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Direct Inhibitory Synaptic Linkage of Pontomedullary Reticular Burst Neurons with Abducens Motoneurons in the Cat

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Summary. 1. Unit spikes of burst neurons were extracellularly recorded in the pontomedullary reticular formation of the cat. These neurons were identified by their burst activity coincident with the quick inhibitory phase of the contralateral abducens nerve during vestibular nystagmus and their antidromic activation from the contralateral abducens nucleus.

2. When the extracellular field potentials in and near the abducens nucleus were triggered by spikes of a contralateral burst neuron, the averaged potential consisted of an early di- or triphasic spike and a late slow positive wave. The early spike was an action current caused by impulses conducting along the axon of the burst neuron.

3. The action potentials of a contralateral burst neuron were employed to trigger a post-spike average of the membrane potential of abducens motoneurons. Then unitary IPSPs with monosynaptic latencies were revealed. This provided direct evidence that the burst neurons are inhibitory in nature. The amplitudes of unitary IPSPs ranged from 18 to 220 μV. Each inhibitory burst neuron branched widely in the abducens nucleus and was estimated to make inhibitory connections with approximately 60% of the motoneuron pool.

4. The post-spike average of compound potentials of the abducens nerve triggered by action potentials of contralateral single inhibitory burst neurons revealed inhibition of spike activity with latencies and time courses compatible with those of unitary IPSPs in motoneurons. The inhibition was observed with all inhibitory burst neurons tested.

Key words: Burst neuron – Pontomedullary reticular formation – Unitary IPSP – Abducens motoneuron – Vestibular nystagmus.

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A group of neurons in the pontine reticular formation has been reported to exhibit a high frequency burst of spikes prior to fast eye movements and they have been called burst neurons or bursters (Duensing and Schaefer, 1957; Sparks and Travis, 1971; Luschei and Fuchs, 1972; Cohen and Henn, 1972a, b; Keller, 1974). On the basis of close functional correlation of activity of burst neurons with eye movement, these neurons were presumed to project to the ocular motor nuclei either directly or through a few synapses and therefore to provide an excitatory motor command signal for fast eye movements. Earlier anatomical works with Golgi methods suggested reticulo-oculomotor connection (Lorente de Nó, 1933; Scheibel and Scheibel, 1958; Szentágothai, 1964). Recent studies with horseradish peroxidase and radioactive tracer techniques have provided evidence for the projection of pontomedullary reticular neurons to the abducens nucleus (Büttner-Ennever and Henn, 1976; Maciewicz et al., 1977; Graybiel, 1977). Physiological studies indicated that electrical stimulation of the paramedian pontine reticular formation induced mono- or disynaptic postsynaptic potentials in IIIrd nucleus motoneurons (Highstein et al., 1974) and VIth nucleus motoneurons (Grantyn and Grantyn, 1976; Highstein et al., 1976).

A different approach to this problem was undertaken by Hikosaka et al. (1977) who recorded presynaptic axon spikes within the abducens nucleus in order to correlate their activity with postsynaptic potential changes of motoneurons. Axons exhibiting a burst of spikes related to vestibular nystagmus were invariably activated at the quick inhibitory phase of motoneurons on which the axons were presumed to terminate. It was suggested that this group of axon is inhibitory in nature and contributes to active inhibition of abducens motoneurons at the quick phase. The presence of IPSPs at the quick phase was demonstrated by intracellular recording from motoneurons (Maeda et al., 1972; Baker and Berthoz, 1974). Hikosaka and Kawakami (1977) explored the pontine and medullary reticular formation to find the origin of inhibitory burst axons projecting to the abducens nucleus. The candidate neurons named inhibitory burst neurons (IBNs) were located in the dorsomedial part of the pontomedullary reticular formation mainly caudal to the level of the abducens nucleus and exclusively contralateral to the abducens nucleus to which their axons projected. Electrical stimulation applied to the region where IBNs were densely located induced monosynaptic IPSPs in contralateral abducens motoneurons, suggesting the inhibitory nature of IBNs. However, the possibility could not be excluded that direct stimulation of the IBN region would activate passing fibers or collateral axons of inhibitory neurons located elsewhere which might terminate on abducens motoneurons.

To confirm that burst neurons are *inhibitory* premotor neurons, direct evidence is required for monosynaptic inhibitory connections of individual IBNs with abducens motoneurons. An elegant technique has been developed to demonstrate monosynaptic connection between a pair of neurons by the use of post-spike averaging. The latter technique reveals the postsynaptic potential correlated with the presynaptic spike (Mendell and Henneman, 1971; Jankowska and Roberts, 1972b; Asanuma and Rosén, 1973; Kirkwood and Sears, 1973, 1974; Scott and Mendell, 1976; Stauffer et al., 1976; Watt et al.,

1976; Rapoport et al., 1977). We have used this technique and have extended it to detect a decrease in firing probability of a population of abducens motoneurons with a time course compatible with unitary IPSPs induced by single IBN activity.

Methods

The experiments were performed on 29 adult cats. Operational procedures, the preparation and the care for conditions of the animal were described in detail elsewhere (Maeda et al., 1972; Hikosaka et al., 1977).

