Patterns of Canal and Otolith Afferent Input Convergence in Frog Second-Order Vestibular Neurons

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Straka, H., S. Holler, and F. Goto. Patterns of canal and otolith afferent input convergence in frog second-order vestibular neurons. J Neurophysiol 88: 2287–2301, 2002; 10.1152/jn.00370.2002. Second-order vestibular neurons (2°VN) were identified in the isolated frog brain by the presence of monosynaptic excitatory postsynaptic potentials (EPSPs) after separate electrical stimulation of individual vestibular nerve branches. Combinations of one macular and the three semicircular canal nerve branches or combinations of two macular nerve branches were stimulated separately in different sets of experiments. Monosynaptic EPSPs evoked from the utricle or from the lagena converged with monosynaptic EPSPs from one of the three semicircular canal organs in ∼30% of 2°VN. Utricular afferent signals converged predominantly with horizontal canal afferent signals (74%), and lagener afferent signals converged with anterior vertical (63%) or posterior vertical (37%) but not with horizontal canal afferent signals. This convergence pattern correlates with the coactivation of particular combinations of canal and otolith organs during natural head movements. A convergence of afferent saccular and canal signals was restricted to very few 2°VN (3%). In contrast to the considerable number of 2°VN that received an afferent input from the utricle or the lagena as well as from one of the three canal nerves (∼30%), smaller numbers of 2°VN (14% of each type of 2°otolith or 2°canal neuron) received an afferent input from only one particular otolith organ or from only one particular semicircular canal organ. Even fewer 2°VN received an afferent input from more than one semicircular canal or from more than one otolith nerve (∼7% each). Among 2°VN with afferent inputs from more than one otolith nerve, an afferent saccular nerve input was particularly rare (4–5%). The restricted convergence of afferent saccular inputs with other afferent otolith or canal inputs as well as the termination pattern of saccular afferent fibers is compatible with a substrate vibration sensitivity of this otolith organ in frog. The ascending and/or descending projections of identified 2°VN were determined by the presence of antidromic spikes. 2°VN mediating afferent utricular and/or semicircular canal nerve signals had ascending and/or descending axons. 2°VN mediating afferent lagener or saccular nerve signals had descending but no ascending axons. The latter result is consistent with the absence of short-latency macular signals on extraocular motoneurons during vertical linear acceleration. Comparison of data from frog and cat demonstrated the presence of a similar organization pattern of maculo- and canal-ocular reflexes in both species.

INTRODUCTION

Components of linear and angular head acceleration are detected separately by the different otolith and semicircular canal organs in the labyrinth. These signals are mediated in parallel pathways by afferent nerve fibers to the vestibular nuclei, the brain stem reticular formation, and the cerebellum. Afferent nerve fibers from individual semicircular canal as well as from otolith organs terminate in all major vestibular nuclei and overlap to a large extent in nonmammalian (Birinyi et al. 2001; Dickman and Fang 1996; Highstein et al. 1992; McCormick and Bradford 1994; Meredith and Butler 1983; Suarez et al. 1985; Will et al. 1985) as in mammalian species (Büttner-Ennever 1992, 1999; Gacek 1969; Siegborn et al. 1991; Stein and Carpenter 1967). From these overlapping termination areas of afferent nerve fibers, a considerable convergence of afferent input signals onto second-order vestibular neurons (2°VN) could be possible.

However, intracellular studies employing selective electrical stimulation of individual semicircular canal nerves in frog (Holler and Straka 2001; Straka et al. 1997), pigeon (Wilson and Felpel 1972), and cat (Kasahara and Uchino 1974; Sans et al. 1972) have shown that monosynaptic canal nerve responses typically originated from only one semicircular canal. Thus 2°VN select among the available semicircular canal nerve inputs and a convergence of signals from more than one canal is limited. Relatively little is known so far about a possible convergence pattern of afferent canal-otolith or afferent otolith-otolith signals in 2°VN in frog.

