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Cervical Effects on Abducens Motoneurons and Their Interaction with Vestibulo-Ocular Reflex

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Summary. The effect of neck afferents on abducens motoneurons and their interaction with the vestibulo-abducens reflex were examined in chloralose-anesthetized or unanesthetized, decerebrate cats. The test reflex elicited in the abducens nerve by stimulation of the contralateral vestibular nerve was inhibited by contralateral and facilitated by ipsilateral cervical dorsal root or neck joint stimulation. These reciprocal effects were obtained by stimulation at the level of C2 and C3, but not from C5 or lower. Contralateral and ipsilateral cervical stimulation induced IPSPs and EPSPs, respectively, in abducens motoneurons. The latencies were 2.8—6.0 msec for the IPSP and 2.8—5.3 msec for the EPSP after stimulation of the dorsal root.

The labyrinthine-induced disynaptic IPSP or EPSP was facilitated by conditioning stimulation of the contralateral and ipsilateral cervical dorsal root, respectively. It is thus postulated that the cervico-abducens and vestibulo-abducens reflex pathways converge upon common inhibitory or excitatory interneurons in the vestibular nuclie. Labyrinthine- and cervical-induced responses of the presumed interneurons in the vestibular nuclei or those of their axons recorded in the abducens nuclie were consistent with the above view.

Lesion experiments in the brain stem indicated that afferent volleys from the neck joint ascend ipsilaterally in the spinal cord, cross to the contralateral side in the brain stem, and eventually project to the vestibular nuclei, thus interacting with the vestibulo-ocular reflex activity.

A possible functional role of the cervical effects on the ocular motoneuron was briefly discussed.

Key words: Cervical afferents — Abducens motoneuron — Interneuron — Vestibular nuclei

Introduction

Since the experiments of Magnus and his collaborators (Magnus and de Kleijn, 1913; Magnus and Storm van Leeuwen, 1914; Magnus, 1924), there have been several investigations on the role of neck proprioceptive mechanisms in postural adjustment (Cohen, 1961; Abrahams and Falchetto, 1969) and their

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close relation to vestibular function (Frenzel, 1928; Fredrickson *et al.*, 1966). Besides the body orientation reflex, the neck proprioceptors play an important role in control of eye position (Magnus, 1924). Extraocular muscle tension is influenced by spinal nerve stimulation in the rabbit (Suzuki and Takemori, 1971). Twisting of the neck and body induces eye deviation in the rabbit as well as in man (Takemori and Suzuki, 1969). Frenzel (1928) studied the influence of neck torsion on nystagmus in man and reported a shift of the beating field of nystagmus. There have been few studies, however, on the neuronal organization between cervical afferents and ocular motoneurons.

The purpose of the present experiment is to elucidate the effects of neck afferents upon abducens motoneurons and their interaction with the vestibulo-ocular reflex. The elementary vestibulo-ocular reflex arc has been analyzed by anatomical and physiological studies (Lorente de Nó, 1933; Szentágothai, 1950; McMaster *et al.*, 1966; Tarlov, 1970). It has been revealed that the reflex arc is composed of excitatory and inhibitory pathways, which act on the abducens motoneurons disynaptically from the primary vestibular nerve (Baker *et al.*, 1969). Similar organization has been found for trochlear (Precht and Baker, 1972) and oculomotor neurons (Highstein *et al.*, 1971). It will be shown here that neck afferents act on the abducens motoneurons through the vestibular nuclei and that both the cervico-abducens and vestibulo-abducens reflex pathways have common excitatory or inhibitory interneurons in the vestibular nuclei.

Methods

Preparation

The present results were obtained with 58 adult cats. Surgical procedures were completed under ether anesthesia. Stimulating electrodes (a fine Ag-AgCl wire) were placed on the vestibular nerve (Shimazu and Precht, 1965) and also, in some experiments, on the region of the upper neck joints (see Fig. 1G) after removal of dorsal neck muscles. In other experiments the cervical dorsal roots were stimulated, and for this a laminectomy was performed. The abducens nerve was detached from the lateral rectus muscle on each side, and a fine Ag-AgCl electrode was placed on the nerve for either stimulation or recording.

The medial part of the cerebellum was aspirated in order to expose the floor of the fourth ventricle. During this procedure the anterior lobe and fastigial nuclei of the cerebellum were removed. In 37 preparations chloralose was administered intravenously (50 mg/kg) and ether was discontinued. In the remaining 21 experiments the animals were decerebrated at the intercollicular level and were maintained without anesthesia. The animals were immobilized with intermittent i. v. injection of gallamine triethiodide (Flaxedil, Teikoku-kagaku) under continued artificial respiration with room air. A pneumothorax was made bilaterally in order to reduce movements of the brain tissue. The systolic blood pressure was maintained at 110—130 mm Hg by intravenous injection of pressor agents, if necessary, and the rectal temperature was kept at 36—37.5° C by a heating pad throughout the experiment.

For the purpose of histological studies, the animal was sacrificed after each experiment by i. v. administration of a large dose of pentobarbital sodium. The brain tissue was removed after injecting 10% formalin solution into the carotid artery.

Stimulation

The vestibular and abducens nerves were stimulated with rectangular pulses of 0.1 msec duration. Single shocks or pulse trains (2.5 msec intervals) were applied to the cervical dorsal roots (less than 2—3 volts) and neck joints (less than 10 volts) once every 2 to 3 seconds. The total number of pulses in a train varied up to 20. Adequate stimulation of the end organ in the horizontal semicircular canal was applied by horizontal rotation of the turn-table.

