

POSTSYNAPTIC INHIBITION UNDERLYING SPIKE SUPPRESSION OF SECONDARY VESTIBULAR NEURONS DURING QUICK PHASES OF VESTIBULAR NYSTAGMUS

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SUMMARY

Synaptic mechanisms of spike suppression of vestibular neurons during quick phases of vestibular nystagmus were investigated by intracellular recording in the rostralateral part of the cat medial vestibular nucleus. When repetitive spike discharges of vestibular neurons were abruptly suppressed at the quick phase, the membrane potential shifted steeply in the hyperpolarizing direction. After the commissural IPSP was inverted into depolarization by intracellular injection of Cl^- ions, the hyperpolarizing deflection of the membrane potential at the quick phase was also inverted into a depolarizing potential. The results indicate that an abrupt generation of IPSPs in vestibular neurons underlies the quick phase suppression of spike activity in these neurons.

Spike activity of a class of neurons in the vestibular nuclei has been found to pause during saccadic eye movements or quick phases of nystagmus [3,4,11,19]. The spike suppression during the quick phase was observed in axons of secondary vestibular neurons recorded in the abducens [9,13] or trochlear nucleus [1] as well, suggesting the premotor role of these vestibular neurons in control of eye movements. The question arises, what is the neuronal mechanism of spike suppression of vestibular neurons at the quick phase? The present study is performed to take a first step toward answering this question by intracellular recording from vestibular nucleus neurons during vestibular nystagmus. Since there are a large number of vestibular neurons whose activity is not related to nystagmic rhythm [6], we have attempted in the early stage of the present experiments to find major location of vestibular neurons which make direct connection with either ipsilateral or contralateral

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abducens motoneurons. This facilitated a successful encounter with nystagmus-related vestibular neurons during intracellular study.

Experiments were performed with adult cats. The animal was anesthetized with ether and mounted on the stereotaxic frame which was fixed to a turntable to identify types of vestibular neurons. The procedures for placement of the electrodes for stimulation of the vestibular and abducens nerves and recording from the abducens nerve were similar to those described elsewhere [9,14]. The animal was decerebrated at the precollicular level or the upper cervical cord was transected. In the latter preparations, all incision and pressure points were repeatedly infiltrated with 4% xylocaine throughout the experiment. The effectiveness of the local anesthetic in controlling pain was carefully checked by observing changes in EEG pattern [9]. The animal was immobilized with gallamine triethiodide. For extracellular recording of vestibular neuron spikes or field potentials in the vestibular nuclei, glass micropipettes were filled with Ringer or 2 M NaCl solution saturated with Fast Green FCF to make dye marks for later histological studies. Double-barrelled microelectrodes were also used: one barrel was filled with the above solution for recording from single vestibular neurons and the other with a solution of 1 M Na glutamate to activate the neuron by its spontaneous leakage or iontophoresis. The microelectrode for intracellular recording from abducens motoneurons or vestibular neurons was filled with 3 M KCl or 2 M K citrate solution. The intracellular potentials of abducens motoneurons were averaged, when necessary, with a digital averaging computer.

Spikes of single vestibular neurons were recorded extracellularly and identified as type I neurons by observing an increase in firing frequency with ipsilateral horizontal angular acceleration and a decrease in frequency with contralateral acceleration [3]. The spikes were evoked by single shocks to the ipsilateral vestibular nerve and superimposed on the N_1 field potential [17]. After completing the identification of type I vestibular neurons, intracellular recording was made from ipsilateral or contralateral abducens motoneurons which were identified by antidromic activation following stimulation of the abducens nerve. The post-spike averaging method [10,16] was utilized to demonstrate monosynaptic connection between simultaneously recorded single vestibular neurons and abducens motoneurons. The vestibular neuron shown in Fig. 1A was activated monosynaptically from the ipsilateral vestibular nerve (Fig. 1A(d)) and antidromically from the contralateral abducens nucleus with a latency of 0.6 msec (not illustrated). The membrane potential in a contralateral abducens motoneuron was triggered from repetitive spikes of the single vestibular neuron and averaged over a 10-msec sweep duration after spikes. The averaged intracellular potential revealed an unitary EPSP (Fig. 1A(b)) compared with the averaged extracellular field potential as a control (Fig. 1A(c)). The latency of the unitary EPSP measured as the interval between the foot of the triggering spike and the onset of depolarization was 0.9 msec. This value was in a monosynaptic range when compared with the latency of antidromic spikes of the vestibular neuron, 0.6 msec. Monosynaptic unitary EPSPs in contralateral abducens motoneurons were obtained with 8 vestibular type I neurons. Recording sites of vestibular neurons identified as excitatory premotor neurons were marked by electrophoretic ejection of

