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Functional Organization of the Vestibular Afferents to the Cerebellar Cortex of Frog and Cat

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Summary. 1. Field and unitary potentials evoked in the vestibulo-cerebellum of frog and cat following vestibular nerve stimulation were recorded with microelectrodes and correlated with their site of origin in the various layers of the cerebellar cortex.

2. In the frog, primary vestibular fibers project both as mossy and as climbing fibers onto the cerebellar auricular lobe. Secondary vestibulo-cerebellar fibers seem to end exclusively as mossy fibers in the auriculum. As a consequence of this dual projection, extra- and intracellular recordings from Purkinje cells in the auricular lobe show two kinds of responses to vestibular nerve stimulation: a) graded, repetitive firing mediated through mossy fiber-granule cell-parallel fiber pathways, and b) all-or-none burst responses caused by monosynaptic impingement of vestibular climbing fibers on Purkinje cells.

3. The field and unitary potentials evoked in the cat nodulus, flocculus and uvula following vestibular nerve stimulation are shown to be generated by mossy fibers exclusively. Considerable convergence of the two labyrinthine mossy fiber inputs to a given cerebellar area was found.

4. Interaction of contralateral and ipsilateral mossy fiber input at the level of the flocculus suggests that Golgi cell inhibition might operate not only as a simple inhibitory feedback loop, but also as a complex gating operator at the granule layer.

5. No short latency climbing fiber activation of Purkinje cells was observed following VIIIth nerve stimulation. Stimulation of the contralateral inferior olive evoked short latency climbing fiber EPSPs in Purkinje cells of the vestibulocerebellum. Suggestions are made as to the possible role of mossy and climbing fiber inputs to this area of the cerebellum.

Key Words: Climbing fibers — Mossy fibers — Vestibulo-cerebellar input — Cat — Frog — Golgi cell inhibition

Introduction

Comparative anatomical studies have demonstrated a close relationship between the vestibular apparatus and the cerebellum. In fact, the cerebellum originates from the rhomboidal lip of the IVth ventricle by an upward and medial expansion of both octavolateral areas, a process which finally results in a cerebellar arc covering the roof of the IVth ventricle (Herrick 1924; Larsell 1923, 1925, 1929, 1934). A close inter-relationship between the vestibular system and

the cerebellum is further suggested by the fact that important primary and secondary vestibular projections to the cerebellum are present in all vertebrates so far studied (for reviews see BRODAL and TORVIK 1957; BRODAL and HOIVIK 1964). In lower vertebrates, the vestibular fibers constitute the main source of afferents to the cerebellar auricular lobe, an area which is located at the lateral border of the corpus cerebelli. As the cerebellum develops further, the auricular lobe maintains its functional relation with the vestibular apparatus and becomes the floccular lobe of birds and mammals (LARSELL 1923, 1925). More detailed studies of the distribution of vestibulo-cerebellar fibers in higher vertebrates showed that the term "vestibulo-cerebellum" includes a wider area of the cerebellum than had been originally assumed on the basis of comparative anatomical studies. Thus besides the flocculonodular lobes, the ventral part of the uvula and certain parts of the paraflocculus also belong to the vestibular area of the cerebellar cortex (BRODAL and HOIVIK 1964). In general vestibulo-cerebellar relations are also characterized by the fact that most subdivisions of the vestibulo-cerebellum send, in turn, fibers to the vestibular nuclei (DOW 1936; ANGAUT and BRODAL 1967) or even, as in the case of the frog, to the sensory cells of the vestibular labyrinth (LLINÁS, PRECHT and KITAI 1967 b; and LLINÁS and PRECHT 1968).

Although numerous morphological studies of the vestibulo-cerebellar system have been performed, very little work has been done concerning the detailed physiological analysis of this input (DOW 1939). The experiments to be described here were undertaken in order to provide an analysis of the mode of termination of primary and secondary vestibular fibers in the cerebellar cortex of the frog and cat. A comparative physiological approach has been chosen in order to study whether any changes in the synaptic organization of this old part of the cerebellum have occurred as it developed from lower to higher forms. A preliminary report of the frog data has been presented by LLINÁS, PRECHT and KITAI (1967 a).

Methods

Bullfrogs *(Rana catesbiana)* were anesthetized with pentobarbital sodium (60 mg/kg of body weight). A craniotomy was performed exposing the cerebellum and its auricular lobe. The labyrinthine cavity was carefully opened by a dorsal approach to allow extracranial dissection of the anterior and posterior branches of the vestibular nerve. Bipolar concentric stimulating electrodes were gently placed on the surface of the vestibular nerve branches at lateralmost positions. A stimulating electrode was also placed on the surface of the auricular lobe (Loc in Fig. 1) and was used to stimulate the parallel fiber system. In several experiments the membranous labyrinth containing the endolymph was also stimulated mechanically by a fine stylus attached to a manipulator. Such stimulation is known to generate endolymph movements which are able to influence the discharge of afferent vestibular nerve fibers in a fashion similar to that of a physiological stimulation (TRINKER 1964).

Most of the cats were anesthetized with pentobarbital sodium (30 mg/kg of body weight). Several control experiments were performed with decerebrate, unanesthetized animals in order to eliminate possible errors due to barbiturate anesthesia. In the early stage of the experiments the vestibulo-cerebellum was approached stereotaxically along a dorso-ventral track following a restricted craniotomy of the occipital bone. This approach, however, did not prove to be very favorable since the recording microelectrode had to penetrate the entire depth of cerebellum in order to reach the nodulus or flocculus; for this reason, in most of the experiments on the nodulus a ventral approach was adopted. After a low tracheotomy was performed, trachea and esophagus were transected and their ends deflected rostrally and caudally so as to expose the base of the skull between the tympanic bullae. The bone covering the ventral surface of the brain stem was removed and after opening the dura the microelectrodes were in-

serted in a ventro-dorsal direction through the brain stem into the nodulus. The exposed area of the brain stem and the adjacent bony edges were covered by agar gel. A direct approach was developed for recording from the floccular lobe. Following a lateral occipital craniotomy, the caudal end of the cerebral occipital lobe was extirpated and the lateral part of the ten-

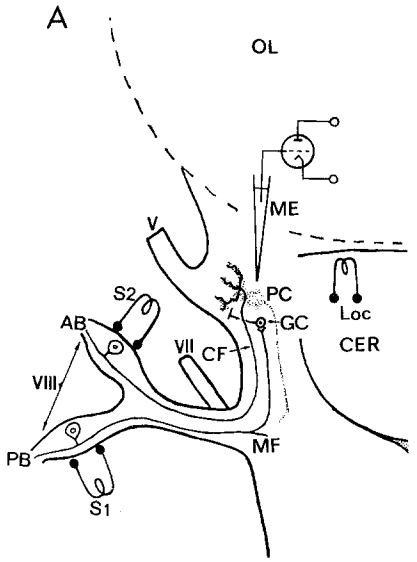

Fig. 1. *Diagram of frog brain stem and experimental arrangement.* CER, cerebellum; CF, climbing fiber; GC, granule cell; LOC, surface stimulating electrode; ME, recording microelectrode; MF, mossy fiber; OL, optic lobe; PC, Purkinje cell in the auricular lobe; S_1 and S_2, peripheral nerve stimulating electrodes; V, trigeminal nerve; VII, facial nerve; VIII, stato-acoustic nerve (AB and PB, anterior and posterior branches, respectively)

torium was carefully removed. Parts of the surface of the flocculus were exposed by gentle aspiration of the lateral paraflocculus under a continuous drip of warm Ringer solution. After the microelectrodes had been placed in the proper recording position, the exposed cerebellar surface was covered by agar gel. The stimulation of the vestibular nerve was carried out by means of silver wires (0.1 mm in diameter), insulated except for the rounded tips and gently placed on the vestibular nerves, bilaterally, after they had been exposed by a ventral approach through the bulla tympanica. The indifferent electrode was placed on the surface of the nearby bony edge. Both electrodes were fixed to the edge of the bulla tympanica with dental cement. The cavity was covered with a warm semi-solid paraffin-vaseline mixture, and the nerve electrically stimulated through an isolation unit which delivered rectangular current pulses of 0.1 msec duration at rates of 1—5/sec. In some experiments a bipolar concentric stimulating electrode was inserted deep in the inferior cerebellar peduncle close to the fastigial nuclei in order to activate the Purkinje cells antidromically (ECCLES, LLINÁS and SASAKI 1966b). Glass microelectrodes filled with 3M KCl or 4M potassium citrate were used for intracellular recordings, while extracellular recordings were carried out with micropipettes filled with 4M NaCl. Direct current amplification was used in both instances.

Following the recording of vestibular-evoked responses in the flocculo-nodular lobe, the microelectrodes were cut and left *in situ* for histological control of the recording sites. At the end of the experiment the animals were sacrificed by an overdose of pentobarbital sodium. The brain was fixed by carotid artery perfusion with 5% formalin, and the brain stem and cerebellum were removed from the skull, care being taken not to move the ends of the microelectrodes. After submersion in formalin for 24 hours, a thin sagittal slide containing the recording microelectrodes was dehydrated and made transparent with methylsalicylate (WALL 1962).

As shown in Fig. 5 the white matter, granular layer, and molecular layer can be easily differentiated without additional staining, and the location of the microelectrodes in the cerebellum can be accurately determined. The average response computation of the field potentials was carried out by means of a Fabri-Tek 1064 computer.

