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Inhibitory interneurons in the reticular formation and their relation to vestibular nystagmus

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During voluntary and visual- or vestibular-induced eye movements, unit spikes of various types of neurons have been recorded in the brain stem, i.e. the pontine reticular formation[5,6,14,15,18,22], prepositus hypoglossi nucleus[2] and vestibular nuclei[7,9,16,17]. These neurons have been classified by their discharge patterns related to oculomotor activity. Connections between the ocular motor nuclei and the pontine reticular formation or the prepositus hypoglossi nucleus have recently been investigated anatomically[4,11] as well as physiologically[1,10,12,13]. However, connections of individual reticular neurons, which are functionally characterized with respect to eye movements, with ocular motoneurons remain to be studied .The present study aims to throw light on the functional organization of the reticulo-oculomotor complex related to vestibular nystagmus and will provide evidence for the presence of presumably inhibitory reticular neurons which exhibit burst discharges and are directly related to inhibition of abducens motoneurons in the quick phase of nystagmus.

The results in this study were obtained with 18 cats. Under ether anesthesia with artificial respiration a portion of occipital bone was removed and the medial part of the cerebellum was aspirated in order to expose the floor of the fourth ventricle. The upper cervical cord was transected and all incisions and pressor points were infiltrated with 4% Xylocaine. After ether was discontinued, the local anesthesia was carefully maintained throughout the experiment. Nystagmus was induced by repetitive stimulation of the vestibular nerve and the alternating rhythmic activities of the abducens nerves were monitored. Techniques for recording from the abducens nerve and for stimulation of the vestibular nerve followed those in a previous paper[20]. Spike potentials of brain stem neurons were recorded extracellularly with a microelectrode filled with 2 *M* NaCl solution saturated with Fast green FCF, having an electric resistance of 1–2 MΩ. Another glass microelectrode was filled with 2 *M* NaCl agar and was inserted into the abducens nuclei or surrounding regions for microstimulation or recording extracellular field potentials. The same type of electrode was used for microstimulation at the region where burst neurons (see below) were recorded. The tip diameter of the agar electrode was 4–10 μm and the electrical resistance was 0.7–1.5 MΩ. The stimulating microelectrode was electrically shielded to reduce stimulus arti-

facts except for 5 mm at the tip end, the shield being connected to the ground[8]. Rectangular cathodal currents of 0.1 msec in duration were passed through the microelectrode for stimulation. Stimulus currents applied were 1–30 μA. More than 30 μA current was not used to minimize spread of stimulating currents. Field potential in the abducens nucleus or compound action potentials of the abducens nerve in response to microstimulation of the brain stem were fed into an averaging computer (Signal Processor-7T07, San-ei Co.). For averaging the nerve discharges, they were recorded monopolarly from the cut end of the nerve with a DC amplifier. Recording sites for brain stem neurons were marked by electrophoretic ejection of Fast green through the recording microelectrode[23]. Sites of stimulation were marked by passing currents through the stimulating electrode.

Spike discharges of those neurons specifically related to the quick phase of horizontal vestibular nystagmus were recorded in the medial part of the brain stem at the levels between 3 mm rostral and caudal to the abducens nucleus. All the spikes examined in the present paper had a negative polarity and were 0.3–1.0 mV in amplitude. During advancement or withdrawal of the recording microelectrode, the single unit spikes could be detected to the extent of 100–200 μm from the site where the spikes were maximally recorded. These spike characteristics indicate that the spikes were recorded in the vicinity of cell bodies and not from axons of passage. Fig. 1A$_a$ exemplifies unit spikes of a neuron which exhibited a high frequency burst precisely at the moment of abrupt suppression of contralateral abducens nerve discharges (Fig. 1A$_b$). When the direction of nystagmus was reversed, its spontaneous spikes were almost completely silent during both the slow and quick phase of nystagmus (Fig. 1B). The on-direction of this neuron was thus ipsilateral with respect to the horizontal vector of nystagmus. The duration of the single burst was mostly 60–80 msec and the maximum firing rate was 600–700/sec. The recording site was marked and is illustrated in Fig. 2G.

According to Maeda et al.[19,20], abrupt cessation of abducens nerve activity at

Fig. 1. Activity of a burst neuron related to vestibular nystagmus. A and B: nystagmus in right and left direction, respectively. In both A and B, a: extracellular spike potentials of a burst neuron located in the right dorsomedial reticular formation. b and c: left and right abducens nerve activity, respectively. Dotted line in A indicates the onset of each burst of the neuron.

