Canal-Specific Excitation and Inhibition of Frog Second-Order Vestibular Neurons

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Straka, H., S. Biesdorf, and N. Dieringer. Canal-specific excitation and inhibition of frog second-order vestibular neurons. J. Neurophysiol. 78: 1363–1372, 1997. Second-order vestibular neurons (2[VNs]) were identified in the in vitro frog brain by their monosynaptic excitation following electrical stimulation of the ipsilateral VIIIth nerve. Ipsilateral disynaptic inhibitory postsynaptic potentials were revealed by bath application of the glycine antagonist strychnine or of the γ-aminobutyric acid-A (GABA_A) antagonist bicuculline. Ipsilateral disynaptic excitatory postsynaptic potentials (EPSPs) were as well. The functional organization of convergent monosynaptic and disynaptic excitatory and inhibitory inputs onto 2[VNs] was studied by separate electrical stimulation of individual semicircular canal nerves on the ipsilateral side. Most 2[VNs] (88%) received a monosynaptic EPSP exclusively from one of three semicircular canal nerves; fewer 2[VNs] (10%) were monosynaptically excited from two semicircular canal nerves; and even fewer 2[VNs] (2%) were monosynaptically excited from each of the three semicircular canal nerves. Disynaptic EPSPs were present in the majority of 2[VNs] (68%) and originated from the same (homonymous) semicircular canal nerve that activated a monosynaptic EPSP in a given neuron (22%), from one or both of the other two (heteronymous) canal nerves (18%), or from all three canal nerves (28%). Homonymous activation of disynaptic EPSPs prevailed (74%) among those 2[VNs] that exhibited disynaptic EPSPs. Disynaptic inhibitory postsynaptic potentials (IPSPs) were mediated in 90% of the tested 2[VNs] by glycine, in 76% by GABA, and in 62% by GABA as well as by glycine. These IPSPs were activated almost exclusively from the same semicircular canal nerve that evoked the monosynaptic EPSP in a given 2 VN. Our results demonstrate a canal-specific, modular organization of vestibular nerve afferent fiber inputs onto 2[VNs] that consists of a monosynaptic excitation from one semicircular canal nerve followed by disynaptic excitatory and inhibitory inputs originating from the homonymous canal nerve. Excitatory and inhibitory second-order (2) vestibular interneurons are envisaged to form side loops that mediate spatially similar but dynamically different signals to 2 vestibular projection neurons. These feedforward side loops are suited to adjust the dynamic response properties of 2 vestibular projection neurons by facilitating or disfacilitating phasic and tonic input components.

INTRODUCTION

Vestibular nerve afferent fibers exhibit a range of diameters from thin to thick. Correlated with this gradient in size are differences in the peripheral innervation pattern, in the discharge rate at rest, in the physiological response properties, and in the biochemical composition. Goldberg and Fernández (1971) and Fernández and Goldberg (1976) classified vestibular nerve afferent fibers according to the regularity of their discharge rate at rest into regularly and irregularly discharging units. These differences, as well as the response dynamics of vestibular afferents, are closely related to their fiber diameter and their location in the sensory epithelium (Baird et al. 1988; Goldberg et al. 1990). Thicker, more irregularly discharging units tend to supply more central regions of a given endorgan and to exhibit more phasic response dynamics than thinner, more regularly discharging units.

A similar classification of vestibular afferents also holds for nonmammalian species, independent of whether type I hair cells are present in addition to type II hair cells (amniotes) or not (anamniotes). Thicker vestibular nerve afferent fibers tend to exhibit more phasic-tonic response properties and to innervate more central regions of the sensory epithelium than thinner fibers (Baird and Lewis 1986; Baird and Schuff 1994; Caston et al. 1977; Honrubia et al. 1981, 1989; Lowenstein and Saunders 1975; Macadar et al. 1975; Yamashita and Ohmori 1990). In addition, a subpopulation of thick vestibular nerve afferents fibers in rat and frog by the colocalization of glutamate and glycine immunoreactivity (Reichenberger and Dieringer 1994). In the frog this subpopulation of thick vestibular afferents was further characterized by a selective uptake and retrograde transport of radioactively labeled glycine (Straka et al. 1996c).

