

Organization of Excitatory Inputs from the Cerebral Cortex to the Cerebellar Dentate Nucleus

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ABSTRACT: Intracellular recording was made from dentate nucleus neurons (DNNs) in anesthetized cats, to investigate cerebral inputs to DNNs and their responsible pathways. Stimulation of the medial portion of the contralateral pericruciate cortex most effectively produced EPSPs followed by long-lasting IPSPs in DNNs. Stimulation of the pontine nucleus (PN), the nucleus reticularis tegmenti pontis (NRTP) and the inferior olive (IO) produced monosynaptic EPSPs and polysynaptic IPSPs in DNNs. The results indicate that the excitatory input from the cerebral cortex to DNNs is at least partly relayed via the PN, the NRTP and the IO. Intraaxonal injection of HRP visualized the morphology of mossy fibers from the PN to the DN and the cerebellar cortex. The functional significance of the excitatory inputs from the PN and the NRTP to the DN is discussed in relation to the motor control mechanisms of the cerebellum.

RÉSUMÉ: Organisation des influx excitateurs originant du cortex cérébral vers le noyau dentelé. Des enregistrements intracellulaires ont été effectués dans des neurones du noyau dentelé (NNDs) chez les chats anesthésiés, pour investiguer les influx cérébraux vers les NNDs et les voies qui en sont responsables. La stimulation de la portion interne du cortex péricrucial contralatéral est la plus efficace pour produire des potentiels post-synaptiques excitateurs (PPSEs) suivis par des potentiels post-synaptiques inhibiteurs (PPSIs) dans les NNDs. La stimulation du noyau du pont (NP), du noyau du toit du quatrième ventricule (NTQV) et de l'olive inférieure (OI) produit des PPSEs monosynaptiques et des PPSIs polysynaptiques dans les NNDs. Ces résultats indiquent que les influx excitateurs provenant du cortex cérébral vers les NNDs sont transmis, au moins partiellement, par le NP, le NTQV et l'OI. L'injection intra-axonale de HRP a permis de visualiser la morphologie des fibres moussues du NP vers le ND et le cortex cérébelleux. Nous discutons de la signification fonctionnelle des influx excitateurs du NP et du NTQV vers le ND, en relation avec les mécanismes du contrôle moteur du cervelet.

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The cerebellar cortex receives two types of afferent fibers from outside the cerebellum; the mossy fibers and the climbing fibers.¹ Both of these afferent fibers mediate excitatory inputs to the cerebellum.² Deep cerebellar nucleus neurons and vestibular nucleus neurons give rise to output fibers that convey excitatory signals to other targets. These nuclei are impinged upon by inhibitory inputs from Purkinje cells in the cortex.^{3,4} It has been generally assumed that the excitatory inputs to the deep cerebellar nuclei are supplied through collaterals of afferents to the cerebellar cortex. In spite of a wealth of anatomical papers reporting the afferent pathways to the cerebellar cortex, there were far fewer studies on afferent projection to the deep cerebellar nuclei until recently.^{2,5-8} As for the projections from the precerebellar nuclei to the dentate nucleus (DN),^{9,10} the existence of the projection from the pontine nucleus (PN) to the DN is most controversial. Dietrichs et al.¹¹ could not positively demonstrate the afferent projection from the PN to the DN with a wheat germ agglutinin-horseradish peroxidase (HRP) injection

method. Therefore, they doubted the results obtained using HRP as a retrograde tracer, since massive fibers projecting to the cerebellar cortex in and outside the DN damaged by an injection needle might take up HRP leaking along an electrode track. Cerebral inputs to the DN and the interpositus nucleus (IN) have been examined by Allen et al.,^{12,13} using an extracellular recording method. Although both facilitation and depression of spike activities were induced in dentate nucleus neurons (DNNs) by stimulation of various parts of the cerebral cortex, it was not possible to determine whether facilitation of spike activities was caused by either excitatory postsynaptic potentials (EPSPs) or disinhibition, and their depression by either inhibitory postsynaptic potentials (IPSPs) or disfacilitation. The only previous intracellular recording from DNNs was reported by Ito et al.⁴ They found that stimulation of the ipsilateral PN produced monosynaptic EPSPs with a very small amplitude in 5 DNNs in the cat. Dietrichs et al.¹¹ were doubtful of that electrophysiological result, since it was not clear from that report whether

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stimulus effects were localized in the PN. The cerebellar projection from the contralateral PN and nucleus reticularis tegmenti pontis (NRTP) is dominant and the fibers from these nuclei pass through the ipsilateral PN. Therefore, the origin of the monosynaptic EPSPs evoked by stimulation of the ipsilateral PN remains to be determined.

The present study was undertaken to analyze excitatory inputs to DNNs from the cerebral cortex and to determine the pathways from the cerebral cortex to the DN, especially to determine the presence or absence of the excitatory projection from the PN, the NRTP and the inferior olive (IO) to the DN. The results show that monosynaptic connections indeed exist from the contralateral PN, NRTP and IO to the DN, and that the excitatory inputs from the cerebral cortex are conveyed to the DN via these precerebellar nuclei. A part of this study was reported before.¹⁴

METHODS

Experiments were performed on cats weighing 2.8 - 4.3 kg. The animals were anesthetized with sodium pentobarbital (Nembutal, Abbott, Switzerland; 35 mg/kg and supplemented as required), and were mounted in a stereotaxic apparatus. The animals were paralyzed by intravenous injection of pancuronium bromide (Mioblock, Organon Co.) and artificially ventilated. A

craniotomy was performed to expose the cerebral cortex, and stimulating electrodes (concentric bipolar metal electrodes) were inserted into the red nucleus (RN), and the cerebral peduncle (CP) or areas 4 and 6 on the left side (Figure 1 inset). The occipital bone was removed and stimulating electrodes of the same type were stereotaxically placed in the PN and the IO on the left side.