Unit spikes of IBNs were recorded extracellularly with glass micropipettes filled with 2 M NaCl solution saturated by Fast Green FCF, having electrical resistance of 1–2 MΩ. Double-barrelled microelectrodes were also used. One barrel was filled with the above solution (resistance 1–2 MΩ) for extracellular recording of IBN spikes. The other was filled with a solution of 1 M Na glutamate and was used for activation of the IBN by its spontaneous leakage or iontophoresis. IBNs were identified by their firing characteristics during vestibular nystagmus and their antidromic activation from the contralateral abducens nucleus (Hikosaka and Kawakami, 1977). The stimulation electrode in the abducens nucleus consisted of a micropipette filled with 2 M NaCl agar and electrically shielded to reduce stimulus artifacts. Rectangular cathodal currents of 0.1 msec duration were passed through the microelectrode for stimulation. Stimulus currents monitored by the voltage drop across a 100 Ω resister were restricted to less than 20 μA. Once a single IBN was identified, the stimulation electrode in the abducens nucleus was replaced by a glass microelectrode filled with 3 M KCl or 2 M K citrate solution (resistance 20–40 MΩ) for intracellular recording from abducens motoneurons.

Systematic survey of single IBN-related extracellular field potential in the abducens nucleus and adjacent structures was made with a glass-insulated tungsten microelectrode (Stoney et al., 1968), having electrical resistance of 0.5–1 MΩ. During the survey of field potentials this electrode was also used as a stimulating electrode for antidromic activation of the IBN.

Abducens nerve activity was recorded monopolarly at the cut end of the nerve with a Ag-AgCl hook electrode and was fed into a d.c. amplifier. This electrode was used for antidromic stimulation of the abducens nerve as well. The vestibular nerve on both sides was stimulated by placing Ag-AgCl electrodes on the round and oval windows in order to keep the receptors intact.

Spike potentials of a single IBN were used to trigger a digital averaging computer with a time resolution of 0.04 msec (Signal Processor, 7T07, San-ei Co., Tokyo). Three kinds of activity related to a single IBN were summed over a 10 msec sweep duration; (1) extracellular activity in and near the abducens nucleus, (2) intracellular potentials in abducens motoneurons, and (3) compound action currents of the whole abducens nerve. The time constant of the recording system for averaging was 100 msec. Since the earlier parts of triggering spikes were not recorded in the computer in most cases, the spikes were reconstructed from the records of spontaneous spikes obtained with another oscilloscope. The latency of the post-spike events was measured from the foot of the reconstructed spike. In some cases an averaging program was so designed that the records could be obtained before and after triggering spikes (see Fig. 2C).

Recording sites of IBNs were marked by electrophoretic ejection of Fast Green FCF from the recording microelectrode (Thomas and Wilson, 1965). At the end of each experiment a lethal dose of pentobarbital was injected intravenously and the brain stem was removed and fixed in 10% formalin solution. Dye marks were studied histologically with Nissl-stained serial sections.

Results

Unit spikes were recorded in the dorsomedial part of the reticular formation at the level caudal to the abducens nucleus where IBNs were most densely located (cf. Hikosaka and Kawakami, 1977). During horizontal rotation of the turn table, those units which exhibited burst spikes at the quick phase of vestibular

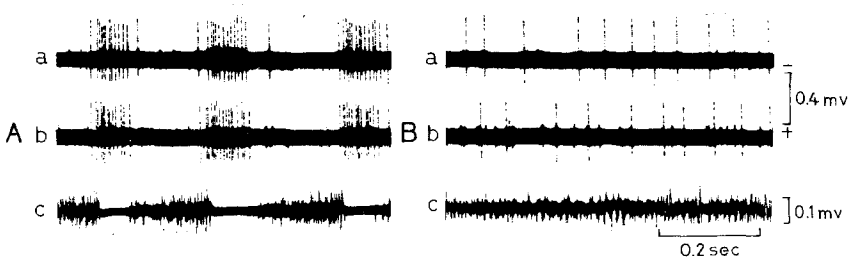

Fig. 1. Simultaneous recording of unit spikes of two IBNs. **A** During nystagmus. **B** Under non-nystagmic condition. a and b, spikes of a pair of IBNs on the right side. c, abducens nerve discharges on the left side. All records were taken with a time constant of 1.7 msec

nystagmus were selected. In the above area the spike burst was always induced at the quick inhibitory phase of contralateral abducens motoneurons. The spikes had a negative polarity with an amplitude of 0.3–1.0 mV and could be recorded while moving the micromanipulator over 300–500 μm, indicating that recording sites were near cell somata of IBNs.

For post-spike averaging to detect synaptic effects of a single IBN on abducens motoneurons, spikes occurring in a burst fashion could not be used for the following reasons. (1) The interspike intervals in the burst were usually less than 2–3 msec, which were much shorter than the sweep duration of 10 msec. (2) Most IBN spikes occurred during a steep progression of the motoneuronal membrane potential in the hyperpolarizing direction (Maeda et al., 1972), which caused a steep negative-going inclination of the baseline of the averaged potential and made it difficult to detect relatively small postsynaptic events correlated to spikes of a single IBN. Therefore, in the present experiments IBNs have first been identified by the occurrence of burst activity during induced nystagmus (Fig. 1A) and then their low frequency spontaneous discharges (usually less than 20/sec) under non-nystagmic condition have been used for postspike averaging. In some cases spike frequency for triggering was increased by electrophoretic ejection of glutamate ions from a pipette of the double-barrelled recording microelectrode. When spontaneous discharges of IBNs tended to fire with a burst fashion in association with spontaneous, phasic change of abducens nerve activity, a small amount of ether was temporarily introduced to abolish burst activity of IBNs and make the nerve activity steady and tonic (Fig. 1B). Under these conditions, cross-correlation between simultaneously recorded two IBNs were examined in twelve pairs and no correlation was found in any pair in the analysis interval of 10 msec. On the basis of these statistical analyses, it was presumed that the averaged postsynaptic events described below were evoked via the axon terminals of single IBNs.