In cat, the convergence of afferent canal-otolith or afferent otolith-otolith signals was studied in different series of experiments by separate electrical stimulation of two labyrinthine nerve branches in various combinations (Kushiro et al. 2000; Sato et al. 2000; Zakir et al. 2000; Zhang et al. 2001). These studies showed some degree of convergence of canal- and otolith-otolith signals. However, an organ-specific canal- or otolith-otolith convergence pattern was not detected. Either such a pattern does not exist in cat or it could not be detected because only two of five individual vestibular nerve branches were stimulated in a given set of experiments.

In the present study, we analyzed the convergence patterns of afferent canal- and otolith-otolith signals in the isolated frog brain and took advantage of the fact that the labyrinthine organs in frog are located in a bony bulla and not embedded in a bone as in mammalian species. Therefore all organs and their nerve supply became visible and accessible after opening of the bulla. The brain together with the VIIIth nerves and their side branches were isolated and maintained for in vitro experimen-
tation for \( \geq 4 \) days. \( 2^\circ \)VN were identified by the presence of monosynaptic excitatory postsynaptic potentials (EPSPs) after separate stimulation of individual canal and otolith nerve branches of the VIIIth nerve. Due to technical limitations, the three semicircular canal nerve branches and one particular otolith nerve or combinations of two otolith nerves were stimulated separately in different sets of experiments. Identified \( 2^\circ \)VN were further classified as projection neurons by the presence of an antidromic spike after stimulation of the cervical spinal cord and/or of the midbrain close to the oculomotor nuclei.

Preliminary results have been published in abstract form (Goto and Straka 2001; Holler and Straka 2000).

METHODS

In vitro experiments were performed on the isolated brains of 33 grass frogs (\textit{Rana temporaria}) and complied with the “Principles of Animal Care”. Publication No. 86-23, revised 1985 by the National Institutes of Health. Permission for these experiments was granted by Regierung von Oberbayern (211-2531-31/95). 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; MS-222) and perfused transcardially with iced Ringer solution [containing (in mM) 75 NaCl, 25 NaHCO\(_3\), 2 CaCl\(_2\), 2 KCl, 0.1 MgCl\(_2\), and 11 glucose; pH 7.4]. Thereafter, the skull and the bony labyrinth were opened by a ventral approach. After dissecting the three semicircular canals on either side, the brain was removed with all labyrinthine end organs attached to the VIIIth nerve on either side. Subsequently the brain was submerged in iced Ringer, and the dura, labyrinthine end organs and the choroid plexus covering the IVth ventricle were removed. In all experiments, the cerebellum remained attached to the brain stem while the forebrain was removed. Brains were used \( \leq 4 \) days after their isolation and were stored overnight at 6\(^\circ\)C in continuously oxygenated Ringer solution with a pH of 7.5 ± 0.1. For the experiments, the brain stem was fixed with insect pins to a silicone elastomer (Sylgard) floor of a chamber (volume, 2.4 ml), which was continuously perfused with oxygenated Ringer solution at a rate of 1.3–2.1 ml/min. The temperature was electronically controlled and maintained at 14 ± 0.1\(^\circ\)C.

The convergence pattern of afferent otolith and canal nerve inputs in \( 2^\circ \)VN was studied in separate sets of experiments because electrical stimulation of all six labyrinthine nerve branches in one experiment was not possible due to limited space for placing the stimulation and recording electrodes. Therefore either one otolith nerve or all three canal nerves or a combination of two otolith nerves were stimulated separately in different sets of experiments. For electrical stimulation, single constant current pulses (0.2 ms; 1–15 \( \mu \)A) across suction electrodes (diameter: 120–150 \( \mu \)m) were used. The use of suction electrodes facilitated the isolation of an individual nerve branch and allowed a separate stimulation of each of the canal and otolith nerves, thereby minimizing false negative or false positive results concerning the convergence of afferent inputs from more than one labyrinthine nerve in \( 2^\circ \)VN (see Straka et al. 1997). For the antidromic identification of \( 2^\circ \)VN with ascending axons, a concentric bipolar electrode (tip diameter: 25 \( \mu \)m; Science Products GmbH) was placed in the midline between the bilateral oculomotor nuclei in the midbrain. For the antidromic identification of \( 2^\circ \)VN with descending axons, a pair of Teflon-coated silver wires with a chlorided tip (diameter: 250 \( \mu \)m) was inserted into the ventro-lateral spinal cord on the left and right side at the C1/C2 level. For brevity and clarity, \( 2^\circ \)VN with antidromic spikes from the midbrain will be labeled as \( 2^\circ \)vestibulo-ocular neurons, and those with antidromic spikes from the spinal cord will be labeled as \( 2^\circ \)vestibulo-spinal neurons. For stimulation, single constant current pulses (0.2 ms; 30–150 \( \mu \)A) were used. All stimulation pulses were produced by a stimulus isolation unit (WPI A 360) at a repetition rate of 0.5 Hz. Glass microelectrodes used for extra- and intracellular recordings were fabricated with a horizontal puller (P-87 Brown/Flaming). Electrodes for extracellular field potential recordings were beveled (30°, 20 \( \mu \)m tip diameter) and filled with 2 M sodium chloride (∼1 M\( \Omega \)). Electrodes for intracellular recordings were filled with a mixture of 2 M potassium acetate and 3 M potassium chloride (10:1); this gave a final resistance of ∼90–110 M\( \Omega \).

