Excitatory and Inhibitory Inputs From Saccular Afferents to Single Vestibular Neurons in the Cat

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Uchino, Y., H. Sato, and H. Suwa. Excitatory and inhibitory inputs from saccular afferents to single vestibular neurons in the cat. J. Neurophysiol. 78: 2186–2192, 1997. Connections from saccular afferents to vestibular neurons were studied by means of intracellular recordings of excitatory (E) and inhibitory (I) postsynaptic potentials (PSPs) in vestibular neurons after focal stimulation of the saccular macula in decerebrated cats. Focal stimulation was given to the saccular macula in two ways, in which the polarity of stimulus current via a pair of electrodes was changed. In group A, one of the electrodes was inserted into the ventral and the other into the dorsal edge of the saccular macula. The focal stimulation was across the striola so that the reversal of morphological polarization in hair cells was bridged by the pulse stimulus. In 22/36 vestibular neurons tested, the stimulation of the saccular macula evoked monosynaptic (1.2 ms) EPSPs, including EPSP-IPSP sequences, with one polarity of stimulation, and disynaptic (1.5 ms) IPSPs when the polarity of the stimulus current was changed. In 14/36 neurons, the response pattern was the same regardless of the stimulus polarity; EPSPs (12/36) or IPSPs (2/36). In group B, a pair of electrodes was inserted into the dorsal edge of the saccular macula, so that the striola was not bridged by the current stimulus. In all of the vestibular neurons tested, the response pattern was always the same regardless of the polarity: mono- (22/31) and disynaptic (3/31) IPSPs or disynaptic EPSPs (6/31). In addition, the saccular nerve was stimulated after removing the macula in some cats (group C). The stimulation of the saccular nerve evoked EPSPs in 62 vestibular neurons (including EPSP-IPSP sequences in 31 neurons) and IPSPs in 19 vestibular neurons. Convergence between the saccular nerve and other vestibular nerves was studied by the intracellular recording of PSPs. Fifty-six percent (18/32) of the saccular-activated neurons had excitatory and/or inhibitory potentials evoked after stimulation of the utricular nerve and the horizontal and anterior semicircular canal nerves, and 44% (19/43) of the neurons received inputs from the posterior semicircular canal nerve. The results support the hypothesis that saccular afferents from one population of hair cells activate vestibular neurons monosynaptically and that afferents from another population of hair cells located on the opposite side of the striola appear to project to the same vestibular neurons disynaptically via inhibitory interneurons. Neural circuits from saccular afferents to vestibular neurons, which we term cross-striolar inhibition, thus may provide a mechanism for increasing the sensitivity to vertical linear acceleration. The circuit described is provided not only with high sensitivity but also with input noise-resistant characteristics.

INTRODUCTION

In mammals, the otolith organs of the inner ear sense linear acceleration. These organs consist of two receptors, the saccular macula and the utricular macula, oriented in planes perpendicular to each other. The saccular macula senses mainly vertical, and the utricular macula senses mainly horizontal, linear acceleration. The mechanisms of excitation of these receptors are fundamentally similar. Hair cells in the receptors are arranged geometrically with morphological polarity and a directional kinocilium (Flock 1964; Lindeman 1973; Wersall 1956). Two groups of oppositely polarized hair cells are divided by the striola in the middle of the receptor. The striola is shaped sigmoidally in the saccus, and by any given direction of linear acceleration, the hair cells will be depolarized (Goldberg et al. 1990; Shotwell et al. 1981). At the same time, some hair cells on the opposite side of the striola will be hyperpolarized.