Field Potentials in the Abducens Nucleus Induced by Single IBN Activity

During recording of extracellular spikes of a single IBN, a glass-insulated tungsten microelectrode was inserted into the brain stem through the abducens

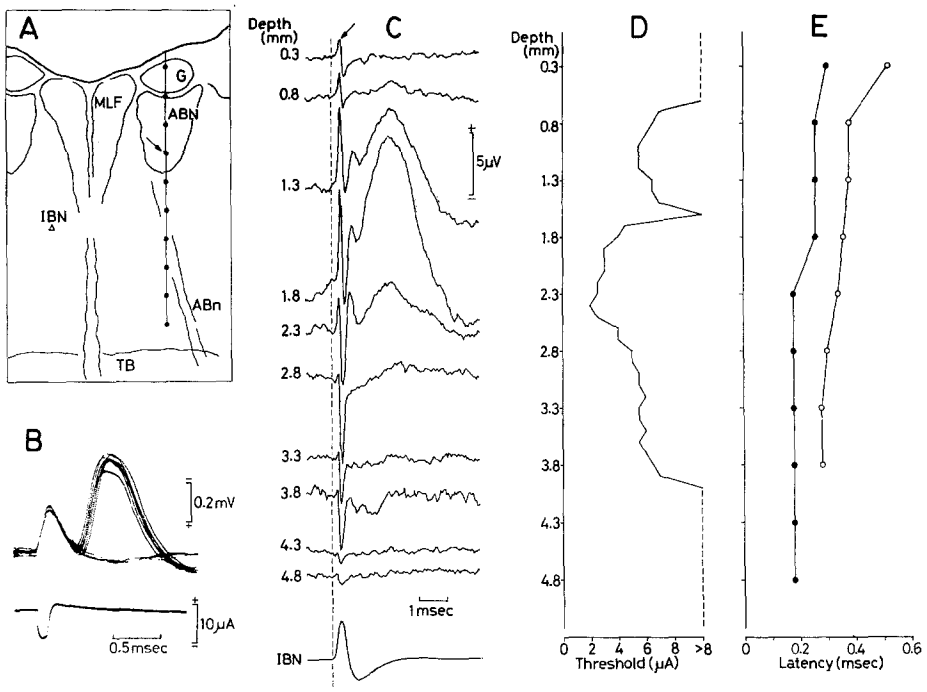

Fig. 2. Correlated survey of field potential averages triggered by a single IBN and antidromic activation of the IBN. **A** Line drawing of a frontal section of the brain stem through the abducens nucleus. An electrode track for recording and stimulation is shown by the vertical line. Dots indicate recording sites of intervals of 500 μm from 0.3 mm to 4.8 mm in depth. A dot with arrow (1.8 mm in depth) represents the spot marked by passing current through the microelectrode. IBN indicated by a triangle was recorded in the frontal plane 1.3 mm caudal to the electrode track through the abducens nucleus. ABN: abducens nucleus, ABn: abducens nerve, G: genu of the facial nerve, MLF: medial longitudinal fasciculus, TB: trapezoid body. **B** Antidromically evoked spikes of the IBN at threshold-straddling intensity (upper trace) and stimulus current (lower trace). **C** Traces of averaged extracellular field potentials triggered by spikes of the IBN (bottom) (3500 sweeps). Records were taken at each site shown by dot in A. Vertical broken line indicates the onset time of IBN spike. **D** Thresholds for antidromic activation of the IBN measured at intervals of 100 μm along the electrode track in A. Thresholds above 8 μA are shown by broken line. **E** Latencies of antidromic spikes of the IBN measured with stimulus currents of 1.5 times the threshold (open circles) and intervals between the onset of IBN spike and the initial positive peak of field potentials in C (filled circles)

nucleus (Fig. 2A) for recording extracellular field potentials at every 500 μm (Fig. 2C) and for stimulation at every 100 μm to evoke antidromic spikes in the IBN (Fig. 2B). The field potential was summed over a 10 msec sweep duration from 5 msec before to 5 msec after the IBN spike. The IBN-induced averaged field potential in and near the abducens nucleus consisted of an early di- or triphasic spike (positive-negative or additional slight positivity) and a late slow positive wave (Fig. 2C, 1.3–2.3).

The early spike was well developed at the site where microstimulation induced an antidromic spike in the IBN with a relatively low threshold (Fig.

2D). It was presumably action current caused by impulses conducting along the axon of the IBN. Assuming that the initial positive peak (turning point from the initial positive-going to the negative-going potential, arrow in Fig. 2C) represents the time of arrival of impulses, the orthodromic conduction time from the IBN to each recording site was estimated by the interval between the foot of IBN spike and the initial positive peak of the averaged field potential (Fig. 2E, filled circles). In the region ventral to the abducens nucleus, the conduction time was 0.18 msec over 2.3–4.8 mm distance from the surface of the brain stem. The apparent uniformity of the conduction time along these distances may be due to the relatively rough time resolution (0.04 msec) of the computer employed in this experiment. In the region within and dorsal to the abducens nucleus, it was prolonged up to 0.26–0.3 msec, suggesting that the axon of the IBN projects to the contralateral abducens nucleus from the deeper area in the tegmentum. The predominance of the initial positivity of the early spike in the abducens nucleus in contrast to the predominance of the second negativity in the deeper area supports this view. The sequence of changes in the orthodromic conduction time according to depth was similar to that of latency changes of antidromic spikes in the IBN in response to microstimulation at each site (Fig. 2E, open circles). The time difference between the orthodromic conduction time and the antidromic latency was 0.10–0.22 msec (mean: 0.13 msec) depending on the site of recording and stimulation. These values presumably correspond to the latency of antidromic spike initiation in the axon at the stimulated site plus a possible delay of antidromic conduction time at the sites of branching of the axon (Hikosaka and Kawakami, 1977).