Glutamate or a related substance is the putative transmitter of vestibular nerve afferent fibers (see de Waele et al. 1995 for a review). Postsynaptic activation of second-order vestibular neurons (2VN) is mediated by different glutamate receptor subtypes [i.e., α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) receptors (Doi et al. 1990; Kinney et al. 1994; Straka and Dieringer 1993; Takahashi et al. 1994) and N-methyl-D-aspartate (NMDA) receptors (Kinney et al. 1994; Straka et al. 1996b; Takahashi et al. 1994)]. Whereas all vestibular nerve afferent fibers appear to activate AMPA receptors, the activation of NMDA receptors was shown to originate in frogs predominantly or even exclusively from the subpopulation of thick vestibular nerve afferent fibers (Straka and Dieringer 1996; Straka et al. 1996b). In the same species, a disynaptic uncrossed inhibitory input was pharmacologically revealed to superimpose apparently pure excitatory postsynaptic potentials (EPSPs) in most of the recorded 2[VNs] (Straka and Dieringer 1996). A similar disynaptic inhibitory input was revealed by current injections in about half of the investigated 2[VNs] in the squirrel monkey (Goldberg et al. 1987).

The functional organization of the excitatory and inhibitory inputs of 2[VNs] is only poorly understood. Following electrical stimulation of individual semicircular canal ampullae, most of the ipsilateral 2[VNs] were activated by only
one of the three canal ampullae in the pigeon (Wilson and Felpel 1972), as in the cat (Kasahara and Uchino 1974; Sans et al. 1972). As far as the uncrossed disynaptic inhibition of 2 VN is concerned, results in squirrel monkey (Goldberg et al. 1987) and in frog (Straka and Dieringer 1996) were collected after VIIIth nerve stimulation and the specificity of these input signals was so far unknown. To study both of these interrelated aspects of the functional organization of semicircular canal inputs onto ipsilateral 2 VN, we took advantage of the isolated frog brain, stimulated individual semicircular canal nerves separately, and studied their projection patterns by means of intracellular recordings from 2 VN on the ipsilateral side. Inhibitory inputs were reversibly unmasked by the application of specific receptor antagonists.

Preliminary parts of this study have been published in abstract form (Straka et al. 1996a).

METHODS

In vitro experiments were performed on the isolated brains of 22 grass frogs (Rana temporaria). As described in previous studies (Cochran et al. 1987; Straka and Dieringer 1993), the animals were deeply anesthetized (0.1% 3-aminobenzoic acid ethyl ester) and perfused transcardially with iced Ringer solution (composition, in mM: 75 NaCl, 25 NaHCO3, 2 CaCl2, 2 KCl, 0.1 MgCl2, and 11 glucose, pH 7.4). Thereafter, the skull and the bony labyrinth were opened by a ventral approach. After the three semicircular canals on either side were cut, the brain was removed with the labyrinthine endorgans attached to the VIIIth nerve. Subsequently the brain was submerged in iced Ringer solution and the dura, labyrinthine endorgans, and choroid plexus covering the IVth ventricle were removed. The forebrain was disconnected in all preparations and the cerebellum was removed in those preparations that were used for pharmacological investigations. Brains were used up to 5 days after their isolation and were stored overnight at 6°C in continuously oxygenated Ringer solution, pH of 7.5 ± 0.1 (SD).

For the experiments the brain stem was glued with cyanoacrylate glue to a plastic net with the ventral side down. This net was fixed with insect pins to the Sylgard floor of a chamber (volume 2.4 ml) that was continuously perfused with oxygenated Ringer solution at a rate of 1.3–2.1 ml/min. Changes in the evoked responses due to the presence of a given antagonist occurred after ~5 min and reached a plateau after ~10–20 min. As a rule, synaptic potentials were measured ~15 min after the addition of the pharmacological agent to the Ringer solution in the bath.

Single sweeps of the responses were digitized (CED 1401), stored on computer, and analyzed off-line (SIGAVG). SF potentials were analyzed from averages of 10–30 single sweeps after electronic subtraction of the extracellular field potential recorded in the vicinity. To determine the parameters of synaptic potentials blocked by pharmacological agents, we electronically subtracted the potentials recorded in the presence of blocking substances from control responses in a given neuron.

Statistical analyses were performed with the aid of commercially available computer software (INSTAT; Graphpad, San Diego, CA). Statistical differences in latencies, amplitudes, areas, and times-to-peak before and after the application of pharmacological agents were calculated according to the Wilcoxon signed-rank test (test for paired parameters). Statistical differences of parameters of neurons or field potentials from different sets of experiments were calculated according to the Mann-Whitney U test (test for unpaired parameters). Regression lines were fitted by computer according to the least-squares method. Graphic presentations were performed with the aid of commercially available computer software (origin, Microcal Software, Northampton, MA; designer, Micrografx, Richardson, TX).