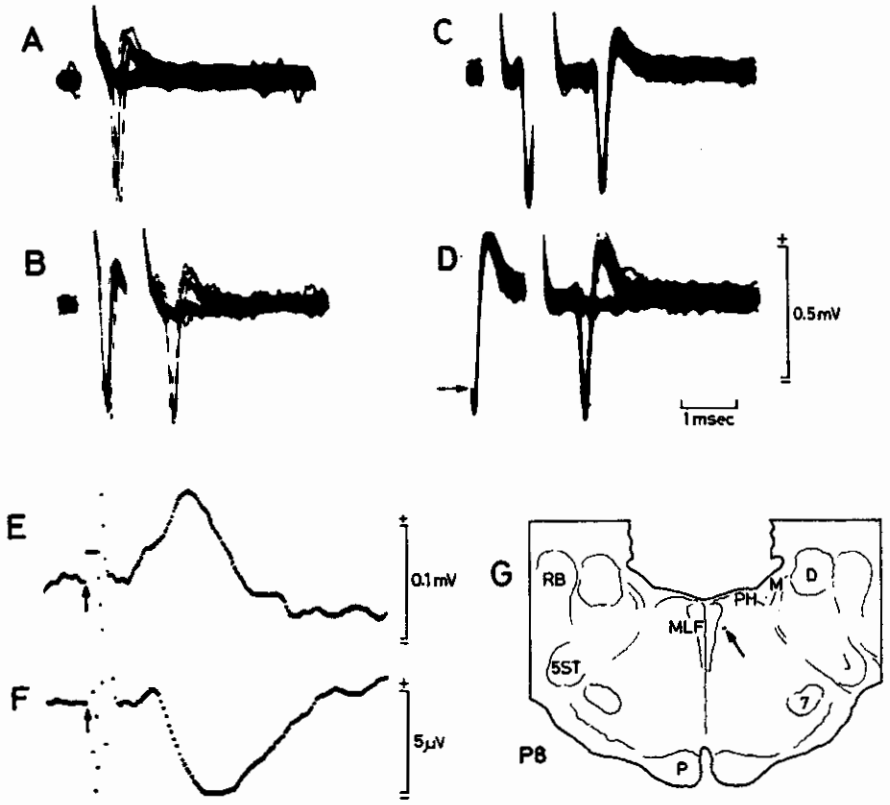

Fig. 2. Location and projection of burst neurons. A–D: responses of the same neuron as in Fig. 1 (A and B) and another neuron (C and D) to stimulation of the contralateral abducens nucleus. A: response to single pulse stimuli (8 μA) at threshold-straddling intensity. B and C: responses to double shocks at an intensity near the threshold for the second spikes. D: collision test between spontaneous spikes and evoked spikes. The sweeps were triggered by spontaneous spikes (arrow) which were followed by stimulation of the contralateral abducens nucleus. E: positive field potential in the left abducens nucleus in response to stimulation (30 μA) of the dorsomedial reticular formation on the right side (dot pointed by arrow in G). Average of 50 sweeps. F: depression of left abducens nerve activity by single shocks (10 μA) to the same site as in E. The discharges were recorded at the cut end of the abducens nerve in the orbit and averaged with 400 sweeps. In this case the amplitude of spontaneous activity of the whole nerve was approximately 100 μV. Arrows in E and F indicate the moment of stimulation. G: line drawing of the brain stem in a transverse section at the level of P8. Dot with arrow indicates the recording site for the neuron in Figs. 1 and 2A and B and the stimulated site in Fig. 2E and F. Time scale in D applies to A–F. Voltage calibration in D applies to A–D. Abbreviations: D, Deiters nucleus; M, medial vestibular nucleus; MLF, medial longitudinal fasciculus; P, pyramidal tract; PH, prepositus hypoglossi nucleus; RB, restiform body; 5ST, trigeminal spinal tract; 7, facial nucleus.

the quick phase of vestibular nystagmus is caused by steep IPSPs in motoneurons and the onset of the IPSPs is synchronous with the onset of EPSPs in contralateral abducens motoneurons. The following two experiments were performed in an attempt to find whether the above-described burst neurons connect directly with abducens motoneurons on either side and cause IPSPs in the contralateral motoneurons or EPSPs in the ipsilateral motoneurons in the quick phase.