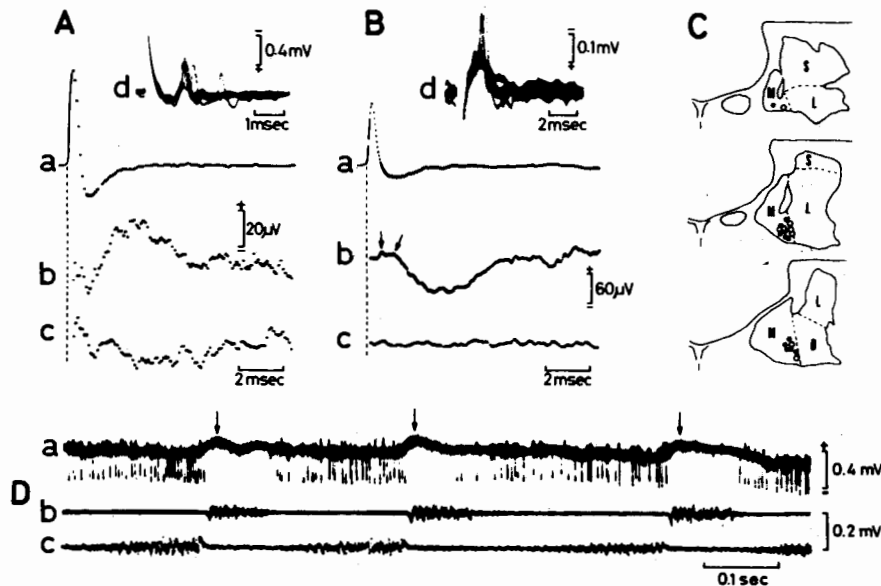


Fig. 1. A: post-spike averaging to detect monosynaptic excitatory connection of a single vestibular type I neuron with a contralateral abducens motoneuron. (a) Averaged vestibular neuron spike used for triggering the sweep. Initial part of the spike drawn by a solid line was reconstructed from spontaneous spikes recorded with another oscilloscope. (b) Unitary EPSP in the motoneuron (average of 400 sweeps with a time resolution of 0.1 msec). (c) Averaged extracellular field potential. Vertical broken line indicates the foot of triggering spike. (d) Activation of the vestibular neuron in response to stimulation of the ipsilateral vestibular nerve. B: Same arrangement as in A, but showing monosynaptic inhibitory connection of another type I neuron with an ipsilateral abducens motoneuron. (b) Unitary IPSP in the motoneuron (average of 300 sweeps with a time resolution of 0.02 msec). First arrow indicates the arrival of the presynaptic impulse and second arrow the onset of IPSP. C: Locations of excitatory (open circles) and inhibitory (filled circles) premotor type I neurons projected to three transverse sections of the brain stem through the vestibular nuclei. S, M, L and D; superior, medial, lateral and descending nucleus. D: Discharge pattern of a type I neuron identified as the inhibitory premotor neuron (a) and ipsilateral (b) and contralateral (c) abducens nerve activities during nystagmus. Arrows indicate positive deflection of the field potential.

Fast Green from the recording microelectrode. They were located mainly in the rostral and ventro-lateral part of the medial vestibular nucleus, and some were on the border between the medial and lateral nuclei (Fig. 1C, open circles).

Similar experiments were performed to detect monosynaptic connection between vestibular type I neurons and ipsilateral abducens motoneurons. Fig. 1B exemplifies the post-spike average of unitary IPSPs in an abducens motoneuron. The latency of the IPSP after triggering spikes was 1.2 msec. A small positive potential (first arrow in Fig. 1B(b)) observed before the averaged synaptic potential presumably represents a presynaptic spike arriving near the target motoneuron [8,10,18,20]. The interval between the positive peak of the presynaptic spike and the onset of unitary IPSP (second arrow in Fig. 1B(b)) was 0.5 msec which corresponded to a single synaptic delay time. Monosynaptic unitary IPSPs in ipsilateral abducens motoneurons were ob-

tained with 12 vestibular type I neurons. Thus identified inhibitory premotor neurons were located in the rostral and ventrolateral part of the medial vestibular nucleus and intermingled with excitatory neurons described above (Fig. 1C, filled circles).

In the superior vestibular nucleus, 29 type I neurons were identified by horizontal rotation. None of them were antidromically activated from the ipsilateral or the contralateral abducens nucleus. Post-spike averaging of membrane potentials in abducens motoneurons revealed no unitary synaptic potentials in any pair of type I neurons in the superior nucleus and abducens motoneurons.

The locations of excitatory and inhibitory vestibular neurons projecting to the abducens nuclei are in agreement with previous suggestion obtained by stimulation experiments [2,7] as well as anatomical studies with retrograde transport of horseradish peroxidase [5,12]. It was noted that identified synaptic actions of single vestibular neurons on abducens motoneurons were always ipsilaterally inhibitory and contralaterally excitatory and that the reverse relation was never found in any pair of type I neurons and motoneurons.