Three classes of afferents (calyx, bouton, and dimorphic) are present in the mammalian cristae and utricular macula (Baird et al. 1988; Fernández et al. 1988). Calyx units have thick fibers, and they are seen only in the central (striolar) zones. Bouton units have thin axons that ramify fine branches in peripheral (extrastriolar) zones and are provided with bouton endings on several cylindrically shaped type II hair cells (Goldberg et al. 1990). Dimorphic units, which comprise the majority of the afferents, are found in all parts of the neuroepithelium and supply both types of hair cells (Goldberg et al. 1990). The peripheral innervation patterns have been studied (Goldberg et al. 1990; Yamashita and Ohmori 1990), but the axonal projections of afferents innervating in different parts of the macula to vestibular neurons have not been studied by physiological or morphological approaches.

The course and termination of saccular afferents in mammals have been demonstrated previously by the degeneration method (Gacek 1969; Stein and Carpenter 1967) and by labeling with horseradish peroxidase (HRP) in monkeys (Carleton and Carpenter 1984), chinchillas (Lee et al. 1992), guinea pigs (Didier et al. 1987; Gstoettner and Carl-tellieri 1992), and gerbils (Kevetter and Perachio 1986). The saccular afferents terminate predominantly in the lateral and descending vestibular nuclei. We recently studied electrophysiologically identified single primary afferents originating from the sacculus by use of intracellular staining with HRP and demonstrated that saccular afferents project mainly in the descending and the ventral lateral vestibular nuclei (Imagawa et al. 1996). However, we did not determine their innervation area in the saccular macula.

We also have studied the responses of vestibular nucleus neurons and neck motoneurons (Uchino et al. 1997) to the selective stimulation of saccular afferents (Sato et al. 1997; Uchino et al. 1994b, 1997). During that series of experiments, we often recorded monosynaptic excitatory postsynaptic potentials (EPSPs) followed by large amplitude inhibitory postsynaptic potentials (IPSPs) in the vestibular neurons after selective stimulation of the saccular nerve. We
hypothesized that primary afferents innervating hair cells on one side of the striola terminate monosynaptically on vestibular neurons and that primary afferents innervating hair cells on the other side disynaptically project on the same neurons via inhibitory interneurons. The sensitivity of the neurons to linear acceleration then should increase by a “disinhibitory mechanism.” In the present experiments, we studied input patterns from different parts of the saccular macula on single vestibular neurons, to determine whether the disinhibitory mechanism exists. The preliminary results were reported previously in abstract form (Uchino et al. 1996b–d).

METHODS

Experiments were performed on 23 cats in conformity with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences, (The Physiological Society of Japan 1988). Each cat was anesthetized initially with an intramuscular injection of ketamine hydrochloride (Ketalar, Parke-Davis; 15–20 mg/kg) followed by halothane (Fluothane, Zeneca)—nitrous oxide by inhalation. The cat then was decerebrated at the intercollicular level. The rectal temperature was maintained between 36 and 38°C. Blood pressure was monitored from the femoral artery and maintained >100 mmHg by an intravenous infusion of 5–10% glucose solution. The animals were paralyzed with pancuronium bromide (Mioblock, Organon; 3 mg/h) and artificially ventilated.

The utricular nerve and the ampullary nerves of the horizontal and the anterior semicircular canals were transected at the entrance zone into the bone in the left inner ear (Uchino et al. 1997). The ampulla of the posterior semicircular canal (PC) also was removed from the inner ear. The inner ear was drained of liquid using a small piece of twisted cotton. A pair of fine silver electrodes (acupuncture needles; stem diameter, 50–100 μm), whose tips were sharpened electrolytically (tip diameter; ~40 μm) and insulated except for ~250–1,000 μm (usually 500 μm) at the tip, were inserted into the rostro-ventral and caudo-dorsal parts of the saccular macula (group A, see text). Utricular nerve and the ampullary nerves of the horizontal and the anterior semicircular canals (UHA) and the posterior semicircular canal (PC) nerves also were stimulated.