Cerebral influences on DNNs were investigated with intracellular recording from DNNs by stimulating the cerebral cortex and the CP. An array of 9 concentric electrodes was positioned in the gray matter of the precruciate gyrus and the lateral portion of the postcruciate gyrus (Figure 2A). For PN stimulation, 2 or 3 concentric electrodes were inserted stereotaxically from the dorsal surface of the cerebellar cortex into the target ($A = 2.0$ mm - $P = 2.0$ mm; $L = 0.3 - 1.5$ mm) at an angle of about 30° inclined posteriorly in the sagittal plane. For systematic mapping of the effective stimulating sites in the PN and NRTP, a contact array of five fine wires was made by gluing together $200 \mu\text{m}$ wires insulated except for the exposed tips (0.3 mm exposure), with their exposed ends separated from one another by $0.6 - 1.3$ mm longitudinally. Two arrays of these multipolar electrodes were positioned in the PN and NRTP (see Figure 3C). The stimulating electrode positions in the PN were verified histologically.¹⁵ To examine the influence of the IO on DNNs, two concentric

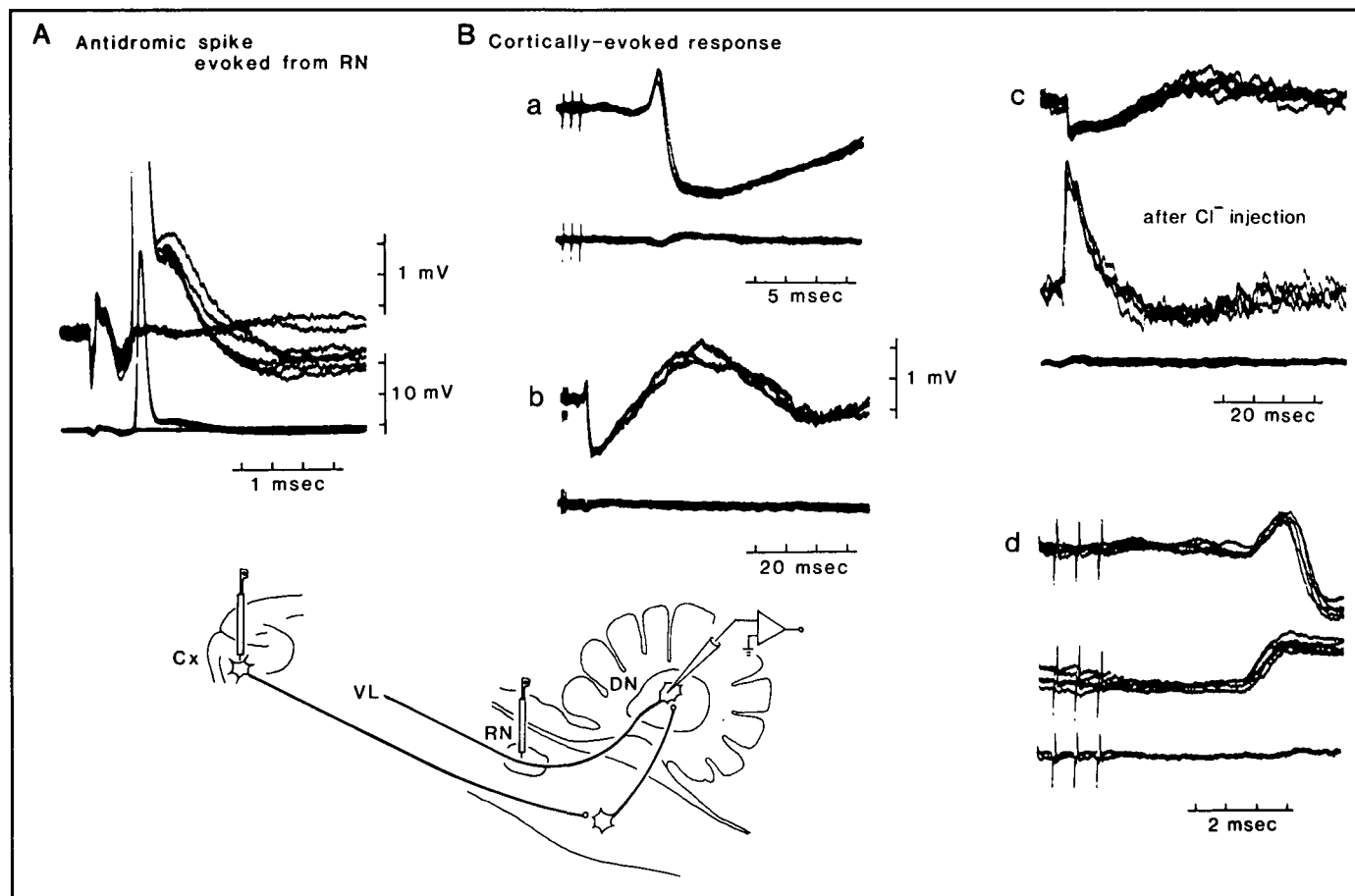


Figure 1 — Cerebral inputs to single DNNs. **A:** Antidromic spikes evoked in an all-or-none manner in response to threshold straddling stimulation ($200 \mu\text{A}$) in the RN. **B:** Intracellular records from a DNN following stimulation of the cerebral cortex. *a-b:* Postsynaptic potentials of depolarization-hyperpolarization sequence evoked from the contralateral area 6 at fast (*a*) and slow sweep speeds (*b*) (stimulating site 3 in Figure 2A). **C:** Reversed IPSPs recorded after Cl^- injection into the cell by hyperpolarizing current. **D:** Early EPSPs remained unchanged after Cl^- injection.

electrodes were positioned in the center or the medial border of the IO.

For stimulation, rectangular negative pulses of 0.2 msec duration were applied bipolarly through individual concentric electrodes placed in the cerebral cortex, the CP, the RN, the PN and the IO or monopolarly through each electrode of the electrode arrays in the PN and the NRTP relative to an indifferent electrode in the temporal muscles. For cortical stimulation, single pulses of 0.5 - 1 mA were not always effective to evoke responses in DNNs, and so double or triple pulses at 1.5 msec intervals were usually used. For stimulation of the PN and the NRTP, the currents of 500 μ A or less were applied with concentric electrodes, and the currents of 300 μ A or less with monopolar electrodes. Intracellular recording was made from DNNs on the right side with glass micropipettes filled with 3M KCl (15 - 25 M Ω in resistance).

At the end of each experiment, electrolytic lesions were made by passing negative currents of 30 μ A for 20 sec through

each stimulating electrode. The animal was perfused with 10% formaldehyde and the brain was removed for later histological examination. The locations of lesions were determined on 100 μ m serial frozen sections of the brain stem stained with cresyl violet. In HRP staining experiments, the animal was perfused with 0.1 M phosphate buffer solution and then with a mixture of 1% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4).¹⁶ Serial coronal frozen sections of 100 μ m thickness were treated for HRP, using the diaminobenzidine method. Three dimensional reconstruction of the neuronal trajectory was performed on these serial sections.

