Electrophysiological Studies on Plasticity of Cerebellothalamic Neurons in Rats Following Neonatal Hemicerebellectomy

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Abstract Changes in the cerebello-thalamo-cerebral projection following hemicerebellectomy were investigated in 16 rats by laminar field potential analysis of cerebello-cerebral responses in the cerebral cortex and by unitary recording of antidromic responses of cerebellar nuclear neurons to stimulation of the thalamus. In contrast to the occurrence of an exclusively contralateral cerebello-cerebral response in intact rats, a significant bilateral cerebello-cerebral response could be evoked in all 8 rats hemicerebellectomized before 6 days of age (early hemicerebellectomy). On the other hand, in all 5 rats operated on at 8 to 15 days of age (late hemicerebellectomy) and in the 3 rats operated on in adulthood, the cerebello-cerebral response was evoked only contralaterally just as in intact rats. Unitary recordings of antidromic responses of cerebellar nuclear neurons revealed that bilateral or ipsilateral cerebellothalamic projection neurons were remarkably more numerous in early hemicerebellectomized rats than in controls ($P<0.001$). Such neurons were less frequently found in late hemicerebellectomized rats, but they were still more numerous than in controls ($P<0.05$). Absence of the ipsilateral cerebello-cerebral response in late hemicerebellectomized rats indicates that even though bilateral or ipsilateral cerebellothalamic projection neurons may exist in these rats more numerously than in intact rats, they may not be sufficient in number or in efficiency to mediate a detectable response.

Previous studies (KAWAGUCHI et al., 1979a, b) provided corroborating evidence for axonal sprouting of cerebellothalamic neurons in kittens following neonatal hemicerebellectomy, which accounts for the occurrence of a marked ipsilateral cerebello-cerebral response in the operated animals in contrast to absence of such a response in intact animals. LIM and LEONG (1975), LEONG (1977), and CASTRO (1978) reported morphological findings that destruction of the deep...
cerebellar nuclei or removal of one half of the cerebellum in rats in an early postnatal period induced aberrant cerebellothalamic and cerebellorubral projections. The present report describes an electrophysiological investigation of such an aberrant cerebellothalamic projection in neonatally hemicerebellectomized rats.

MATERIALS AND METHODS

Twenty-two rat pups (Wistar strain) ranging in age from 0 to 15 days old were anesthetized with ether vapor combined with hypothermia. A craniotomy was made from the foramen magnum and the exposed cerebellum was sectioned sagittally at the midline by a small sharp scalpel. The left half of the cerebellum was then removed by suction under a dissecting microscope. After the operation, these animals were suckled by their own mothers. Three adult rats were also hemicerebellectomized under pentobarbital anesthesia (35 mg/kg, i.p.). After 29–280 postoperative days, these animals were subjected to electrophysiological experiments. The animals were anesthetized with pentobarbital (initial dose 35 mg/kg, i.p.). The trachea was cannulated and a fine polyethylene tube was introduced into the saphenous vein. Via this venous cannula pentobarbital was intermittently administered to maintain an adequate level of anesthesia during the experiments. Usually the animals were allowed to breathe naturally, but they were immobilized with gallamine triethiodide when units of cerebellar nuclear neurons were recorded. The level of anesthesia was monitored by the electrocorticogram. Laminar field potentials in the cerebral cortex were recorded from 16 hemicerebellectomized rats by stimulating the interpositus or the lateral nucleus of the spared hemicerebellum in the same manner as described elsewhere (YAMAMOTO et al., 1979). Antidromic responses of cerebellar nuclear neurons were recorded extracellularly by stimulating the thalamic ventrolateral and the ventromedial nuclei in a manner similar to a previous study (KAWAGUCHI et al., 1979a) in 9 hemicerebellectomized rats and in 4 intact adult rats. Concentric electrodes (0.3 mm in outer diameter and 0.2 mm in interpolar distance) were used for stimulation of the cerebellar nuclei or the thalamic nuclei. Usually a brief, monophasic, single pulse of constant current (0.2–0.5 mA in strength and 0.05–0.5 msec in duration) was delivered through the electrodes. At times a train of two or three pulses of a short time interval was employed. A glass micropipette filled with Fast Green FCF in 2 M Na-acetate was used for the recording of laminar field potentials and extracellular units. Recording sites were marked with Fast Green FCF by passing 10 μA of cathodal current for 10 min through the micropipette.