Results

Field Potentials Evoked by Vestibular Nerve Stimulation in the Auricular Lobe of the Frog

The cerebellar auricular lobes, while relatively large in tadpoles, undergo a reduction in size in adult frogs; this is due to the fact that the lateral line system disappears as the animal changes from aquatic to terrestrial life. As a result of this development the input to the auricular lobes is almost exclusively vestibular in nature (LARSELL 1923, 1925), while the corpus cerebelli is under the dominant influence of the spino-cerebellar system (HERRICK 1924). Two types of vestibular afferents are found to terminate in the frog's cerebellar cortex: firstly, a direct system arising from the bipolar cells of the vestibular ganglion and secondly, an indirect projection originating from the vestibular nuclei. The primary or direct vestibular fibers reach the cerebellum via the anterior and posterior branches of the eighth nerve. Once they enter the brain stem, they divide into both ascending and descending fascicles, the latter ending in the vestibular nuclei. The ascending bundle terminates for the most part in the ipsilateral auricular lobe and gives off some fibers to the vestibular commissure which ends in the contralateral auricular lobe as well as in the corpus cerebelli. Secondary vestibular fibers originating in the vestibular nuclei also terminate in the auriculo-cerebellar cortex. In order to activate vestibulo-cerebellar fibers in a selective manner, the electrical stimulation of the VIIIth nerve was restricted almost exclusively to its anterior branch. It is known from BURLET'S (1929) studies that this branch is purely vestibular in origin, its fibers arising from the cristae ampullares of the anterior and lateral semicircular canals and from the macula utriculi and sacculi. On the other hand, the posterior branch — being composed of fibers from the posterior crista ampullaris, papilla lagena, papilla basilaris and papilla amphibiorum — contains auditory as well as vestibular afferents. In the experiments where the posterior branch was also stimulated, the field potentials recorded in the auricular lobe appeared to be of smaller amplitude as compared to the potentials recorded in response to stimulating the anterior branch.

The field potentials evoked at different depths from the surface of the auriculum after electrical stimulation of the anterior branch of the VIIIth nerve are shown in Fig. 2A. The electrical stimulus strength used in the present study ranged between one and three times the threshold for nerve activation. The field potential complex illustrated in Fig. 2 consists of an early fast negative deflection with a latency of approximately 0.5 msec, followed by a slower negative wave. The early negativity is assumed to be generated by the compound action currents evoked by the primary vestibular afferents which are found throughout the depth of the auricular lobe. The antidromic activation of Purkinje cells following the vestibular nerve stimulation can also contribute to this early potential (LLINÁS, PRECHT and KITAI 1967b). At 200—300 μ from the surface (this depth corresponds approximately to the Purkinje cell layer) another small sharp negativity is often seen to

follow immediately the first potential. This potential has been interpreted as generated by the antidromic invasion of those Purkinje cells which project to the peripheral vestibular sensory organ (LLINÁS, PRECHT and KITAI 1967b; LLINÁS and PRECHT 1969). Both of these early potentials follow high frequency or double shock stimulation (Fig. 2C) without significant decrease of their amplitudes. Furthermore, action potentials superimposed on these fields were able to follow double shock activation at short intervals.

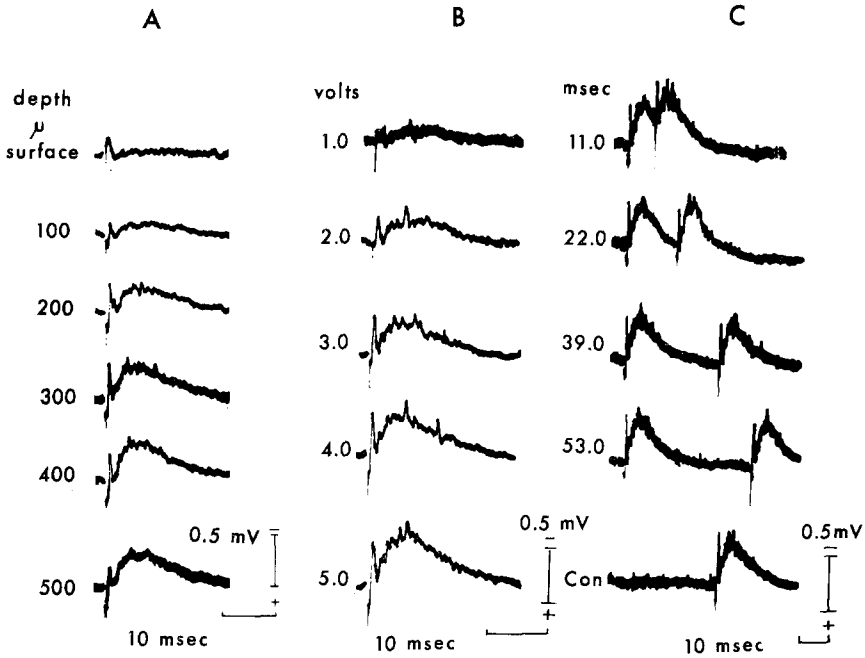

Fig. 2. *Field potentials generated by stimulation of the VIIIth nerve and recorded in the auricular lobe of the frog.* A, field potentials recorded at different depths in the auricular lobe after stimulation of the ipsilateral anterior branch. B, the field recorded at a depth of 250 μ (Purkinje cell layer) as the intensity of stimulation increases from top to bottom. C, double vestibular nerve stimuli were given at various time intervals (indicated in msec on the left of each record) and the resulting field potentials were recorded in the ipsilateral auricular lobe at a depth of 300 μ from the surface. Con, the control response to the second shock alone

In accordance with the anatomical distribution of the primary vestibular fibers in the auriculum, the early negativity was recorded at all depths as shown in Fig. 2A. The second slow potential wave decreased its amplitude during high frequency stimulation and following injection of barbiturates. This potential may be ascribed to the synaptic and action currents generated by the granule cells and Purkinje cells following their activation via mossy and climbing fibers, respectively. As can be seen in Fig. 2A and B, spike potentials can be observed at depths which correspond to the Purkinje cell layer. Further support for this interpretation will be given in the next section. In Fig. 2C double vestibular nerve stimuli were applied at various time intervals. None of the slow negative waves generated by the test volley show a measurable decrease of their amplitudes after conditioning

volleys at various intervals. In the uppermost record in C, the potential generated by the second stimulus (given after a delay of 11.0 msec) shows a slight increase of its amplitude, suggesting that the frog auricular lobe, as the corpus cerebelli, lacks the long-term inhibition of Purkinje cells (LLINÁS and BLOEDEL 1967) as well as the Golgi cell inhibition at the granular layer (LLINÁS 1969).

Activation of Frog Purkinje Cells Via Mossy Fiber-Granule Cell Pathway

The extracellular records shown in Fig. 4A—D and the intracellular records of Fig. 3 were obtained from Purkinje cells at depths of about 250 μ from the surface of the auricular lobe and demonstrate the activation of Purkinje cells by ipsilateral vestibular nerve stimulation, most likely through the mossy fiber-granule cell pathway. As the stimulus strengths increased (Fig. 4 from A—D and Fig. 3 from A—C), the cells were caused to discharge repetitively. In this respect

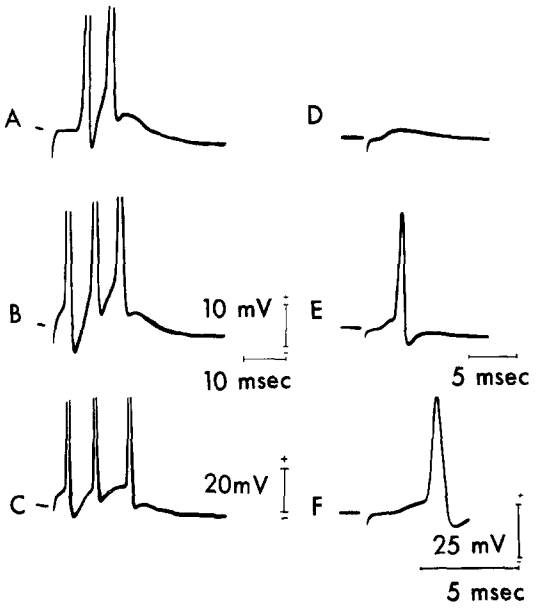

Fig. 3. *Intracellular records from frog Purkinje cells.* Activation of individual Purkinje cells (A—C, D—F) located in the auricular lobe of the frog cerebellum at depths of approximately 250—300 μ from the surface following stimulation of the anterior branch of the ipsilateral VIIIth nerve. As the strength of stimulation increases from A—B, the unit shows graded repetitive firing. D shows generation of an EPSP in a Purkinje cell by a vestibular nerve stimulus. As the intensity of stimulation is increased in E and F, the EPSP reaches firing level

the Purkinje cells of the frog cerebellum definitely differ from those of the cat (Fig. 11) where activation of mossy fibers generally produces a single spike in Purkinje cells (ECCLES, LLINÁS and SASAKI 1966b) and only occasionally double activation. Graded repetitive activation of individual Purkinje cells in the frog occurs due to the absence of long-term inhibition in the cerebellum of this species (LLINÁS and BLOEDEL 1967). Furthermore, no IPSPs were ever evoked in Purkinje cells following cerebellar cortex or vestibular stimulation.