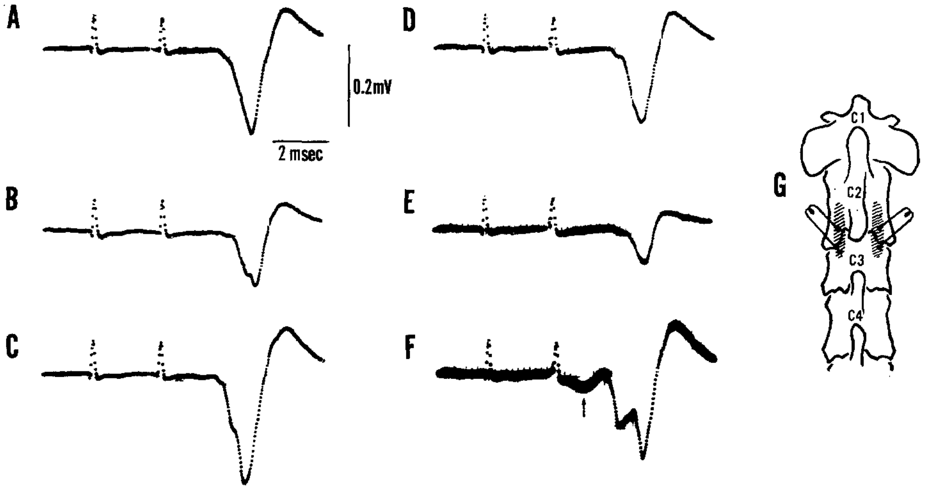

Fig. 1. *Conditioning effects of cervical afferent volleys on vestibulo-abducens reflex.* A and D: test responses in left abducens nerve to double shocks to right vestibular nerve (average over 5 sweeps). Control for B—C and E—F, respectively. B and C: conditioning single shocks (not shown on trace) were applied to right (contralateral) (B) and left (ipsilateral) (C) dorsal root at C2. Interval between conditioning shock and second test shock was 8 msec. E and F: conditioning triple shocks were applied to right (E) and left (F) neck joint at C2—C3. Interval between 3rd conditioning shock and 2nd test shock was 7 msec. G: hatched area represents the region of upper neck joint where stimulating electrodes were placed. Time constant of the recording system was 100 msec

Recording

Glass micropipettes containing 3 M KCl or 2 M K-citrate were used for intracellular recording from abducens motoneurons and for recording from axons of the vestibular neurons within the abducens nuclei. The electrode resistance was 15—30 MΩ. A conventional input stage was used for recording and for passing current through the microelectrode. For extracellular recording of the spikes of the vestibular neurons, glass micropipettes filled with Ringer solution, with d.c. resistances of 5—8 MΩ were inserted in the dorsoventral direction into the vestibular nuclei. The potentials were fed into a dual-beam oscilloscope (Tektronix, type 565) after d. c. amplification.

The action potential of the abducens nerve was averaged over 5—10 sweeps using an electronic computer (ATAC 501, Nihon Kohden). The frequency of occurrence of vestibular unit spikes before and after cervical stimulation was counted with the computer over 200–400 sweeps in order to yield the histogram of spike distribution (Kasahara *et al.*, 1968).

Results

I. Influence of Cervical Somatic Afferent Volleys Upon Vestibulo-Abducens Reflex

Effects of Dorsal Root Stimulation

Action potentials were induced in the abducens nerve when a single shock was applied to the contralateral vestibular nerve. Amplitudes of the reflex response, which will be called the test reflex, often fluctuated appreciably in successive trials. In these cases double shocks (the intensity of the first shock being so adjusted as to be subthreshold for the reflex response) were applied to the vestibular nerve to make the amplitude of the test response more constant. The test reflex was averaged over 5—10 sweeps in order to increase even further the reliability of measurement of the amplitudes of the test reflex as an indicator of the conditioning effects.

Figure 1A shows the control test response in the left abducens nerve. Prior to the test shocks the right dorsal root at the level of C2 or C3 segment was stimulated by a single shock, which will be called "contralateral conditioning" shock. The amplitude of the test response was thereby markedly reduced (Fig. 1B), and this inhibitory effect was evident throughout a wide range of test response amplitude. On the other hand, it was remarkably increased by conditioning stimulation of the left (ipsilateral) C2 or C3 dorsal root (Fig. 1C). No appreciable discharges were evoked by the conditioning shock alone, though they were occasionally induced when strong stimulation of more than 10 pulses was applied.

Conditioning shocks at the level of C2 or C3 segment were most effective for provoking these effects, whereas those applied to the dorsal roots caudal to the fifth segment induced neither facilitation nor inhibition.

Effect of Cervical Deep Somatic Stimulation

Stimulation was applied to the nerve innervating mainly the ipsi- or contralateral biventer cervicis and complexus muscles. This did not evoke any suppression or facilitation of the test reflex with the exception of slight bilaterally induced facilitation in a few cases, even though the stimulus strength was increased up to ten times the threshold for the nerve potential.

After removal of the dorsal neck muscles, stimulating electrodes were placed on the region of upper neck joints (Fig. 1G). Electric currents applied through the electrodes may excite afferents from the ligaments, capsules and periostea around the intervertebral joints. Figure 1D—F represent results in an experiment in which the test reflex was conditioned by volleys arising from the neck joints at the level of C2—C3. Contralateral conditioning shocks inhibited the test reflex (compare Fig. 1D and E), whereas ipsilateral conditioning shocks facilitated it (Fig. 1F). It was noted that the first test volleys alone, which did not produce any response in the control record (Fig. 1D), induced a clear response (Fig. 1F, arrow) after ipsilateral conditioning shocks to the neck joints. The effects induced by a conditioning shock to the contralateral or ipsilateral neck joint were similar to those produced by dorsal root stimulation described above. Double or multiple shocks lowered the threshold intensity for the conditioning effect. The lowering of the threshold for the inhibition or the facilitation with multiple volleys may indicate the importance of temporal summation along the pathways from the neck joint to the abducens nuclei.

Figure 2 represents effects of 1% procaine locally applied to the neck joint region where the stimulating electrodes were placed. The control effects of the conditioning stimulation (compare Fig. 2B with A) was slightly reduced 5 min after the application (Fig. 2C) and completely abolished 15 min later (Fig. 2D). Ten minutes after washing out procaine by Ringer solution the effect began to reappear (Fig. 2E), and then returned to the original value (Fig. 2F) 15 min later. This finding excludes the possibility that the conditioning effects were induced by a spread of stimulating currents to the dorsal roots or directly to the spinal cord,

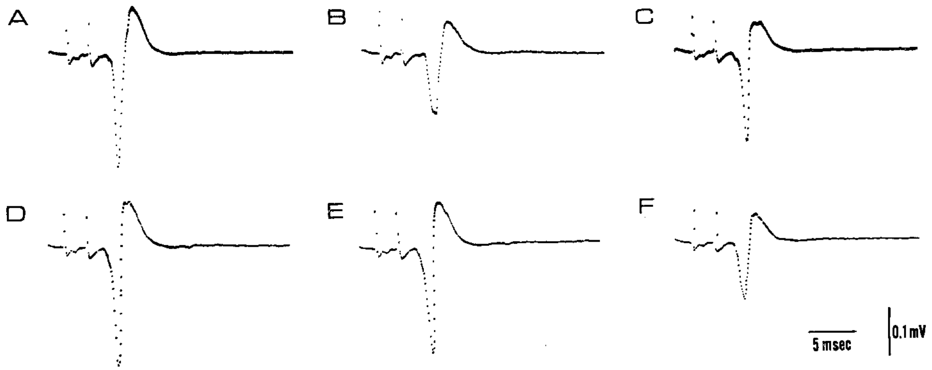

Fig. 2. *Effect of local application of procaine around neck joint region where stimulating electrodes were placed.* A: control test response in left abducens nerve. B: inhibition of the test response induced by stimulation of right neck joint (conditioning artifact not shown). C: record taken 5 min after procaine application around stimulating electrodes, indicating slight reduction of inhibition. D: complete abolition of inhibitory effect 15 min later. E: record taken 10 min after washing procaine by Ringer solution. F: 15 min later. Inhibition recovered to the original level. Each trace was obtained by averaging over 10 sweeps