After the experiments, animals were perfused with 10% formalin solution via the ascending aorta and the tracks of stimulating electrodes as well as the recording sites marked with Fast Green FCF were histologically examined.
RESULTS

**Bilateral cerebello-cerebral responses following neonatal hemicerebellectomy**

Stimulation of the cerebellar nucleus in intact rats (Yamamoto et al., 1979) as in cats (Sasaki et al., 1972) and kittens (Kawaguchi et al., 1979a) induced a marked response in the cerebral cortex only contralaterally. By contrast, in all 8 rats whose hemicerebellum had been removed before 6 days of age, a significant cerebello-cerebral response could be evoked bilaterally on stimulation of the lateral or the interpositus nucleus of the spared hemicerebellum. On the other hand, in all 8 rats hemicerebellectomized after 8 days of age, the cerebello-cerebral response was evoked only contralaterally just as in intact animals. Ages at the time of surgery (A) and days after surgery at the time of recording (B) in these 16 animals, expressed as A OP B, were as follows: 0 OP 29, 0 OP 32, 0 OP 95, 1 OP 127, 3 OP 84, 3 OP 104, 3 OP 123, 6 OP 120, 8 OP 180, 9 OP 279, 11 OP 103, 13 OP 124, 15 OP 280, adult OP 138, adult OP 145, and adult OP 151.

Examples of the cerebello-cerebral response in 3 hemicerebellectomized rats are illustrated in Fig. 1: they are laminar field potentials recorded from the cerebral cortex contralateral (C-Cx) or ipsilateral (I-Cx) to the spared hemicerebellum. Records of the left and the middle panel were obtained from 2 rats. In these rats the hemicerebellum was removed at 3 and 6 days of age, and record-

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**Fig. 1.** Laminar field potentials of the cerebellar-induced response in the cerebral cortex in 3 rats of different ages at the time of hemicerebellectomy. Animal's age at the time of operation (A) and days after the operation (B) are indicated as A OP B. Records are from the motor cortex contralateral (C-Cx) and ipsilateral (I-Cx) to the spared hemicerebellum. Calibrations are applicable to every record.

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Fig. 2. Distribution of the cerebello-cerebral responses summarized from 8 rats hemicerebellec- 
tomized at 0 to 6 days of age. Reference point for coordinates of recording 
sites is bregma (b) and each recording site is 1 mm apart on the rostrocaudal and 
mediolateral axis. Filled circles indicate responsive areas and the diameter of circles 
signifies the relative amplitude of the response. The amplitude of the response was 
largest at 2 mm lateral to the midline at the level of bregma. It was $0.71 \pm 0.09$ mV 
on the contralateral side and $0.54 \pm 0.08$ mV on the ipsilateral side. Amplitude of the 
response was measured with respect to the negative peak at a depth of 0 $\mu$m and the 
positive peak at a depth of 1,500 $\mu$m. Asterisks indicate unresponsive areas.

ings were made on the 123rd and the 120th postoperative day, respectively. Both 
rats showed clear responses in the ipsilateral cortex. Illustrated in the right panel 
are responses obtained from another rat operated on at 8 days of age and kept 
alive for 180 days thereafter. No response was evoked ipsilaterally whereas the 
responses evoked contralaterally were just the same as in intact animals (YAMA-
moto et al., 1979). The ipsilateral cerebello-cerebral response in the 8 rats operated 
on before 6 days of age was detectable as early as 29 days after surgery and its 
depth profiles were similar to those of the contralateral cerebello-cerebral response.