In measuring the latencies of activation of a large number of extra- and intracellularly recorded action potentials (Fig. 3), the shortest value found was 1.8 msec. The later responses may have latencies of up to 10 msec or more. The synaptic activation of Purkinje cells after vestibular nerve stimulation can be ascribed to impulses arriving along primary vestibular fibers ending as mossy fibers on the granule cells of the auricular lobe which in turn would excite Purkinje cells via the parallel fibers, thus establishing a disynaptic pathway connecting the labyrinth and the cerebellar Purkinje cells. Longer latencies for Purkinje cell activation following vestibular nerve shocks may be evoked through the activation of mossy fibers arising from cells of the vestibular nuclei. It has been shown by PRECHT and SHIMAZU (1965) that many vestibular neurones of the cat show only long latency polysynaptic activation after vestibular nerve stimulation. Those neurones could give rise to vestibulo-cerebellar mossy fibers and thus account for the long latencies of activation found in many Purkinje cells after vestibular stimulation. It has been shown anatomically (LLINÁS, PRECHT and KITAI 1967a; HILLMAN, personal communication) that primary vestibular fibers end as mossy fibers in the granule cell layer of the auricular lobe. This finding is in perfect agreement with the interpretation given for the short latency "mossy" fiber responses described above. On the basis of the present physiological data, we would postulate that in the frog secondary vestibulo-cerebellar fibers end also as mossy fibers in the auricular lobe. Similar long latency responses were obtained in the frog by CHANG and KOSTYUK (1960).

Activation of Frog's Purkinje Cells by Vestibular Climbing Fibers

It has long been a matter of controversy whether vestibulo-cerebellar fibers end as mossy or climbing fibers. As shown in the preceding section, there is anatomical and physiological evidence for vestibular mossy fibers in the frog. Since it is relatively easy to recognize activation of Purkinje cells by climbing fibers in the corpus cerebelli (MATTHEWS, PHILLIPS, RUSHWORTH 1958; LLINÁS and BLOEDEL 1966/1967), climbing fiber responses should be also found in the auricular lobe if primary vestibular fibers were to end as climbing fibers on Purkinje cells. Both in the cat (ECCLES, LLINÁS and SASAKI 1966c) and in the frog (LLINÁS and BLOEDEL 1966/1967) climbing fiber activation of Purkinje cells is characterized by all-or-none bursts of spikes extracellularly. After a Purkinje cell is penetrated, an all-or-none long-lasting series of action potentials is recorded, the latter being produced by a large all-or-none synaptic depolarization. The all-or-none character of this EPSP reflects the all-or-none character of the action potential in the presynaptic fiber, there being a one-to-one relation between climbing fibers and Purkinje cells (RAMÓN Y CAJAL 1904). In Fig. 4E—H typical extracellular all-or-none Purkinje cell bursts were recorded at the auricular lobe from two different cells (E—F and G—H respectively) following stimulation of the anterior branch of the vestibular nerve. The all-or-none character of these potentials is shown in E and G where the intensities of the stimuli were near to the thresholds for these cells. In comparing the graded repetitive action potentials evoked by vestibular nerve stimulation in Fig. 4A—D with the responses shown in E—H, the fundamental difference between the two types of Purkinje cell responses becomes immediately apparent. The shortest latency of climbing fiber activation

after vestibular nerve stimulation was 0.8 msec and the longest one found in the present study was 1.6 msec. These responses had a shorter latency than has been observed in the more medial regions of the cerebellum by stimulating the underlying white matter (MATTHEWS, PHILLIPS and RUSHWORTH 1958; LLINÁS and

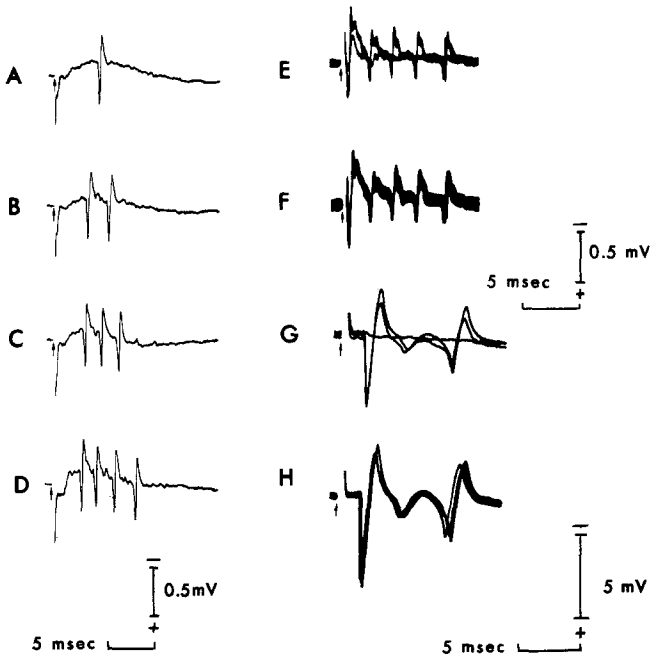

Fig. 4. *Extracellular recordings from frog's Purkinje cells.* A—D, auricular Purkinje cell spikes (270 μ from the surface) generated by stimulation of the anterior branch of the VIIIth nerve. As stimulus intensity gets larger from A—D, number of action potentials increases and their latency becomes smaller. E—H, monosynaptic climbing fiber activation of Purkinje cells in response to stimulation of the anterior branch of the VIIIth nerve. E and F, Purkinje cell in the auricular lobe (300 μ from the surface); in E the all-or-none character of the response is shown by the failure of the cell to respond to one of the three superimposed stimuli; F, superposition of five traces with slightly higher stimulus strength. G and H, vestibular climbing fiber activation of a Purkinje cell at 250 μ from the surface. The all-or-none character of the burst response is exemplified in G; a negligible latency fluctuation is shown in H

BLOEDEL 1966/1967). The differences in conduction time between vestibular climbing fibers and those evoked by white matter stimulation can be explained if the former are assumed to have a higher conduction velocity. Given the fact that the longest latency for climbing fiber activation of auricular Purkinje cells from the VIIIth nerve was 1.6 msec, which is less than the shortest latency for mossy fiber excitation of Purkinje cells in the same area, it may be readily assumed that these burst responses are monosynaptically evoked by primary vestibular fibers ending as climbing fibers on auricular Purkinje cells. On the other hand, the lack of long-latency climbing fiber activation of Purkinje cells after VIIIth nerve stimulation may imply that secondary vestibulo-cerebellar fibers end purely as mossy fibers in the granular layer of the auricular lobe. The physiological evidence for

a monosynaptic connection between the vestibular nerve and the auricular Purkinje cells by means of climbing fibers has been fully confirmed anatomically in a parallel series of studies (HILLMAN, personal communication; LLINÁS, PRECHT and KITAI 1967a). Nauta stains, as well as electron microscopical studies, have shown degenerating fibers in the molecular layer of the auricular lobe following section of the VIIIth nerve extracranially. These fibers, which contact directly the dendrites of Purkinje cells at their smooth branch level, have been identified as typical climbing fibers. Since the frog vestibular climbing fiber activation is restricted almost exclusively to the auricular lobe, other sources must exist in the frog to provide climbing fibers to the Purkinje cells of the rest of the cerebellar cortex. These additional sources are not known at the present stage. In several instances, climbing fiber activation of Purkinje cells did not only occur in response to electrical stimulation with the characteristic short latency but the burst responses could also be elicited by means of gentle mechanical stimulation of the walls of the membranous labyrinth, a stimulus which is known to cause movements of the endolymph which in turn stimulate the vestibular hair cells. This finding demonstrates beyond reasonable doubt the peripheral origin of the climbing fiber activation of many of the Purkinje cells in the auricular lobe.

Analyses of the Potential Fields in Cat's Vestibulo-Cerebellum Produced by VIIIth Nerve Stimulation

Numerous anatomical studies in the past have demonstrated the presence of primary and secondary vestibulo-cerebellar fibers in various vertebrates (for summary of the literature, see BRODAL and TORVIK 1957; BRODAL and HOIVIK 1964). In a recent experimental study with silver impregnation methods by BRODAL and HOIVIK (1964), the areas of termination of primary vestibular fibers within the cerebellar cortex were confirmed. Degenerating vestibular nerve terminals are found in the nodulus, flocculus, parts of the uvula and in the ventral paraflocculus, and it was suggested that these parts of the cortex be referred to as the vestibulo-cerebellum. Another question of paramount interest has been answered recently by means of anatomical methods. As originally suggested by SNIDER (1936), the primary vestibular fibers in the cat were found to terminate exclusively as mossy fibers in the granular layers of the areas described above (BRODAL and HOIVIK 1964). There is also good histological evidence for the hypothesis that secondary vestibulo-cerebellar fibers end as mossy fibers in the granular layers of the vestibulo-cerebellum (SZENTÁGOTHAI and RAJKOVITS 1959). Thus there is, in contrast with our findings in the frog, no anatomical evidence for primary or secondary vestibular fibers ending as climbing fibers on Purkinje cells of cat's cerebellum. During the course of the present physiological study, therefore, particular care has been taken not to overlook any possible vestibular climbing fiber projection to the cerebellum.

The vestibular nerves on both sides were stimulated in the way described above. As has been shown by SHIMAZU and PRECHT (1965) and PRECHT and SHIMAZU (1965), a characteristic field potential complex can be recorded in the ipsilateral vestibular nuclei after vestibular nerve stimulation. In order to have a standard control of the stimulus, the intensity of vestibular nerve stimulation was expressed as multiples of N_1 threshold; only occasionally were stimuli with

intensities larger than three times threshold used. In most cases the field potentials in the cerebellum were evoked by stimuli of two times N_1 threshold since this magnitude has been shown to be subthreshold for the activation of the reticular formation of the brain stem and, thus, mainly activates the primary and secondary vestibulo-cerebellar pathways.

