Pflügers Arch. 355, 85—94 (1975)

Responses of Neurons of Lizard's, Lacerta viridis, Vestibular Nuclei to Electrical Stimulation of the Ipsi- and Contralateral VIIIth Nerves

A. Richter, W. Precht, and S. Ozawa

Neurobiologische Abteilung, Max-Planck-Institut für Hirnforschung, Frankfurt (Main) (FRG)

Received October 21, 1974

Summary. Field and intracellular potentials were recorded in the vestibular nuclei of the lizard following stimulation of the ipsi- and contralateral vestibular nerves. The field potentials induced by ipsilateral VIIIth nerve stimulation consisted of an early negative or positive-negative wave (presynaptic component) followed by a slow negativity (transsynaptic component). The spatial distribution of the field potential complex closely paralleled the extension of the vestibular nuclei. Mono- and polysynaptic EPSPs were recorded from vestibular neurons after ipsilateral VIIIth nerve stimulation. In some neurons early depolarizations preceded the EPSPs. These potentials may be elicited by electrical transmission. Often spike-like partial responses were superimposed on the EPSPs. It is assumed that these potentials represent dendritic spikes.

Contralateral VIIIth nerve stimulation generated disynaptic and polysynaptic IPSPs in some neurons and EPSPs in others. The possible role of commissural inhibition in phylogeny is discussed.

In a group of vestibular neurons stimulation of the ipsilateral VIIIth nerve evoked full action potentials with latencies ranging from 0.25—1.1 msec. These potentials are caused by antidromic activation of neurons which send their axons to the labyrinth.

Key words: Reptile Vestibular Neurons — Vestibular Efferents — Commissural Inhibition.

Previous studies of the functional organization of the vestibular input into the vestibular nuclei in lower (Precht *et al.*, 1974; Ozawa *et al.*, 1974) and higher vertebrates (Shimazu and Precht, 1965, 1966; Precht and Shimazu, 1965; Mano *et al.*, 1968; Ito *et al.*, 1969; Kawai *et al.*, 1969) have shown that the phylogenetic differences in neuronal circuitry of the vestibular system are much greater than is usually assumed. Thus, the commissural inhibition present in higher vertebrates is missing in amphibia. Amphibian vestibular neurons unlike their mammalian counter parts participate in the efferent system projecting to the sensory hair cells. These were characterized by their ability to generate dendritic spikes in response to various inputs, as well as by the frequent occurrence of electrotonic coupling (Precht *et al.*, 1974; Ozawa *et al.*, 1974).

The present study of the vestibular system of a reptile—the lizard —was undertaken to further elucidate the comparative physiology of the vestibular nuclei. Although some anatomical studies of the vestibular nuclei of reptiles (Weston, 1936; Larsell, 1967; Mehler, 1972), are available nothing is known about their functional organization. Although many similarities between reptile and amphibian vestibular systems were observed, the presence, however, of the commissural inhibition in reptiles indicates a higher differentiation of vestibular circuitry in reptiles as compared to amphibia which have no crossed inhibition (Ozawa *et al.*, 1974).

Methods

The experiments were performed on lizards (lacerta viridis) weighing 15—40 g and anesthetized with pentobarbital sodium (35—50 μg/g i.p.). The region of the vestibular nuclei on both sides of the brain stem was exposed by a craniotomy. The bilateral labyrinthine cavities were opened by a dorsal approach to allow extracranial dissection of the vestibular nerves. A pair of stainless steel wires (40 μ in diameter) insulated except for the cut ends were used for stimulation of the VIIIth nerve. The stimulating electrodes were gently placed on the surface of the VIIIth nerves on both sides.

The field potentials and extracellular unitary potentials induced by electrical stimulation of the ipsilateral VIIIth nerve were recorded with the aid of micropipettes filled with 2 M NaCl saturated with fast green. Their resistances were 5—10 MΩ. Recording sites were confirmed by electrophoretic application of fast green (20 μA, 10 min) (Thomas and Wilson, 1965). For intracellular recording, the micropipettes were filled with 3 M KCl or 2 M K-citrate solution resulting in resistances of 15—30 MΩ. A conventional bridge circuit was used for recording and passing current through the microelectrode. All records were taken at low gain DC and high gain AC (300 msec time constant). A FabriTek computer of average transients was also used for clearer presentation of both extra- and intracellular recordings. The brains were fixed with 10% formalin and serial sections, 30 μ thick, of the brain stem were obtained with a freezing microtome and stained with the Nissl method.