At the beginning of each recording session, field potentials in the ipsilateral vestibular nuclei were recorded after electrical stimulation of individual semicircular canal nerve branches, otolith nerve branches, the midbrain, and the spinal cord. A standard recording site for these field potentials was located 0.4 mm caudal to the entry of the VIIIth nerve at a depth of 0.4 mm, except for the SA-evoked field potential, which was recorded at the same depth but at the level of the entry of the VIIIth nerve. The amplitude of the labyrinthine nerve branch evoked negative \( N_1 \) field potential component (which represents the monosynaptic activation of \( 2^\circ \)VN) (see Precht et al. 1974) served to determine the threshold for the postsynaptic excitation of these neurons. Intracellular single-cell recording experiments were started unless the semicircular canal or otolith nerve-evoked \( N_1 \) component was <0.15 mV. The stimulus threshold intensity (\( T \)) for the \( N_1 \) component as for canal and otolith nerve-evoked monosynaptic EPSPs was ∼1.2–3.3 \( \mu \)A and was similar for each of the nerve branches. The position of the stimulation electrode in the midbrain and spinal cord was optimized by maximizing the amplitude of the short-latency antidromic field potentials recorded at the standard recording site in the vestibular nuclei. The amplitude usually ranged between 0.3 and 0.5 mV. The stimulus threshold intensity (\( T \)) for this field potential was ∼10–20 \( \mu \)A and was similar for the spinal and for the midbrain stimulation electrode. During intracellular recordings, the stimulation intensity to evoke an antidromic spike was limited to 4 × \( T \). Vestibular neurons were recorded between 0.5 mm rostral and 0.8 mm caudal to the entry of the VIIIth nerve in a depth between 0.05 mm and 0.8 mm below the dorsal surface of the brain stem. This recording area included the superior, lateral, and descending vestibular nuclei and excluded the most medial parts of the medial vestibular nucleus (see Straka et al. 2000). All vestibular neurons recorded in the isolated frog brain in this study were not spontaneously active as in earlier studies (Straka and Dieringer 1996, 2000; Straka et al. 1997), and only those with a membrane potential more than −55 mV (range: −55 to −81 mV) and amplitudes of evoked action potentials >60 mV (range: −60 to −90 mV) were included in this study.

The known distance of antidromically identified \( 2^\circ \)VN relative to the midbrain or spinal stimulation electrodes and the latency of evoked field potentials or of antidromic spikes were used to calculate the conduction velocity of the ascending or descending axon, respectively. To compensate for the recruitment time of the stimulation, 0.5 ms was subtracted from the latency of the antidromic spikes before calculation of the conduction velocity (see Straka et al. 1997). Single sweeps of the responses were digitized (CED 1401, Cambridge Electronic Design), stored on computer, and analyzed off-line (SIGNAL, Cambridge Electronic Design). Synaptic potentials were analyzed from averages of 20–30 single sweeps after electronic subtraction of the extracellular field potential recorded in the vicinity. Statistical differences in parameters were calculated according to the Mann-Whitney \( U \) test (Prism, Graphpad Software). Graphical presentations were performed with the aid of commercially available computer software (Origin, Microcal Software; Corel Draw, Corel Corporation).