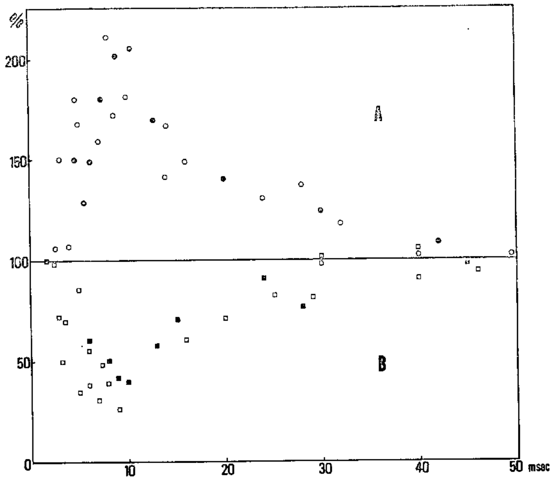

Fig. 3. *Time course of facilitation (A) and inhibition (B) of the test reflex by conditioning shocks to cervical dorsal root or neck joint.* The effect of conditioning on the test reflex is expressed on the ordinate as per cent of control amplitude. Abscissa represents time interval between conditioning and test shocks. When multiple pulses were used for conditioning or test stimulation, the interval between the effective shocks of each stimulation was plotted. Filled circle and square were obtained in one preparation with ipsi- and contralateral dorsal root stimulation, respectively. Open circle and square were from four preparations with ipsi- and contralateral neck joint stimulation, respectively

and indicates that the inhibitory effects originate from the neck joint region. The facilitatory influence from the ipsilateral neck joint was also blocked by procaine application to the stimulated region.

The reciprocal effect on abducens activity, that is, facilitation from the ipsilateral and inhibition from the contralateral dorsal root or neck joint, was obtained in all of the 37 preparations anesthetized with chloralose. Among 21 decerebrate, unanesthetized animals, the above reciprocal effect was obtained in 11 preparations, whereas the test reflex was facilitated in 8 animals and was inhibited in 2 animals by both ipsilateral and contralateral cervical stimulation. The difference of the results between anesthetized and unanesthetized animals might be related to different sensitivities of reciprocal and non-reciprocal (diffuse) pathways to anesthetic agents (Shimazu *et al.*, 1962). The present paper deals with an analysis of neural mechanisms mediating the reciprocal effects.

Time Course of Inhibition and Facilitation

To determine the time course of the effects of conditioning cervical stimulation on the test response, the test stimulus strength was adjusted so as to give about one-third of the maximal response.

In Fig. 3 amplitudes of the test response expressed as per cent of the control value were plotted against the time interval between conditioning and test volleys. As the interval between the conditioning and the test shocks was progressively increased, inhibition from the contralateral (Fig. 3B) as well as facilitation from the ipsilateral cervical afferents (Fig. 3A) was first detected at approximately 3 msec, attained its maximum at 8—10 msec, and then regressed slowly over up to 30—50 msec. The latency of inhibition could not be accurately determined because of small but inevitable fluctuations of the test response amplitudes.

When long train pulses such as 10—20 shocks were applied as the conditioning stimulation, the time course of inhibition and facilitation were considerably prolonged. The former lasted over about 80 msec and the latter more than 150 msec.

II. Postsynaptic Potentials Evoked in Abducens Motoneurons by Cervical Stimulation

The abducens motoneurons impaled were identified by their antidromic responses to stimulation of the ipsilateral abducens nerve (Fig. 4A). The data were sampled from the intracellular records of 76 abducens motoneurons in which the membrane potential ranged from –40 to –70 mV.

Stimulation of Contralateral Dorsal Root and Neck Joint Region at C2—C3

Contralateral dorsal root stimulation produced a hyperpolarizing potential in the abducens motoneurons. With increased stimulus intensity, or increased number of pulses, the hyperpolarization became larger in amplitude and longer in duration (Fig. 4D—F). The amplitude attained 5 mV and the duration 20–40 msec with single to triple shocks. The duration was approximately consistent with that of the inhibition curve described above (Fig. 3B). In order to clarify the nature of synaptic events in the abducens motoneurons underlying the hyperpolarizing potential, Cl^- ions were electrophoretically injected into the cell by passing hyperpolarizing currents through the recording micro-electrode. Under this condition, the hyperpolarization induced from the contralateral dorsal root was inverted into a depolarizing potential (Fig. 4G—I). The reversal of the hyperpolarization to a depolarization was obtained during intracellular passage of hyperpolarizing current as well. Thus the hyperpolarization should represent an

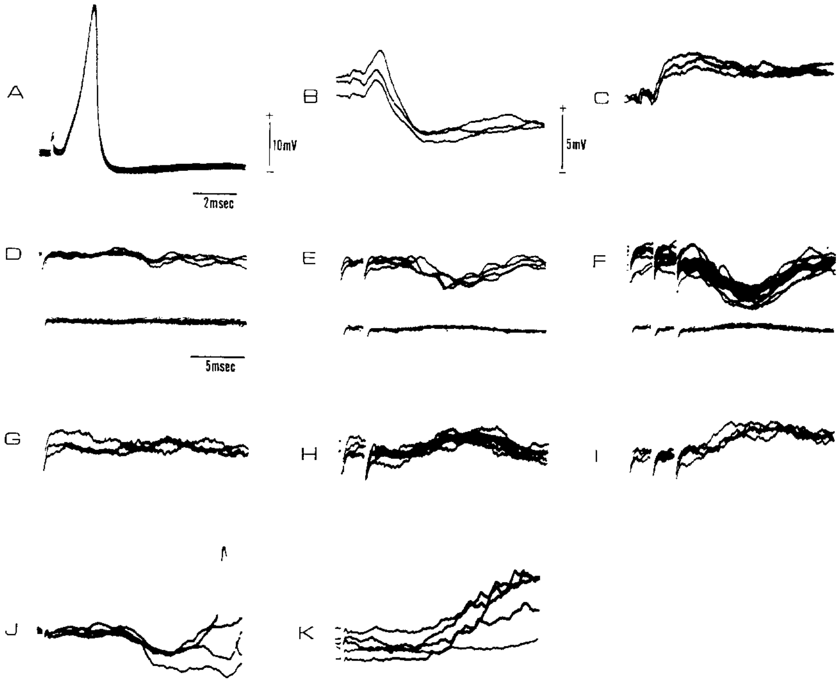

Fig. 4. *Intracellular records from abducens motoneurons in response to contralateral cervical stimulation.* Records A—I were obtained from one motoneuron and records J—K were from another. A: antidromic response to stimulation of left abducens nerve. B: IPSPs induced by single shocks to left vestibular nerve. C: same as in B but after Cl^- injection into the cell. D—F: IPSPs induced by right dorsal root stimulation at C2 with increased number (single to triple) of stimulus pulses. Lower traces in each record represent extracellular field potentials. G—I: same as in D—F but records obtained during the same period when reversal of the vestibular-induced IPSP (C) occurred due to Cl^- injection. J and K: IPSPs in response to single shocks to right neck joint at C2—C3 (J) and those recorded after Cl^- injection (K). Voltage calibration for B also applies to C—K. Time scale for A also applies to B and C, and that for D to E—K