Results

Field Potentials Recorded in the Vestibular Nuclei and Evoked by Stimulation of the VIIIth Nerve

In all experiments the ipsilateral VIIIth nerve was stimulated electrically to obtain the spatial distribution of the vestibular-evoked field potentials in the brain stem which represented an approximate analogon of the actual dimensions of the vestibular nuclear complex in each animal. As shown in Fig. 1A the field potentials consisted of an early sharp negative or positive-negative deflection (latency ca. 0.2—0.3 msec), followed by a slower negative wave (latency ca. 1.2—1.3 msec). The early potential followed double shock activation at short intervals and was resistant to anesthesia. Conversely, the slower negative potential which failed to follow closely spaced double shocks was sensitive to barbiturates.

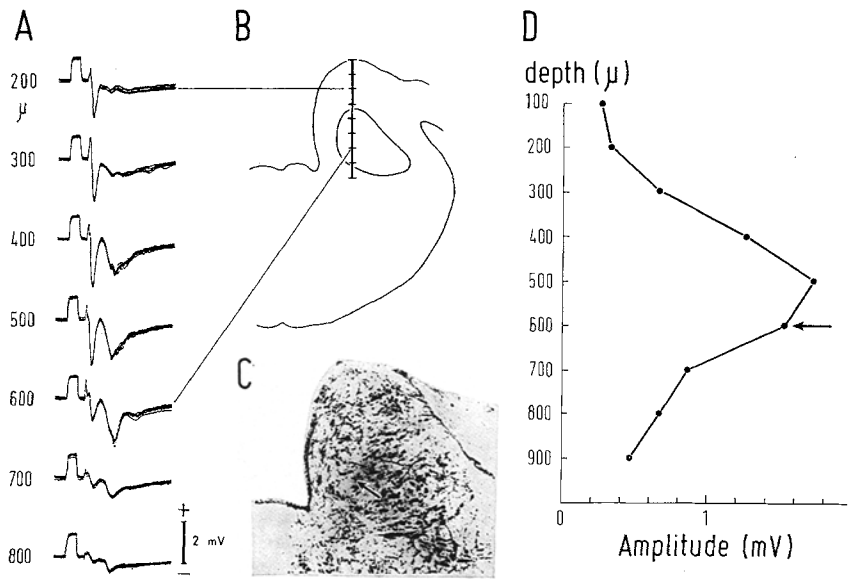

Fig. 1A—D. Field potentials recorded in the vestibular nuclei after stimulation of the ipsilateral VIIIth nerve. (A) Field potentials recorded at different depths (μ) from the surface of the brain stem. Calibration signal: 1 msec, 1 mV. (B) Line drawing of histological section of the brain stem partially shown in (C). Arrow in (C) indicates site of fast green mark 600 μ from surface). (D) Diagram showing relative amplitudes of the second negativity of field potentials shown in (A)

Fig. 1A depicts the field potentials that were evoked at different depths (200—800 μ) along a dorsoventral track through the vestibular nuclei (Fig. 1B) after stimulation of the ipsilateral VIIIth nerve. The early negativity was prominent in dorsal regions, whereas the second potential wave was more prominent in deeper recording positions (Fig. 1A,D). It is this region that corresponds to the vestibular nuclei (see Fig. 1B,C). A fast green mark was placed at the site at which the maximum slow negativity was recorded. As shown in Fig. 1C its location is within the borders of the vestibular nuclei. A similar distribution of the field potentials was found in more rostral, caudal or lateral tracks.