RESULTS

To analyse cerebral inputs to the DN and their pathways, intracellular recording was made from 201 efferent DNNs, in which postsynaptic potentials (PSPs) were examined by stimulation of various portions of the cerebral cortex and the

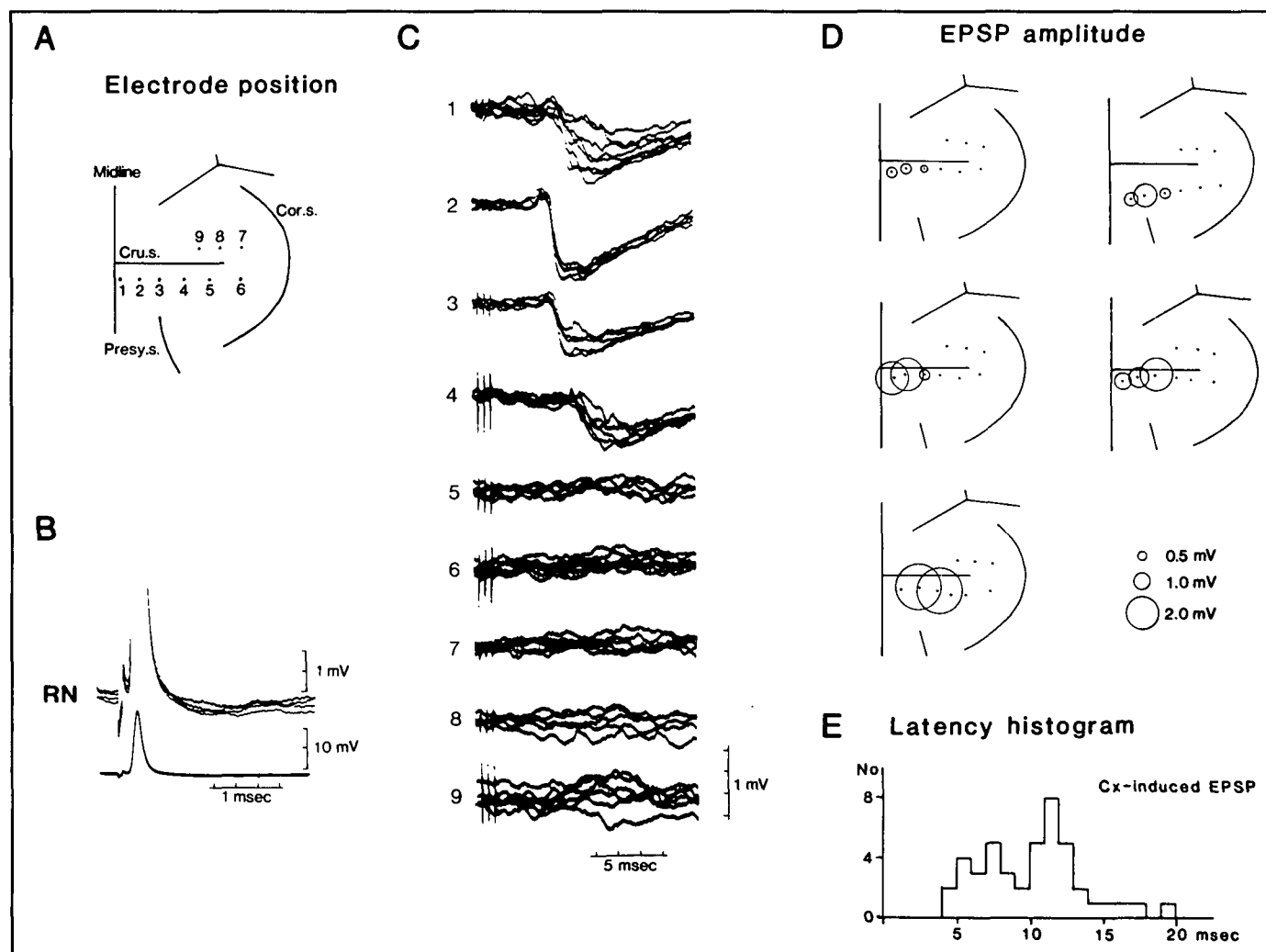


Figure 2 — Distribution of effective stimulating sites in the cerebral cortex to induce PSPs in DNNs. A: Arrangement of 9 stimulating electrodes in the pericruciate cortex. cru.s.; cruciate sulcus. presy.s.; presylvian sulcus. cor.s.; coronal sulcus. B: Antidromic spikes evoked by stimulation of the RN at suprathreshold. C: Distribution of cortical inputs to a single DNN. Responses to stimulation of the cortical sites (1-9) indicated in A. PSPs were only evoked from the most medial four electrodes. D: Diagrams showing the distribution of effective sites for evoking EPSPs in 5 DNNs. The diameter of a circle is proportional to the amplitude of an EPSP evoked from each cortical site. Dots without circles represent the sites from which we failed to evoke EPSPs. A train of three pulses at 1 mA was used to evoke EPSPs. E: Latency histograms of cerebral-evoked EPSPs in DNNs.

precerebellar nuclei. Efferent DNNs were identified by their antidromic responses to stimulation of their output fibers within the contralateral RN (Figure 1A).^{17,18} Out of 143 DNNs examined on their caudal projection, 69 DNNs were activated antidromically only from the RN, 54 (37.8%) DNNs were activated antidromically from the PN or the NRTP, but not from the IO and 20 (14.0%) DNNs were antidromically activated from the IO.

Cerebral Inputs to Dentate Nucleus Neurons

Cerebral effects on DNNs were investigated by stimulating areas 6 and 4 on the contralateral side. Stimulation of the medial portion of the precruciate gyrus produced depolarization followed by long-lasting hyperpolarization (Figure 1Ba). This hyperpolarization was further followed by long-lasting depolarization (Figure 1Bb). Iontophoretic injection of Cl⁻ into a DNN reversed the hyperpolarizing potential to the depolarizing poten-

tial and its following depolarizing potential to the hyperpolarizing potential (Figure 1Bc), but the early depolarization remained unaffected (Figure 1Bd). Therefore, we concluded that the early depolarization was an EPSP, the long-lasting hyperpolarization was an IPSP and the late depolarization was disinhibition.¹⁹