inhibitory postsynaptic potential (IPSP) (Coombs *et al.*, 1955; Eccles, 1964). Similar IPSPs were produced by stimulation of the contralateral neck joint (Fig. 4J and K). The reversal of polarity of the IPSP induced by cervical stimulation and that of the IPSP produced by ipsilateral vestibular nerve stimulation (Baker *et al.*, 1969) (Fig. 4B and C) always occurred concurrently with Cl^- injection, a dissociation of polarity of the two IPSPs induced from the different sources never being observed.

Latencies of the IPSPs induced from the contralateral dorsal root and the neck joint were determined by superimposing the original IPSPs either on those inverted by Cl^- injection or on the extracellular field potential. When a single shock did not produce any detectable IPSP, double or triple shocks were applied. In these cases its latency was measured from the effective shock. The latencies ranged from 2.8 to 6.0 msec ($n = 24$, mean 4.1, S.D. 0.9) from the dorsal root and 3.0 to 7.5 msec ($n = 28$, mean 4.6, S.D. 1.1) from the neck joint. Figure 5 represents histrogams of latencies of the IPSPs induced by stimulation of the

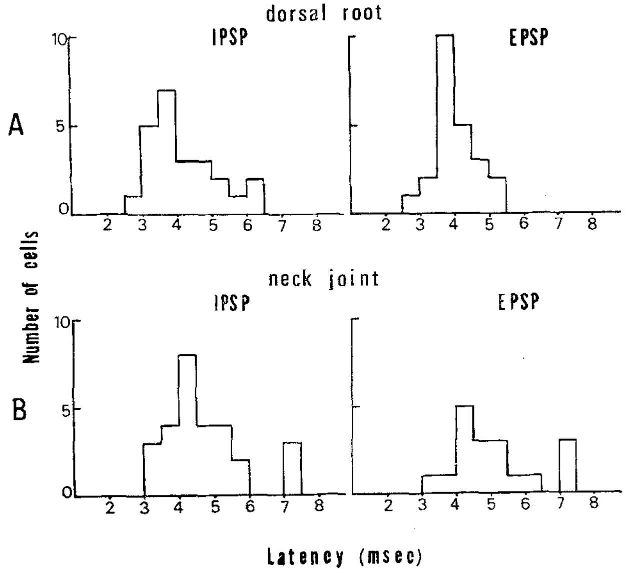

Fig. 5. *Histograms of latencies of IPSPs and EPSPs evoked in abducens motoneurons by stimulation of cervical dorsal roots (A) and neck joint (B).* Left histograms: distribution of latencies of IPSPs induced by contralateral stimulation. Right histograms: that of EPSPs induced by ipsilateral stimulation

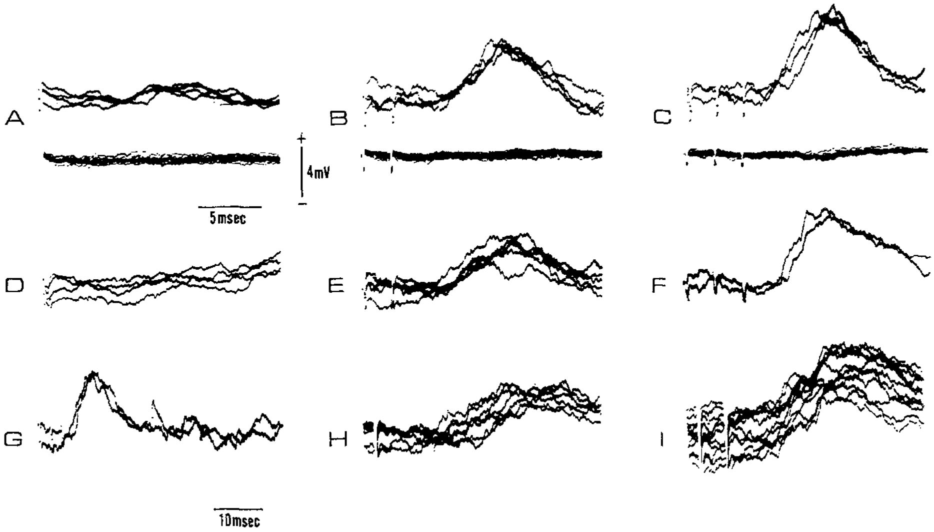

Fig. 6. *Intracellular records from abducens motoneurons in response to ipsilateral cervical stimulation.* Records A—G were obtained from one motoneuron and records H—I were from another. A—C: EPSPs induced by ipsilateral dorsal root stimulation at C2 with single to triple shocks. Lower traces in each record indicate extracellular field potentials. D—F: same as in A—C but after Cl^- injection that was sufficient to reverse the vestibular-induced IPSP to depolarization. G: same as in C but recorded with slower sweep speed. H and I: EPSPs induced by single and double shocks to ipsilateral neck joint at C2—C3. Calibration for A applies to other traces, Time scale for A applies to other traces except for G

contralateral dorsal root (Fig. 5A, left) and the neck joint (Fig. 5B, left). The histograms reveal a tendency for the IPSPs produced from the dorsal root to start slightly earlier than neck joint-induced IPSPs. The latency difference, 0.5 msec on the average, was statistically significant at the level of $P < 0.1$ (t-test). It may be attributed to the time required for the impulse propagation along the distance from the stimulated spots at the neck joint to the dorsal root.

