity, when allowed to impinge on spinal centers, modulate spinal cord excitability changes that form the basis of fixation. Examination of these higher brain center/spinal cord interactions may prove valuable for a more complete understanding of the fixation process. The present studies provide additional information concerning cerebellar induced spinal fixation. In Experiment 1, muscle afferents to the spinal cord were severed to evaluate the necessity of muscle feedback for maintenance of a fixed cerebellar lesion induced asymmetry. Experiment 2 provides evidence for fixation with cerebellar stimulation. This new preparation should prove valuable for future studies designed to evaluate cerebellar-spinal interactions present during the fixation process.

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EXPERIMENT 1

Previous studies have demonstrated consistent display of cerebellar lesion-induced fixation when at least 45 min are allowed between brain lesion and midthoracic spinal section (Steinmetz et al., 1981). Experiment 1 was conducted to determine if ongoing input from flexor muscle and other afferents is necessary to maintain a cerebellar lesion induced fixation. In brief, dorsal root rhizotomies were given to disrupt muscle afferent connections with the spinal cord. The rhizotomies were performed after cerebellar lesion, a 45-min waiting period, and at the time of a midthoracic spinal cord section.

Method

Nine male Long-Evans hooded rats were used in Experiment 1. The rats weighed 400-500 g, received food and water ad lib, and were maintained on 12-/12-h light/dark cycles. All experimental procedures were performed during light portions of the light/dark cycles.

Each rat was anesthetized with pentobarbital (50 mg/kg, ip) and placed in a Kopf stereotaxic head-holding apparatus. The anterior cerebellum was then visually located after removal of the interparietal bone. Next, a laminectomy was performed between the sixth thoracic (T-6) and first sacral (S-1) vertebrae and the exposed spinal cord bathed with saline to prevent it from drying. While the head remained secured in the stereotaxic device, the animal was suspended by the tail and supported by a sling that surrounded the abdomen. This apparatus allowed free movement of the hindlimbs while firmly restraining the animal for subsequent experimental procedures. Stereotaxically guided unilateral lesions were then placed in the cortex or underlying nuclear regions of the left anterior cerebellum by delivering 18 sec of 10-mA anodal current through an insulated stainless steel electrode. The lesions resulted in postural asymmetries of the hindlimbs typically characterized by flexion of one limb and extension of the opposite limb. The flexion was at times contralateral and at times ipsilateral to the lesion, depending upon the exact lesion placement. Those animals that failed to show an immediate, discernible asymmetry after four lesions were not used in the study. Of the nine animals included in the study, four required two lesions to produce postural asymmetry and the remaining five required only one.

Following the lesion, each rat was allowed a 45-min waiting period. To assess the degree of postural asymmetry at the end of the waiting period, weights were suspended from the flexed leg until the hindlimbs were in a symmetric position. The weights were then removed, the spinal cord sectioned at T-7, and all dorsal roots between T-7 and S-1 cut near their entry point into the cord. Five minutes were allowed for outward signs of spinal surgery to subside (e.g., leg-twitching), and weights were again placed on the flexed leg to measure asymmetry present following the combined spinal section and dorsal rhizotomy. If the limb failed to return to a flexed position after the weights were removed, post-section asymmetry was recorded as zero. To assess long-lasting effects of the cerebellar lesion, asymmetry was measured a third time, 1 h after spinal section and rhizotomy.

The animals were then sacrificed, perfused through the heart with .9% saline followed by 10% Formalin, and the cerebellum and brainstem saved for histological examination of lesion placement. Visual checks for confirmation of complete spinal transection and dorsal rhizotomy were also conducted at this time.