To determine the effective cortical area able to induce responses in DNNs, the stimulating electrodes were arranged in such a way as shown in Figure 2A and the effective stimulating sites were determined from which EPSPs could be evoked at a stimulus intensity of 1 mA. In a typical example shown in Figure 2C, shortest-latency and largest-amplitude EPSPs and IPSPs were evoked from the position 2, and slightly smaller- and longer-latency EPSPs and IPSPs were evoked from the adjacent positions 1 and 3. Much longer-latency IPSPs were evoked from the position 4. In five other examples shown in Figure 2D, the effective stimulating sites were mainly found in the medial portion of the precruciate gyrus, which corresponds

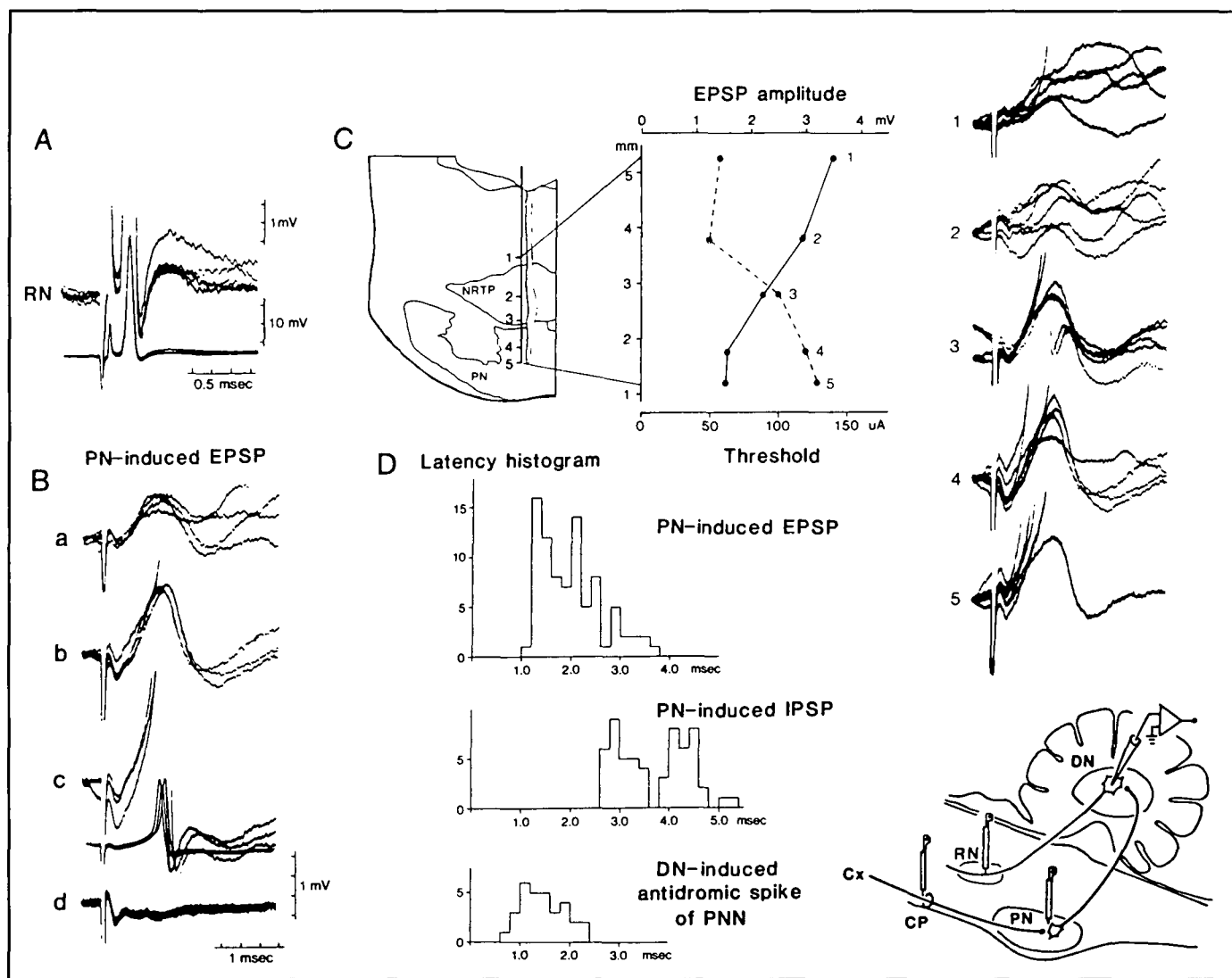


Figure 3 — Inputs from the PN to DNNs. **A:** Antidromic spikes in response to stimulation in the RN. **B:** Effects of stimulus strength on the responses of a DNN. Stimulus intensity increased from 100 μ A (a) to 200 μ A (b) and 300 μ A (c). **C:** Transverse section through the pons angled at 38° posteriorly to the vertical plane, showing the relationship between the amplitude (dotted line) and the threshold (solid line) of the EPSPs relative to stimulating sites in the PN and NRTP. **D:** Latency histograms of EPSPs and IPSPs evoked in DNNs from the PN and extracellular spikes of PNNs evoked from the DN.

to area 6a β and its adjacent area 4 γ of Hassler and Muhs-Clement,²⁰ although weak responses were evoked from the lateral portion of the precruciate gyrus in some DNNs. The shortest latency of the cortically-evoked EPSPs for individual DNNs ranged from 4.0 to 20.4 msec (mean \pm S.D., 9.7 ± 3.5 msec, $N = 44$) (Figure 2E) and that of the cortically-evoked IPSPs ranged from 8.0 to 27.0 msec (13.4 ± 2.6 msec, $N = 49$).

Inputs From the PN and the NRTP to Dentate Nucleus Neurons

To determine the pathways from the cortex to the DN, we searched for the precerebellar nuclei to mediate the cortical inputs to the DN. Effects of PN stimulation on DNNs will be described first. Stimulation of the contralateral PN evoked short-latency EPSPs followed by IPSPs (Figure 3B). The EPSPs had latencies of 1.1 - 3.6 msec (mean \pm S.D., 2.0 ± 0.6 msec, $N = 84$) and the IPSPs had latencies of 2.6 - 5.3 msec (3.7 ± 0.7 msec, $N = 58$) (Figure 3D). To identify the PN as a site responsible for generation of the EPSPs in DNNs, systematic mapping for effective stimulating sites in the PN and the NRTP was performed. Figure 3C shows the stimulated sites in the PN and NRTP. When a DNN was penetrated, the most effective site in the PN or the NRTP was sought by measuring the amplitudes of EPSPs evoked at the same stimulus intensity, and the thresholds for the EPSPs in individual stimulating sites. In the DNN exemplified in Figure 3C, stimulation of the NRTP produced small-amplitude EPSPs in the DNN and the thresholds for the EPSPs were high. When the more ventral portion of the PN was stimulated, larger EPSPs were evoked and the thresholds for the EPSPs became much lower. In this way, the lowest threshold site and the maximum response site to evoke EPSPs were identified within the PN for 39 DNNs.