Stimulation of Ipsilateral Dorsal Root and Neck Joint Region at C2—C3

A depolarizing potential was induced in abducens motoneurons by ipsilateral dorsal root stimulation. The depolarization became larger in amplitude and longer in duration with increased intensity and pulse number of the stimulation (Fig. 6A—C). The depolarizing potential was not appreciably changed after Cl^- injection into the motoneuron (Fig. 6D—F), thus indicating that the depolarization was mainly due to the excitatory postsynaptic potential (EPSP). Similar EPSPs were produced after ipsilateral neck joint stimulation (Fig. 6H and I). The latencies of the EPSPs induced from the ipsilateral dorsal root and the neck joint were determined by superimposing the EPSPs on the extracellular field potentials. The latencies ranged from 2.8 to 5.3 msec ($n = 23$, mean 3.9, S.D. 0.6) from the dorsal root and 3.2 to 7.3 msec ($n = 18$, mean 4.9, S.D. 1.2) from the neck joint. The difference between latencies of EPSPs induced from the two different sources was statistically significant (t-test, $P < 0.001$). Figure 5 depicts latency histograms of the EPSPs produced by stimulation of the ipsilateral dorsal root (Fig. 5A, right) and neck joint (Fig. 5B, right). Inspection of the histograms in Fig. 5 reveals that the latencies of the EPSPs and IPSPs distribute within approximately the same range (compare left with right histogram in A and B, respectively), suggesting that the pathways mediating the excitation and the inhibition from the cervical afferents are not very different in complexity.

Durations of EPSPs in abducens motoneurons produced by single to triple shocks to the ipsilateral dorsal root or neck joint were about 20—40 msec (Fig. 6G), which were similar to that of the facilitation of the test reflex (Fig. 3A).

Interaction of Vestibular and Cervical Effects Upon Abducens Motoneurons

Stimulation of the ipsilateral and contralateral vestibular nerve produces an IPSP and EPSP, respectively, in the abducens motoneurons and these PSPs are disynaptically evoked from the nerve, the interneurons in the reflex pathway being located mainly in the rostral part of the medial vestibular nucleus (Baker *et al.*, 1969). An examination was made to detect whether there is any interaction between labyrinthine and cervical volleys at the internuncial level in the vestibular nuclei. Figure 7A shows a control disynaptic IPSP (reversed in polarity after Cl^- injection) in an abducens motoneuron evoked by ipsilateral vestibular nerve stimulation. When conditioned by contralateral cervical stimulation that was so adjusted as to produce only a small IPSP (Fig. 7C), the same test vestibular volley induced a larger disynaptic IPSP (Fig. 7B) than the algebraical summation of the control response and the response to the conditioning volley alone (Fig. 7B, dotted line). This finding indicates that the contralateral cervical volleys converge on and facilitate interneurons that mediate disynaptic inhibition from the ipsilateral vestibular nerve.

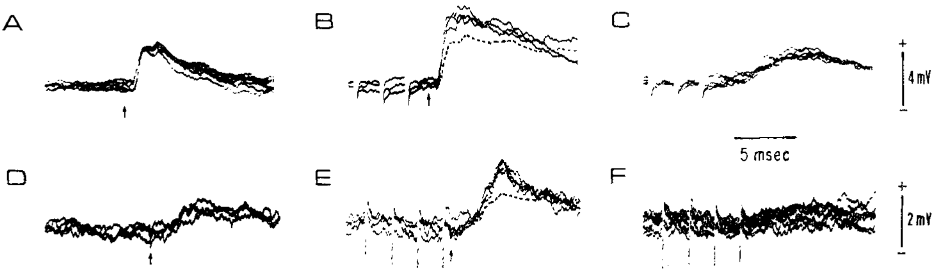

Fig. 7. *Facilitation of the vestibular-induced disynaptic IPSPs and EPSPs by cervical stimulation.* A—C: intracellular records from a left abducens motoneuron. A: disynaptic IPSPs (reversed in polarity after Cl^- injection) evoked by left vestibular nerve stimulation. B: the same test stimulation as in A was conditioned by triple shocks to the right dorsal root. Dotted line indicates an algebraical summation of record A and C. C: response to dorsal root stimulation alone. D—F: Intracellular records from another left abducens motoneuron. D: disynaptic EPSPs evoked by right vestibular nerve stimulation. E: the same test stimulation as in D was conditioned by 4 shocks to the left dorsal root. Dotted line indicates an algebraical summation of record D and F. F: response to dorsal root stimulation alone. Upward arrows in A, B or D, E indicate left or right vestibular nerve stimulation, respectively

Figure 7D—F shows a facilitatory convergence in the excitatory pathway to an abducens motoneuron from the labyrinth and from the neck. A control disynaptic EPSP was evoked in the abducens motoneuron by contralateral vestibular nerve stimulation (Fig. 7D). When the vestibular nerve stimulation was conditioned by shocks to the ipsilateral dorsal root which themselves produced a barely detectable EPSP (Fig. 7F), it induced a larger disynaptic EPSP (Fig. 7E). These results indicate that the facilitatory interaction occurred at the level of the excitatory interneurons in the vestibular nuclei.

If the sites of labyrinthine and cervical interaction are within the vestibular nuclei for both the facilitatory and inhibitory influences on abducens motoneurons, then the vestibular nuclei neurons projecting to the abducens nuclei should exhibit responses to neck stimulation as expected from the above considerations. The results will be described in the following section.

III. Activity of Secondary Vestibular Neurons in Response to Cervical Volleys

Axonal Spikes of Vestibular Neurons Recorded within the Abducens Nucleus

Impulse activity was recorded within the abducens nucleus from axons that are presumed to have their origin in the vestibular nuclei. Identification of these unit spikes followed the criteria described previously (Maeda *et al.*, 1971).

Figure 8C exemplifies a unit that was activated monosynaptically from the ipsilateral vestibular nerve. Cervical effects were found in 28 units. Of these, 25 units were excited by *contralateral* dorsal root or neck joint stimulation (Fig. 8D). but not by ipsilateral cervical stimulation (Fig. 8E). When these units were spontaneously active, they were even inhibited by the latter stimulation. The remaining 3 units were activated by cervical stimulation on both sides. The latencies of the evoked spikes ranged from 2.0 to 3.9 msec ($n = 12$, mean 2.9, S.D. 0.6) from the contralateral dorsal root (Fig. 8F) and 2.1 to 4.3 msec ($n = 13$, mean 3.3, S.D. 0.6) from the neck joint (Fig. 8G). The mean latency was significantly shorter from the dorsal root than from the neck joint (t-test, $P < 0.001$).