RESULTS

Identification of second-order vestibular neurons

In the first three sets of experiments, the three ipsilateral semicircular canal nerves and in addition one of the three otolith nerves were stimulated. In sets number four to six, combinations of two otolith nerves were stimulated. At the beginning of each experiment, the thresholds of the responses to stimulation of each of the
nerve branches were determined to assess and if necessary to correct the effectiveness of the different stimulation electrodes. To that end pre- (N0) and postsynaptic (N1) negative field potentials were recorded in the vestibular nuclei following separate stimulation of the ipsilateral utricular (UT), lagena (LA), saccular (SA), or individual semicircular canal nerve branches. In addition, the shortest onset latencies (N1) for monosynaptic EPSPs were established. Only minor differences were observed between different preparations. The mean latencies of LA nerve-evoked responses (N0; 1.1 ± 0.2 ms; N1; 2.9 ± 0.2 ms; n = 17) were significantly shorter (P ≤ 0.0001) than the corresponding latencies of canal nerve-evoked responses (N0; 1.6 ± 0.3 ms; N1; 3.4 ± 0.4 ms; n = 57) or UT nerve-evoked responses (N0; 1.6 ± 0.2 ms; N1; 3.4 ± 0.4 ms; n = 19). This difference was explained by a distance between the stimulation electrode of the LA nerve and the brain stem that was shorter than the distance between the stimulation electrodes of any of the three canal nerves or the UT nerve branch and the brain stem. The latencies of the SA nerve-evoked responses (N0; 1.4 ± 0.3 ms; N1; 3.2 ± 0.4 ms; n = 12) ranged between those of the LA and the canal nerve-evoked components and were not significantly different to corresponding values from other nerve branches. The latter finding was consistent with a position of the stimulation electrode on the SA nerve branch that was located between those on the horizontal, anterior canal, and UT nerve and that on the LA nerve. The difference between N0 and N1 latencies (between 1.7 and 1.8 ms) represented a synaptic delay at a temperature of 14°C as reported earlier (Straka et al. 1997). Accordingly, intracellular recorded EPSPs after semicircular canal or UT nerve stimulation were defined as monosynaptic if their latency ranged between 3.4 ms (average onset of the N1 field potential after stimulation of a particular nerve branch) and 5.2 ms (earliest possible onset of disynaptic EPSPs after adding another synaptic delay time of 1.8 ms). The corresponding values were slightly shorter for SA nerve-evoked responses (between 3.2 and 5.0 ms) or for LA nerve-evoked responses (between 2.9 and 4.7 ms) due to the earlier average onset of the N1 field potentials, respectively (see preceding text).

The average latency of monosynaptic EPSPs was similar for responses evoked by horizontal (HC), anterior vertical (AC), or posterior vertical canal (PC) nerve stimulation (mean: 3.7 ± 0.6 ms; n = 551), by UT nerve stimulation (mean: 3.7 ± 0.6 ms; n = 208), or by SA nerve stimulation (mean: 3.6 ± 0.5 ms; n = 92). LA nerve stimulation evoked monosynaptic responses (mean: 3.3 ± 0.6 ms; n = 221) that had significantly (P ≤ 0.0001) shorter latencies. With these parameters, the recorded neurons were identified as second-order vestibular neurons (2°VN). Further subdivision in second-order canal (2°canal), utricular (2°UT), lagena (2°LA), and saccular (2°SA) neurons and in combined second-order otolith + canal neurons followed according to the presence of monosynaptic EPSPs from vestibular nerve branches. Neurons with converging monosynaptic inputs from different vestibular nerve branches, for instance, from the UT and one of the canal nerves were designated as 2°UT + AC, 2°UT + PC, or 2°UT + HC neurons.