To demonstrate that cerebral signals are conveyed to DNNs via PN neurons (PNNs), we used a conditioning-testing paradigm and examined the interaction between CP-evoked and PN-evoked EPSPs. The existence of spatial facilitation was examined in the DNNs with monosynaptic EPSPs evoked from the PN. Stimulus intensities to the CP and the PN were decreased and the time of the PN stimulus was delayed so that the EPSPs from the CP and the PN were evoked in the DNN at the same onset. Combined stimulation of the CP and the PN evoked much larger EPSPs than the sum of the control EPSPs evoked separately by the CP and the PN (not illustrated). This spatial facilitation should occur only when the PN stimulus activates cell bodies of PNNs rather than their axons. Similar spatial facilitation was observed in 5 out of 8 DNNs. Therefore, we conclude that there is an excitatory input to DNNs from the cerebral cortex by way of the PN.

To examine whether neurons in the NRTP terminated on DNNs or not, effective stimulating sites were carefully sought in the NRTP in the same experimental arrangement as in the PN. In an example shown in Figure 4F, the thresholds of EPSPs were plotted at individual stimulating sites for 13 DNNs. The most effective stimulating site for the monosynaptic EPSP was found in the NRTP and/or the PN in individual DNNs. Twenty-one DNNs were identified as receiving EPSPs from the NRTP, since they were evoked from the electrode in the NRTP with the lowest threshold. The latencies of the EPSPs were 1.1 - 2.5 msec (1.6 ± 0.4 msec, $N = 21$) and those of the IPSPs were 2.7 - 4.5 msec (3.4 ± 0.7 msec, $N = 11$). The spatial facilitation

between CP-evoked EPSPs and NRTP-evoked monosynaptic EPSPs was observed in 3 out of 5 DNNs.

Inputs From the IO to Dentate Nucleus Neurons

Stimulation of the contralateral IO produced long-latency depolarization followed by long-lasting hyperpolarization in a DNN (Figure 4Aa, Ba). Injection of Cl⁻ into the cell reversed the late long-lasting hyperpolarization to the depolarizing potential, but the early depolarization remained unchanged (Figure 4Ab and Bb). Therefore, these potentials were considered to be EPSPs and IPSPs, respectively. Around the peak of the IPSPs, notches were occasionally found. This indicates that there are at least two synchronous inhibitory presynaptic bombardments to DNNs. With the increase of stimulus intensity, the amplitude of EPSPs gradually increased and orthodromic spikes were generated, when the EPSPs exceeded firing threshold (Figure 4C). The latencies of the EPSPs, however, remained constant even near the threshold, strongly suggesting that these EPSPs were induced monosynaptically from the contralateral IO. The latency of the EPSPs evoked from the IO ranged from 2.0 to 5.9 msec (mean \pm S.D., 3.4 ± 0.8 msec, $N = 121$). In order to determine that some of the EPSPs evoked from the cerebral cortex were induced by way of the IO, interaction of IO-evoked EPSPs and CP-evoked EPSPs was examined in a DNN. Conditioning stimulation of the CP facilitated the monosynaptic EPSPs evoked from the IO (not illustrated). The existence of this spatial facilitation of the inputs strongly suggested that the CP-evoked EPSPs were partly mediated by way of the IO. The latency of the IPSPs evoked from the contralateral IO ranged from 3.6 to 8.4 msec (5.5 ± 0.9 msec, $N = 133$). Many IPSPs had latencies between 5.0 and 7.0 msec. These IPSPs were assumed to be produced by way of Purkinje cells, since climbing fibers originating from the IO excite Purkinje cells in the cerebellar cortex² and Purkinje cells, in turn, project to cerebellar nucleus neurons.⁴

Single DNNs were often found to receive convergent inputs from the PN, the NRTP and the IO as shown in Figure 4D - E. The input from the IO was most prominent and most stable among three inputs and the latencies of the EPSPs evoked from the NRTP (Figure 4D) or the PN (Figure 4E) were much shorter than those from the IO (Figure 4C) in individual DNNs.

The Morphology of Single PN Axons in the DN

To confirm the existence of mossy fiber projections from the PN to the DN morphologically, mossy fibers were visualized by intraaxonal staining with HRP.¹⁶ Single axons were penetrated in the white matter of the cerebellum and were identified electrophysiologically as PNN axons by their direct responses to stimulation of the contralateral PN, their synaptic activation from the contralateral cerebral cortex and the decrease in threshold for evoking direct spikes in stimulation of the PN by conditioning stimuli applied in the cerebral cortex. After this electrophysiological identification, HRP was iontophoretically injected into the axons. Figure 5 shows an example of the axonal trajectory of axon collaterals of a mossy fiber arising from the contralateral PN. The stem axon of this PNN was traced to the granular layer of the cerebellar cortex and it terminated there as typical mossy fiber rosettes. On its way, the axon collaterals were given off to the DN. The diameters of these collaterals were very thin compared with the large diameter of the parent

axon. These results provided morphological evidence for the existence of the projection from the PN to the DN.

DISCUSSION

Excitatory Inputs From the Precerebellar Nuclei to the DN

The present study demonstrated that at least three precerebellar nuclei, the PN, the NRTP and the IO, convey excitatory inputs to DNNs. Localized stimulation of the PN and the NRTP evoked short-latency EPSPs in DNNs (Figures 3 and 4). These EPSPs were monosynaptic for the following reasons; 1) short latencies ranging from 1.1 to 2.7 msec (Figures 3D and 4D, E), 2) constant latencies even at near-thresholds (Figure 3B) and 3) latencies that were compatible with antidromic latencies of PNNs evoked from the contralateral DN (0.7 - 2.4 msec), making an allowance of 0.3 - 0.4 msec for a synaptic delay (Figure 3D). The most effective stimulus sites in the PN and NRTP were determined by measuring thresholds for evoking EPSPs and amplitudes of the EPSPs and the threshold minima were found

in the PN or the NRTP or both and the lowest thresholds were as low as 20 μ A. Taken together, the results indicated that neurons in the PN and the NRTP project to the DN. The morphological study confirmed the projection from the PN to the DN (Figure 5),¹⁶ and further showed that this projection is by way of collaterals of mossy fibers terminating in the cerebellar cortex.