The late slow positivity was found in the region of and closely ventral to the abducens nucleus in the track shown in Figure 2A. The duration of the positive field potential was 2.5–3.0 msec and was within the range of the time-to-peak of the unitary IPSP (see next section). The onset time of the positive wave could not be accurately measured, since its initial part appeared to be masked by the tail of the early spike.

Monosynaptic Inhibitory Connection of IBN with Contralateral Abducens Motoneurons

During extracellular recording from an IBN, localized stimulation was applied to the contralateral abducens nucleus to confirm its axonal projection to the nucleus (Fig. 3A). In Figure 3D, spikes of an IBN were induced at a fixed latency of 0.6 msec in an all-or-none fashion and followed double shocks at an interval of 1 msec. Collision of these evoked spikes with spontaneous IBN spikes was demonstrated in a previous paper (Hikosaka and Kawakami, 1976), indicating their antidromic activation. The intracellular microelectrode was inserted into the abducens nucleus to impale a motoneuron which was identified by its antidromic spikes induced by stimulation of the abducens nerve (Fig. 3C). Immediately after penetration most motoneurons fired repetitively, but they often ceased to fire spontaneously or by slight manipulation of the microelectrode. In the following experiments the membrane potential was

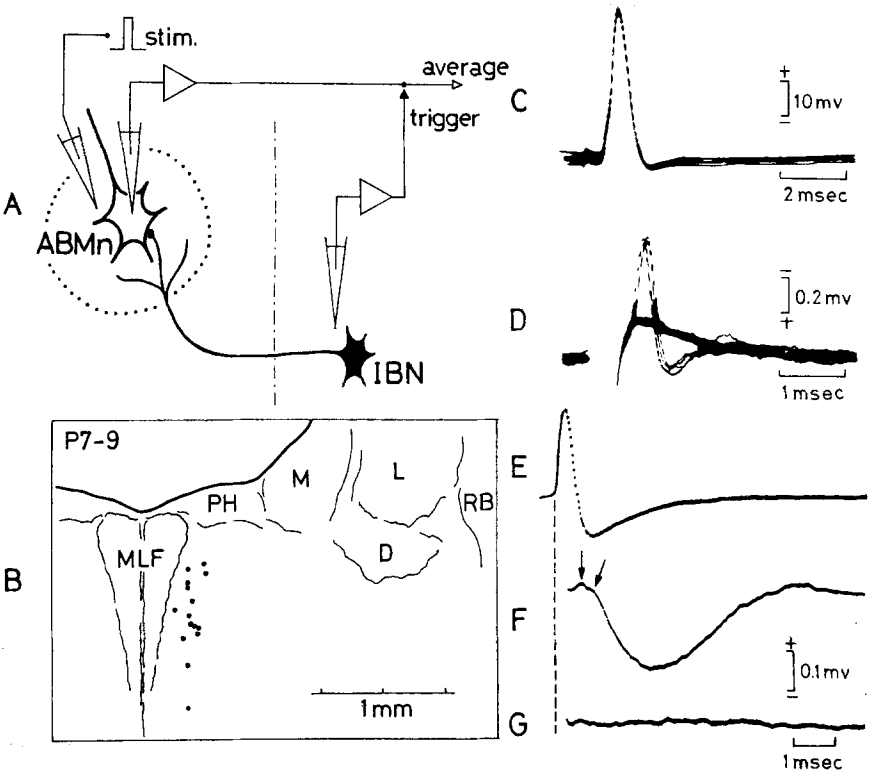

Fig. 3. Experimental procedures to detect IBN unitary IPSPs in abducens motoneurons and locations of identified IBNs. **A** Schematic representation of experimental arrangements. Vertical broken line indicates the midline. ABMn: abducens motoneuron. **B** Locations of IBNs (dots) which were identified as making monosynaptic inhibitory connections with contralateral abducens motoneurons. The IBNs located in a rostro-caudal extent from P7 to P9 are shown by projecting on a frontal plane. MLF: medial longitudinal fasciculus, PH: prepositus hypoglossi nucleus, M, L, D: medial, lateral and descending vestibular nucleus, RB: restiform body. **C** Intracellular spike potential in an abducens motoneuron identified by antidromic activation from the abducens nerve. **D** Extracellular spikes of an IBN activated antidromically from the contralateral abducens nucleus. **E** Averaged IBN spike used for triggering the sweeps. Initial part of the spike drawn by a solid line was reconstructed from spontaneous spikes recorded with another oscilloscope. **F** Averaged unitary IPSP triggered by IBN spikes (360 sweeps). First arrow indicates the arrival of the presynaptic impulse and second arrow the onset of IPSP. **G** Averaged extracellular field potential recorded in the vicinity of the motoneuron (360 sweeps). Vertical broken line indicates the onset of triggering IBN spike

maintained at –40–60 mV without any spike activity. The membrane potential trace was triggered from IBN spikes (Fig. 3E) and summed over a 10 msec sweep duration after the spike (Fig. 3F). The summed potential revealed a membrane hyperpolarization as compared to the summed extracellular field potential as a control (Fig. 2G). When the early part of the triggering spike was reconstructed from records of spontaneous spikes (solid line in Fig. 3E), the

latency of the hyperpolarization (second arrow in Fig. 3F) measured from the estimated onset of the spike was 0.8 msec. Since the latency for spike generation in the axon at the stimulated site was 0.1–0.2 msec (see Fig. 2E; cf. also Jankowska and Roberts, 1972a), the conduction time along the axon was estimated to be 0.4–0.5 msec. Thus, the latency of the hyperpolarization in the motoneuron, 0.8 msec, was in a monosynaptic range.