FIG. 1. A: ventrolateral view of the cat inner ear showing the electrode position for focal stimulation of the saccular macula and the other vestibular nerves. Bipolar silver electrodes were inserted into the rostro-ventral and caudo-dorsal parts of the saccular macula (group A, see text). Utricular nerve and the ampullary nerves of the horizontal and the anterior semicircular canals (UHA) and the posterior semicircular canal (PC) nerves also were stimulated. B: schematic diagram of the saccular macula showing the morphological polarity of hair cells. →, direction of kinocilia (thickest and longest hair in each hair cell shown in D and C). Stimulus current was applied so that the rostral-ventral electrode ”a” was negative. Size of the saccular macula is ~2.5 mm rostro-caudally and ~1.0 mm ventro-dorsally. C: cross-section of the saccular receptor (at ± ± ± in B) and afferent connections, and the cross-striolar inhibition predicted from the results (see text). Saccular afferents were depolarized near the negative electrode and hyperpolarized near the positive electrode. Therefore the firing rate of afferent d1 decreased, whereas that of afferent d2 increased. D: cross-section of the saccular receptor showing functional pseudo-equivalence of the focal stimulation in C. If the acceleration is upward, the displacement of hairs is downward; hair cells under the striola then depolarize (red) and those above it hyperpolarize (dark blue).

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were inserted for focal stimulation of the saccular macula. The interelectrode distance was \( \approx 0.8 \) mm.

Three groups of animals were used in different experimental sessions. The group A animals (\( n = 7 \)) had electrode placement across the striola such that one electrode was in the rostro-ventral part of the macula and the other was in the caudo-dorsal part (Fig. 1A, B; Fig. 2A5). A small constant current (usually <30 \( \mu \)A) was applied to activate a small area of the macula; the activated area could be changed by reversing the polarity of the stimulus current. In group B (\( n = 2 \)), a pair of electrodes was placed on the dorsal-lateral edge of the saccular macula, not across the striola (Fig. 3C). In group C (\( n = 14 \)), the saccular macula was damaged during surgery, so that a pair of electrodes was inserted into the saccular nerve (Uchino et al. 1994b). Stimulating electrodes also were inserted into the PC nerve in 10 cats (4 group A, 6 group C) and into the cut ends of the utricular nerve and the ampullary nerves of the horizontal and the anterior semicircular canals (UHA) nerves in 3 cats (2 group A, 1 group C; Fig. 1A) to test for convergence between the saccular nerve and the PC nerve or the UHA nerves on single vestibular neurons. The electrodes were fixed to the occipital bone with dental cement. To prevent the spread of stimulus current, the nerves and electrodes were covered with a warm semisolid paraffin-petroleum jelly (Vaseline) mixture. Cathodal or anodal current pulses of 200-\( \mu \)s duration were applied to the saccular macula, PC nerve, and UHA nerves at a rate of 2–3 Hz.