First, axonal projections of these neurons were examined by microstimulation of the abducens nuclei. While recording extracellular spikes of single burst neurons, another glass micropipette was inserted into the abducens nuclei or surrounding regions for stimulation. Fig. 2A and B show spikes of the same neuron as in Fig. 1 in response to stimulation of the contralateral (left) abducens nucleus. At threshold-straddling intensity (8 μA) of stimulation, spikes were evoked with a fixed latency of 0.5 msec in an all-or-none manner (Fig. 2A). The spikes followed double shocks with an interval of 0.9 msec at suprathreshold intensity for the first spikes, and the second spikes also had a fixed latency in an all-or-none manner (Fig. 2B). These results indicate that the spikes were induced antidromically. The latencies of thus characterized antidromic spikes ranged from 0.3 to 0.8 msec. For those burst neurons which were activated with relatively long latencies (0.7 msec for the first spikes in Fig. 2C), their antidromic nature was further confirmed by the collision test. The sweeps were triggered by spontaneous spikes and stimulation at the contralateral abducens nucleus was delivered at various intervals after the spontaneous spike. As shown in Fig. 2D, the smallest interval between the spontaneous spikes and evoked spikes was 2.0 msec. This value was much longer than the refractory period of the neuron tested by double shocks to the abducens nucleus, 1.2 msec (Fig. 2C). These results exclude the possibility of orthodromic activation of the neuron by abducens nucleus stimulation and suggest that the activation is antidromic in nature. The smallest interval in Fig. 2D, 2.0 msec, is reasonably explained by the sum of the orthodromic and antidromic conduction times and the refractory period of the axon at the stimulated site. The effective sites for evoking antidromic spikes of burst neurons were highly localized to the contralateral abducens nucleus with stimulating currents less than 30 μA. All the 15 burst neurons tested were activated antidromically from the contralateral abducens nucleus and none was activated from the ipsilateral abducens nucleus. These results suggest that the axons of burst neurons project to the contralateral and not to the ipsilateral abducens nucleus.

Second, effects of stimulation at the region of burst neurons were examined on contralateral abducens motoneurons. Fig. 2E shows the positive field potential recorded in the contralateral abducens nucleus in response to stimulation at the site of the burst neuron in Fig. 2G. The positive potential was localized within the contralateral abducens nucleus and its latency was 0.8 msec. Since the shortest latency of antidromic activation of burst neurons was 0.3 msec from the contralateral abducens nucleus, the positive field potential is likely to be the extracellular counterpart of monosynaptic IPSP in abducens motoneurons. This idea was supported by the fact that spontaneous abducens nerve activity on the contralateral side was depressed by microstimulation (10 μA) at the same site as above with a latency of 1.3 msec (Fig. 2F). The difference in latencies between the positive field potential and depression of nerve activity was 0.5 msec. This value was attributed to the conduction time from the abducens nucleus to the recording site for nerve activity, since the shortest conduction time was 0.43 msec as determined by antidromic activation of motoneurons from the orbit[3]. Effective sites to produce a positive field potential in the contralateral abducens nucleus were fairly localized in the region where burst neurons were located. Thus, the

burst neurons are postulated to be inhibitory interneurons which make monosynaptic connections with contralateral abducens motoneurons. This postulate accords with the finding that their burst discharges are consistently in phase with the silent period of contralateral abducens nerve activity (Fig. 1A).

Unitary spikes were recorded from 34 neurons which exhibited burst discharges closely related to the quick phase of nystagmus directed to the ipsilateral side as shown in Fig. 1. Twenty-six of them were located in a relatively localized area in the dorsomedial part of the reticular formation caudal to the abducens nucleus and close to the medial longitudinal fasciculus; i.e. rostrocaudal level: P7.5–8.5[21], lateral distance from the midline: 0.6–1.2 mm, depth: 0.5–3.0 mm. The remaining 8 neurons were distributed rostrally up to P5 as well as caudally to P9. The marked spots were found outside the prepositus hypoglossi nucleus. When we recorded in the prepositus hypoglossi nucleus, spikes of most neurons related to horizontal nystagmus were induced during both the slow and quick phases of abducens nerve activity on the ipsilateral side. These neurons may correspond to burst-tonic neurons recorded during voluntary horizontal eye movements in the cat[2]. No neurons of purely burst type as shown in Fig. 1 were found in this nucleus. Spikes of the burst neurons in the present study were evoked by intense shocks to the ipsilateral vestibular nerve (more than 3 times the threshold for activation of secondary vestibular neurons) with fluctuating, long latencies such as 5–10 msec, while contralateral vestibular nerve stimulation had no excitatory effects. This also differentiated these neurons from prepositus neurons[1].

The firing characteristics of the burst neurons described here were similar to those of burst units[5,15], especially of medium-lead burst units[18], in the pontine reticular formation in alert monkeys.

In conclusion, burst neurons in the dorsomedial part of the reticular formation caudal to the abducens nucleus send their axons to the contralateral abducens nucleus. These neurons are postulated to be inhibitory in nature and their burst discharges are likely to cause monosynaptic IPSPs in abducens motoneurons at the quick inhibitory phase of vestibular nystagmus.

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