RESULTS

CANAL-SPECIFIC MONOSYNAPTIC EXCITATION OF 2 VN. Field potentials recorded at the standard recording site (see METHODS) in the VNC consisted of a presynaptic N0 and a postsynaptic N1 component (Fig. 1, A1–A4). The latency of the N0 component following VIIIth nerve stimulation (0.9 ± 0.2 ms; n = 29) was significantly shorter (P < 0.001) than that following stimulation of a particular semicircular canal nerve (latency ~ 1.7 ms; n = 29; Fig. 1C) because of the difference in the distances between the standard recording site and thestimulation sites (see METHODS). The latencies of the N0 components evoked by separate stimulation of the three semicircular canal nerves were not significantly differ-
ent (Fig. 1C). For a given stimulation site, the latency of the \( N_0 \) component increased with the distance from the recording site (Fig. 1B). Assuming a utilization time of 0.5 ms (Fig. 1B), the conduction velocity of the fastest vestibular afferents was \( \approx 2.7 \text{ m/s} \). The slope of the linear regression (\( r^2 = 0.85; n = 5 \)) was significantly different from zero (\( P \approx 0.0001 \)).

The latency of the VIIIth-nerve-evoked \( N_1 \) component (2.6 \( \pm \) 0.4 ms; \( n = 29 \)) differed significantly (\( P \approx 0.0001 \)) from that of the \( N_1 \) component (\( \approx 3.6 \) ms; \( n = 29 \)) that was evoked by stimulation of one of the semicircular canal nerves (Fig. 1C). No significant difference in the latencies of the \( N_1 \) components was found between \( N_1 \) components evoked by different semicircular canal nerves. With respect to the corresponding \( N_0 \) component, the \( N_1 \) component was delayed by 1.8–1.9 ms (Fig. 1C). This value represents the synaptic delay between vestibular afferent fibers and 2\(^\circ\) VN's at a temperature of 14\(^\circ\)C (see Straka and Dieringer 1993).

Stimulation of the VIIIth nerve was used to search for neurons in the ipsilateral vestibular nuclei with a monosynaptic excitation (2\(^\circ\) VNs). The resting membrane potential of the recorded neurons (\( n = 181 \)) ranged between \(-51\) and \(-85\) mV. Electrical stimulation of the VIIIth nerve evoked a chemically transmitted EPSP (Fig. 2, A1, B1, and C1) that was preceded in 25 of 181 neurons by an early depolarization (0.9 \( \pm \) 0.3 ms; \( n = 25 \)) independent of the stimulus intensity and represented an electrically transmitted EPSP component as described for in vivo (Precht et al. 1974) and in vitro recordings (Babalian and Shapiro 1984; Cochran et al. 1987; Straka and Dieringer 1996). The threshold of the electrical EPSP was as low as the threshold of the chemical EPSP in a given neuron. At variance with the chemically evoked EPSPs (see below), the amplitude of the electrically evoked EPSP increased only little with stronger stimuli and saturated in different neurons between 0.3 and 1.5 mV. The low thresholds and the saturation at moderate stimulus intensities suggest a preferred activation of coupling potentials via thick vestibular afferent fibers, a finding compatible with earlier results (Straka and Dieringer 1996).

The latency of the chemically mediated EPSPs ranged between 2.4 and 3.3 ms (mean 2.7 \( \pm \) 0.4 ms; \( n = 181 \); Table 1). Because the synaptic delay at 14\(^\circ\)C is \( \approx 1.8 \) ms, these EPSPs were monosynaptic in nature. The amplitudes of the chemically mediated EPSPs gradually increased with stronger stimuli and reached up to 10 mV, with an average rise time of 5.2 \( \pm \) 2.3 ms (\( n = 181 \); Table 1). Similar values were reported for in vivo experiments (Dieringer and Precht 1979). At higher stimulus intensities, partial spikes occasionally appeared on top of the EPSPs (see Precht et al. 1974), and at even higher stimulus intensities, full action potentials with an amplitude of 60–100 mV were evoked. The mean latency of the earliest of these action potentials was 4.3 \( \pm \) 1.3 ms (\( n = 10 \)). The evoked bursts of action potentials at even higher stimulus intensities were limited to two to three spikes, as reported for in vivo (Dieringer and Precht 1979) and in vitro studies (Straka and Dieringer 1996).