As shown in Figure 4A–C, the summed hyperpolarization (B) was inverted into a depolarizing potential (C) by injection of Cl^- ions into the motoneuron through the recording microelectrode, indicating that the hyperpolarization was an inhibitory postsynaptic potential (IPSP) (Eccles, 1964). This IPSP will be called IBN 'unitary' IPSP in the sense described by Jankowska and Roberts (1972b). When the IBN unitary IPSP was inverted by Cl^-, disynaptic IPSPs evoked by stimulation of the ipsilateral vestibular nerve were also inverted to a depolarizing potential (insets of Fig. 4C; cf. Baker et al., 1969b). The reversal of IBN unitary IPSP occurred usually in parallel with the reversal of vestibular-induced IPSP during the course of intracellular Cl^- injection, though precise comparison was often difficult because of spontaneous diffusion of Cl^- from the orifice of the microelectrode. When the inverted unitary IPSPs were large in amplitude, they were visualized with simple superimposition of faint traces in spite of large fluctuation of the membrane potential caused by spontaneous synaptic inputs from different sources (Fig. 4E). During membrane depolarization with a K citrate electrode, the IBN unitary IPSP and the vestibular-induced IPSP became larger in amplitude together (Fig. 4G–I). No significant difference was found between IBN unitary IPSP and vestibular-induced IPSP in amplitude or polarity change in the course of various degree of membrane polarization. In Figure 4G–I, latter parts of the averaged potential trace were not uniform but fluctuated. The ripple fluctuation was presumably due to contamination by some synaptic noise unrelated to the IBN activity concerned. Since brain stem neurons had to be kept spontaneously active to induce nystagmus for identification of IBNs, large fluctuation of the membrane potential caused by spontaneous synaptic bombardments could not completely be eliminated even with the averaging technique.

During recording from a single IBN, several motoneurons were impaled and those which exhibited vestibular-induced IPSPs of more than 1 mV in amplitude were selected for construction of post-spike averages. This criterion may not absolutely be required, but in view of parallelism of two kinds of IPSP, it was employed as an indication that the penetration was appropriate to detect, if there were any, IBN unitary IPSPs. Seventy-six pairs of IBNs and abducens motoneurons, consisting of 29 IBNs and 76 motoneurons, were investigated. Forty-five of the 76 pairs (60%) revealed unitary IPSPs. The latencies of these IPSPs ranged from 0.4 to 1.1 msec (Fig. 5A) and the mean value was 0.68 msec ($\pm$ 0.18, S.D). The latencies of antidromic spikes in the IBNs ranged from 0.3 to 0.8 msec (mean and S.D.: 0.51 $\pm$ 0.15 msec) from the abducens nucleus. The orthodromic conduction time was given by subtracting 0.13 msec (see above) from the mean antidromic latency and the value of 0.38 msec was obtained. The mean synaptic delay for the unitary IPSP was thus estimated to be 0.30 msec on the average.

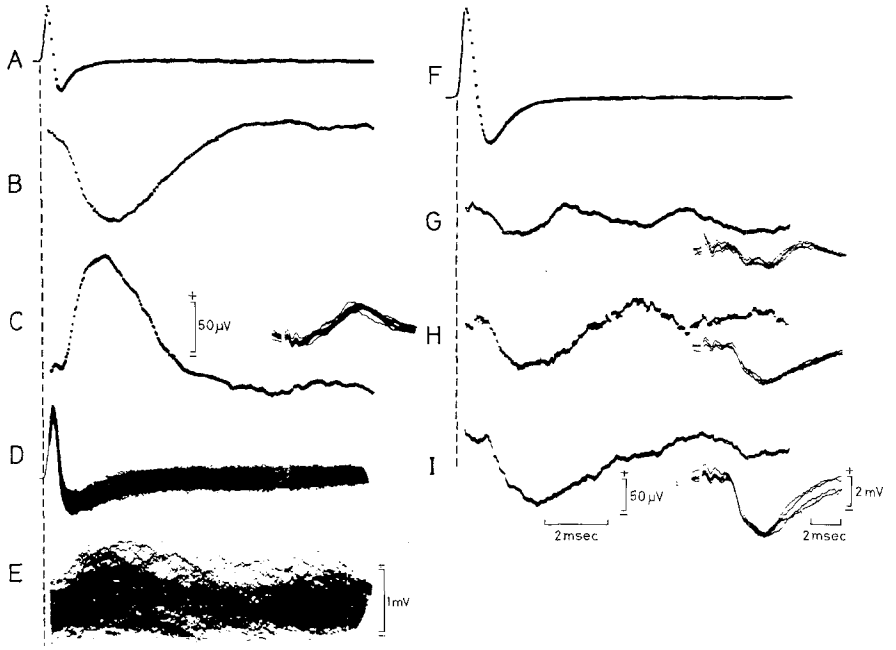

Fig. 4. Effects of Cl^- injection into the cell and membrane polarization on IBN unitary IPSP. **A** Triggering IBN spike for B and C. **B** Averaged unitary IPSP recorded with a KCl electrode (2500 sweeps). **C** Same as in B, but recorded after Cl^- injection into the motoneuron (2500 sweeps). Inset record in C shows inverted IPSP induced by stimulation of the ipsilateral vestibular nerve and recorded immediately before averaging in C. **D** and **E** Superimposed traces of triggering IBN spikes (D) and motoneuronal inverted IPSP (E). **F** Triggering IBN spike for G–I. **G** Averaged unitary IPSP as a control recorded with a K-citrate electrode (300 sweeps). **H** and **I** Same as in G, but recorded during passage of depolarizing currents of 12 nA (H) and 21 nA (I) obtained with 200 sweeps, respectively. Inset traces in G–I show vestibular-induced IPSPs recorded under respective conditions of membrane polarization. Vertical broken lines indicate the onset of IBN spikes. Time scale in I applies to A–H. Amplitude calibration in C applies to B and that in I to G–H. Time scale and amplitude calibration for inset record of I apply to all insets