**Convergence of UT and canal nerve afferent inputs onto second-order vestibular neurons**

The convergence of monosynaptic afferent UT and semicircular canal signals onto 2°VN (n = 242) was investigated in a first set of experiments. Separate electrical stimulation of each one of the three canal nerves and of the UT nerve evoked excitatory responses that differed in latencies, rise times, and amplitudes (Fig. 1, A and B). The amplitudes of monosynaptic EPSPs evoked by canal or UT nerve stimulation gradually increased with stronger stimuli (Fig. 1, A1 and B, 1 and 4) and reached up to 7 mV. At higher stimulus intensities partial spikes with amplitudes of 2–6 mV occasionally appeared on top of the EPSPs (Fig. 1B4, *) (see Precht et al. 1974) and at even higher stimulus intensities full action potentials with an amplitude of 60–90 mV were evoked (not shown). In a large number of recorded neurons, the monosynaptic EPSP was followed by a disynaptic EPSP at higher stimulus intensities as reported earlier (Straka et al. 1997). In most cases, the latter one was separated from the monosynaptic EPSP by a marked notch (Fig. 1B1, →). Di- and oligosynaptic EPSPs (Fig. 1, A, 2–4, and B, 2 and 3) from labyrinthine nerve branches from which no monosynaptic EPSP was evoked had smaller amplitudes and longer rise times than monosynaptic EPSPs (Fig. 1, A1 and B, 1 and 4). These EPSPs did not reach spike threshold. However, in some neurons, stimulation of a particular nerve branch evoked oligosynaptic EPSPs that reached amplitudes of up to 7 mV (e.g., Fig. 1A2), although partial spikes or full action potentials were not triggered by these inputs.

About 43% of the recorded 2°VN in this set of experiments (101 of 242 neurons) received a monosynaptic excitation following stimulation of the UT nerve branch (Fig. 2A). About one-quarter of these neurons (23 of 101 neurons) received a monosynaptic EPSP from the UT nerve but not from any one of the three canal nerves (2°UT neurons; Figs. 2A). The remaining three quarters of these 2°VN (78 of 101 neurons) received a monosynaptic EPSP from one (n = 65) or from two or more canal nerves (n = 13) in addition to the UT nerve-evoked monosynaptic EPSP (2°UT + canal, 2°UT + mult. canal neurons; Fig. 2A).

The canal nerve input in 2°UT neurons originated predominantly from the HC nerve (74%; Fig. 2B). In the remaining 2°UT neurons, the monosynaptic EPSPs originated in about equal proportions from the AC or the PC nerve (Fig. 2B). This predominance in the convergence with HC canal inputs was not related to a possible bias in the recording sample. In fact, this predominance of HC over AC and PC nerve inputs in 2°UT + canal neurons (Fig. 2B) was counterbalanced by the presence of a large number of 2°AC and 2°PC neurons and a small number of 2°HC neurons (Fig. 2A). Almost equal numbers of 2°VN with a monosynaptic EPSP from the AC nerve (i.e., 2°AC and 2°UT + AC neurons; n = 64), the HC nerve (i.e., 2°HC and 2°UT + HC neurons; n = 64), or from the PC nerve (i.e., 2°PC and 2°UT + PC neurons; n = 59) were encountered (Fig. 2, A and B). Therefore HC nerve and UT nerve afferent inputs converged more frequently than AC or PC nerve and UT nerve afferent inputs.

The amplitudes of EPSPs recorded in 2°UT + canal neurons differed between neurons as well as between different inputs in a given neuron even though comparable stimulus intensities were used. Converging inputs from the UT nerve and from the canal nerves were distinguished according to which one had the larger amplitude as major or minor inputs, described earlier for 2°canal neurons (Straka et al. 1997). In 2°UT + canal and in 2°UT + mult. canal neurons, the minor input(s) reached 47 ± 19% (n = 94) of the amplitude of the major input,
independent of the number of monosynaptic inputs of a given 2°VN or its origin(s). In most 2°UT + HC (40 of 48 neurons) and 2°UT + PC neurons (6 of 9 neurons), the major input originated from the HC and PC nerve, respectively. In the remaining neurons (8 of 48 2°UT + HC neurons; 3 of 9 2°UT + PC neurons), the major input originated from the UT nerve. Equal numbers of 2°UT + AC neurons received their major input from the AC nerve (4 of 8 neurons) or from the UT nerve (4 of 8 neurons). In 2°UT + mult. canal neurons, the major input originated either from the AC nerve (2 of 13 neurons), from the HC nerve (4 of 13 neurons), from the PC nerve (5 of 13 neurons) or from the UT nerve (2 of 13 neurons).