Intracellular Potentials Recorded from Vestibular Neurons in Response to Ipsi- and Contralateral VIIIth Nerve Stimulation

Ipsilateral Stimulation. As described above, the arrival of the microelectrode tip in the vestibular nuclei was signalled by the appearance of the typical field potential complex evoked by VIIIth nerve stimulation. Stable intracellular recordings from more than 100 vestibular neurons were obtained during the course of these experiments. The resting mem-

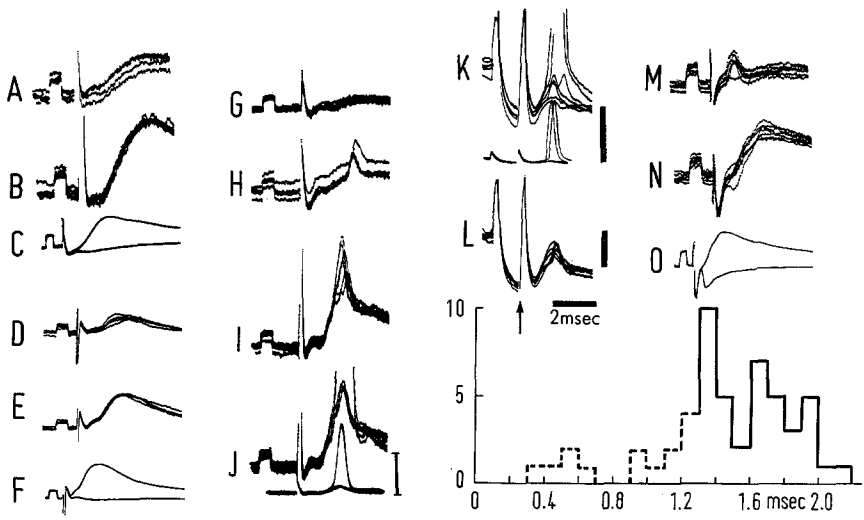

Fig. 2 A—O. Synaptic potentials in vestibular neurons evoked by ipsilateral VIIIth nerve stimulation. (A—B) EPSPs evoked in a vestibular neuron with increasing stimulus strengths. (C) Superposition of intra- and extracellular traces of the same neuron averaged by computer (16 averaged traces). (D—F) Same for other neuron but showing early depolarizations and EPSPs. (G—J) EPSPs, partial and full spikes evoked in vestibular neuron with increasing stimulus strengths. Low gain, DC recording in (J). (K, L) Early depolarization and partial responses evoked by VIIIth nerve stimulation, and the effects of hyperpolarizing pulses of different strengths. (M) All-or-none early depolarization evoked with low intensity of stimulation. (N) Early depolarization plus EPSP after strong stimulation recorded in the same neuron. (O) Computer averaged intra- and extracellular traces of (N). Calibration pulses: 1 mV, 1 msec. Lower trace in J (40 mV) and K (50 mV); L and upper trace in K 2 mV. Histogram shows frequency distribution of early depolarization (dashed line) and EPSPs (solid line)

brane potentials of the neurons selected for further measurements were 40—60 mV (74 neurons). Following ipsilateral VIIIth nerve stimulation excitatory postsynaptic potentials were recorded from 54 neurons. The exact latencies of these EPSPs were determined by superimposing them on the extracellular traces obtained after withdrawal of the microelectrode from the cell (Fig. 2C, F, O). Since the latencies varied with stimulus strength and strong stimulation may cause current spread to the brain stem nuclei, measurements were performed with weak stimulation (less than 2× threshold for the field potential). The latencies of the EPSPs ranged from 1.25—2.2 msec (see histogram Fig. 2). Occasionally EPSPs with longer latencies were also encountered.

In 12 neurons the EPSPs were preceded by early depolarizing potentials even when the stimulus intensity was very low. Their latencies

ranged from 0.3 to 1.1 msec (see histogram of Fig. 2). These early depolarizing potentials could be i) graded and combined with a later EPSP at all stimulus intensities (Fig. 2D—F) or ii) predominantly all-or-none in nature with low intensity (Fig. 2M) and combined with a later EPSP with increasing stimulus strength (Fig. 2N, O). The effects of hyperpolarizing currents on these early depolarizing potentials were examined. Occasionally early all-or-none partial spikes and graded components were masked by action potentials occurring with similar latencies and were revealed only when the spike potentials were blocked by hyperpolarizing currents (Fig. 2K). As indicated by their different latencies, shapes and amplitudes these potentials can not be m-spikes only. Increasing the hyperpolarizing current across the membrane did not block these early depolarizing potentials (Fig. 2L). Similarly the graded early potentials were unaffected by intracellular application of current through the recording electrode.