Results

All nine animals displayed presection postural asymmetries after the 45-min waiting period had elapsed (mean = 19.4 ± 1.1 g). With the exception of

one rat, asymmetries persisted 5 min after spinal section and dorsal rhizotomy (mean = 7.2 ± 1.5 g) and 1 h after spinal section (mean = 5.6 ± 1.0 g). Correlated *t* tests revealed a significant decrease in asymmetry after spinal section and rhizotomy [$t(8) = 13.91$, $p < .01$] and a significant reduction in asymmetry over the 1-h period that followed spinal cord section [$t(8) = 2.00$, $p < .05$]. Histological examination of the brain tissue revealed lesion sites that included either large areas of the left anterior cerebellar cortex or more discrete areas involving the left fastigial nucleus.

In brief, these data indicate that asymmetry created by cerebellar lesions generally outlasts spinal cord section and dorsal rhizotomy when 45 min are allowed to elapse between brain lesion and spinal surgery. The significant decreases in asymmetry observed after spinal section and over the 1-h waiting period have previously been observed (Steinmetz et al., 1981). Reduction of asymmetry after spinal section most likely resulted from the elimination of input from the asymmetry-initiating source (i.e., the injured cerebellum), whereas the decrease in asymmetry 1 h after spinal section probably represents a gradual diminution of the spinal cord's ability to support the fixated postural change. Even though asymmetry decreases were noted, the fixation effect was still evident 1 h after spinal section. More importantly, the fixated asymmetry was maintained without ongoing neural activity generated by higher brain centers and in the absence of continuous sensory input from muscle afferents. While it is possible that afferent input contributed some to the ongoing flexion prior to rhizotomy, the results are clear in showing that this contribution is not critical to obtain the fixation effect. It is therefore apparent that spinal alterations, independent of other sources of neural input, are able to support fixation of a cerebellar lesion induced postural asymmetry for at least 1 h after midthoracic spinal cord section.

EXPERIMENT 2

The results of Experiment 1 are important in that it now seems clear that neural alterations at the spinal level, in the absence of ongoing higher center input or muscle feedback, are sufficient to support a fixated, centrally induced, postural asymmetry. Similar evidence has been obtained for peripherally induced fixation (e.g., Steinmetz, Cervenka, et al., 1982). The basic issue that remains to be investigated, however, is precisely how descending input from the injured cerebellum alters spinal cord excitability during the waiting period that follows the brain lesion. Unfortunately, cerebellar lesioning methods do not afford the experimenter a great deal of control over the fixation process because effective lesions for producing postural asymmetries are often extensive and their placement is variable between subjects. Stimulation

methodology, however, could provide a relatively greater amount of experimental control for evaluating neural events that underlie centrally induced fixation. Experiment 2 was an attempt to obtain the fixation effect using cerebellar stimulation instead of cerebellar lesions to produce postural asymmetry.

Method

The subjects were 14 male Long-Evans hooded rats. They weighed 300–500 g and were housed and cared for in the manner described for Experiment 1. Each rat was anesthetized with pentobarbital (50 mg/kg, ip) and placed in a stereotaxic head-holding apparatus. Then the anterior cerebellar cortex was exposed by removal of the interparietal bone, a laminectomy was performed between T-6 and T-8, and the animal was suspended in the restraint apparatus described in Experiment 1.

Cerebellar stimulation was delivered by a Grass SD-5 stimulator through an insulated stainless steel electrode with a 50–75- μ exposed tip. The electrode was stereotactically guided toward one of two stimulation sites—the left fastigial nucleus or the left anterior cerebellar cortex. Stimulation consisted of 70-Hz, 7-msec repetitive pulses with voltage (1–7 V) manually adjusted to produce at least 2 cm of hindlimb asymmetry. If asymmetry was not obtained with 7 V of stimulation, the electrode was repositioned. Animals were discarded from the study if criterion asymmetry was not obtained after three electrode placements. Stimulation-induced asymmetry involved strong ipsilateral flexion with varying degrees of contralateral extension. The strong flexion was present for the duration of the stimulation periods in all animals. After criterion hindlimb asymmetry was obtained, the rats were randomly assigned to two groups that received either 20 or 50 min of cerebellar stimulation. To assess presection asymmetry at the end of the respective stimulation periods, weights were suspended from the flexed limb. The rats then received a spinal section at T-7 and were allowed 5 min for outward signs of spinal surgery to subside. Then, to assess postsection asymmetry, weights were again suspended from the flexed limb. Finally, a third asymmetry measure was recorded 1 h after spinal section in three animals (two animals from the 50-min group and one animal from the 20-min group).