Convergence of LA and canal nerve afferent inputs onto second-order vestibular neurons

The convergence of monosynaptic afferent LA and semicircular canal signals onto 2°VN (n = 228) was investigated in a second set of experiments. Separate electrical stimulation of each one of the three canal nerves and the LA nerve evoked either excitatory responses (Fig. 3, A, 1, 2, and 4, B, 1–4, and C, 1–4), with similar parameters as those described in the first set of experiments or no response (Fig. 3A3). Fast rising monosynaptic canal or LA nerve-evoked EPSPs (e.g., Fig. 3, A1, B, 1 and 2, and C4) were superimposed in many neurons by disynaptic EPSPs at higher stimulus intensities, separated from the first ones by a notch (Fig. 3, B2 and C4, ↓). With few exceptions (Fig. 3A2) di- and oligosynaptic EPSPs from one of the remaining nerve branches (Fig. 3, A4, B, 3 and 4, and C, 1–3) had smaller amplitudes and longer rise times than monosynaptic EPSPs.

About 50% of the 2°VN identified in this set of experiments (113 of 228 neurons) received a monosynaptic excitation following stimulation of the LA nerve (Fig. 4A). About one-quarter of these neurons (28 of 113 neurons) received a monosynaptic EPSP exclusively from the LA nerve (2°LA neurons; Fig. 4A), while the remaining three-quarters of these neurons (85 of 113 neurons) received in addition a monosynaptic EPSP from one (n = 71) or more (n = 14) canal nerves (2°LA + canal, 2°LA + mult. canal neurons; Fig. 4A). Interestingly, the monosynaptic canal nerve input of 2°LA neurons originated from one of the vertical but not from the horizontal semicircular canal nerve (Fig. 4B). More 2°LA + AC neurons (63%) than 2°LA + PC neurons (37%; Fig. 4B) were found. However, the absolute numbers of 2°VN with a monosynaptic input from the AC nerve (i.e., 2°AC and 2°LA + AC neurons; n = 64) or from the PC nerve (i.e., 2°PC and 2°LA + PC neurons; n = 65) were almost identical. Therefore the percentage of convergence between afferent AC and LA signals was larger than between afferent PC and LA signals. In most of the 2°LA + mult. canal neurons (13 of 14 neurons), an AC nerve-evoked monosynaptic EPSP was present. This predominance of affer-
CONVERGENCE OF CANAL AND OTOLITH AFFERENT SIGNALS

FIG. 2. Convergence pattern of utricular and canal nerve afferent inputs in identified 2nd-order vestibular neurons. A: distribution of neurons (n = 242) classified by their monosynaptic canal nerve and/or utricular inputs. B: 2nd-order neurons (n = 65) with converging inputs from utricular and canal nerve afferents were subdivided into 2nd-order utricular + horizontal canal (2°UT + HC), 2nd-order UT + anterior vertical canal (2°UT + AC), and 2nd-order UT + posterior vertical canal (2°UT + PC) neurons. Numbers of neurons are indicated in parentheses; mult., multiple.

ent AC nerve inputs in 2°LA + mult. canal neurons, corroborated the pronounced convergence of afferent LA and AC nerve-evoked EPSPs in 2°VN.

The amplitudes of the convergent monosynaptic EPSPs recorded in 2°LA + canal neurons and in 2°LA + mult. canal neurons differed even though comparable stimulus intensities were used. Independent of its origin the minor input reached 44 ± 21% (n = 103) of the amplitude of the major input. In most 2°LA + AC neurons (37 of 45 neurons), the major input originated from the AC nerve. In the remaining neurons (8 of 45 neurons), the major input originated from the LA nerve. About equal numbers of 2°LA + PC neurons received their major input from the PC nerve (12 of 26 neurons) or from the LA nerve (14 of 26 neurons). In 2°LA + mult. canal neurons, the major input originated either from the AC nerve (6 of 14 neurons), from the PC nerve (5 of 14 neurons), or from the LA nerve (3 of 14 neurons) but in none of these neurons from the HC nerve.