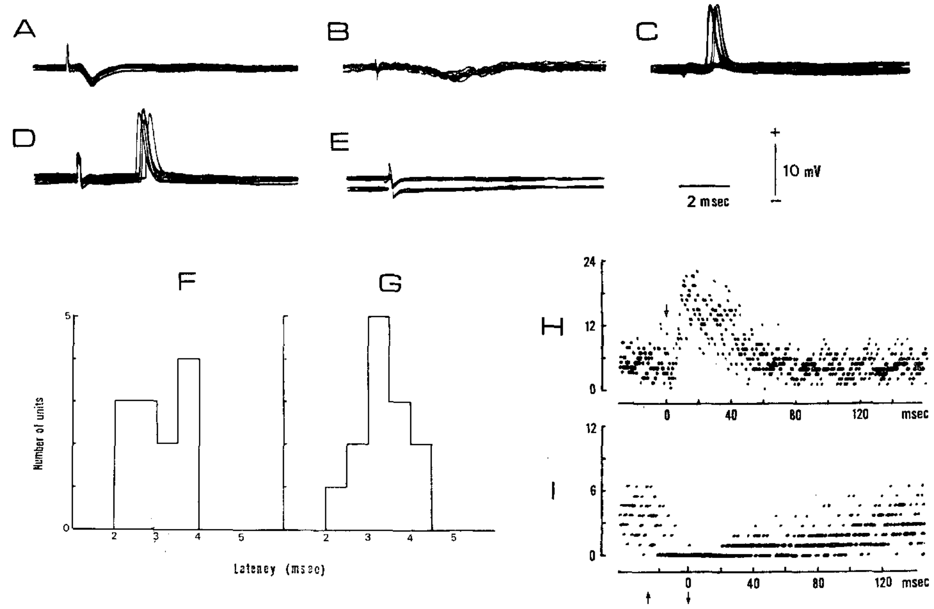

Fig. 8. *Activity of secondary vestibular neurons in response to cervical volleys.* A—E: responses of an axon of secondary vestibular neuron recorded within right abducens nucleus. A: supramaximal antidromic stimulation of the abducens nerve did not evoke spikes but only field potentials. B: single shocks to left vestibular nerve induced only negative field potentials. C: single shocks to right vestibular nerve induced spikes with monosynaptic latencies. D: spikes were also induced by left dorsal root stimulation at C2. E: no responses to right dorsal root stimulation. F and G: histograms of the latencies of axonal spikes after shocks to the contralateral dorsal root (F) and neck joint (G). All of these neurons were activated monosynaptically by ipsilateral vestibular nerve stimulation. H: spike distribution for a type-1 neuron constructed from 300 sweeps, indicating facilitation by single shocks (arrow) to contralateral neck joint. I: that for another type 1 neuron (400 sweeps), indicating inhibition by ten shocks (400/sec) to ipsilateral neck joint. Upward and downward arrows indicate onset and cessation of stimulation, respectively. Ordinate: number of spikes counted at each interval of 0.4 msec. Abscissa: time after stimulation

The units activated by contralateral vestibular nerve stimulation were also influenced by cervical stimulation. The effects examined in 4 units were consistently an activation from the *ipsilateral* dorsal root or neck joint with a latency range similar to that described above.

Vestibular Type-1 Neurons Identified by Horizontal Rotation

Neurons in the vestibular nuclei were identified by horizontal angular acceleration and deceleration. The present experiments deal with type-1 neurons as defined by their frequency responses to horizontal rotation in parallel with receptor activities in the ipsilateral horizontal canal (Gernandt, 1949; Duensing and Schaefer, 1958).

The type-1 neuron was distinctly facilitated by single shocks to the contralateral neck joint (Fig. 8H). In Fig. 8I, on the other hand, long train shocks to the ipsilateral neck joint produced an inhibition of spontaneous discharges of the

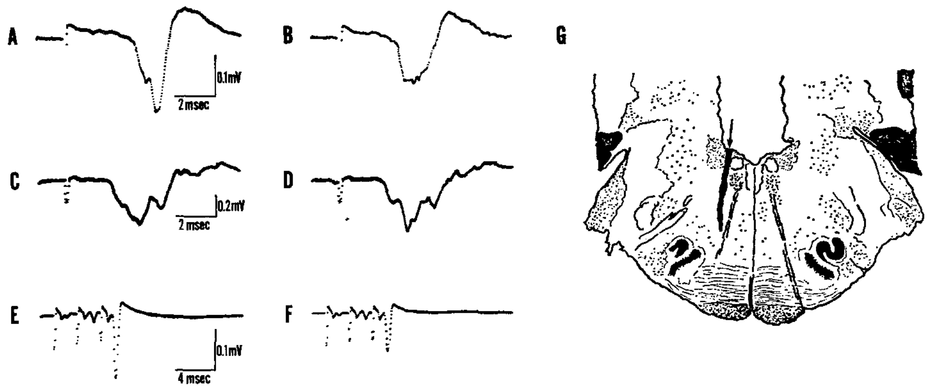

Fig. 9. *Effect of interruption of connections between the vestibular and abducens nuclei.* A: test response in left abducens nerve to right vestibular nerve stimulation. Control for B. B: 5 msec prior to test shock, conditioning triple shocks (not shown) were applied to right neck joint. C and D: same as in A and B, respectively, but records made after longitudinal incision in the brain stem (downward arrow in G) from level of the inferior colliculus to 1 mm caudal to the abducens nucleus with approximately 4 mm in depth. E: response in right abducens nerve to direct stimulation of the brain stem in the vicinity of left MLF. Control for F. F: the test response was inhibited by conditioning stimulation of left neck joint. Each trace was obtained with average over 10 sweeps. Time scale and calibration for A, C and E apply to B, D and F, respectively. G: drawing of the brain stem at the level of the abducens nuclei, showing longitudinal incision (arrow) between the left vestibular and abducens nuclei

type-1 neurons, though single to triple shocks were not effective. Of 16 type-1 neurons tested, 8 neurons exhibited similar responses to those in Fig. 8 H and I, 3 neurons were activated bilaterally, one neuron was inhibited bilaterally and the remaining 4 neurons received no effects.

IV. Effects of Brain Stem Lesions on the Pathway of Cervico-Abducens Reflex

According to anatomical investigations in the cat, fibers from the rostral portion of the medial vestibular nucleus pass medially into the ipsilateral and the contralateral abducens nuclei (Tarlov, 1970). This finding is consistent with physiological studies in the cat showing that both excitatory and inhibitory neurons are located in the rostral part of the medial vestibular nucleus and that they project to the contralateral and ipsilateral abducens motoneurons, respectively (Baker *et al.*, 1969).