After recording the final asymmetry measure, all animals received a lesion to mark electrode placement. The lesions were made through the stimulating electrode by passing 3 sec of 2-mA anodal current. The animals were then sacrificed and perfused through the heart with .9% saline followed by 10% Formalin. The cerebellum and brainstem were saved for histological verification of stimulation sites. Visual checks for confirmation of complete spinal transection were also conducted at this time.

Results

Pre- and postsection asymmetries for the two groups of rats were compared with independent *t* tests. Comparisons of presection asymmetries revealed a significant difference between groups [$t(12)=3.78$, $p < .01$], with 50-min animals (mean = 12.1 ± 1.5 g) displaying significantly greater amounts of presection asymmetry than 20-min animals (mean = 4.0 ± 1.6 g). Similarly, analysis of postsection asymmetries revealed significant group differences [$t(12)=3.36$, $p < .01$], with greater amounts of postsection asymmetry present in the 50-min group (mean = 5.7 ± 1.3 g) than in the 20-min group (mean = $.7 \pm .7$ g). Six of the seven 50-min animals showed persisting postsection asymmetry, whereas only one of the seven 20-min animals demonstrated postsection asymmetry. Furthermore, examination of asymmetry in those animals observed 1 h after spinal section re-

vealed asymmetry persistence in the two 50-min animals but not in the 20-min animal.

To test the possibility that stimulation voltage was different for the two groups, a *t* test was conducted on the voltages used to create hindlimb asymmetry. No statistical difference between the 50-min (mean = 4.1 ± 1.0 V) and 20-min (mean = 4.3 ± 0.7 V) groups was found. Correlational analyses (Pearson's *r*) further demonstrated a lack of relationship between stimulation voltage and presection asymmetry and stimulation voltage and postsection asymmetry for the two groups. The correlations between voltage and presection asymmetry for the 50- and 20-min groups were 0.11 and -0.08 , respectively, and the respective correlations between voltage and postsection asymmetry for the 50- and 20-min groups were 0.24 and -0.29 .

Histological examination of effective stimulation sites revealed electrode placements in one of three areas: (1) the left fastigial nucleus (three of the 50-min animals and two of the 20-min animals), (2) left central lobuli I or II of vermal cortex (three of the 50-min animals and three of the 20-min animals), and (3) a cerebellar area between the left vermal lobe and left fastigial nucleus (one of the 50-min animals and two of the 20-min animals). Close examination of the data revealed no clear relationship between amounts of pre- and postsection asymmetry, stimulation voltage, and effective stimulation site. See Figure 1 for a representation of effective stimulation sites.

It is apparent from these data that the fixation effect can be obtained with stimulation of the anterior

Figure 1. The three areas found to effectively produce hindlimb asymmetry when stimulated are represented in this drawing. Area A encompasses portions of central lobuli I and II of vermal cortex, area B is a region that includes vermal cortex and portions of the fastigial nucleus, and area C represents stimulation sites confined to the fastigial nucleus. Actual anterior-posterior placement of area C was .5–1 mm posterior to the representative section pictured here. The drawing was derived from the Paxinos and Watson (1982) atlas.

cerebellar cortex or the fastigial nucleus. Lack of differences between stimulation voltages and effective stimulation sites for the two groups of rats argues against the possibility that group differences were caused by discrepancies in stimulation procedures. It is more likely that differences in the amount of persisting postsection asymmetry were the result of differential durations of central stimulation.