Convergence of SA and canal nerve afferent inputs onto second-order vestibular neurons

The convergence of afferent SA and semicircular canal signals onto 2°VN (n = 164) was investigated in a third set of experiments. Separate electrical stimulation of each one of the three canal nerves and the SA nerve evoked excitatory responses that were similar to those described in the first two sets of experiments. In 2°VN identified by a monosynaptic canal nerve input usually di- and oligosynaptic EPSPs were evoked from the remaining two canal nerves but only occasionally from the SA nerve (not shown). The monosynaptic SA nerve-evoked EPSPs (Fig. 5A) were similar in its parameters to those evoked by canal nerve, UT nerve, or LA nerve stimulation (see 1st and 2nd set of experiments), including the presence of superimposed disynaptic EPSPs at higher stimulus intensities. In these 2°SA neurons, the canal nerve inputs were evoked either long latency EPSPs with a long rise time and a very small amplitude (Fig. 5B) or no response (Fig. 5, C and D).

About 28% of the identified 2°VN (46 of 164 neurons) received a monosynaptic EPSP from the SA nerve (Fig. 5E). In the majority of these 2°VN (41 of 46 neurons), additional canal nerve-evoked monosynaptic EPSPs were absent (2°SA neurons; Fig. 5E). Only few of these 2°VN (5 of 46 neurons) received converging afferent inputs from the SA nerve and from one of the three canal nerves (2°SA + canal neurons; Fig. 5E). In these neurons, the canal nerve-evoked monosynaptic EPSP originated either from the AC (n = 3) or from the PC nerve (n = 2). In these few 2°SA + canal neurons that were encountered, the monosynaptic EPSPs evoked at comparable stimulus intensities could be divided in a major and a minor input, as described for 2°UT + canal nerve or for 2°LA + canal neurons. In all 2°SA + canal neurons, the SA nerve-evoked monosynaptic input was small and represented the minor input with 35 ± 12% (n = 5). Apart from the fact that only very few 2°canal neurons received an additional afferent EPSP from the SA nerve, di- and oligosynaptic EPSPs originating from the SA nerve were also rare.

Convergence of canal nerve afferent inputs onto second-order vestibular neurons

In each of the three sets of experiments described so far, a large number of 2°VN was monosynaptically excited from one (Fig. 3C4) or more semicircular canal nerves but not from an otolith nerve branch. The overall percentage of these 2°canal neurons (59%) among the total number of recorded neurons (374 of 634 neurons) was similar in the UT-canal convergence experiments (58%; Fig. 2A) and in the LA-canal convergence experiments (51%; Fig. 4A) but slightly higher in the SA-canal convergence experiments (72%; Fig. 5E). In the majority of these 2°canal neurons (325 of 374 neurons; 87%), the monosynaptic EPSP originated from only one canal nerve (see 2°AC, 2°HC, 2°PC neurons in Figs. 2A, 4A, and 5E) as reported earlier (Holler and Straka 2001; Straka et al. 1997). A minority of 49 neurons received a monosynaptic EPSP from two (47 of 374 neurons; 12%) or all three ipsilateral canal nerves (2 of 374 neurons; 1%). In the latter 2°mult. canal neurons a major and a minor input could be distinguished. The amplitude of the minor input in these 2°mult. canal neurons reached 42 ± 17% (n = 53) of the amplitude of the major EPSP.

Convergence of otolith nerve afferent inputs onto second-order vestibular neurons

Separate stimulation of the UT and the LA nerve identified 2°VN (n = 153) by the presence of a monosynaptic EPSP from
one or both otolith organs in a fourth set of experiments. About
equal numbers of 2° VN received a monosynaptic EPSP either
from the UT nerve or from the LA nerve (Fig. 6A). A small
number of neurons (17 of 153 neurons; 11%) received a mono-
synaptic EPSP from the UT (B1) as well as from the LA nerve (B2). This neuron received in
addition an oligosynaptic excitation from the PC (B3) and HC (B4) nerve, respectively. C, 1–4: 2nd-order horizontal canal (2°HC)
neuron, identified by a monosynaptic EPSP from the HC nerve (C4). In addition, an oligosynaptic excitation from the LA (C1),
AC (C2), and from the PC nerve (C3) were present. □, the onset of superimposed disynaptic EPSPs. Stimulus intensity is expressed
in multiples of the stimulus threshold intensity (× T) of the 1st negative postsynaptic field potential (N1) on the ipsilateral side. ☐, the mean ± SD of the onset latency of field potentials after stimulation of the indicated nerve branch. - - - - baselines; ▲, ▼, stimulus onset. Each record represents the average of 24 responses.