Following VIIIth nerve stimulation spike-like partial responses of different amplitudes were additionally superimposed on the EPSPs in vestibular neurons (Fig. 2H—J). When the intensity of vestibular nerve stimulation was increased, full action potentials were evoked in most cells (Fig. 2J). They appeared to be generated from partial spikes of different amplitudes. As with the partial spikes responses evoked in synchrony with the early depolarizations (Fig. 2K, L), the all-or-none responses superimposed on the EPSPs were generally not affected by hyperpolarizing currents applied across the cell membrane by means of the recording electrode.

Contralateral Stimulation. Following stimulation of the contralateral VIIIth nerve EPSPs were evoked in one group of neurons, while in other cells only IPSPs were produced. It was noted that it was more difficult to maintain good intracellular recordings in those neurons which showed IPSPs than in those with EPSPs. EPSPs evoked by contralateral stimulation (Fig. 3A, B) had latencies ranging from 3.0—6.2 msec. Their times to peak were much greater than with ipsilaterally evoked EPSPs (Fig. 3C, D). Action potentials were never superimposed on contralateral EPSPs even on supramaximal or double shock stimulation.

Examples of contralaterally evoked IPSPs are shown in Fig. 3E, G, H, J. Intracellular injection of chloride ions into the neuron resulted in a reversal of the hyperpolarizing into a depolarizing PSP (Fig. 3J). The latencies of the IPSPs fell into 2 groups: one with a range of 2.2—4.4 msec and the other with a range of 7.3—11 msec. In most neurons in which contralateral stimulation generated IPSPs, ipsilateral stimulation produced EPSPs.

Antidromic Activation by Ipsilateral VIIIth Nerve Stimulation. The VIIIth nerve stimulation of 16 vestibular neurons induced action po-

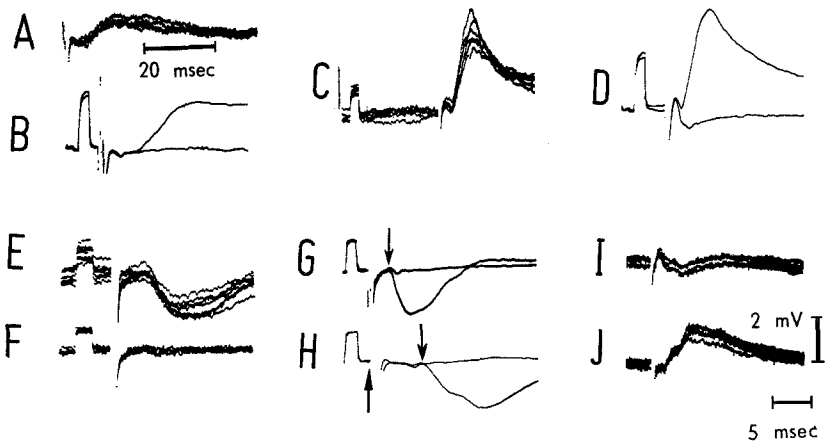

Fig. 3 A—J. Synaptic potentials in vestibular neurons evoked by contralateral VIIIth nerve stimulation. (A) and (C) Intracellular recordings of EPSPs in vestibular neuron after contra- and ipsilateral VIIIth nerve stimulation, respectively. (B) and (D) Superposition of intra- and extracellular computer-averaged traces of (A) and (C). (E—H) Intracellular recordings of IPSPs and their extracellular counterparts. (I, J) Original recording and chloride-reversed counterparts of IPSPs

tentials with very short latencies (0.25—1.1 msec). At the threshold stimulus intensity the action potentials arose abruptly from the baseline with no prepotential (Fig. 4 A). As the stimulus intensity was slightly increased, an early action potential occurred in each trial and a later synaptic activation was also observed (Fig. 4 B). The occurrence of synaptic activation as well as the occasional presence of clear IS—SD inflexions indicated that the recordings were obtained from second order vestibular neurons and not from primary fibers. In some neurons the threshold intensity for antidromic activation was lower than that for synaptic activation, while in others the opposite was observed. Since the distance between the recording site in the vestibular nucleus and the stimulating site in the nerve was approximately 3—4 mm, the possibility of direct excitation of vestibular neurons by spread of stimulating current should carefully be ruled out. The following findings strongly suggest antidromic activation of efferent neurons: i) the excitation was obtained with stimulus intensities close to the threshold for the field potential which was confined to the vestibular nucleus; ii) activation included a distinct latency, and iii) after crushing or coagulating the VIIIth nerve at the site proximal to the stimulating electrode, the stimulus intensity necessary for direct activation of vestibular neurons was increased up to 10 times the threshold of the intact nerve.