Nystagmus was induced by high frequency stimulation (400/sec) of the vestibular nerve on one side. Vestibular neurons identified as excitatory or inhibitory premotor neurons exhibited nystagmus-related spike activity: tonic discharges during the slow phase were abruptly suppressed at the quick phase of abducens nerve activities (Fig. 1D). It was noted that DC recording of vestibular field potentials revealed a positive deflection during the quick phase (Fig. 1D, arrows). This suggests the presence of active inhibition of vestibular neurons at the quick phase, in view of previous findings obtained in the abducens nucleus during nystagmus [14].

To confirm this possibility, we attempted to record intracellularly from vestibular neurons. Since the above results indicated that the premotor neurons located mainly in the rostro-lateral area in the medial vestibular nucleus exhibited nystagmus-related spike activity, we made a large number of intracellular recording in this region and obtained successful penetration in six neurons which showed spike suppression during quick phases. In Fig. 2A, the membrane potential was maintained at a depolarized level or proceeded slightly in the depolarizing direction during the slow phase. Spikes were induced repetitively during this period. In this particular case, the discharge frequency was much higher than that of extracellular spikes (Fig. 1D), probably due to depolarizing effects of penetration. When spike discharges were abruptly suppressed at the time of cessation of contralateral abducens nerve activity (Fig. 2A, vertical broken lines), the membrane potential shifted steeply in the hyperpolarizing direction. The alternating rhythmic changes in the membrane potential levels clearly correlated with activation and suppression of spike activity.

In order to examine the possible contribution of IPSPs to the steep hyperpolarizing deflection of the membrane potential at the quick phase, Cl^- ions were injected into the cell through the recording microelectrode. The effectiveness of Cl^- injection was judged by reversal of the commissural IPSP [15] into a depolarizing potential (Fig. 2B). Under this condition the depolarization

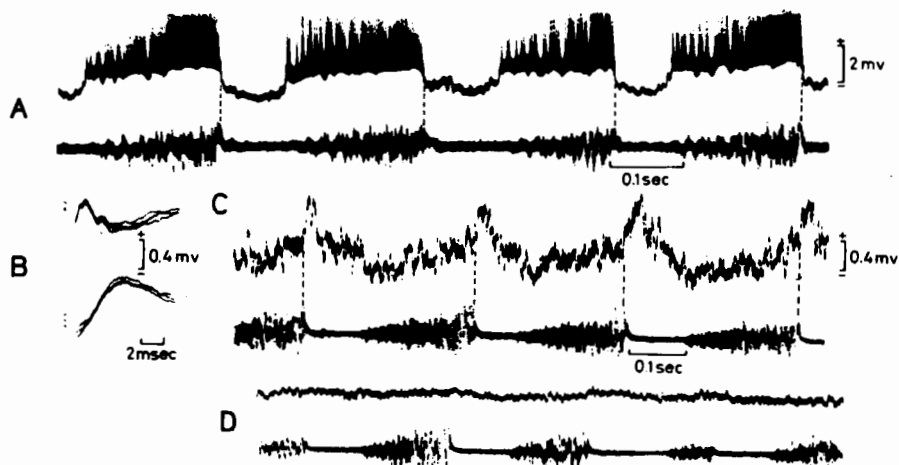


Fig. 2. A: membrane potential changes and spike activity of a vestibular neuron (top) associated with nystagmic modulation of contralateral abducens nerve activity (bottom). B: commissural IPSP induced by stimulation of the contralateral vestibular nerve (top) and its reversal to depolarization by injection of Cl^- ions into the cell (bottom). C: nystagmus-related changes of the membrane potential after reversal of the IPSP. Vertical broken lines in A and C indicate the onset of the quick phase. D: extracellular field potential as a control for C.

during the slow phase was followed by an additional, steep depolarization at the beginning of the quick phase (Fig. 2C, vertical broken lines), instead of the hyperpolarizing shift consistently found in the control record. These potential changes were almost entirely attributed to the transmembrane potential on the basis of control recording of the extracellular field potential (Fig. 2D). Thus, the synaptic mechanism, having produced a hyperpolarization at the quick phase in the control, induced a depolarization when the IPSP was inverted into a depolarizing direction. These results provide evidence that an abrupt production of the IPSP in vestibular neurons underlies the suppression of spike activity in these neurons during quick phases.

Periodic suppression of spike activity of a neuron could be initiated either by an intrinsic mechanism of the neuron causing frequency adaptation of spikes or by extrinsic inhibitory synaptic input generated through more or less remote neural pathways. In the present study there is no sign of frequency adaptation of spikes before the moment of production of steep IPSPs. It is therefore suggested that an abrupt inhibitory input is primarily responsible for spike suppression of vestibular neurons during quick phases of vestibular nystagmus.

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