The animal was suspended by hip pins and a clamp on the spinal process of the T1 vertebra. The caudal part of the cerebellum was aspirated to expose the floor of the fourth ventricle. Field potentials were recorded from the vestibular nuclei with glass micropipettes containing 2 M NaCl saturated with Fast Green dye. The intracellular recording from the lateral and descending vestibular nucleus neurons was done by use of micropipettes filled with 2 M K citrate and having a resistance of 3–10 M\( \Omega \). The thresholds of the N1 field potential, which is due to the monosynaptic activation of secondary vestibular neurons (Precht and Shimazu 1965), evoked in the ventral part of the lateral vestibular nucleus by stimulation of the saccular nerve ranged from 4 to 45 \( \mu \)A [17 ± 11 \( \mu \)A (mean ± SD), \( n = 32 \)]. These thresholds were comparable with those reported in previous papers (Sasaki et al. 1991; Uchino et al. 1994a,b, 1996a, 1997).
an IPSP after a stimulus at an intensity of \( \sim 0.7-2.0 \times N_1 \) threshold. Similarly, an IPSP was preceded by an EPSP in 3 of these 22 neurons after a stimulus of reversed polarity at an intensity of \( \sim 0.5-1.0 \times N_1 \) threshold. The typical pattern of PSPs evoked by focal stimulation of saccular macula is shown in Fig. 2A. After focal stimulation of the macula (Fig. 2A5), an EPSP followed by an IPSP was recorded in a vestibular neuron (Fig. 2A1, electrode “a” negative and electrode “b” positive). When the stimulus current was increased, the amplitudes of both the components became typical examples of a monosynaptic EPSP and disynaptic A1, A2, electrode “b” positive) . When the stimulus current was increased, the amplitudes of both the components became typical examples of a monosynaptic EPSP and disynaptic A1, A2, electrode “b” positive) . When the stimulus current was increased, an EPSP was evoked before the IPSP in a vestibular neuron (Fig. 2A4). The late IPSP evoked by the former polarity of stimulation and the early EPSP evoked by the latter polarity are presumably due to stimulus spread. In the remaining 14 neurons in group A, however, the pattern of PSPs was not changed irrespective of stimulus polarity; EPSPs in 12 neurons, including EPSP-IPSP sequences in 5 neurons, and IPSPs in 2 neurons were recorded (Fig. 2B2). The latencies of the majority of the EPSPs were monosynaptic (\( \leq 1.2 \text{ ms} \), Fig. 2B1), whereas those of the IPSPs were disynaptic (\( \geq 1.5 \text{ ms} \), Fig. 2B1) (Goldberg et al. 1987; Kasahara and Uchino 1974; Precht and Shimazu 1965; Uchino et al. 1994a; Wilson and Melvill Jones 1979). Thus it appears that a group of saccular afferents had monosynaptic excitatory contacts with vestibular neurons, and another group of afferents that originated from hair cells with an opposite morphological polarity had disynaptic connections with the same vestibular neurons through an inhibitory interneuron (Fig. 1C).

Typical examples of a monosynaptic EPSP and disynaptic IPSP evoked by focal stimulation of the dorsal-lateral edge of the saccular macula (group B) are illustrated in Fig. 3. A and B. In these cells, the response pattern was always the same regardless of the polarity of the stimulus current at an intensity of \( \sim 2.0 \times N_1 \) threshold. When the stimulus current was increased, an EPSP was evoked before the IPSP in a few neurons. In the cell illustrated in Fig. 3A, a monosynaptic EPSP ( latency of 0.9 ms ) was evoked by both stimulus polarities with a threshold around the N1 threshold. In the other cell (Fig. 3B), a disynaptic IPSP ( latency of 2.3 ms ) was evoked by both stimulus polarities with a threshold of \( < 2 \times N_1 \) threshold. In 31 neurons located in the ventral lateral and the rostral descending vestibular nuclei in two cats, the same response pattern remained when the stimulus...
polarity was changed: EPSPs (including EPSP-IPSP sequences in 5 neurons) in 25/31 neurons and IPSPs in 6/31 neurons. The latencies of the majority of the EPSPs were monosynaptic ($\leq 1.2$ ms, Fig. 3D), whereas those of the IPSPs were disynaptic ($\leq 1.5$ ms, Fig. 3D).