The cerebello-cerebral responses were recorded using coordinates after HALL 
and LINDHOLM (1974); the reference point was placed on the bregma and each 
recording site was 1 mm apart on the rostrocaudal and mediolateral axis. The 
amplitude of the responses was measured with respect to the negative peak at a 
depth of 0 $\mu$m and the positive peak at a depth of 1,500 $\mu$m. Of the 8 rats hemi-
cerebellecetomized before 6 days of age, amplitude of the response at each record-
ing site was averaged and illustrated in Fig. 2. Distribution and amplitude of the 
contralateral cerebello-cerebral response did not differ from those observed in 
intact rats (cf. YAMAMOTO et al., 1979, Fig. 2). The responsive areas of the ipsi-
lateral cerebello-cerebral response were restricted within the medial portion of the 
cortex: they correspond to the forelimb and vibrissae areas (HALL et al., 1974).
The ipsilateral cerebello-cerebral response was always smaller than the contralateral cerebello-cerebral response of the corresponding site.

**Neuronal connections mediating the ipsilateral cerebello-cerebral response**

As would be expected from previous studies in kittens (Kawaguchi et al., 1979a, b), cerebellar nuclear neurons projecting bilaterally or ipsilaterally to the thalamus were remarkably more numerous in the neonatally hemicerebellectomized rats than in controls. This was evidenced by unitary recordings of the cerebellar nuclear neurons with antidromic activation from stimulation of the thalamus. Illustrated in Fig. 3A and B are two examples of the bilateral projection neuron which was activated antidromically on stimulation of the thalamic ventrolateral nucleus of either side (S1 or S2 in the inset diagram) with the absolute refractory period of 0.73 msec (A) and 0.64 msec (B). The antidromic response was identified on the basis of fixed latency and faithful follow to double shock stimulation at an interval of less than 1 msec. Owing to axonal bifurcation, these neurons showed impulse collision between stimulation of S1 and S2 at a certain time interval (1.11 msec for A and 1.43 msec for B). With bilateral projection neurons, conduction times of an axon before and after the branch point ($t_1$, $t_2$, and $t_3$ in the...
inset diagram) were calculated from the absolute refractory period and the maximal time interval of stimulation for impulse collision in the same manner as described in the previous study (Kawaguchi et al., 1979a). The values (mean ± S.D.) calculated from 7 neurons were 0.38 ± 0.25 msec for $t_1$, 0.50 ± 0.16 msec for $t_2$, and 0.66 ± 0.15 msec for $t_3$. The branch point is supposed to lie more distally to the soma than would be expected from these values since the conduction velocity is presumably much slower in the branch than in the stem axon (Shinoda et al., 1976).

Cerebellar nuclear neurons with antidromic activation were sampled from 9 hemicerebellectomized rats and 4 intact adult rats. For the 9 rats, ages at the time of hemicerebellectomy and postoperative days at the time of recording were as follows: 0 OP 67, 0 OP 74, 0 OP 106, 1 OP 82, 4 OP 111, 8 OP 131, 9 OP 270,

![Fig. 4. Latency distributions of antidromic response of cerebellar nuclear neurons. A, 5 rats hemicerebellectomized before 4 days of age; B, 4 rats hemicerebellectomized at 8 to 13 days of age; C, 4 intact adult rats. In each group, neurons activated contralaterally, those activated ipsilaterally, and those activated bilaterally are plotted in the upper, middle, and bottom rows, respectively. With bilateral neurons, latencies of contralaterally- and ipsilaterally-induced antidromic responses are distinguished as open and stippled columns, respectively.](image-url)
11 OP 150, and 13 OP 270. These animals were divided into 3 groups: group A consisted of 5 rats hemicerebellectomized at 0 to 4 days of age; group B, 4 rats hemicerebellectomized at 8 to 13 days of age; group C, 4 intact adult rats. The number of neurons sampled and latency distribution of the antidromic response in the 3 groups are illustrated in Fig. 4A-C. In each group, neurons activated only contralaterally, those activated only ipsilaterally, and those activated bilaterally are distinguished. Of the 120 units sampled in group A, 84 (70%) were activated only contralaterally, 28 (23.3%) only ipsilaterally, and 8 (6.7%) bilaterally. Eighty-nine (83.2%) of the 107 units from group B were activated only contralaterally, 12 (11.2%) only ipsilaterally, and 6 (5.6%) bilaterally. In group C, only one (0.8%) of the 118 sampled units was activated ipsilaterally, and the remaining 117 units (99.2%) were activated only contralaterally. The ratio of neurons activated bilaterally or ipsilaterally was significantly larger in group A (P<0.001) and in group B (P<0.05) than in group C. The difference between group A and group B was also significant (P<0.02).