A third source of the excitatory input to the DN is the IO. Electrophysiologically, Ito et al.⁴ found that stimulation of the contralateral IO produced EPSPs in DNNs. The present study confirmed their result and also the result obtained by Amatuni et al.²¹ with an extracellular recording method. These EPSPs are most likely to be mono-synaptic for the following reasons. 1) The latencies of the EPSPs were constant, when the stimulus strength was varied (Figure 4C). 2) Antidromic spikes of olivary neurons were evoked from the cerebellar cortex at a latency from 2.8 to 5.5 msec.²² Judging from the small diameter of climbing fibers and the slow conduction velocity of the antidromic spikes, the latency of the IO-induced EPSPs (2.0 - 5.9 msec) is compatible with the latency of the monosynaptic

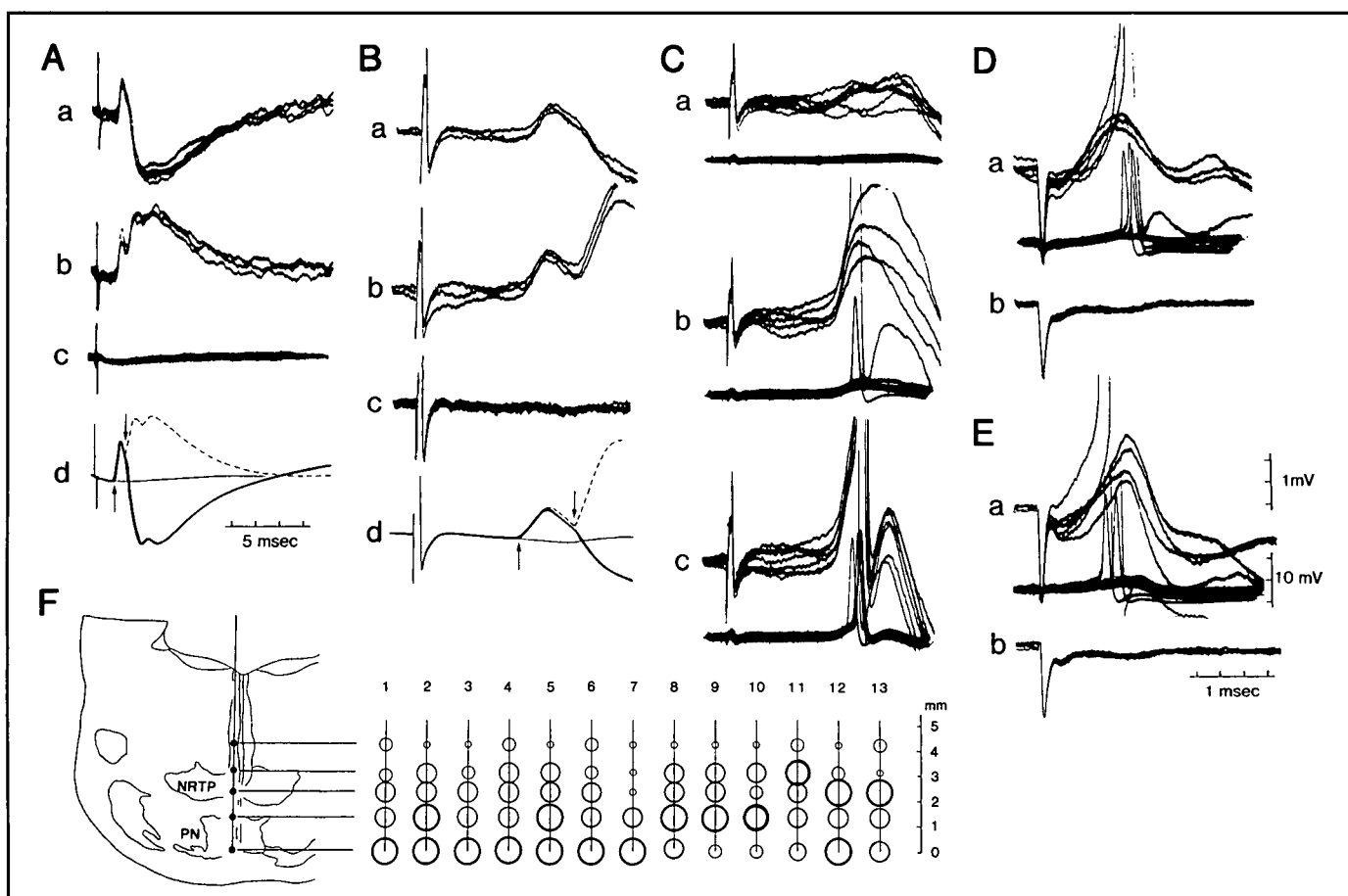


Figure 4 — Intracellular responses of DNNs to stimulation of the IO. **A:** Responses to IO stimulation at a long time scale. *a:* Control. *b:* Reversal of hyperpolarization after iontophoretic injection of Cl⁻. *c:* Field potential recorded just outside the cell. *d:* Superposition of three traces (*a*, *b*, *c*) to determine the onsets of EPSPs and IPSPs. The upward and the downward arrows indicate the onsets of the EPSPs and the IPSPs, respectively. **B:** Long-latency IPSPs evoked from the IO. *a:* Intracellularly-recorded IO-evoked EPSPs and IPSPs. *b:* The reversed IPSPs after Cl⁻ injection. *c:* Field potentials. *d:* Determination of the onsets of the EPSPs (upward arrow) and the IPSPs (downward arrow) by superimposing the three traces shown above. **C:** Responses evoked by stimulation of the contralateral IO and effects of the stimulus strength on PSPs. Stimulus intensities; 600 μ A(*a*), 650 μ A(*b*), and 900 μ A(*c*). Time calibration in E is applied to all traces except for A. **D-E:** Stimulation of the NRTP (D) and the PN (E) evoked EPSPs followed by IPSPs with superimposed orthodromic spikes in some traces (*a*). *b:* Juxtacellular field potentials. **F:** Effects of stimulation of the PN and the NRTP in 13 DNNs. Five sites illustrated in the pons on the left were stimulated. The largest and the smallest circles indicate the thresholds for the EPSPs at 50 μ A and 300 μ A, respectively.