In group C, stimulation of the saccular nerve at an intensity of $\approx 1 \times N1$ threshold evoked EPSPs in 62 vestibular neurons (including EPSP-IPSP sequences in 31 neurons) and IPSPs in 19 neurons (Fig. 3E). The latency of the PSPs shown in Fig. 3E reflects the findings that the majority of the EPSPs, including the EPSP-IPSP sequences, were monosynaptic (latencies $\leq 1.2$ ms) and the majority of the IPSPs were disynaptic (latencies $\approx 1.5$ ms). These synaptic potentials could not be evoked by current spread to other branches of the vestibular nerve because the patterns and the latencies of the responses were different from those of the responses to the other nerve stimulation (Fig. 4B, a and b). That is, the responses to saccular nerve stimulation were EPSPs, EPSP-IPSP sequences, or IPSPs, whereas those to UHA or PC stimulation were mainly EPSPs; disynaptic IPSPs were very rare (Fig. 3, F and G). When the patterns of synaptic potentials evoked by stimulation of the saccular and other nerves were the same and their latencies were within the same range, we performed occlusion (convergence) tests between saccular- and UHA-evoked or saccular- and PC-evoked PSPs to determine whether the response was partly or entirely due to current spread. An example of the occlusion test is shown in Fig. 4A. The saccular nerve was stimulated at supramaximal intensity, and the PC nerve was stimulated so as to evoke PSP comparable with that of a saccular-activated PSP. If the excitation change during upward acceleration was the same as in circuit A, the excitability would increase by 2 in the upper afferent and decrease by 2 in the lower afferent compared with the resting condition. Consequently, output excitability becomes 4, and the increment of excitability is 4, which is twice as large as that in circuit A. The converse is true in the case of downward acceleration, because the final output in circuit B is twice as large as that in circuit A.

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**FIG. 4.** A: summation (occlusion) test between intracellular responses after stimulation of the saccular nerve and the PC nerve. Conditioning shock was applied to the saccular nerve at 150 $\mu$A ($6 \times N1$ threshold, maximal intensity (Uchino et al. 1997) (a), and the test shock to the PC nerve at 8 $\mu$A ($2 \times N1$ threshold) (b). Bottom traces in a and b indicate juxtacellular records. Interval between the shocks was shortened in c and the stimuli were nearly simultaneous in d. B: summary of the convergence test: convergence between the saccular nerve and the UHA nerves (Bu), and that between the saccular nerve and PC nerve (Bb). $\bullet$, excitatory inputs; $\blacksquare$, inhibitory inputs; and $\square$, no inputs.

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**FIG. 5.** Schematic diagram for mechanism for increasing sensitivity. A: cascade circuit. Numbers indicate excitability. For brevity of explanation, excitability was expressed from a stationary state. In the resting condition, suppose excitability to be 2, therefore output is 2. Suppose excitability to increase by 2 during upward acceleration, so output excitability becomes 4. Therefore, the increment of excitability was 2. B: proposed circuit, termed cross-striolar inhibition, from the present data. Filled neurons are inhibitory, and the open ones excitatory. In the resting condition, the input from the inhibitory neuron and that from the excitatory afferent cancel in the output neuron (input noise-resistant characteristics). If the excitability change during upward acceleration was the same as in circuit A, the excitability would increase by 2 in the upper afferent and decrease by 2 in the lower afferent compared with the resting condition. Consequently, output excitability becomes 4, and the increment of excitability is 4, which is twice as large as that in circuit A. The converse is true in the case of downward acceleration.
inputs from the UHA nerves, and 19 of the 43 neurons tested (44%) received them from the PC nerve. The majority of neurons that received inputs from saccular afferents with monosynaptic latencies produced no visible potentials in response to stimulation of the UHA nerves, but some neurons produced an EPSP or an IPSP with a longer latency by UHA nerve stimulation (Fig. 4Ba). Most neurons in which an IPSP with a longer latency was elicited by saccular afferent stimulation produced an EPSP with a monosynaptic latency after stimulation of the UHA nerves (Fig. 4Ba). A similar convergence pattern was observed between the saccular afferents and PC nerve inputs (Fig. 4Bb).

**Discussion**

The major finding of the present study is that the stimulation of afferents from the two sides of the saccular macula divided by the striola can evoke monosynaptic EPSPs and disynaptic IPSPs in the same vestibular neurons. This result was obtained when focal stimulation was given across the striola and the polarity of the stimulus current was changed via a pair of electrodes (group A) and when a pair of electrodes was inserted into one side of the saccular macula so that the striola was not bridged by the stimulus current (group B). This push-pull circuit (Fig. 5B), which we named cross-striolar inhibition, unlike the simple connection shown in Fig. 5A, provides the hypothesis for the increasing sensitivity to vertical linear acceleration presented in the Introduction (Fig. 1C). This circuit not only has high sensitivity but also noise-resistant input characteristics. Common mode noise is rejected, as shown in Fig. 5B. The present results suggest that two groups of oppositely polarized hair cells in the striola are extremely important to the functions of light. Response latencies ranged from 16.4 to 18.5 ms (Bush et al. 1993).