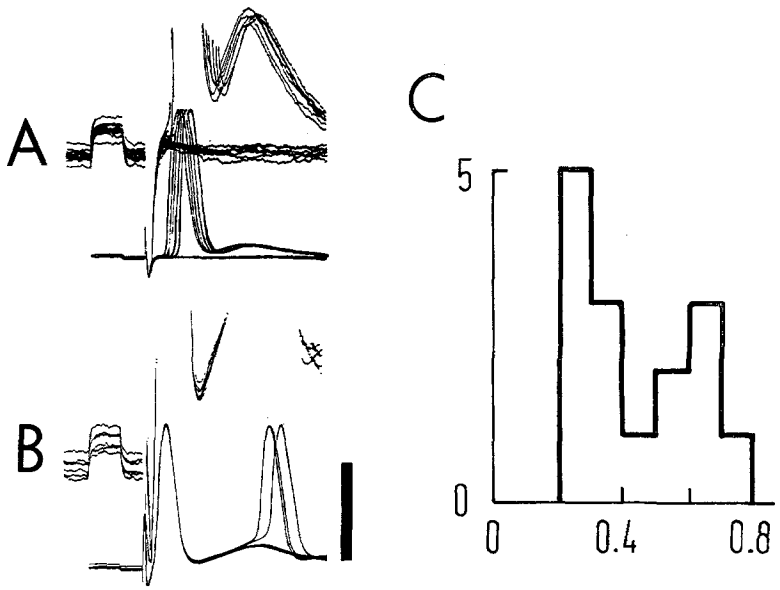

Fig. 4 A—C. Antidromic activation of vestibular neuron after ipsilateral VIIIth nerve stimulation. Intracellular records taken at threshold straddling intensity of the axon of the impaled neuron. (B) Same but with higher stimulus strength. Note additional synaptic activation. (C) Histogram showing latency distribution of antidromically activated action potentials recorded from vestibular neurons. Calibration pulses: 1 mV, 1 msec; vertical bar in (B) (50 mV) indicates gain for lower traces in (A) and (B)

Discussion

Field Potentials

The prominent field potential complex that was recorded in the vestibular region of the brain stem following VIIIth nerve stimulation may be divided into an early and late negativity which represent the pre- and postsynaptic components of the field potential. This statement is based on the finding that axonal spikes frequently occurred on the early negativity and that these spikes followed double shock stimulation at close intervals and were resistant to anesthesia. In addition the early negativity is composed of the action currents of efferent neurons excited antidromically. The transsynaptic nature of the second field component is revealed in the finding that it is sensitive to double shock stimulation and anesthesia and that the latencies of the EPSPs and action potentials paralleled the time course of the slow negativity. Anatomical studies of the reptile's brain stem show that the VIIIth nerve passes dorso-ventrally through the brain stem to reach the vestibular portion of the statoacoustic complex which can be clearly distinguished from the more superficially located acoustic region (Fig. 1 C).

The spatial distribution of the vestibular-evoked field potentials reflected these morphological features in that the amplitude of the early negativity was large in the dorsal region and decreased as the electrode approached the vestibular nucleus, and also in that the second negativity displayed the opposite amplitude distribution. Furthermore this field potential profile indicated that in the present experiments the electrical stimulation excited primarily vestibular fibers and that coexcitation of acoustic fibers was negligible.

EPSPs and Early Depolarizations

The time of arrival of vestibular impulses at the vestibular nucleus may be indicated by the latency of the peak of the positivity of the early positive-negative wave which occurred 0.2—0.3 msec after the stimulus. Given that in cold blooded animals the synaptic delay is in the order of 1 msec (Brookhart and Fadiga, 1960) the earliest EPSPs which occurred 1.2 msec after the stimulus may be monosynaptic. Later EPSPs, however, are polysynaptic in origin. Similar mono- and polysynaptic activation of vestibular neurons was reported in the cat (Precht and Shimazu, 1965; Ito *et al.*, 1969; Kawai *et al.*, 1969) and frog (Precht *et al.*, 1974).