Following stimulation of one of the semicircular canal nerves, the latency of the \( N_1 \) component of the field potential was 3.6 \( \pm \) 0.8 ms (\( n = 29 \)) and determined the earliest onset of chemically mediated monosynaptic EPSPs. Disynaptic potentials must be expected to be delayed by another 1.8 ms (see above). Therefore EPSPs with a latency of \(<5.5 \) ms after semicircular canal nerve stimulation (i.e., 3.6 ms for \( N_1 \) component plus 1.8 ms for 1 synaptic delay) were considered to be monosynaptic in nature.

Most 2\(^\circ\) VNs (159 of 181, i.e., 88\%) received a monosynaptic EPSP from only one of the three ipsilateral semicircular canal nerves (Fig. 2, A2, B3, and C4). About equal numbers of neurons were monosynaptically activated from the anterior canal nerve, from the horizontal canal nerve, or from the posterior canal nerve (Fig. 3A) and are designated 2\(^\circ\) ACN, 2\(^\circ\) HCN, or 2\(^\circ\) PCN. Some 2\(^\circ\) VNs (19 of 181, i.e., 10%; Fig.
Typically, in a given recording session monosynaptic EPSPs were evoked by stimulation of anterior canal nerve (2°ACN) could be evoked from each of the three semicircular canal nerves in 2°VNs. Although the responses of most 2°VNs displayed a high degree of specificity, the possibility must be considered that there exists convergence with second-order inputs that exhibit a high-threshold of activation. We tested for this in 119 cells that responded with a monosynaptic EPSP to low-intensity stimulation of one semicircular canal nerve by stimulation of each of the other two semicircular canal nerves with strong stimuli (up to 15 × T). Such stimulation had no effect on the results as far as monosynaptic potentials are concerned. However, in the majority of these neurons disynaptic responses were already present at low stimulus intensities following stimulation of each of the three semicircular canal nerves. These results indicate that the observed distribution of canal nerve inputs in different 2°VNs is not simply related to inefficient electrical stimulation of particular nerve branches.

The monosynaptic chemical EPSP of 2° canal neurons was preceded by a short-latency depolarization in 21 of 181 neurons. The mean latency (1.8 ± 0.5 ms; n = 21) of early depolarizations was similar for responses evoked by stimulation of the anterior (n = 7), horizontal (n = 6), or posterior canal nerve (n = 8). The thresholds of these coupling potentials were as low as those of the chemical EPSPs. Their amplitude increased only a little with stimulus intensity and saturated between 0.4 and 1.2 mV in different neurons.

The latencies of the chemically mediated EPSPs (mean 4.1 ± 0.7 ms; n = 159) were similar between 2°ACN, 2°PCN, and 2°HCN. This is consistent with the results that depolarizations following electrical stimulation of 1 of 3 ipsilateral semicircular canal nerves in 2°VNs is not simply related to inefficient electrical stimulation of particular nerve branches.
2°HCN, and 2°PCN, but were significantly longer ($P \leq 0.0001$) than the latencies of VIIIth-nerve-evoked EPSPs (Table 1). The amplitudes of these EPSPs gradually increased with stimulus intensity and usually saturated at stimulus intensities between 3 and $4 \times T$ (mean $4.7 \pm 2.3$ mV; $n = 102$). The times-to-peak of the semicircular-canal-nerve-evoked monosynaptic EPSPs were significantly longer than those of VIIIth-nerve-evoked EPSPs (Table 1). Such a difference in the rise times was expected as a result of the larger temporal dispersion of canal nerve inputs when compared with VIIIth-nerve-evoked inputs because of the longer distances between stimulation and recording sites. Stronger stimuli ($>4 \times T$) triggered partial spikes on top of the EPSPs with an amplitude of 3–7 mV or full action potentials with an amplitude of 60–90 mV. The mean latency for the onset of the earliest action potential was $5.8 \pm 1.0$ ms ($n = 12$).

The latencies and the thresholds of monosynaptic EPSPs activated in a given neuron by more than one semicircular canal nerve were similar between each other as well as between different neurons. The mean latencies of monosynaptic inputs converging from two or from three semicircular canal nerves ($4.0 \pm 0.8$ ms; $n = 47$) onto a given 2°VN were not different from the mean latency of the monosynaptic input in neurons with only one semicircular canal nerve input ($4.1 \pm 0.7$ ms; $n = 159$). However, major and minor inputs could be differentiated in a given neuron with respect to the amplitudes of the EPSPs evoked at comparable stimulus intensities. Minor inputs reached $\sim 45 \pm 20\%$ ($n = 25$) of the amplitudes of the major inputs in neurons activated monosynaptically from two or three semicircular canal nerves. The times-to-peak of major and minor EPSPs were statistically not different from those measured in neurons with a monosynaptic input from only one semicircular canal nerve.