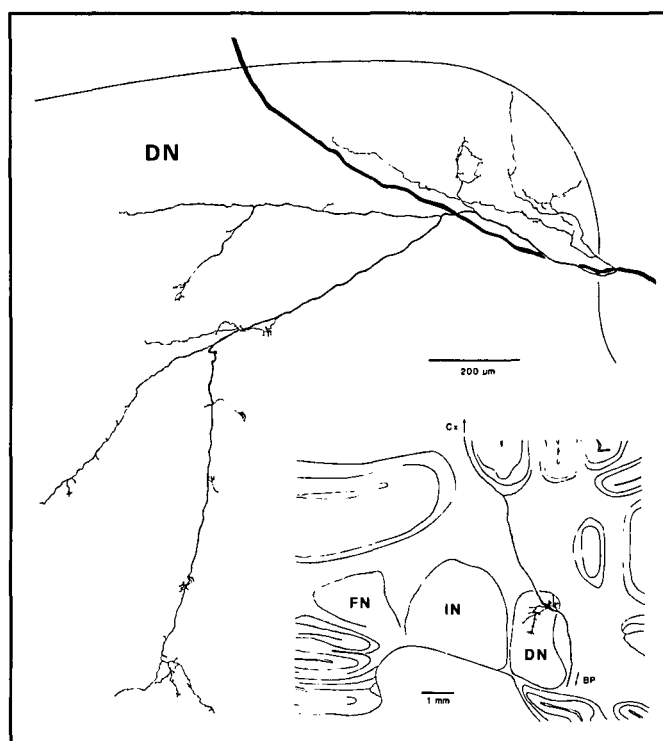


Figure 5 — Reconstruction of two axon collaterals arising from a single node of Ranvier of a parent PN axon stained intraaxonally with HRP. The inset on the right shows the axonal trajectory of the parent mossy fiber axon.

EPSPs. In fact, the projection from the IO to the DN has been anatomically suggested⁸ and recently confirmed with the autoradiographic method.²³ This projection is completely contralateral,²⁴ and there seems to be a topographical arrangement between the IO and the cerebellar nuclei; the DN receives the input from the principal olive and the posterior IN and the anterior IN receive inputs from the dorsal and the medial accessory olives, respectively.⁵⁻⁷

Cerebral Inputs to the DN

The present study showed that DNNs receive inputs mainly from the medial portion of the precruciate gyrus (area 6aβ) and its adjacent middle portion of the precruciate gyrus (area 4γ). This result is partly in agreement with the results obtained in the monkey,¹³ in that the DN and the IN receive main inputs from the premotor and the supplementary motor regions of area 6, and the sensorimotor cortex (area 4 and the first somatosensory cortex), respectively. Allen et al.¹³ also found that there was a great deal of convergence of the inputs from areas 4 and 6 to DNNs. However, convergence of the inputs from areas 4 and 6 to single DNNs was rare in the present study. This discrepancy between our results and Allen et al.'s result might be due to several factors (see the details in Shinoda et al.¹⁴).

The neural pathways mediating EPSPs and IPSPs to the DN are assumed to be multifold. The PN receives cerebral inputs from all cortical areas and there is a topographical relationship.²⁵⁻²⁷ The NRTP also receives inputs from all cortical areas.^{28,29} Cortical inputs to PNNs were extensively analysed electrophysiologically.³⁰⁻³³ The majority of PNNs are innervated by axon collaterals of PTNs.³² In previous studies, differential

inputs from areas 4 and 6 to single PNNs have not been analysed. In a recent study of corticofugal neurons in areas 4 and 6, it became clear that there are two groups of PN-projection neurons; corticopontine neurons (CPNs) and PTNs with axon collaterals to the PN.³⁴ CPNs in areas 6 and 4 make up 64.6% and 30.4% of the total PN-projecting neurons, respectively. The latency of EPSPs evoked in DNNs from area 6 was fairly long (4.0 - 20.5 msec) (Figure 2F). This long latency of the EPSPs is partly explained by the finding that, among PN-projecting neurons, slow CPNs are predominant in area 6.³⁴ Furthermore, slow PNNs receive inputs from slow PN-projecting corticofugal neurons and fast PNNs receive inputs from fast PN-projecting neurons.^{31,33} Consequently, PNNs receiving the input from area 6 must have slow conducting axons. Therefore, PNNs with slow conducting axons may be another cause for the longer latency of the EPSPs produced in DNNs following cortical stimulation. The IO also receives cortical inputs.³⁵⁻³⁷ Recent studies suggested the relatively greater contribution of area 6 to olivary afferents compared with area 4.^{38,39} Stimulation of the precruciate cortex induces spikes in inferior olivary neurons at a latency of 8 - 20 msec.^{22,40} This response may be mediated by two pathways; a direct cortico-olivary and an indirect cortico-rubro-olivary pathway.⁴⁰ Since olivary neurons are activated at a long latency from the cortex, the EPSPs and the IPSPs evoked from the cerebral cortex with the shorter latencies could not be mediated via the IO, but those with longer latencies might be mediated via the IO.

Functional Consideration of Excitatory Inputs to the DN

During the slow tracking movement of the wrist flexion and extension, both Purkinje cells⁴¹ and neurons in the DN and IN⁴² were found to show a bidirectional increase of spike activities rather than a reciprocal pattern. In rapid alternating movements of a forelimb, nuclear cells showed reciprocal changes of activities above and below a resting mean frequency,^{43,44} whereas Purkinje cells had a bidirectional discharge pattern (predominantly bidirectional increase).⁴¹ The neural mechanism of this increase of discharge frequency in cerebellar nuclear cells is an unsolved important question of cerebellar physiology. There are two possible pathways to produce this facilitation (Figure 6). If there is no excitatory input to the DN, nuclear cell firing rates will be caused to change solely by changes in Purkinje cell firing rates; an increase in nuclear cell discharge will be caused by disinhibition through Purkinje cells (Figure 6A). On the other hand, if there is an excitatory input of extracerebellar origin to the DN, it will cause an initial increase of nuclear cell discharge (Figure 6B). Since Purkinje cells are inhibitory,³ a decrease of their activity will produce an increase in nuclear cell discharge by disinhibition. However, most Purkinje cells increase their activity at the onset of movement.^{41,43} Accordingly, other excitatory inputs of extracerebellar origin must exist to increase nuclear cell discharge at the onset of movement. In many textbooks, the projections of axon collaterals of mossy fibers or climbing fibers to the deep cerebellar nucleus had been described in spite of no experimental data until recently. Allen and Tsukahara⁴⁵ were very thoughtful, because they did not include the projection of mossy fibers to the deep cerebellar nucleus in their diagrams after very extensive review of the literature. The existence of the projections of mossy fibers to the cerebellar nucleus had been controversial.^{11,46-48} However, we demonstrated that