Post-spike average of intracellular records revealed a presynaptic spike arriving near the target motoneuron in 9 cases (Fig. 3F, first arrow). In other cases, the presynaptic activity was not clearly recorded probably because the high resistance of the intracellular microelectrode (20–40 MΩ) distorted the high frequency characteristics of the recording system. In the 9 successful cases, the interval between the positive peak of presynaptic spike and the onset of unitary IPSP ranged from 0.25 to 0.50 msec and the mean value was 0.38 msec (± 0.09, S.D.). Assuming that the observed presynaptic spike represents the spike induced near the axon terminal synapsing with the motoneuron recorded from, the value of 0.38 msec gave the single synaptic delay time and was close to the above value obtained by indirect measurement. The synaptic delay of IBN unitary IPSP is similar to the values obtained by direct measurement in other systems; on the average 0.33 msec for Ia inhibitory interneurons (Jankowska and Roberts, 1972b) and 0.4 msec for vestibulocollic neurons (Rapoport et al., 1977), and most frequently 0.4 msec for Ia muscle afferents (Watt et al., 1976).

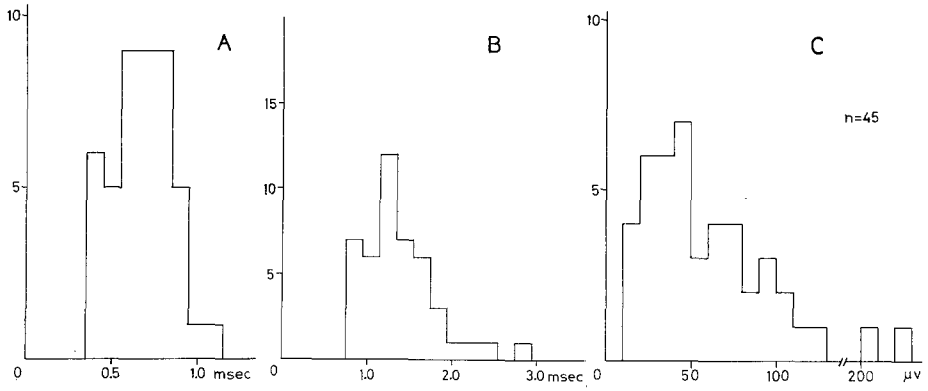

Fig. 5. Characteristics of IBN unitary IPSPs. Histograms of the latency (**A**), time to peak (**B**) and amplitude (**C**)

The histograms in Figure 5B and C show the time-to-peak and the amplitude of IBN unitary IPSPs, respectively. The time-to-peak ranged from 0.8 to 2.8 msec (mean and S.D.: 1.40 ± 0.44 msec) and the amplitude from 18 to 220 μV. Spontaneous synaptic bombardments unrelated to a triggering IBN spike may reduce the accuracy of the measurement; e.g., some of the unitary IPSPs less than 20 μV in amplitude were often difficult to discriminate from the baseline noise. These uncertain results were discarded in the present study and this may have caused an underestimation of the number of small amplitude values in Figure 5C.

Recording sites of the IBNs identified as inhibitory premotor neurons were marked by electrophoretic ejection of the dye from the recording microelectrode (Fig. 3B). They were located in the dorsomedial reticular formation caudal to the abducens nucleus (0.6–1.0 mm from the midline), the location being consistent with the results reported by Hikosaka and Kawakami (1977). Anatomical studies with horseradish peroxidase (Maciewicz et al., 1977) and radioactive tracer technique (Graybiel, 1977) confirm that cells in the region of IBN project to the contralateral abducens nucleus.

Inhibitory Effect of Single IBN Activity on Motoneuron Spikes

It was investigated whether the motoneuronal unitary IPSP induced from a single IBN actually had an inhibitory effect on motoneuron spikes. Before testing this possibility, preliminary experiments were performed to examine the validity of the technique employed. Compound action currents of the whole abducens nerve were monopolarly recorded at its cut end in the orbit with a d.c. amplifier (Fig. 6A). The spikes thus recorded were positive-going monophasic deflections and whole abducens nerve activity was composed of the summed positive spikes. An increase in nerve activity was therefore accompanied by a

positive shift of the d.c. potential level (Fig. 6Ba and Ca). The postsynaptic potentials in abducens motoneurons did not contribute to the d.c. shift of the nerve potential, since no appreciable potential shift was found prior to spike activity at the quick phase of nystagmus (Fig. 6C) although motoneuronal EPSPs precede spike generation by 5–10 msec (Maeda et al., 1972). The absence of spread of the soma potential to the recording site was reasonable in view of the relatively long distance between the recording site and the motoneuron somata, approximately 4 cm. The spread of potential along a myelinated nerve fiber is governed by the attenuation factor, 0.3–0.5, per internodal distance (2–2.5 mm for the frog nerve fiber) (Tasaki, 1955).