**TABLE 2.** Distribution of disynaptic EPSPs in identified 2° canal neurons following stimulation of homo- or heteronymous semicircular canal nerves on the ipsilateral side

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<th>2° ACN</th>
<th>2° HCN</th>
<th>2° PCN</th>
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<tr>
<td>AC nerve</td>
<td>44%</td>
<td>26%</td>
<td>47%</td>
</tr>
<tr>
<td>HC nerve</td>
<td>14%</td>
<td>55%</td>
<td>39%</td>
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<tr>
<td>PC nerve</td>
<td>21%</td>
<td>21%</td>
<td>51%</td>
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$N$, number of neurons. ACN, AC neuron; HCN, HC neuron; PCN, PC neuron. For other abbreviations, see Table 1. Bold numbers: disynaptic excitatory inputs from homonymous canal nerves.

![FIG. 4. Excitatory postsynaptic responses in 2° vestibular neuron following electrical stimulation of 1 of 3 ipsilateral semicircular canal nerves. Stimulation of horizontal canal (HC) nerve evoked multiple excitatory potentials (A1). Onset of the 1st and 2nd of these potentials was monosynaptic, respectively (B1). Stimulation of anterior (A2) or posterior canal nerve evoked (A3) EPSPs with disynaptic delay (B2 and B3). Horizontal dashed lines: baselines. First vertical dashed line in B1–B3: stimulus; 2nd dashed line in B1: onset of monosynaptic responses; 3rd dashed line in B1–B3: onset of disynaptic responses. Arrowheads: stimulus onset. Each record represents average from 20 responses.](http://jn.physiology.org/doi/10.1210/jn.54.11.1367)
number of disynaptic EPSPs triggered by heteronymous canal nerves in 2º PCN (Table 2; Fig. 5). About one-third of the recorded neurons exhibited no clearly visible disynaptic EPSPs (Fig. 5D). From those 2º VNs that were disynaptically activated, ~74% received a disynaptic input from the homonymous canal nerve. The mean latencies of disynaptic EPSPs triggered by the homonymous (7.4 ± 0.9 ms; n = 79) and by the heteronymous canal nerves (7.5 ± 0.9 ms; n = 95) were similar.

2º canal neurons with monosynaptic EPSPs from two semicircular canal nerves (n = 19) received a disynaptic excitation that originated from the same semicircular canal nerve that provided the major monosynaptic input (5 of 19), the minor monosynaptic input (9 of 19), or no monosynaptic input (11 of 19) in a given neuron. Polysynaptic excitatory or inhibitory inputs (latency >9 ms) were observed in ~80% of the recorded neurons. Most of these inputs were excitatory (90%) and only a few were inhibitory (10%) in nature. Stimulation of the horizontal canal tended to activate more polysynaptic components than did stimulation of the posterior canal nerve. However, these inputs were not analyzed in detail.

**CANAL-SPECIFIC DISYNAPTIC INHIBITION IN 2º CANAL NEURONS.** Comparison of VIIIth-nerve-evoked EPSPs before and during the application of bicuculline (0.5 µM) or strychnine (0.5 µM) revealed superimposed short-latency inhibitory postsynaptic potentials (IPSPs) in most of the 2º VNs recorded from (bicuculline: 13 of 17; strychnine: 14 of 18). The thresholds of the strychnine- or bicuculline-sensitive IPSPs were low and similar to the threshold of the N1 field potential component, suggesting that both potentials were triggered by stimulus-evoked activity in thick vestibular afferent fibers. The latencies of these IPSPs (Table 3A) were within the limits for disynaptic potentials (see above) and similar to those reported earlier (Straka and Dieringer 1996).