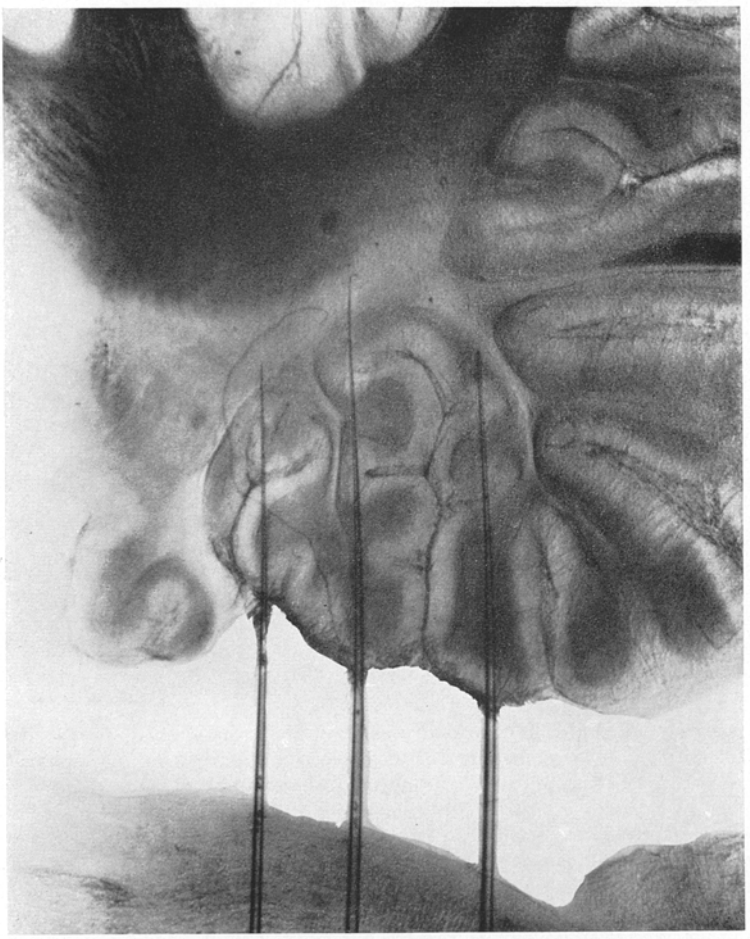

Fig. 5. *Microphotograph of the posterior vermis of the cat.* Microelectrodes are shown in the nodulus and the ventral uvula (electrode on the right). Molecular and granular layers and white matter can be differentiated. Brain tissue fixed with formalin and treated with methylsalicylate. Microelectrode tip to the right was broken during slicing of the cerebellum

The field potentials which can be recorded in the vestibulo-cerebellum after vestibular nerve stimulation are exemplified in Fig. 6 which illustrates the potential fields evoked in the different layers of the nodulus. The line drawing in Fig. 6A is taken from the actual microphotograph shown in Fig. 5, and the recordings are those obtained with the middle electrode. As this micropipette penetrates through the nodulus along a slightly para-sagittal track, it records the potentials generated

in an alternative manner at molecular (Fig. 6B, D) and granular layers (Fig. 6C, E). Physiological studies by ECCLES, LLINÁS and SASAKI (1966b) and ECCLES, SASAKI and STRATA (1967a) have provided a detailed analysis of the field potentials generated in the cerebellar cortex by a mossy fiber volley and the potentials have been correlated with the anatomical structures of the cortex. The great similarity between the mossy fiber field potentials reported by these authors and the fields generated in the nodulus as a result of vestibular nerve stimulation be-

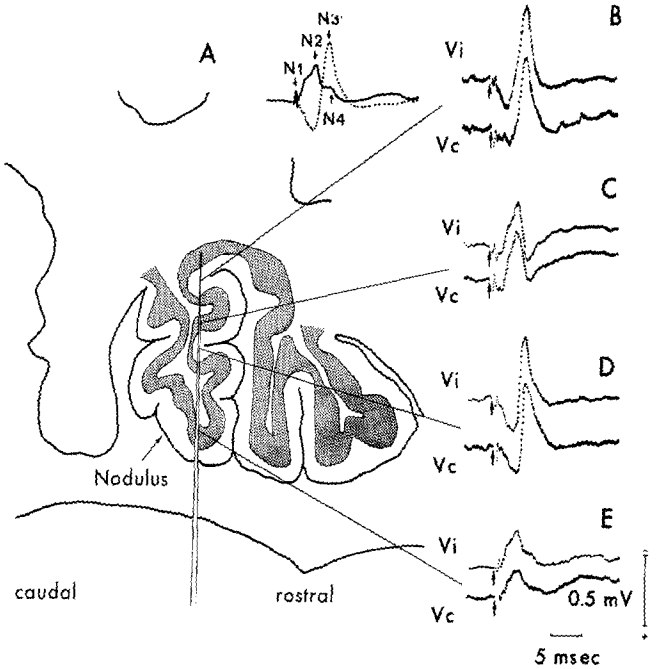

Fig. 6. *Correlation of nodulus field potentials with microelectrode location.* A, line drawing of a parasagittal section of cat's cerebellum with microelectrode in the nodulus (1 mm paramedian). The microelectrode has been inserted in a ventrodorsal direction and cut *in situ* after recordings were obtained. Dotted areas correspond to the granular layers, outer white area the molecular layer. The innermost white space corresponds to the white matter. B—E, averaged field potentials generated by sixteen shocks to the ipsilateral (Vi) and contralateral (Vc) VIIIth nerve and recorded from the nodulus along electrode track shown in A. Lines correlate the field potentials (B—E) with the recording sites in A. The potentials in B and D and C and E were recorded in the molecular and granular layers, respectively. Inset line drawing at the top of A shows superimposition of the field potentials in the molecular (dotted line) and granular (solid line) layers indicating the various negative field components (N_1—N_4). Arrows indicate stimulus artefact

comes immediately apparent. In the molecular layers of the nodulus the field potentials consisted mainly of a small N_1 and a larger N_3 negative component (Fig. 6B, D). With recordings obtained from the granular layer (Fig. 6C, E), N_1, N_2 and N_4 negative potentials were most prominent. In most experiments a small negativity was seen to occur between N_1 and N_2. These different potentials are clearly shown in a superimposed drawing of molecular fields (dotted) and granular fields (solid line) at the top of Fig. 6. Purkinje cell action potentials were recorded

in most cases at the junction between molecular and granular layers, which correspond to the Purkinje cell layer (Fig. 11).

The interpretation of the field potentials generated by mossy fiber activation has been previously described by ECCLES, LLINÁS and SASAKI (1966b) and ECCLES, SASAKI and STRATA (1967a); a similar form of interpretation will be adopted in this study. The N_1 potential will be attributed to the compound action current generated as the primary vestibular fiber volley reaches the granular layer. This potential follows high frequency stimulation and is resistant to barbiturate anesthesia. The small negative potential interposed between N_1 and N_2 potentials will be mainly ascribed to the compound action current of secondary vestibulocerebellar fibers, although part of it might be generated by primary vestibular fibers having slower conduction velocities. The small amplitude of this second fiber potential does not necessarily imply that the number of secondary vestibulocerebellar fibers is small since it is possible that some of the cells of origin of those fibers do not receive primary vestibular fibers and hence are not excited by vestibular nerve stimulation (see discussion). The N_2 wave is ascribed to the synaptic and action currents produced by vestibular fibers in the granule and Golgi cells of the granular layer. The N_3 wave represents the action currents in parallel fibers and the synaptic and action currents which these fibers generate in Purkinje cells and in the interneurones of the molecular layer. The impulses evoked in Purkinje cells travel down their axons and generate the N_4 potential at the granular layer.

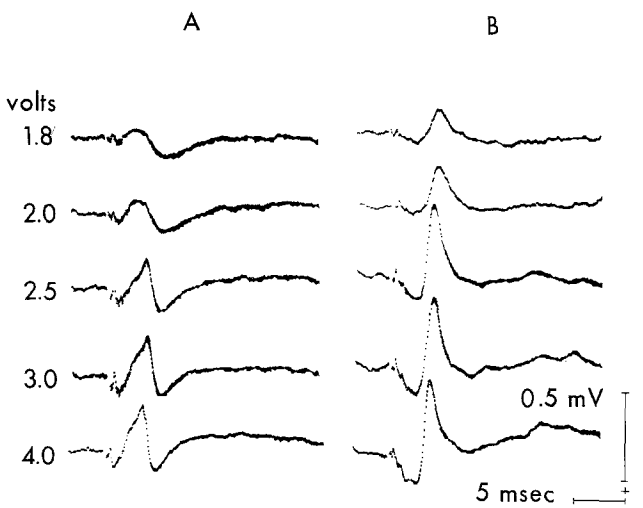

Fig. 7. *Field potentials evoked by VIIIth nerve volleys and recorded from the granular and molecular layers of the ipsilateral nodulus.* A, field potentials recorded from the granular layer of the nodulus. Stimulus strength increases from the top to the bottom. B, field potentials obtained from the molecular layer of the nodulus in response to same stimuli applied in A. Each record represents the average of the responses. Arrows indicate stimulus artefact

Potential fields of similar configuration were also recorded from the flocculus and ventral parts of the uvula and are in very good agreement with the anatomical distribution of the vestibulo-cerebellar fibers. Likewise the latencies measured from the stimulus artefact to the beginning of the negativity for the different

components were in the same range, i. e. 0.6—0.75 msec for the N_1, 1.6—1.7 msec for the N_2 and 3.3—3.5 msec for the N_3. The short latency of the N_1 potential wave (0.6—0.75 msec) also indicates that this potential must be generated by the action currents of the primary vestibular fibers terminating in the cerebellum since the earliest transsynaptic activation of neurones in the vestibular nuclei occurs at about 1.0 msec after the stimulus (PRECHT and SHIMAZU 1965). As shown in Fig. 7, a stepwise increase of the stimulus strength causes by larger vestibular fiber recruitment an increase in the amplitude of N_1 and N_2 in the granular layer (A) and N_1 and N_3 in the molecular layer (B) of the nodulus. With stronger stimuli the N_2 potential usually had two peaks which have been described by ECCLES, SASAKI and STRATA (1967a) as representing the impulse discharge of Golgi cells followed by granule cells.