In conformity with these data, a longitudinal incision of the dorsal brain stem was made in four cats between the left vestibular nuclei and the left abducens nucleus, from the level of the inferior colliculus to 1 mm caudal to the abducens nucleus, in order to interrupt the connections between them (Fig. 9G). The extent and depth of the incision were adjusted by monitoring the disappearance of inhibition of test abducens activity caused by stimulation of the left vestibular nerve and were confirmed by histological examination after the experiment. After the incision was made, inhibition of the test response in the left abducens nerve by conditioning stimulation of the right neck joint (compare Fig. 9B with A) was completely abolished (compare Fig. 9D with C). Note that the test abducens response was remarkably augmented after the incision (see different calibration

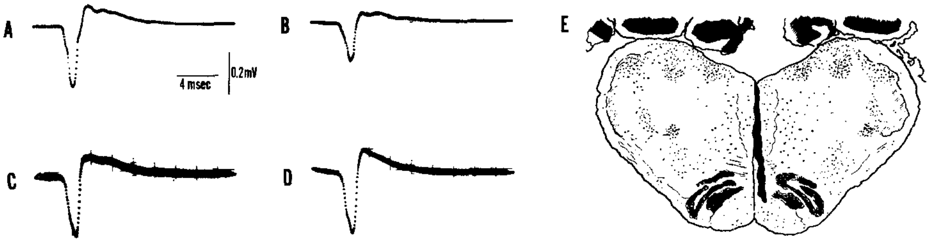

Fig. 10. *Effect of brain stem lesion.* A: test response in right abducens nerve induced by left vestibular nerve stimulation. Records were taken after transverse hemisection (right half) of the spinal cord at the level of its rostral end (obex). B: same test response as in A was still inhibited by triple shocks to left neck joint. C: test response recorded after an additional midline incision from the obex to the level of rostral end of the inferior olive nucleus. Note that amplitude of the test response was not changed (compare C with A). D: conditioning triple shocks were applied to left neck joint. Inhibitory effect was completely abolished (compare D with C). Each trace was obtained with average over 10 sweeps. E: drawing of the brain stem at a level 400 micra caudal to the rostral end of the midline incision

scales), probably because of a decrease of tonic inhibition coming from the left vestibular nuclei. Abolition of the neck-induced inhibition after the incision cannot be ascribed to general depression of brain stem activity due to the surgical procedures, because right abducens nerve discharges induced by stimulation through electrodes located medially to the incision were still clearly inhibited by left neck jnoit stimulation (compare Fig. 9F with E). These findings support the view that the inhibitory effects on abducens motoneurons from the neck joint are mediated through the ipsilateral vestibular nuclei. These considerations hold for the neck-induced facilitation, because the facilitatory effects were also remarkably decreased after interrupting the vestibulo-abducens excitatory pathway.

Since prolonged supression or facilitation produced by long train shocks (such as 20 pulses) to the neck joint did not change clearly after the incision, other structures than the vestibular nuclei may mediate the prolonged effect. Involvement of the forebrain in the pathway was excluded by the present experimental condition (decerebration).

Transverse hemisection of the spinal cord was performed in three cats at the level of its rostral end. After the hemisection the ipsilateral facilitatory effect on the test reflex by neck-joint stimulation was abolished on the side of hemisection, while the inhibitory effect from the contralateral neck joint was still as apparent as before injury (compare Fig. 10B with A). When a longitudinal midline incision of the brain stem was additionally made from the obex to the level of the rostral end of the inferior olive nucleus (Fig. 10E), the inhibition of the test reflex from the contralateral neck joint was completely abolished (compare Fig. 10D with C).

Discussion

Origin of Cervical Effects

With respect to the receptive area responsible for neck-induced motor activities, Magnus and de Kleijn (1913) limited the receptive field for the tonic neck reflex to the distribution of the first three cervical nerves. According to McCouch *et al.* (1951) it was located in the region of the upper neck joints, especially at the atlanto-axial and atlanto-occipital joints. Biemond and De Jong (1969) reported that positional nystagmus in rabbits was caused by interruption of cervical sensory fibers and suggested that the main receptors of their origin were in the intervertebral joints and ligaments.

The present experiments have shown that abducens motoneurons receive inhibition from the contralateral and excitation from the ipsilateral cervical dorsal roots or neck joints. The effects were obtained from the level of C2 and C3, but not from the level below C5. The possibility of direct spread of stimulating current to the spinal cord was carefully examined and ruled out. Local application of procaine to the stimulated area in the upper neck joint abolished both the inhibitory and the facilitatory effects on the vestibulo-abducens reflex. Furthermore, latencies both of the PSPs in the abducens motoneurons and of presumed presynaptic axonal spikes were slightly but significantly longer after stimulation of the neck joint than after dorsal root stimulation. This is expected if the impulses originate from the neck joint and conduct to the dorsal roots. Thus, in agreement with the previous studies the effective volleys may be attributed to those from the upper neck joint, probably intervertebral mechanoreceptors, though some contribution of muscle or fascial afferents cannot completely be excluded.

Cervical and Labyrinthine Convergence

Several workers have suggested that there is an interaction between labyrinthine and cervical influences on motor performance. Magnus and Storm van Leeuwen (1914) stated that the deficits in cats caused by cutting the dorsal roots from C1 to C3 resemble those produced by a bilateral labyrinthectomy. Cohen (1961) noted even more marked labyrinthine deficit-like disturbances in monkeys following section or local anesthesia of the C1 to C3 dorsal roots. With respect to cervical influences on the vestibular neurons, anatomical studies have shown that cervical afferents project to the vestibular nuclei (Corbin *et al.*, 1935; Brodal *et al.*, 1962). Projection of spinal ascending route to the Deiters neurons has been demonstrated physiologically (Ito *et al.*, 1964; Wilson *et al.*, 1966; Allen *et al.*, 1972a, b). Fredrickson *et al.* (1966) have shown convergence of cervical and labyrinthine influences on single units in the vestibular nuclei, as will be discussed below.

Figure 11 illustrates a schematic representation of cervico-vestibular interaction for explanation and interpretation of the present results. It has been shown above that stimulation of the neck joint facilitates the labyrinthine-induced disynaptic IPSP and EPSP in the abducens motoneurons (Fig. 7). Facilitation of the IPSP was induced from the neck joint contralateral to the abducens motoneurons recorded from, while facilitation of the EPSP was from the ipsilateral neck joint. According to Baker *et al.* (1969), inhibitory interneurons located in the vestibular nuclei project to the ipsilateral abducens motoneurons and excitatory interneurons to the contralateral abducens motoneurons. Thus, it is most likely that the inhibitory pathway to the abducens motoneurons from the contralateral neck joint and that from the ipsilateral labyrinth converge on common inhibitory interneurons in the vestibular nuclei. Likewise, the excitatory pathway to the abducens motoneurons from the ipsilateral neck joint and contralateral labyrinth converge on common excitatory interneurons in the contralateral vestibular nuclei.