In contrast, the early depolarizing potentials may not be mediated by chemical synaptic transmission, since their shortest latencies (0.3—1 msec, are well within the range of the latency of the afferent volley. It is suggested that early potentials are generated by electrical transmission which may occur between primary afferents and vestibular neurons and/or via electrotonic coupling between vestibular neurons and efferent neurons. Since the presumed coupling potentials were small and since occasionally only all-or-none potentials of similar latencies were seen the electrical transmission probably occurred at a distance from the soma, and the partial spikes were probably generated by coupling potentials occurring at dendritic sites. Similar results have been obtained in the vestibular neurons of the frog (Precht *et al.*, 1974) and the pigeon (Wilson and Wylie, 1970). In the cat, however, no such early potentials following VIIIth nerve stimulation have been noted.

As in the frog (Precht *et al.*, 1974) the vestibular neurons of the lizard exhibited spike-like partial responses which occurred spontaneously as well as in response to VIIIth nerve stimulation at various levels of the postsynaptic depolarization. Hyperpolarizing currents applied across the membrane through the recording microelectrode generally failed to abolish these responses, and depolarizing currents failed to evoke any response. These findings suggest that the sites of origin of these spike-like partial responses are located at some distance away from the soma, presumably in the dendrites. Partial responses similar to those described here were recorded in central neurons of different vertebrates (Eccles

et al., 1958; Spencer and Kandel, 1961; Kuno and Llinás, 1970; Llinás and Nicholson, 1971; cf. Purpura, 1967). In all these cases it may be assumed that some areas of the dendrites are electrically excitable. As a result dendritic spike generation would increase the efficacy of synaptic excidation occurring at distal parts of the neurons.

Crossed Effects

Contrary to the frog in which only crossed excitation was found (Ozawa *et al.*, 1974) stimulation of the contralateral VIIIth nerve of the lizard produced EPSPs in one group of neurons and IPSPs in another. The finding of crossed inhibition in the lizard is similar to the cat in which second order neurons of the semicircular canals receive precisely organized inhibitory projections from the contralateral labyrinth via the commissural pathway (Shimazu and Precht, 1966; Mano *et al.*, 1968). As in the cat (Mano *et al.*, 1968) the short latencies of some of the IPSPs of the first group of lizard vestibular neurons (2.2—4.4 msec) may indicate that there exists a disynaptic inhibitory pathway across the midline which is supplemented by additional polysynaptic inhibitory paths. In the cat it has been shown that commissural inhibition will enhance the sensitivity of second order canal neurons to rotatory stimulation (Shimazu and Precht, 1966) and may play a role in the regulation of the vestibulo-ocular reflex. Since in contrast to the frog reptiles have rather highly developed eye movements including vestibular nystagmus the occurrence of commissural inhibition in reptiles may be a step toward the functional differentiation of the vestibular system as it develops from lower to higher forms or as it is required to perform more complex functions.

Crossed excitation which has been described in the frog (Ozawa *et al.*, 1974) and cat (Shimazu and Precht, 1966; Shimazu and Smith, 1971) was also found in a group of vestibular neurons in the lizard. Whereas contralateral excitation in the cat mainly affects the central otolith neurons (Shimazu and Smith, 1971) the canal neurons in the frog are likewise affected (Ozawa *et al.*, 1974). Studies employing natural labyrinthine stimuli are required to clarify which mode characterizes contralateral organization in the lizard.

Efferent Vestibular System

As was previously demonstrated in the frog (Precht *et al.*, 1974) the present experiments in lizard demonstrate that neurons located in the vestibular nuclei participate in the efferent innervation of the sensory epithelium. Efferent neurons also showed synaptic excitation after VIIIth nerve stimulation. This finding implies the existence of a feedback loop between labyrinth and vestibular neurons on the same side. Recent anatomical work by Gacek and Lyon (1974) has shown that in the cat unlike frog and the lizard efferent neurons are located outside but in close proximity to the vestibular nuclear complex.

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Priv. Doz. Dr. W. Precht
Neurobiologische Abteilung
Max-Planck-Institut für Hirnforschung
D-6000 Frankfurt (Main), Deutschordenstr. 46
Federal Republic of Germany