Further support for the assumption that these potential fields are evoked exclusively by mossy fiber activation is given by the records shown in Fig. 8. Two

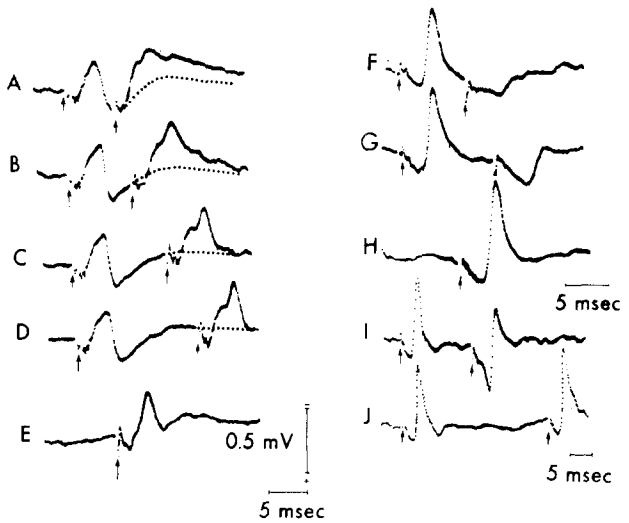

Fig. 8. *Field potentials evoked by contralateral VIIIth nerve stimulation and their conditioning by preceding ipsilateral stimuli.* The time course of the inhibitory action of the preceding ipsilateral stimuli on contralateral test response in the granular (A—E) and molecular layers (F—J) of the nodulus. E and H are control records. Each record represents 16 averaged responses to the stimuli applied at a rate of 2/sec. Note different time scales. Arrows indicate stimulus artefact

vestibular nerve stimuli were delivered at the ipsilateral and contralateral VIIIth nerve at various intervals and the potentials were recorded in the granular layer (A—E) and molecular layer (F—J) of the nodular lobe, E and J being the control records for the stimuli. If the contralateral stimulus is preceded by an ipsilateral shock at short intervals, the spike components of the N_2 potential in the granular layer and the positivity following N_2 are obliterated by the inhibitory action of Golgi cells upon granule cell acitivity (Fig. 8A and B). Thus the remaining part of N_2 represents only the synaptic currents which are produced by vestibular fibers in granule and Golgi cells. Similarly, the N_3 potential in the molecular layer

(F—H) is obliterated by the inhibitory action of the Golgi cells on the mossy fiber-granule cell relay. The full recovery period for the N_3 potential when preceded by another vestibular stimulus is usually in the range of 40—60 msec. Similar results are seen when two consecutive stimuli are delivered to the same vestibular nerve.

On several occasions it was noted, however, that in the flocculus a given field potential was depressed more efficiently by a conditioning volley given to the same eighth nerve (VI—VI or VC—VC in Fig. 9), than by a large overlapping volley

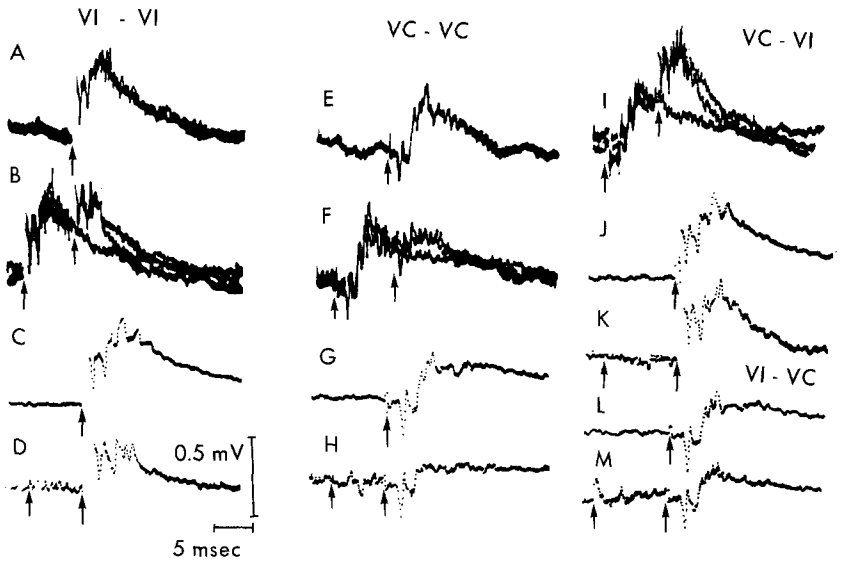

Fig. 9. *Field potentials in the flocculus evoked by VIIIth nerve stimulation and their conditioning by preceding ipsilateral and contralateral VIIIth nerve stimuli.* Records in A, B, E, F and I were obtained by superimposing oscilloscope traces, and each record in C, D, G, H, J and M consists of 16 averaged responses. Arrows indicate location of stimulus artefact. A and C, control records of the field potential generated by ipsilateral VIIIth nerve stimulation in the granular layer of the flocculus. Note small granule cell spikes superimposed on the field potentials. In B and D the test stimuli are preceded by ipsilateral VIIIth nerve stimuli. In D the preceding ipsilateral stimulus (first arrow) has been subtracted by the computer in order to show more clearly the effect on test response. Records in E and H are the same as in A and D except that stimuli were applied to the contralateral vestibular nerve. In I and K the ipsilateral stimulus was preceded by a contralateral shock. The control record for the test stimulus in I is A. In K the preceding contralateral field potential was subtracted. J is control for K. L and M show the contralateral shock preceded by an ipsilateral volley, L being the control for the contralateral test response. In M the contralateral conditioning response has been subtracted. Note the absence of significant inhibition of N_2 in I, K and M and the clear reduction of the N_2 amplitudes in B, D, F and H; N_1 potential is not changed in any of the records

from the contralateral side (VC—VI or VI—VC in Fig. 9). These different effects of preceding ipsi- or contralateral eighth nerve stimuli on a test response are exemplified in Fig. 9 by the recordings of the N_1 and N_2 field potentials taken from the granular layers of the flocculus. It must be noted that the contra- and ipsilateral mossy fiber volleys activate granule cells which are close enough in each case to be recorded from the same microelectrode position and thus a true overlap must

be present. The reduced Golgi cell inhibition where homonymous and heteronymous mossy fibers are interacted can also be shown in the activity of single granule cells. In B and D and in F and H, the number of granule cell spikes evoked by the second homonymous volley is markedly reduced from the control A and C, E and G, respectively. On the other hand, I, K and M show only a slight change in the activity of granule cells following heteronymous pairing of stimuli.

The potentials shown in Fig. 6B—E, 8, 9A and E, and 10, demonstrate a considerable convergence of the ipsi- and contralateral vestibular inputs to a given area of the cerebellar cortex. When the electrodes were inserted parasagittally into the nodulus or into the flocculus on one side, stimulation of the ipsi- and contralateral nerve generated potentials of similar shape and amplitude. This was

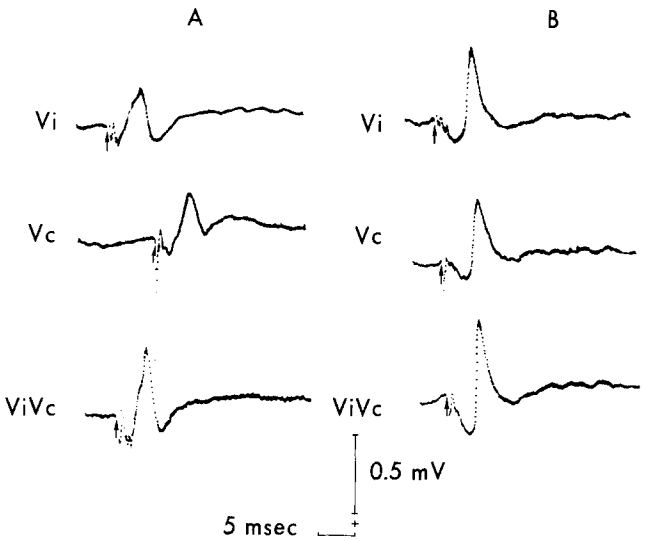

Fig. 10. *Convergence of ipsi- and contralateral vestibular nerve projection in the nodulus.* Field potentials recorded from the granular (A) and molecular (B) layers of the nodulus (1.5 mm paramedian) in response to single shock stimulation of the ipsilateral (Vi) and contralateral (Vc) VIIIth nerve. Sixteen stimuli averaged in each record. In the records at the bottom of A and B, the ipsi- and contralateral stimuli were applied simultaneously

constantly found to be so in the nodulus. Figure 10 shows the potential fields generated by weak ipsi- and contralateral vestibular nerve stimuli and recorded in the granular layer (A) and molecular layer (B) of the nodular cortex along a parasagittal plane (1 mm lateral). The records at the bottom of Fig. 10A and B clearly show the increase of the potential amplitudes caused by simultaneous stimulus of the ipsi- and contralateral vestibular nerve. In the case of the flocculus the potential generated by the contralateral nerve volley was often smaller in amplitude but the latency difference for the N_1 potentials produced by ipsi- and contralateral VIIIth nerve stimulation in the vestibulo-cerebellum was small though present. These differences in latency can be explained by the larger distance that the contralateral volley has to travel in order to reach a given spot in the cerebellar cortex. Finally, the N_1 potentials recorded as a result of contralateral

vestibular stimulation were never found to be reduced in amplitude when conditioned by another vestibular stimulus (Fig. 9H, M) preceding at short intervals. These findings strongly suggest that the impulses initiated in the contralateral vestibular nerve travel mainly along primary fibers to the cerebellum where they send off collateral branches which reach the corresponding part of the opposite side (a small secondary fiber contingent seems to be present also) (Fig. 13). On the basis of the anatomical data (BRODAL and HOIVIK 1964), it would be expected that the contralateral vestibulo-cerebellar projection would be rather feeble. On the contrary, however, the present physiological analysis shows a considerable overlapping of the two vestibular inputs, particularly in the vermis (nodulus and uvula) but also to some degree in the flocculus (Fig. 13).