The amplitudes of the disynaptic IPSPs (between ~0.3 and ~2.1 mV) and the times-to-peak (Table 3B) were similar for IPSPs revealed by bicuculline or by strychnine. Most of the 2º canal neurons identified by the presence of a monosynaptic EPSP from one ipsilateral semicircular canal nerve received, in addition, IPSPs that were reversibly blocked in the presence of bicuculline (Fig. 6A1; 20 of 21 neurons) or strychnine (Fig. 6B1; 21 of 21 neurons). As a rule, this inhibitory input was triggered by stimulation of the same (homonymous) semicircular canal nerve that activated the monosynaptic EPSP in a given 2º canal neuron and was mediated either by GABA (Fig. 7A) or by glycine (Fig. 7B). The latencies of these IPSPs were either disynaptic (bicuculline: 16 of 20 neurons; mean 7.3 ± 1.2 ms; Table 3A; strychnine: 19 of 21 neurons; mean 7.4 ± 1.2 ms; Table 3A) or polysynaptic in nature (bicuculline: 4 of 20 neurons; strychnine: 2 of 21 neurons). No differences in the latencies were detected between IPSPs activated by stimulation of different semicircular canal nerves. As expected, the latencies of the disynaptic IPSPs were significantly (P ≤ 0.0001) delayed by ~1.5–2 ms with respect to the disynaptic IPSPs evoked by stimulation of the VIIIth nerve (Table 3A). The thresholds of disynaptic strychnine- and/or bicuculline-sensitive IPSPs were low and similar to monosynaptic evoked IPSPs. The amplitudes of the disynaptic IPSPs ranged between ~0.3 and ~2.4 mV in different 2º VNs and were similar between strychnine- and bicuculline-sensitive disynaptic IPSPs. The times-to-peak of strychnine-sensitive and the bicuculline-sensitive disynaptic IPSPs were similar with respect to each other (Table 3B).

Disynaptic inhibition of 2º canal neurons with monosynaptic inputs from more than one semicircular canal nerve was investigated with bicuculline in three neurons and with strychnine in four neurons. A disynaptic bicuculline-sensitive IPSP (in all 3 neurons) and a disynaptic strychnine-sensitive IPSP (in 3 of the 4 neurons) was present following

<table>
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<th>Table 3.</th>
<th>Latencies and times-to-peak of disynaptic IPSPs evoked by stimulation of the VIIIth nerve or of an individual homonymous semicircular canal nerve and revealed by bicuculline or strychnine</th>
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<tr>
<td></td>
<td>2º VIIIth Nerve</td>
</tr>
<tr>
<td>A</td>
<td></td>
</tr>
<tr>
<td>Latency</td>
<td>(bicuculline added)</td>
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<td>N</td>
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<td>Latency</td>
<td>(strychnine added)</td>
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<td>Time-to-peak</td>
<td>(bicuculline added)</td>
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<td>Time-to-peak</td>
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Values for latency and time-to-peak are means ± SD, in ms. N. number of neurons. IPSP, inhibitory postsynaptic potential; for other abbreviations, see Table 1. *P ≤ 0.001, †P ≤ 0.05, ‡P ≤ 0.01 with respect to VIIIth-nerve-evoked IPSP data (Mann-Whitney U test).
stimulation of either one of the homonymous semicircular canal nerves. The remaining neuron tested with strychnine exhibited a polysynaptic IPSP superimposed on its major as well as on its minor monosynaptic EPSP. The amplitudes of disynaptic IPSPs evoked by the major semicircular canal nerve were larger than the amplitudes of the disynaptic IPSPs originating from the minor semicircular canal nerve. No differences were observed in the latencies and times-to-peak between disynaptic IPSPs revealed by bicuculline (latency: 6.8 ± 1.6 ms; mean time-to-peak: 5.9 ± 6.6 ms; n = 6) or by strychnine (latency: 7.4 ± 1.3 ms; mean time-to-peak: 5.5 ± 4.1 ms; n = 6).

Some of the identified 2° canal neurons with a monosynaptic EPSP from one canal nerve received IPSPs following stimulation of one or of either of the remaining heteronymous semicircular canal nerves. IPSPs from the heteronymous semicircular canal nerve(s) were unmasked with bicuculline or strychnine in 10 of 21 neurons, respectively. The latencies of these unmasked IPSPs were >9 ms and were therefore considered polysynaptic.

The convergence of inhibitory inputs from GABAergic and from glycinergic 2° vestibular interneurons was tested in 13 identified 2° canal neurons. Simultaneous application of bicuculline (0.5 μM) and of strychnine (0.5 μM) depolarized the membrane potential and facilitated the occurrence of bursts of action potentials even at very low (<1.2 × T) stimulus intensities. These bursts lasted up to a few seconds. To avoid the activation of bursts, we first exposed a given cell to bicuculline (Fig. 6A), removed this drug from the bath after its effect had been established, and subsequently exposed the same cell to strychnine (Fig. 6B). Bicuculline unmasked disynaptic (n = 9) or polysynaptic (n = 3) IPSPs in 12 of 13 neurons following stimulation of the homonymous canal nerve. Strychnine revealed IPSPs in all 13 neurons (disynaptic IPSPs: n = 11; polysynaptic IPSPs: n = 2). A convergence of disynaptic GABAergic and of disynaptic glycinergic IPSPs as in Fig. 6, A1 and B1, was detected in 8 of the 13 neurons tested (Fig. 7C). In the remaining five neurons, one or both of the antagonists revealed either a polysynaptic IPSP (n = 4) or no IPSP (n = 1). The parame-
ters of the disynaptic IPSPs were similar to each other and did not depend on the specific canal nerve that triggered these responses or on the substance, i.e., glycine or GABA, that mediated these responses.