In the semicircular canal system, the ipsilateral afferent inputs to second-order neurons are excitatory, whereas contralateral inhibitory actions are produced by commissural neurons. This circuitry is in keeping with the morphology of the ampullary cristae. Hair cells in the cristae have only one polarity. When hair cells in one semicircular canal depolarize during angular acceleration, those in the coplanar contralateral canal hyperpolarize. Sensitivity then is increased by the convergence of facilitation and disinhibition or by disfacilitation and inhibition, the latter in each case via commissural fibers (Goldberg et al. 1987; Kasahara and Uchino 1974; Mano et al. 1968; Markham 1968; Shimazu and Precht 1966; Uchino et al. 1986). In contrast, there is no commissural inhibition in the otolith system (Shimazu and Smith 1971; Wilson et al. 1978). Hair cells across the striola in the otolith organ have opposite polarities. When hair cells on one side of the striola depolarize during linear acceleration, those on the opposite side hyperpolarize. A facilitation-disinhibition or disfacilitation-inhibition circuit thus could consist unilaterally as the circuit we have described above, i.e., cross-striolar inhibition.

In the present experiments, EPSPs followed by IPSPs (Fig. 2A, 1, 2, and 4) were elicited in some neurons by focal stimulation of the saccular macula; both components of the complex potentials probably were evoked by the spread of the excited area to the other side of the macula. This event would not be unexpected because of the following present findings: the width of the dorsal edge and the ventral edge of the saccular macula is ~1 mm; EPSPs followed by IPSPs were recorded more often after focal stimulation in group A, in which the focal stimulation was across the striola; EPSPs followed by IPSPs were evoked only by stronger shock in group B, in which the striola was not bridged by stimulus current; and pure disynaptic IPSPs without EPSPs were evoked after stimulation of one side of the macula. However, in frogs, some secondary vestibular neurons receive monosynaptic excitatory and disynaptic inhibitory inputs from a single canal nerve (Dieringer et al. 1996; Straka and Dieringer 1996; Straka et al. 1996). Each semicircular canal cristae has hair cells with uniform polarity. Therefore, we cannot rule out the possibility that the hair cells on one side of the striola contribute to this monosynaptic excitatory and disynaptic inhibitory circuit.

Where do these amplified signals go? In the cat, saccular-activated vestibular neurons may target preferentially the spinal cord. One-third of saccular-activated neurons send axons to the upper cervical spinal cord (Sato et al. 1997). Stimulation of the saccular nerve evoked disynaptic EPSP in extensor neck motoneurons (Uchino et al. 1997). Saccular-activated vestibular neurons rarely were activated antidromically after stimulation of the oculomotor nuclei (Sato et al. 1997). Vertical eye movements induced by a brief period of free fall were recorded from monkeys in total darkness immediately after binocular fixation at a target light. Response latencies ranged from 16.4 to 18.5 ms (Bush and Miles 1996). Saccular afferents seem to have mainly polysynaptic linkages to extraocular motoneurons.

Our present data also show that information from the semicircular canal system converges on saccular nerve-activated vestibular neurons (Fig. 4B). This convergence may contribute jointly to the vestibulocollic reflex by sending an excitatory input to neck muscles during combined angular and linear acceleration (Baker et al. 1984; Boyle and Pompeiano 1980; Bush et al. 1993; Kasper et al. 1988; Manzoni et al. 1993; Markham 1968; Schor and Miller 1982). Signals also may be conveyed to higher centers, such as the cerebrum and the cerebellum (Ito 1984).

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