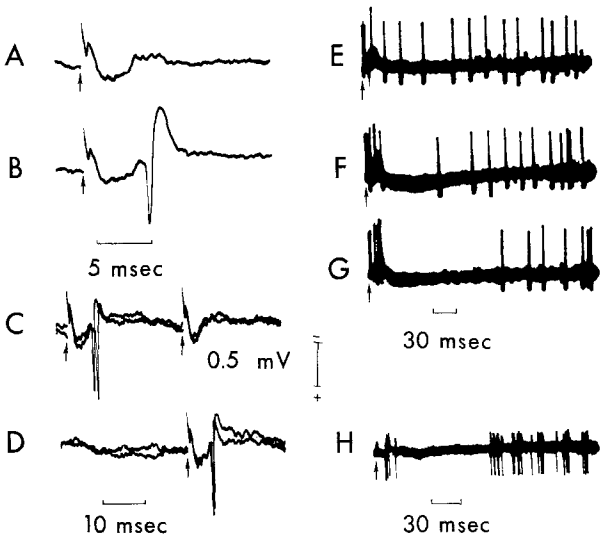

Fig. 11. *Extracellular records of Purkinje cells in the cat's nodulus and flocculus.* A—D, activation of a Purkinje cell in the nodulus by stimulation of the ipsilateral VIIIth nerve. A, subthreshold stimulus generating small field potential. B, stimulus strength 1.5 times N_1 threshold generates a Purkinje cell spike. C, double shocks applied to the ipsilateral VIIIth nerve; the second shock given after 28 msec delay fails to excite the Purkinje cell. D shows control response with the second stimulus alone. E—G, inhibition of spontaneous discharge of Purkinje cell in the flocculus by ipsilateral VIIIth nerve stimulation at a rate of 2/sec with increasing intensity from E—G (4 sweeps superimposed on each record). Note initial activation followed by long term inhibition. H shows similar excitation followed by depression of discharge for a Purkinje cell located in the nodulus after ipsilateral VIIIth nerve stimulation

Recordings of unitary action potentials in the vestibulo-cerebellum give further support for the interpretation that the field potentials evoked by vestibular stimulation are generated by mossy fiber afferents exclusively. The Purkinje cells were characterized by their antidromic invasion following electrical stimulation of the inferior cerebellar peduncle. In Fig. 11A—E, ipsilateral vestibular nerve stimulation generates action potentials in a floccular Purkinje cell. The responses to VIIIth nerve stimulation consisted in all cases of single action potentials with latencies ranging from 3—15 msec. The shortest latency (3 msec) and the occur-

rence of only one or occasionally two action potentials to a single vestibular nerve shock implies activation of the Purkinje cells via mossy fiber-granule cell pathway. When the test stimulus was preceded by a conditioning vestibular stimulus (Fig. 11 C), it failed to generate Purkinje cell activation, presumably because of the inhibitory action of Golgi cells on the granule cells. In Fig. 11 E—H, spontaneously active Purkinje cells are shown to be depressed for as long as 150—200 msec following a vestibular nerve activation, the duration of the depression being related to the stimulus strength, which was increased from E—G. This depression of discharge of the Purkinje cells may be due to the combined inhibitory action of the cerebellar interneurones on Purkinje and granule cells, as well as to a disfacilitatory mechanism initiated by the inhibitory action of Purkinje cell axons on the excitatory background arising in the brain stem nuclei. This latter mechanism is very feasible since direct Purkinje cell projection from the vestibulo-cerebellum (Fig. 13) has been described for all four vestibular nuclei (ANGAUT and BRODAL 1967). Similar inhibition of spontaneous discharge of Purkinje cells has been seen upon stimulation of the underlying white matter (ECCLES, LLINÁS and SASAKI 1966 b).

Climbing Fibers in the Vestibulo-Cerebellum

In none of the experiments carried out in cats was there any indication of potential fields evoked by activation of the climbing fiber system following

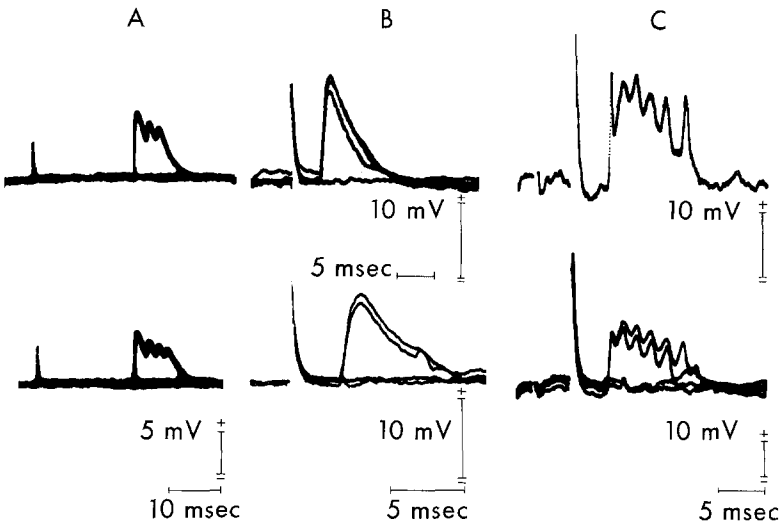

Fig. 12. *Intracellular records from Purkinje cells in the vestibulo-cerebellum of the cat.* A shows activation of an all-or-none climbing fiber EPSP recorded from a Purkinje cell in the nodulus in response to stimulation of the ipsilateral VIIIth nerve (stimulus intensity 4 times threshold of N_1 potential). Note the long latency for activation. B and C, monosynaptic all-or-none climbing fiber EPSPs evoked in a Purkinje cell (nodulus) by stimulation of the contralateral inferior olive in the region of the medial accessory olive

vestibular stimulation. However, in most cases, typical spontaneous climbing fiber activation of Purkinje cells was observed. Vestibular nerve stimulation of the magnitude used for activating vestibular mossy fibers, on the other hand,

never evoked any of these Purkinje cell bursts. When the stimulus strengths were raised by two to three times the usual value, which is known to produce direct activation of other structures besides the vestibular nerve, typical all-or-none burst activations of Purkinje cells were occasionally observed, their latencies being between 15—20 msec or longer (Fig. 12A). These same Purkinje cells usually showed monosynaptic extracellular burst responses following contralateral inferior olive stimulation. When Purkinje cell potentials were recorded intracellularly, large all-or-none EPSPs could be observed on stimulation of the contralateral medial accessory olive (Fig. 12B, C) which is known to give origin to olivocerebellar projections to the nodulus (JANSEN and BRODAL 1954). Similar short latency climbing fiber activation of anterior lobe Purkinje cells following inferior olive stimulation was reported by ECCLES, LLINÁS and SASAKI (1966a) and led them to postulate, in accordance with anatomical findings by SZENTÁGOTHAI and RAJKOVITS (1959), that the climbing fiber system originates from the inferior olive. On the basis of these findings, it may be concluded that primary and secondary vestibulo-cerebellar fibers in the cat project to the cerebellum as a pure mossy fiber input and that the climbing fiber component to the vestibulo-cerebellum must originate from other structures. Furthermore, since the two afferent systems must carry information from different sources to the cerebellar cortex, they should not be taken as true parallel channel systems.