**DISCUSSION**

2°VNs were identified and further characterized as 2° canal neurons by the presence of monosynaptic EPSPs following stimulation of the VIIIth nerve and of individual semicircular canal nerve branches. Short-latency excitatory canal nerve inputs were remarkably specific. Most neurons received a monosynaptic excitatory input from only one canal nerve and a major disynaptic excitatory input from the same semicircular canal nerve. Disynaptic inhibitory inputs mediated by GABA and/or glycine exhibited a similarly high degree of specificity. Most of the identified 2° canal neurons were disynaptically inhibited by interneurons activated from the same semicircular canal nerve.

**Specificity of semicircular canal nerve inputs**

Our results demonstrate considerable specificity of semicircular canal nerve inputs to 2° neurons in the frog vestibular nuclei. At least in theory, this specificity could only be apparent because of a subthreshold stimulation of particular canal nerve branches or a convergence of remote, weakly excitatory inputs. The first possibility is practically excluded, because the results of this study are based on preparations in which stimulation of each of the three canal nerves evoked field potentials in the vestibular nuclei and postsynaptic potentials in 2°VNs. The presence of convergent, weakly excitatory inputs cannot be entirely excluded given that the dendrites of 2°VNs are in part very long and that remote inputs could have been present without being noticed at the presumed somatic recording site. The negative results with strong stimuli argue against the presence of considerable convergence as well as against a possible spread of current at the site of stimulation.

Monosynaptic semicircular canal nerve inputs in the pigeon (Wilson and Felpel 1972) and in the cat (Kasahara and Uchino 1974; Sans et al. 1972) exhibited a specificity rather similar to the one described here for frogs. In each of these studies ~90% of the 2°VNs received monosynaptic inputs from only one semicircular canal nerve. The conformity of the results from these studies suggests to us a common canal-specific organization principle in vertebrates. On the basis of the results of this study, we suggest extending this common organization principle by including canal-specific disynaptic inputs in 2°VNs. The presence of a disynaptic inhibition following electrical stimulation of the VIIIth nerve had been described earlier for a number of species (cat: Ito et al. 1969; squirrel monkey: Goldberg et al. 1987; frog: Straka and Dieringer 1996), but the specificity of these inhibitory inputs had not been investigated so far. Because both the monosynaptic and disynaptic inputs of most of the 2°VNs originate from the same semicircular canal, the spatial coding of angular head velocity in different canal neurons, given by the arrangement of the canal planes in the labyrinth, is preserved in 2°VNs. At least a subset of these 2°VNs with unaltered spatial orientation vectors might be expected to be present to facilitate a central vectorial reconstruction of the direction of actual head movements in space. Other subsets of 2°VNs might be modified by multiple canal (Baker et al. 1984a,b; Fukushima et al. 1990; Graf et al. 1993; Markham and Curthoys 1972) and/or canal-otolith inputs (Angelaki et al. 1993; Bush et al. 1993), mostly via di- or oligosynaptic inputs as a first step in the necessary spatial transformation of signals from sensory to motor coordinates.