DISCUSSION

Two basic findings concerning cerebellar induced spinal fixation are evident from the present studies: (1) Dorsal rhizotomy fails to abolish retention of a cerebellar lesion-induced postural asymmetry. From these data, it is apparent that continuous feedback involving muscle afferents is not required for postsection retention of hindlimb asymmetry. (2) Fifty minutes of cerebellar stimulation is sufficient to produce postsection persistence of postural asymmetry. Effective stimulation sites include the fastigial nucleus and portions of anterior vermal cortex.

The observation that cerebellar lesion-induced postural asymmetry outlasts spinal cord section and dorsal rhizotomy provides additional evidence that neural alterations responsible for maintaining a fixated asymmetry occur at the spinal cord level. This finding is consistent with recent studies that have demonstrated lack of involvement of neuromuscular junctions (Steinmetz, Cervenka, et al., 1982) or cutaneous receptors (Steinmetz, Beggs, Lupica, & Patterson, 1983) in retention of peripheral stimulation initiated fixation. It is possible that feedback from muscle afferents is required during the postlesion waiting period to produce, but not maintain, centrally initiated fixation. This possibility could be tested by performing dorsal rhizotomy prior to cerebellar lesion. However, we have encountered difficulties in producing robust amounts of asymmetry when dorsal root rhizotomy precedes cerebellar lesion (unpublished data). Apparently, muscle afferent innervation of the spinal cord is necessary to at least initially obtain adequate amounts of hindlimb asymmetry. Precisely when, during the postlesion waiting period, alterations at the spinal level become sufficient to maintain abnormal hindlimb posture has yet to be determined.

Data from Experiment 2 indicate that cerebellar stimulation can initiate a time-dependent fixation of postural asymmetry. Compared with cerebellar lesion methods, stimulation produced lesser amounts of pre- and postsection asymmetry (cf. Experiment 1 and Experiment 2 results). However, present data indicate that percentages of presection asymmetry retained after spinal section are similar for the two preparations (35.4% for cerebellar lesioned animals and 42.8% for animals given 50 min of cerebellar stimulation). Apparently, rather robust percentages

of presection asymmetry retention can be obtained with cerebellar stimulation, even though stimulation produces lesser amounts of postural alteration than do lesion methods.

It could be argued that long periods of cerebellar stimulation damage cerebellar tissue, which in turn induces the fixation effect in a manner similar to the delivery of a cerebellar lesion. Data from pilot animals provide evidence against this possibility. Histological sections from four pilot animals revealed no signs of poststimulation damage. Fixated hindlimb asymmetries in these animals were created with 60 min of 7-V, 70-Hz, 7-msec stimulation. After stimulation, the animals were perfused and the cerebellums were examined. Except for signs of the electrode track, no visible evidence of cerebellar damage was found. Because rather extensive cerebellar lesions are required to induce postural asymmetry, it seems highly unlikely (although not impossible) that cerebellar stimulation produced the fixation effect through brain tissue damage.

In addition to the histological evidence, the postural changes associated with the experimental procedures also demonstrate that the mechanisms producing fixation are different in the two experiments. Lesions placed in the cerebellar cortex or the fastigial nucleus produced flexion that was at times ipsilateral and at times contralateral while the opposite limb extended. In contrast, cerebellar stimulation always produced ipsilateral flexion and contralateral extension. Along these lines of investigation, Sprague and Chambers (1953) found that aspiration lesions of cerebellar cortex in cats produced contralateral flexion and ipsilateral extension. This effect could then be reversed by lesioning the fastigial nucleus. Their findings may explain the lateral differences noted here, but further analysis of hindlimb asymmetry produced by cerebellar lesions in rats is required to determine the exact locus of these postural changes.

Once parametric features of the cerebellar stimulation preparation are studied (e.g., temporal relationships, peripheral contributions, and stimulation parameter manipulation), the preparation should prove valuable for assessing modulatory influences exerted by cerebellar centers on the fixation process. Furthermore, cerebellar stimulation, used in conjunction with the peripheral stimulation preparation, should provide a unique opportunity for studying spinal plasticity with simultaneously occurring peripheral and central sources of neural input.

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