Discussion

It has been demonstrated in the preceding sections that, in the frog, vestibulocerebellar fibers end in the auricular lobe as mossy fibers in contact with granule cells and as climbing fibers in contact with Purkinje cells. In the cat, however, evidence has been presented which strongly suggests that the vestibular input to the cerebellum is a pure mossy fiber projection (Fig. 13). Both findings are in perfect agreement with the histological studies by HILLMAN (personal communication) in the frog and by BRODAL and HOIVIK (1964) in the cat. Thus, even the oldest part of the cerebellum undergoes significant changes in its afferent components as it develops from the lower to the higher forms. Possible functional reasons for the loss of the climbing fiber projection from the vestibular system in cats are unknown at present. It is reasonable to assume that mossy and climbing fibers may relay different types of information from the labyrinthine receptors to the cerebellum, and thus that the type of information received by the cerebellar cortex from these receptors differs for frog and higher vertebrates. As the peripheral labyrinth develops from the more primitive form in the frog to the one in higher forms, the functional connections with the central nervous system may also undergo changes so that the climbing fiber input to the vestibulo-cerebellum is no longer required and is thus used by the vestibular apparatus or by other systems as a separate channel to the flocculonodular lobe. Positive evidence for a monosynaptic climbing fiber projection from the medial accessory olive to the contralateral nodulus has been given above. It is known from studies by WALBERG (1956) that the area of origin of olivo-cerebellar fibers to the vestibulo-cerebellum receives mainly descending afferents from higher centers, such as the caudate nucleus. These connections would allow the higher centers to influence vestibular activity in the vestibulo-cerebellum via the inferior olive. In a recent study, BAVA,

SAPIENZA and URBANO (1966) have indeed recorded field potentials in the nodular and floccular cortex upon stimulation of the ectosylvian gyrus with latencies of 5—30 msec. These potentials, which were independent from vestibulo-cerebellar

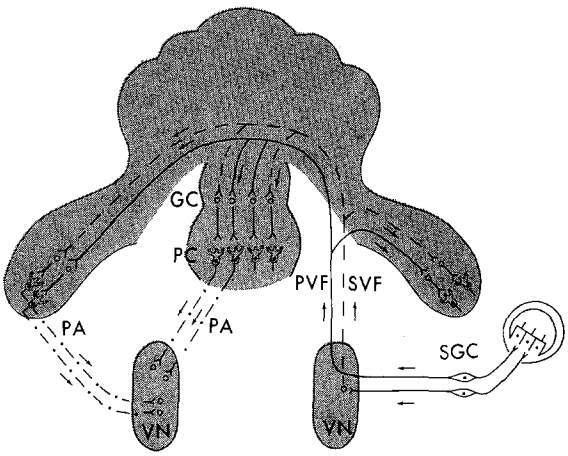

Fig. 13. *Diagrammatic representation of the connections between the vestibular system and the cerebellar cortex in cat.* The floccular lobes are shown laterally, nodulus and uvula in the center. Solid and broken lines represent primary (PVF) and secondary (SVF) vestibulo-cerebellar fibers, respectively. Lines of dots and dashes represent Purkinje cell projection to the vestibular nuclei (VN). Afferent connections to the cerebellar cortex on the right of the diagram, the efferent cortico-vestibular projections on the left. The arrows point in the direction of impulse conduction. Vestibulo-cerebellar projection not indicated. GC, granule cells; PA, Purkinje cell axons; PC, Purkinje cells; PVF, primary vestibulo-cerebellar fibers; SGC, vestibular ganglion cells; SVF, secondary vestibulo-cerebellar fibers; VN, vestibular nuclei

projections, were shown to be mediated through the inferior olive and thus a pathway would be provided for cortical control of cerebellar activity in the vestibular areas. It is feasible that the climbing fiber activation of Purkinje cells which was observed following supramaximal activation of the vestibular nerve is evoked by secondary activation of these higher nerve centers, which would ultimately be channeled into the inferior olive.

The field potentials generated by vestibular nerve stimulation in the vestibulocerebellum of the cat are of the classical mossy fiber type (ECCLES, LLINÁS and SASAKI 1966b; ECCLES, SASAKI and STRATA 1967), and resemble closely the potential fields described recently by SASAKI and STRATA (1967), following stimulation of various extracerebellar mossy fiber sources. Anatomical studies by BRODAL and DRABLØS (1963) have shown that nearly all mossy fiber endings in the flocculonodular lobe differ in their appearance from those in the anterior lobe, the vestibular mossy fiber endings being a more densely packed aggregation of rosettes. Also, the density of Golgi cells in the granular layer appears to be greater in the vestibulocerebellum. The present study did not reveal any significant physiological differences which can be correlated with the above anatomical fact, except for the findings of a certain specificity in Golgi cell inhibition. It must be kept in mind, however, that similar experiments have not been carried out in other cerebellar areas.

At present, it is not known what kind of vestibular receptors are connected to the primary and secondary vestibulo-cerebellar fibers. Given the fact that secondary vestibulo-cerebellar fibers originate partly in the descending vestibular nucleus (BRODAL and TORVIK 1957) which is known to receive afferents from the utriculus (STEIN and CARPENTER 1967; PETERSON 1957), it is possible to assume that at least otolithic influences are carried to the cerebellum along this pathway. This does not exclude the possibility of semicircular canal activity reaching the cerebellum since considerable convergence of various labyrinthine receptors on single vestibular neurones has been described by DUENSING and SCHAEFER (1959). Convergence can be carried out by interposed neurones located in the vestibular nuclei and thus does not require convergence of primary vestibular fibers on a particular cell. Furthermore, the vestibular nuclei are not simply a relay station for vestibular impulses reaching different levels of the nervous system but rather a complex system for integration of various sensory impulses. It is of interest to point out that some of the areas of the vestibular nuclei giving rise to vestibulo-cerebellar fibers (particularly cell group x as shown by BRODAL and TORVIK 1957) receive spinal afferents (POMPEIANO and BRODAL 1957). This may provide a means by which somatic afferent impulses arising at the body's periphery can also reach the vestibulo-cerebellum. Vestibular as well as spinal influences transmitted along mossy fibers can thus interact at the level of the vestibulo-cerebellum with influences from "higher centers" most likely carried also by climbing fibers.

Besides the vestibulo-cerebellar fibers, indirect pathways via the reticular formation may provide other means for vestibular impulses to reach the cerebellum. ANDERSON and GERNANDT (1954) described potentials in the lobulus simplex and culmen following vestibular nerve stimulation that had longer latencies than those recorded in the vestibulo-cerebellum. These potentials are probably mediated through vestibulo-reticulo-cerebellar pathways since no vestibular fibers have been found to terminate in this part of the cerebellum.

Much of the earlier work on the function of the vestibulo-cerebellum has dealt mainly with ablation and stimulation experiments and the behavioral effect produced thereby. A few studies related to vestibulo-ocular mechanisms will be mentioned. Depression of vestibular nystagmus upon electrical stimulation of the vestibulo-cerebellum has been reported by FERNANDEZ and FREDRICKSON (1963). Recent anatomical and physiological studies on the circuitry of the vestibulo-cerebellar system allow us to explain some of the behavioral effects at least at a "first approximation." It is conceivable that in the case of the depression of vestibular nystagmus the inhibitory action of Purkinje axons upon vestibular neurones (ITO and YOSHIDA 1964) plays an important role. As shown anatomically by ANGAUT and BRODAL (1967), Purkinje axons from the vestibulo-cerebellum do indeed reach all vestibular nuclei (Fig. 13). Direct physiological evidence for a monosynaptic inhibitory connection between flocculus and the superior vestibular nucleus has recently been given by ITO (1968). Since the vestibular nuclei are an important link in the transmission of vestibular impulses to the oculomotor system, an inhibitory action of the vestibulo-cerebellum onto vestibular neurones can effectively influence the transmission at this level. On the other hand, ablation of parts of the vestibulo-cerebellum causes prolongation of vestibular-induced nystagmus

(Bauer and Leidler 1912; Fernandez and Fredrickson 1963). This effect could be explained as being due to the removal of the inhibitory regulation of the vestibulo-cerebellum onto vestibular neurones by interruption of the labyrintho-cerebello-vestibular loop. Evidence for a tonic inhibitory action of cerebellar Purkinje cells on vestibular neurones has been obtained by Ito, Kawai, Udo and Sato (1968). Furthermore, removal of the vestibular cortex would also cause disinhibition of the deep cerebellar nuclei which are known to receive primary and secondary vestibular fibers (Brodal and Hoivik 1964; Precht and Llinás 1968) and in turn project to the vestibular nuclei. There is strong evidence for this projection being an excitatory one (Shimazu 1967). In this manner withdrawal of the direct Purkinje cell inhibition on the vestibular neurones, as well as the disinhibition of the excitatory nucleo-vestibular pathways, may combine in the generation of an enhanced response of the brain stem centers upon vestibular stimulation. For the time being, it is impossible to assess any functional differences between the various subdivisions of the vestibulo-cerebellum on the basis of microphysiological studies. More precisely controlled studies of the afferent pathways by means of adequate labyrinthine stimulation and the effects produced thereby in various parts of the vestibulo-cerebellum may reveal functional differences. The anatomical studies of the projection from the vestibulo-cerebellum to the vestibular nuclei by Angaut and Brodal (1967) show that there is a differential distribution in the vestibular nuclei of the Purkinje axons originating in different parts of the cerebellar cortex. Thus, for example, fibers from the nodulus-uvula region reach only the "hindlimb" region of the lateral vestibular nucleus while those from the flocculus go to both the hindlimb and forelimb areas of that nucleus (Angaut and Brodal 1967).

Besides the specific information relating to the vestibulo-cerebellar interaction, several overall conclusions regarding cerebellar function can be drawn from this study. The interaction experiments between ipsilateral and contralateral vestibular volleys in the cat cerebellum suggest that distribution of the Golgi cell axonal plexus may not generate a random inhibitory action on the granule cells but, rather, that it may favor particular patterns of interaction between mossy fiber inputs. In this manner more than an overload-preventing mechanism, a "pattern sensitive" function, can be attributed to this inhibitory feedback, especially since as shown by Hámori and Szentágothai (1966) anatomically, and confirmed functionally by Eccles, Sasaki and Strata (1967b), the mossy fiber itself has a sizable direct relation to the Golgi cells. It is evident that if this were to be the case, the complexity of function of the cerebellar circuitry would become orders of magnitude larger than has been assumed up to now, given that a more or less random distribution for the Golgi cell axons was implicitly taken (Eccles, Llinás and Sasaki 1966b).