With respect to the latency distribution of the antidromic responses in the hemicerebellectomized rats, the ipsilaterally or bilaterally activated neurons differed little from the contralaterally activated neurons. This is similar to the results obtained in the hemicerebellectomized kittens (Kawaguchi et al., 1979a).

The remarkable increase in the number of cerebellar nuclear neurons projecting bilaterally or ipsilaterally to the thalamus appears to account for the occurrence of a significant ipsilateral cerebello-cerebral response in the rats whose hemicerebellum had been removed before 6 days of age. The absence of such a response in the rats operated on after 8 days of age indicates that the increase in the number of bilateral or ipsilateral projection neurons in group B may not be sufficient to mediate a detectable cerebello-cerebral response. Alternatively, a synaptic connection of aberrant ipsilateral cerebellothalamic projection cannot be fully achieved in the late hemicerebellectomized rats.

**DISCUSSION**

The present results agree with morphological observations in rats (Lim and Leong, 1975; Leong, 1977; Castro, 1978) that the ipsilateral cerebellothalamic projection increased following neonatal hemicerebellectomy and are consistent with electrophysiological findings in kittens (Kawaguchi et al., 1979a) that the increased ipsilateral cerebellothalamic projection was responsible for occurrence of a marked ipsilateral cerebello-cerebral response in the operated animals. For abundance of ipsilateral cerebellothalamic projections in the operated animals, the following four possible explanations can be offered. 1) Ipsilateral cerebellothalamic fibers which might exist in a considerable number in the newborn period and normally disappear during development would persist as a result of hemicerebellectomy in an early postnatal period. 2) The pre-existing ipsilateral cerebellothalamic fiber terminals, even though quite sparse, might proliferate in the
thalamus deafferentated from crossed cerebellothalamic fibers. 3) Axon collaterals sprouted from contralateral cerebellothalamic fibers would be distributed ipsilaterally. 4) Some of the developing cerebellofugal axons would change their course and project ipsilaterally to the thalamus. The first possibility appears unlikely since distribution of cerebellar efferents of neonatal animals does not differ from that of adult rats (LEONG, 1977). As to the second possibility, the remarkable increase in the number of cerebellar nuclear neurons activated antidromically from the ipsilateral thalamus is difficult to ascribe to the proliferation of the pre-existing terminals as discussed in the previous study (KAWAGUCHI et al., 1979a). By the analogy of the results to those obtained in kittens (KAWAGUCHI et al., 1979a, b), the third possibility appears most likely, although we have no evidence to rule out the fourth possibility. The latter possibility was first speculated by LIM and LEONG (1975) on the basis of their finding that aberrant ipsilateral cerebellothalamic fibers take courses uncrossed from the cerebellum through the thalamus. However, their finding was disputed by CASTRO (1978) who observed recrossed ipsilateral cerebellothalamic fibers but no uncrossed fibers: the recrossed fibers are crossed once at the decussation of the brachium conjunctivum and recrossed in the thalamic massa interna, in agreement with the results in kittens (KAWAGUCHI et al., 1979a, b).

We wish to express our gratitude to Prof. K. Sasaki for his constant encouragement and helpful advice and to M. Ohara for editing the manuscript.

REFERENCES


Japanese Journal of Physiology