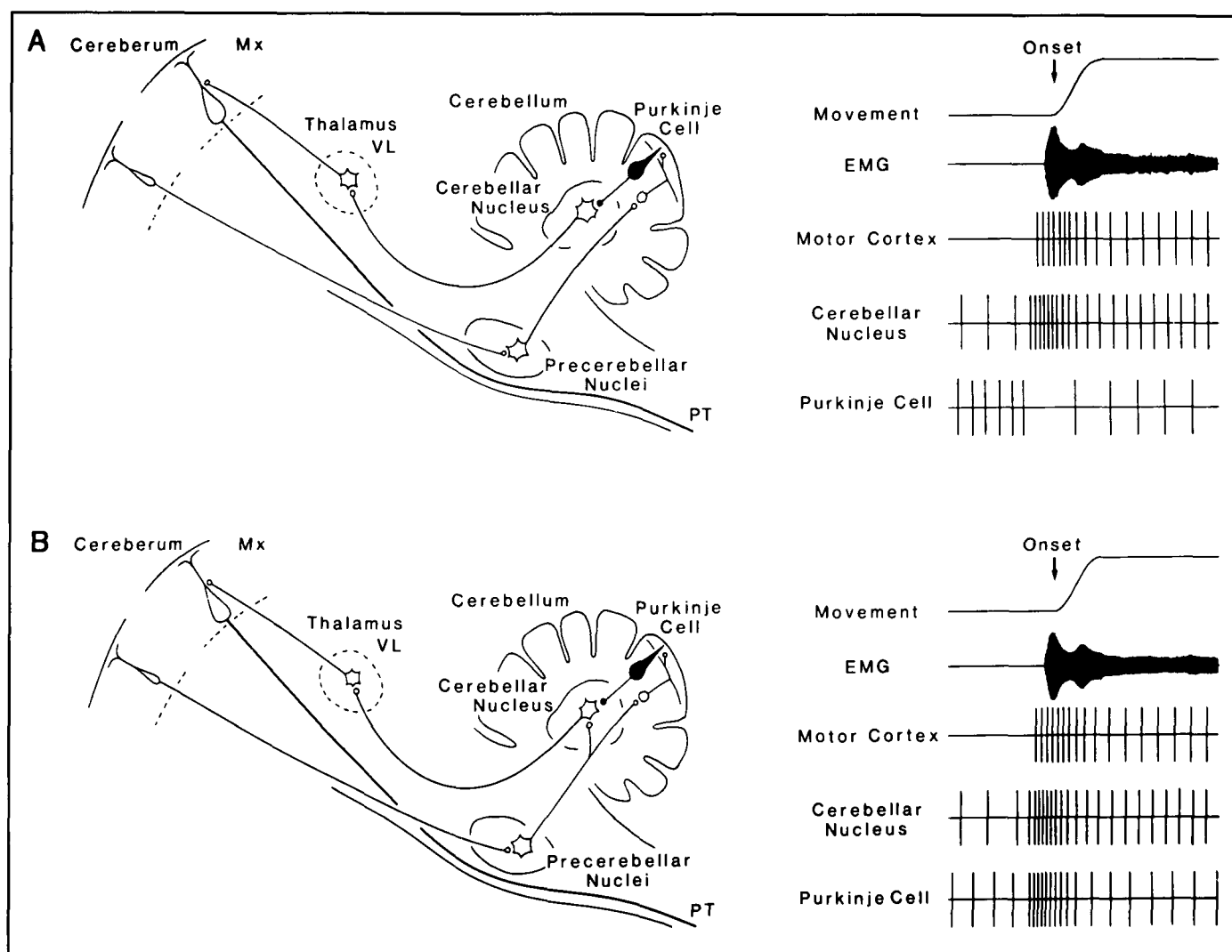


Figure 6 — Two possible operational modes of cerebellar corticonuclear interaction in the DN in relation to mossy fiber inputs from the cerebral cortex.

there are excitatory pathways from the PN, the NRTP, and the IO to the DN.¹⁴ Our study has also given morphological evidence for the existence of mossy fiber collaterals from PN axons to the DN.¹⁶ Gerrits and Voogd⁴⁹ clearly demonstrated the projection from the NRTP to the DN. Therefore, these results suggest that excitatory inputs from the PN and the NRTP to the DN could at least partly contribute to the increase of activity of cerebellar nucleus neurons. In addition to these excitatory inputs, signals conveyed from these precerebellar nuclei exert their modulatory influences onto efferent DNNs via Purkinje cells. These Purkinje cells could impose efficient modulation of activity on nuclear cells by inhibition or disinhibition.

The question remains as to whether the input by way of these mossy fiber collaterals or climbing fibers is strong enough to change neuronal discharge in DN neurons. Spike potentials in output neurons of the DN could be often evoked by stimulation of the cerebral cortex, the PN (Figure 3B) and the IO (Figure 4C) and these spikes were generated by EPSPs.^{13,14} Accordingly, the excitatory input from the PN or the IO is not so weak to generate spike activities in DN neurons. In addition, cerebellar

nucleus neurons have high spontaneous or background activities.⁴³ It has been suggested that the background discharge of cerebellar nucleus neurons is generated by a continuous excitatory barrage along input lines, or nuclear neurons themselves may be generating the discharge without any inputs.⁴³ Recent study in a slice preparation supports the idea of the latter mechanism by the electroresponsive properties of the membranes of nuclear neurons contributes to the background discharge of nuclear neurons.⁵⁰ Furthermore, Llinás and Mühlethaler⁵¹ demonstrated the existence of a powerful low threshold Ca^{2+} -dependent spike which triggered a burst of fast Na^{+} -dependent spikes in cerebellar nucleus neurons. Therefore, the firing properties of cerebellar nucleus neurons may be markedly modulated by their resting membrane potential. On account of the underlying spontaneous activity of cerebellar nucleus neurons, changes of spike activities in cerebellar nucleus neurons can be easily generated by slight modulation of the membrane potential by excitatory or inhibitory inputs. Taken together, the excitatory inputs by way of mossy fiber collaterals of the PN and the NRTP origin are thought to be effective enough to modulate

spike activities of nuclear output neurons. As shown above, inputs from the cerebral cortex are conveyed to the DN by way of several parallel pathways. An important question remains open as to what kinds of information is carried to the DN by way of these individual pathways from the cerebral cortex.

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