Of general interest is the possibility borne out by the differences between vestibulo-cerebellar projection systems in frog and cat, that the climbing and mossy fiber system may represent two distinct information channeling systems (Llinás, Hillman and Precht 1969). The presence of a dual afferent system into the cerebellum could then be interpreted as utilizing the Purkinje cell mantle in a "time-sharing" fashion for quite different functions, and not primarily for interaction at the Purkinje cell level.

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References

ANDERSON, S., and B.E. GERNANDT: Cortical projection of vestibular nerve in cat. Acta oto-laryng. (Stockh.), Suppl. **116**, 10—18 (1954).

ANGAUT, P., and A. BRODAL: The projection of the "vestibulo-cerebellum" onto the vestibular nuclei in the cat. Arch. ital. Biol. **105**, 441—479 (1967).

BAUER, J., u. R. LEIDLER: Über den Einfluß der Ausschaltung verschiedener Hirnabschnitte auf die vestibulären Augenreflexe. Arb. neurol. Inst. Univ. Wien **19**, 155—226 (1912).

BAVA, A., S. SAPIENZA e A. URBANO: Proiezioni discendenti dalla corteccia cerebrale al lobo flocculonodulare del cerveletto di gatto: Ricerche electtrofisiologiche. Arch. Sci. biol. (Bologna) **50**, 63—79 (1966).

BRODAL, A., and P.A. DRABLØS: Two types of mossy fibre terminals in the cerebellum and their regional distribution. J. comp. Neurol. **121**, 173—187 (1963).

—, and B. HOIVIK: Site and mode of termination of primary vestibulo-cerebellar fibres in the cat. An experimental study with silver impregnation methods. Arch. ital. Biol. **102**, 1—21 (1964).

—, u. A. TORVIK: Über den Ursprung der sekundären vestibulocerebellaren Fasern bei der Katze. Eine experimentellanatomische Studie. Arch. Psychiat. Z. ges. Neurol. **195**, 550—567 (1957).

BURLET, H.M. DE: Zur vergleichenden Anatomie der Labyrinthinnervation. J. comp. Neurol. **47**, 155—169 (1929).

CHANG, S.T., and P.G. KOSTYUK: Discharges of single neurons of the toad's cerebellum evoked by stimulating the vestibular nerve. Fiziol. Zh. (Mosk.) **46**, 926—932 (1960).

DOW, R.S.: The fibre connections of the posterior parts of the cerebellum in the cat and rat. J. comp. Neurol. **63**, 527—548 (1936).

— Cerebellar action potentials in response to stimulation of various afferent connections. J. Neurophysiol. **2**, 543—555 (1939).

DUENSING, F., u. K.P. SCHAEFER: Über die Konvergenz verschiedener labyrinthärer Afferenzen auf einzelne Neurone des Vestibulariskerngebietes. Arch. Psychiat. Nervenkr. **199**, 345—371 (1959).

ECCLES, J.C., R. LLINÁS and K. SASAKI: Parallel fibre stimulation and the responses induced thereby in the Purkinje cells of the cerebellum. Exp. Brain Res. **1**, 17—39 (1966a).

— — — The mossy fibre-granule cell relay of the cerebellum and its inhibitory control by Golgi cells. Exp. Brain Res. **1**, 82—101 (1966b).

— — — The excitatory synaptic action of climbing fibres on the Purkinje cells of the cerebellum. J. Physiol. (Lond.) **182**, 268—296 (1966c).

—, K. SASAKI and P. STRATA: Interpretation of the potential fields generated in the cerebellar cortex by a mossy fibre volley. Exp. Brain Res. **3**, 58—80 (1967a).

— — — A comparison of the inhibitory actions of Golgi cells and basket cells. Exp. Brain Res. **3**, 81—94 (1967b).

FERNANDEZ, C., and J.M. FREDRICKSON: Experimental cerebellar lesions and their effect on vestibular function. Acta oto-laryng. (Stockh.), Suppl. **192**, 52—62 (1963).

HÁMORI, J., and J. SZENTÁGOTHAI: Participation of Golgi neuron processes in the cerebellar glomeruli: an electron microscope study. Exp. Brain Res. **2**, 35—48 (1966).

HERRICK, C.J.: Origin and evolution of the cerebellum. Arch. Neurol. Psychiat. (Chic.) **11**, 621—652 (1924).

ITO, M.: The mode of integration at the cerebellar subcortical neurones. Proc. Intern. Union of Physiol. Sci., Vol. VII (1968).

—, N. KAWAI, M. UDO and N. SATO: Cerebellar-evoked disinhibition in dorsal Deiters' neurones. Exp. Brain Res. **6**, 247—264 (1968).

—, and M. YOSHIDA: The cerebellar-evoked monosynaptic inhibition of Deiters' neurones. Experientia (Basel) **20**, 515—516 (1964).

JANSEN, J., and A. BRODAL: Aspects of Cerebellar Anatomy. Oslo: Johan Grundt Tanum 1954.

LARSELL, O.: The cerebellum of the frog. J. comp. Neurol. **36**, 89—112 (1923).

— The development of the cerebellum in the frog *(Hyla regilla)* in relation to the vestibular and lateral-line system. J. comp. Neurol. **39**, 249—289 (1925).

Larsell, O.: The comparative morphology of the membranous labyrinth and the lateral-line organs in their relation to the development of the cerebellum. In: The Cerebellum, pp. 279—328. Baltimore: William and Wilkins 1929.

— Morphogenesis and evolution of the cerebellum. Arch. Neurol. Psychiat. (Chic.) **31**, 373—395 (1934).

Llinás, R.: Functional aspects of interneuronal evolution in the cerebellar cortex. In: The Interneurone, UCLA Forum in Medical Sciences No. 11. Ed. by M. A. B. Brazier. Los Angeles: Univ. of Calif. Press 1969. (in press).

—, and J. Bloedel: Climbing fibre activation of Purkinje cells in the frog cerebellum. Brain Res. **3**, 299—302 (1966/1967).

— — Frog cerebellum: Absence of long-term inhibition upon Purkinje cells. Science **155**, 601—603 (1967).

—, D. E. Hillman and W. Precht: Functional aspects of cerebellar evolution. In: The Cerebellum in Health and Disease. Ed. by W. S. Fields and W. D. Willis, Jr., St. Louis: Warren H. Green 1969. (in press).

—, and W. Precht: The inhibitory vestibular efferent system and its relation to the cerebellum in the frog. Exp. Brain Res. **8**, 16—29 (1969).

— — and S. T. Kitai: Climbing fibre activation of Purkinje cell following primary vestibular afferent stimulation in the frog. Brain Res. **6**, 371—375 (1967a).

— — — Cerebellar Purkinje cell projection to the peripheral vestibular organ in the frog. Science **158**, 1328—1330 (1967b).

Matthews, P. B. C., C. G. Phillips and G. Rushworth: Afferent systems converging upon cerebellar Purkinje cells in frog. Quart. J. exp. Physiol. **43**, 38—52 (1958).

Peterson, B. W.: Effect of tilting on the activity of neurons in the vestibular nuclei of the cat. Brain Res. **6**, 606—609 (1967).

Pompeiano, O., and A. Brodal: Spino-vestibular fibres in the cat. An experimental study. J. comp. Neurol. **108**, 353—382 (1967).

Precht, W., and R. Llinás: Direct vestibular afferents to cat cerebellar nuclei. Proc. Intern. Union of Physiol. Sci., Vol. VII, Abst. 1063, p. 355 (1968).

—, and H. Shimazu: Functional connections of tonic and kinetic vestibular neurons with primary vestibular afferents. J. Neurophysiol. **28**, 1014—1028 (1965).

Ramón y Cajal, S.: La Textura del Sistema Nervioso del Hombre y los Vertebrados. Madrid: Moya 1904.

Sasaki, K., and P. Strata: Responses evoked in the cerebellar cortex by stimulating mossy fibre pathways to the cerebellum. Exp. Brain Res. **3**, 95—110 (1967).

Shimazu, H.: Mutual interactions between bilateral vestibular nuclei and their significance in motor regulation. In: Neurophysiological Basis of Normal and Abnormal Motor Activities. Ed. by M. D. Yahr and D. P. Purpura. Hewlett, N.Y.: Raven Press 1968.

—, and W. Precht: Tonic and kinetic responses of cat's vestibular neurons to horizontal angular acceleration. J. Neurophysiol. **28**, 991—1013 (1965).

Snider, R. S.: Alterations which occur in mossy terminals of the cerebellum following transection of the brachium pontis. J. comp. Neurol. **65**, 417—435 (1936).

Stein, B. M., and M. B. Carpenter: Central projections of portions of the vestibular ganglia innervating specific parts of the labyrinth in rhesus monkey. Amer. J. Anat. **120**, 281—318 (1967).

Szentágothai, J., u. K. Rajkovits: Über den Ursprung der Kletterfasern des Kleinhirns. Z. Anat. Entwickl.-Gesch. **121**, 130—141 (1959).

Trinker, D.: Kennlinien der Receptoren und primären Neurone des nichtakustischen Säuger Labyrinthes. Pflügers Arch. ges. Physiol. **281**, 87 (1964).

Walberg, F.: Descending connections to the inferior olive. An experimental study in the cat. J. comp. Neurol. **104**, 77—173 (1956).

Wall, P. D.: The origin of a spinal-cord slow potential. J. Physiol. (Lond.) **164**, 508—526 (1962).

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