When abducens nerve activity was summed and averaged over a few msec after spikes of an abducens motoneuron, a sharp positive spike was obtained as shown in Fig. 6Db. Its time-to-peak was 0.1–0.2 msec, the duration 0.3–0.4 msec, and the latency of its positive peak 0.4–0.8 msec after the onset of motoneuron spike. The latency values were within the range of conduction time of the abducens nerve from the orbit to the motoneuron soma, 0.43–1.3 msec (Baker et al., 1969a). The above characteristics of the positive spike indicate that it was the action current of the axon originating from a single motoneuron whose spike was used as the triggering signal. Figure 6E shows the relation between the latency of the positive peak and the amplitude of the positive spike obtained with a number of different motoneurons in two preparations. There was a tendency for negative correlation between these two values under similar recording condition in the same preparation (filled circles: $r = -0.75$, $P < 0.02$; open circles: $r = -0.63$, $P < 0.1$, t-test). This negative correlation was expected from the finding that the magnitude of the axonal action current varies as the square of conduction velocity (Clamann and Henneman, 1976). The results suggest that any change in the level of the summed nerve potential is attributable to a change in the density of spikes in the whole nerve, though the contribution of the spike of each axon to the summed nerve potential depends on axonal size.

When the nerve potential trace was triggered from single IBN spikes on the contralateral side and summed over a 10 msec sweep duration after the spike (Fig. 6A), a transient negative deflection of the potential was obtained with a latency of 0.65 msec and a duration of 4 msec (Fig. 6Fb). Judging from the data described above, the negative deflection can be interpreted as a decrease in the density of spikes in the whole nerve. Spikes of all IBNs examined caused a suppression of contralateral abducens nerve activity and never ipsilateral activity (Fig. 6Fc). Latencies and amplitudes of the negative deflections obtained with a large number of IBNs were plotted in Fig. 6G (filled triangle). The latencies ranged from 0.65 to 1.15 msec (mean 0.9 msec) and the magnitude of suppression varied with different IBNs. The interval between the onset of motoneuron spike (Fig. 6Da) and the onset, instead of the peak, of the positive nerve potential (Fig. 6Db) ranged from 0.25 to 0.7 msec (Fig. 6G, open triangles). When the value of 0.25 msec was subtracted from the latencies of inhibitory nerve response, the remaining values, 0.4–0.9 msec, were well within the range of latencies of unitary IPSPs in the motoneuron (Fig. 5A). This leads to the conclusion that motoneuronal spikes are inhibited by IBN unitary IPSPs.

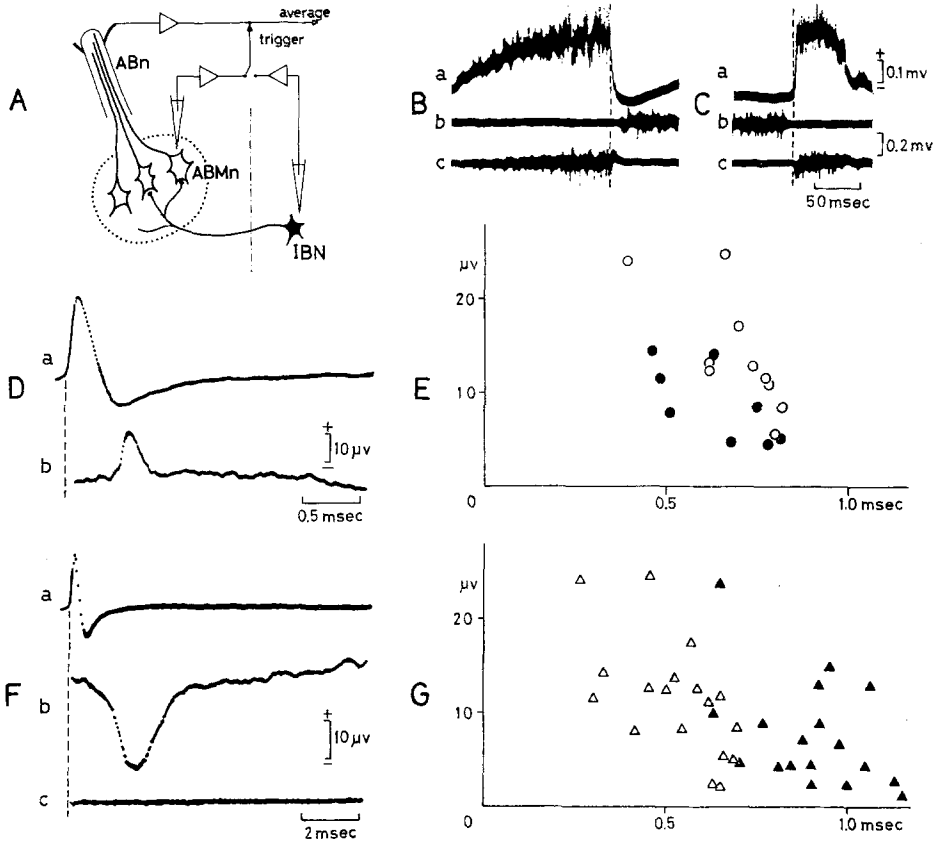

Fig. 6. Responses of abducens nerve potentials triggered by spikes of single abducens motoneurons and IBNs. **A** Schematic representation of experimental arrangements. ABn: abducens nerve, ABMn: abducens motoneuron. **B** and **C** Abducens nerve discharges during slow and quick phases of nystagmus. b and c, left and right nerve discharges recorded with a time constant of 1.7 msec. a, same as in c, but recorded with a time constant of 0.1 sec to show a d.c. shift of the potential level. **D** Averaged nerve potential (b) triggered by spikes of a single abducens motoneuron (a) (2000 sweeps, 0.1 sec time constant for b). **E** Diagram showing amplitudes of the positive spike (Db) and latencies of its positive peak measured from the onset of motoneuron spike. Open and filled circles represent data obtained with each of two different preparations, respectively. **F** Averaged nerve potentials on the left (b) and right side (c) triggered by IBN spikes on the right side (a) (5000 sweeps, 0.1 sec time constant for b and c). **G** Diagram showing amplitudes of negative deflection of nerve potential (Fb) and latencies of its onset measured from IBN spikes (filled triangles). For comparison, open triangles are replotted from the data shown in E, but in abscissa is indicated the interval between the onset of triggering motoneuron spikes and the onset, not the peak, of the positive spike in the nerve potential (Db)