The disynaptic canal-specific inhibition of 2°VNs could be mediated either by recurrent axon collaterals of 2° projection neurons or by local interneurons. At least in the cat, some of the 2°VNs projecting to the ipsilateral abducens nucleus have axon collaterals that project back to the ipsilateral vestibular nucleus in which the somata of these neurons are located (McIver et al. 1980; Ohgaki et al. 1988). Thus, at least in some of the horizontal canal 2°VNs, the disynaptic glycinergic inhibition could originate from recurrent axon collaterals. However, other arguments tend to exclude projection neurons and to implicate interneurons as the origin of the disynaptic inhibition. 1) Most of the neurons analyzed in this study can be assumed to have relatively large cell bodies. Because our records were always obtained at stimulus intensities below the threshold for the activation of action potentials in the recorded neurons (>4 × T), the spike activation threshold of those neurons that mediated the disynaptic inhibition must have been much lower and therefore the size of these neurons was presumably smaller. 2) If disynaptic inhibition were mediated by recurrent axon collaterals of 2° projection neurons, a predominance of glycinergic inhibition might be expected in horizontal-canal-related 2°VNs and a predominance of GABAergic inhibition might be expected in vertical-canal-related 2°VNs, given that uncrossed inhibition of abducens motoneurons is mediated by glycine (cat: Spencer et al. 1989; frog: Straka and Dieringer 1993) and that inhibitory vertical canal neurons projecting to the oculomotor and trochlear motoneurons are GABAergic (rabbit: Ito and Tsuchiya 1970; cat: Precht et al. 1973; frog: Cochran 1992). A recent immunocytochemical study of the frog’s VNC (Reichenberger et al. 1997) described GABA-immunoreactive neurons that were similar in size and in location to those of mammalian species. With respect to glycine immunoreactivity, the same study described small, medium, and large vestibular neurons in the frog that were found in all parts of the VNC. However, the density of GABA- and glycine-immunoreactive cells was complementary along the rostrocaudal extent of the VNC. In the superior vestibular nucleus, a region devoid of these neurons in the cat (Walberg et al. 1990), only very few glycine immunoreactive cells were found in frogs. Interestingly, some vestibular neurons and terminal-like structures express colocalization of GABA and glycine (Reichenberger et al. 1997). Therefore part of the inhibitory disynaptic inputs mediated by GABA as well as by glycine could have been conveyed even by the same interneuron. Such a possibility is compatible with our observation that the parameters of IPSP components mediated by GABA (Fig. 6A1) and by glycine (Fig. 6B1) were virtually identical in some of these neurons.

**Functional implications**

Semicircular canal nerve afferents activate a number of subsets of excitatory and inhibitory vestibular interneurons...
and projection neurons. 2° vestibular interneurons form excitatory and inhibitory side loops through their feedforward projections onto other 2° VNs on the same side (Fig. 8). From a huge number of possible combinations of convergence patterns, only very few are actually realized. Most 2° VNs receive disynaptic inhibitory and excitatory inputs that originate from the same semicircular canal as their monosynaptic excitation. Therefore most of the 2° vestibular interneurons do not modify the spatial orientation of the maximal activation direction of the responses of their target neurons with respect to the canal planes. Rather, these interneurons are suited to facilitate or disfacilitate specific dynamic response parameters of their 2° target neurons, depending on the dynamic range covered by those vestibular nerve afferent fibers that activate these 2° interneurons. The similarities in the thresholds of monosynaptic EPSPs and of disynaptic IPSPs suggest to us that both the recorded and the interposed interneurons were activated among others from thick (i.e., phasic) vestibular nerve afferent fibers. It is important in this context to realize that the disynaptic homonymous inhibition of 2° VNs not only tends to reduce part of the driving force of the monosynaptic phasic input but in addition affects the voltage-dependent activation of NMDA receptors that are activated by thick but not by thin vestibular nerve afferent fibers (Straka and Dieringer 1996; Straka et al. 1996b). It will be important to determine in future experiments the dynamic range conveyed by excitatory and inhibitory vestibular interneurons and to elucidate the functional consequences of these side loops under more natural stimulus conditions.

Interestingly, a synaptic arrangement similar to the one described here for 2° VNs in frogs (Fig. 8) was proposed by Minor and Goldberg (1991) for the squirrel monkey. The absence of a gain and phase change in the horizontal vestibulo-ocular reflex after a selective ablation of irregular afferent nerve fibers was explained by inhibitory vestibular interneurons that are activated by irregular horizontal canal nerve fibers and that remove this response component from the output of their target neurons, i.e., from horizontal 2° vestibulo-ocular neurons. Even though vestibuloocular or vestibulospinal projection neurons were not identified in this study, it is most likely that at least some of the recorded neurons were in fact projection neurons. The similarities in the elabo-
rate control of the response dynamics of 2° VNs between frog and monkey suggest a basic design, probably common to most vertebrates, that is suited to adapt vestibular reflexes, e.g., during development or in a particular behavioral context.

We thank L. Schindler for technical assistance.
This research was supported by Sonderforschungsbereich 462 (Sensomotorik) der Deutschen Forschungsgemeinschaft and by the Friedrich-Baur-Stiftung 44/95.
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Received 10 February 1997; accepted in final form 8 May 1997.

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