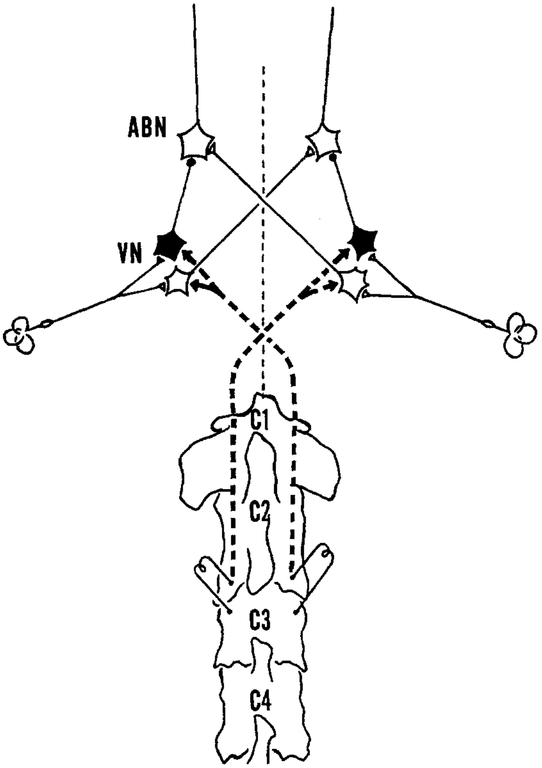

Fig. 11. *Schematic drawing of simplified pathways from neck joints to abducens motoneurons and their interaction with the vestibulo-abducens reflex arc.* Thick broken lines indicate cervical afferent pathways which converge on secondary vestibular neurons and facilitate them. ABN: abducens nucleus. VN: vestibular nucleus. Inhibitory neurons are filled in black and excitatory neurons open. To simplify the drawing commissural inhibitory pathways between right and left vestibular nuclei are not shown

Responses of vestibular neurons to cervical stimulation were also consistent with the inference derived from the scheme in Fig. 11. The axonal spikes recorded within the abducens nuclei and induced monosynaptically by ipsilateral vestibular nerve stimulation were postulated to be inhibitory and those activated from the contralateral vestibular nerve to be excitatory for abducens motoneurons, by comparing their firing phase with motoneuronal activities (Maeda *et al.*, 1971). In the present experiments, as would be expected, presumed inhibitory and excitatory presynaptic fibers in the abducens nuclei were activated by contralateral and ipsilateral cervical stimulation, respectively. Latencies of the activation were slightly shorter than those of the neck-induced PSPs in the abducens motoneuron; thus, it is not unreasonalbe to assume that these fibers are candidates responsible for production of the PSPs in the motoneuron. In the above experiment, however, vestibular neurone which send axons to the abducens nuclei were not identified with respect to their receptor origin in the labyrinth. In some other cases, however, functional identification of vestibular neurons was performed with horizontal rotation of the turn-table. Since the abducens motoneurons are activated or suppressed by horizontal canal activites and these influences are presumably mediated through type-I neurons in the vestibular nuclei (Precht *et al.*, 1967; Richter and Precht, 1968), at least some of type-I neurons are expected to be activated from the contralateral neck joint. In fact such activation was obtained in half of the type-I neurons recorded. These results on vestibular neuron responses provide further support for the view that the facilitatory interaction between the vestibulo-abducens and cervico-abducens reflexes occurs in the vestibular nuclei.

Some comments may be required on the finding that spontaneous discharges of vestibular type-I neurons were inhibited by long train shocks to the ipsilateral neck joint. A possible explanation for this inhibition would be that the ascending volleys from the cervical cord cross the midline and activate the contralateral vestibular type-I neurons which in turn inhibit the ipsilateral type-I neurons through the commissural inhibitory pathway (Shimazu and Precht, 1966). Thus, with reference to Fig. 11, we would like to suggest that tonic inhibitory influences on the right abducens motoneurons from the right vestibular nuclei will be decreased (disinhibition) by long trains of shocks to the right neck joint that also cause increased excitatory effects on the motoneuron from the left vestibular nuclei. In Fig. 6 the disinhibition was actually not evident, probably because single or triple shocks were not effective in inhibiting the ipsilateral vestibular type-I neurons as described above. Likewise, when recording from the right abducens motoneurons after long trains of shocks to the left neck joint, the hyperpolarizing response would consist not only of an IPSP, but also of disfacilitation.

Pathway

On the basis of the convergence experiment of vestibular and cervical input in the vestibular nuclei (Fig. 7), there are evidently crossed excitatory effects on vestibular neurons from the neck joint (Fig. 11). The lesion experiment in the brain stem (Figs. 9 and 10) indicates that afferent volleys from the neck joint ascend ipsilaterally in the spinal cord, cross to the contralateral side in the brain stem at a level or levels caudal to the rostral end of the inferior olive, and eventually project to the vestibular nuclei, thus interacting with the vestibulo-ocular reflex activity. Although the precise tract and the location of synapses along the pathway from the spinal cord to the vestibular nuclei remain to be studied, it may not be a multisynaptic, but a fairly direct route, considering that the shortest latency of evoked spikes of vestibular neurons projecting to the abducens nuclei was 2.0 msec and that of the IPSP in the motoneuron was 2.8 msec after stimulation of the contralateral dorsal root. The pathway concerned may be different from the spinal ascending route projecting to the Deiters nucleus so far studied. According to Ito *et al.* (1964), short latency, presumably monosynaptic, EPSPs were produced in Deiters neurons after spinal cord (C3) stimulation on the ipsilateral, instead of the contralateral, side, whereas the contralaterally induced PSPs had a fairly long latency such as 8 msec. Spinal ascending effects on Deiters neurons found by Wilson *et al.* (1966) were bilateral excitation and may provide these neurons with a generalized facilitatory background.

For the late effects produced by long train shocks to the neck joint, other pathways than those illustrated in Fig. 11 may be involved, since the interruption of vestibulo-abducens connections did not affect them appreciably.

Functional Significance

According to the pioneer work of Magnus (1924), turning the body from side to side with the fixed head position induces eye deviation in the direction contralateral to body turning. Bizzi *et al.* (1971) investigated eye-head coordination by recording from the neck and eye muscles in monkeys. They concluded that the head movement provides by way of vestibular and neck proprioceptors the reflex excitation necessary for the compensatory eye movement, i.e. the eye movement which is counter to the head rotation and compensates for it. The direction of the compensatory eye movement is consistent with the finding of Magnus. Fredrickson *et al.* (1966) found that firing rates of vestibular neurons, which were activated by ipsilateral labyrinthine cathodal polarization, were increased by turning (from side to side) the body in the direction ipsilateral to the vestibular nuclei recorded from and were decreased by contralateral body turning. They suggested that these vestibular neuron responses may lead the compensatory eye movement. Since electric stimulation of the neck joint activates the contralateral vestibular neurons intercalated in the vestibulo-abducens reflex pathway, it may be reasonably assumed that turning the body to the right side activates receptors of the left neck joint presumably due to stretching the joint ligaments and thereby excites the left abducens motoneurons as well as the right vestibular neurons. On the basis of this assumption, the cervico-ocular reflex pathway analyzed here may function conjointly with the vestibulo-ocular reflex to cariy the compensatory eye movement.

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