Discussion

The technique used in the present experiments has been developed to study synaptic connections to spinal motoneurons from group Ia muscle afferents (Mendell and Henneman, 1971; Scott and Mendell, 1976; Watt et al., 1976), group II muscle afferents (Kirkwood and Sears, 1974; Stauffer et al., 1976), Ia inhibitory interneurons (Jankowska and Roberts, 1972b), medullary respiratory

neurons (Kirkwood and Sears, 1973) and vestibulocollic neurons (Rapoport et al., 1977). Evidence for synaptic connections between single neurons in the motor cortex was also provided with this technique (Asanuma and Rosén, 1973). The degree of divergence of a single neuron was estimated in the spinal motoneuron pool. Each Ia fiber has excitatory synaptic connection with all or nearly all homonymous motoneurons (Mendell and Henneman, 1971) and fewer connections with heteronymous motoneurons (Scott and Mendell, 1976; Watt et al., 1976). Each Ia inhibitory interneuron makes synapses with approximately 20% of the pool of target motoneurons (Jankowska and Roberts, 1972b). In contrast, vestibulocollic neurons are suggested to be less divergent than the Ia projection (Rapoport et al., 1977). The present study provides evidence for monosynaptic inhibitory connections of IBNs with contralateral abducens motoneurons. Our sampling of abducens motoneurons was not restricted to the area near the original antidromic stimulation site. Therefore, the results that IBN unitary IPSPs were found in 60% of the pairs examined indicate a wide divergence of each IBN. Since some of small IPSPs less than 20 μV in amplitude may have been overlooked, the actual divergence ratio could be larger than 60%. This is in good agreement with the extensive distribution of axonal branches of single IBN in the abducens nucleus (Hikosaka and Kawakami, 1977).

The averaged amplitudes of IBN unitary IPSPs were similar to those of Ia unitary IPSPs (Jankowska and Roberts, 1972b) and somewhat larger than those of vestibulocollic unitary IPSPs (Rapoport et al., 1977). The IBN unitary IPSPs were easily inverted to a depolarizing potential by Cl^- injection into the cell, suggesting that IBNs make ample synapses fairly proximally on motoneurons. The time-to-peak (1.40 ± 0.44 msec) appeared to be slightly longer than that of Ia unitary IPSP (0.85 ± 0.18 msec, Jankowska and Roberts, 1972b) or vestibulocollic unitary IPSP (0.81 ± 0.15 msec, Rapoport et al., 1977). The delayed peak of IBN unitary IPSPs may partly be attributed to somewhat prolonged action of inhibitory transmitter on abducens motoneurons. Contribution of distally located synapses may also not be excluded, since some IBNs extend their axon branches outside the abducens nucleus, especially in the region ventral to the nucleus (Hikosaka and Kawakami, 1977), where motoneuron dendrites extend (Ramón y Cajal, 1911; Lorente de Nó, 1933). In keeping with the latter possibility, the time-to-peak of the inverted unitary IPSP was often shorter than that of the hyperpolarizing IPSP (compare Fig. 4C with 4B).

Post-spike average of a late slow field potential in the spinal motoneuron pool has been described by Watt et al. (1976) and Taylor et al. (1978). The positive field in the abducens nucleus induced by single IBN activity may be produced mainly by inhibitory postsynaptic currents in a large number of neurons connected with the IBN and in part be attributed to reduction of spike currents during the unitary IPSP.

The inhibitory effects of IBN on abducens nerve potentials were detected with all IBNs examined, though the amplitude of the unitary IPSPs were mostly smaller than 100 μV. When the inhibitory effects were examined on spikes of a single abducens motoneuron, the number of sweeps for averaging to detect clear

inhibition had to be much larger (usually 10 times or more) than that with abducens nerve potentials (Hikosaka, Igusa and Imai, unpublished observation). The easy detectability of inhibition on the whole nerve potential may be due to an extensive divergence of a single IBN, because the present technique corresponds to the summation of records obtained from many abducens axons correlated to the IBN. This greatly improves the signal-to-noise ratio despite of intermingled IBN-unrelated axonal spikes in the whole abducens nerve. The present technique may be similar to that of Fetz et al. (1976) who detected corticomotoneuronal connection of precentral neurons by post-spike average of EMG activity.

The time course of observed inhibition of abducens nerve potential (approximate duration of 4 msec in Fig. 6Fb) was in general faster than that of IBN unitary IPSP. The time course of inhibition of single motoneurons is presumably still shorter than that of nerve potential, because there are considerable differences in conduction time among abducens motor axons (Baker et al., 1969a). This short time course of inhibition may well be explained by relatively stronger inhibitory effects due to an increase in membrane conductance of motoneurons at the initial phase of unitary IPSP (Eccles, 1964).

The widely divergent connection of single IBNs with abducens motoneurons suggests that each motoneuron receives inhibition from a large number of IBNs. This leads to highly synchronous production of IPSPs at the quick phase among all motoneurons (Maeda et al., 1972; Hikosaka et al., 1977), in spite of more or less variable onset times of burst activity in different IBNs. The same argument may explain the fairly uniform feature of spike suppression of each motoneuron at the onset of fast eye movements in off-direction (Fuchs and Lsuchei, 1970; Robinson, 1970).

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