The convergence of UT and SA afferent signals was investi-
gated in a separate subset of 2°VN (n = 48) that were
identified by the presence of a monosynaptic EPSP from the
UT and/or the SA nerve. The vast majority of identified 2°VN
received a monosynaptic EPSP either from the UT nerve or
from the SA nerve (Fig. 6B). Only two neurons (4%) were
recorded that received a monosynaptic EPSP from both the UT
as well as the SA nerve (Fig. 6B). In both neurons, the UT
nerve-evoked EPSP comprised the major input. The small
percentage of UT and SA convergence was further emphasized
by the absence of di- or oligosynaptic EPSPs from the SA nerve in 2° UT neurons or from the UT nerve in 2° SA neurons.

The convergence of LA and SA afferent nerve signals was investigated in a third subset of 2° VN (n = 39) that were identified by the presence of a monosynaptic EPSP from the LA and/or the SA nerve. Most identified 2° VN received a monosynaptic EPSP either from the LA nerve or from the SA nerve (Fig. 6C). Only two neurons (5%) received convergent monosynaptic inputs from the LA as well as from the SA nerve (Fig. 6C), with the major input originating from the LA nerve. Di- or oligosynaptic EPSPs from the SA nerve in 2° LA neurons or from the LA nerve in 2° SA neurons were largely absent as it was observed for the convergence of UT and SA afferent nerve signals.

Ascending and descending projections of second-order vestibular neurons

Stimulation of the spinal cord at the C1/C2 level or of the midbrain evoked short latency negative field potentials in the vestibular nuclei. The onset latencies of the field potentials evoked by stimulation of the spinal cord (mean: 1.0 ± 0.1 ms; n = 19) and of the midbrain (mean: 1.1 ± 0.2 ms; n = 19) indicated the earliest arrival of antidromic action potentials (note that the synaptic delay is 1.8 ms) (Straka et al. 1997).

FIG. 4. Convergence pattern of lagener and canal nerve afferent inputs in identified 2nd-order vestibular neurons. A: distribution of neurons (n = 228) classified by their monosynaptic canal nerve and/or lagener inputs. B: 2nd-order neurons (n = 71) with converging inputs from lagener and canal nerve afferents were subdivided into 2nd-order lagener + anterior vertical canal (2°LA + AC) and 2nd-order lagener + posterior vertical canal (2°LA + PC) neurons. Note the absence of 2nd-order lagener + horizontal canal neurons. Numbers of neurons are indicated in parentheses; HC, horizontal canal; mult., multiple.

These mean onset latencies were therefore used to determine the conduction velocity for the fastest vestibulo-spinal and -ocular fibers. The mean conduction velocity, calculated from the latency, the known recruitment time (0.5 ms) (Straka et al. 1997), and the known distance between the recording and the stimulation electrodes was significantly higher (P ≤ 0.0001) for the fastest vestibulo-spinal axons (16.0 ± 5.2 m/s; n = 19) than for the fastest vestibulo-ocular axons (4.6 ± 1.9 m/s; n = 19).

Intracellularly recorded action potentials were considered to represent antidromic action potentials if there was an all-or-nothing response in the absence of an underlying prepotential at a threshold stimulus intensity (see insets in Fig. 7, A1, B1, and C, 1 and 2). The presence of antidromic action potentials evoked from the spinal cord and/or from the midbrain was used to classify vestibular neurons as vestibulo-spinal (VSP; Fig. 7A), vestibulo-ocular (VOR; Fig. 7B), or vestibulo-ocular-spiral (VOS; Fig. 7C) neurons. In the latter neurons, a collision test was performed and resulted in the extinction of the second spike at short-latency intervals (not shown). Monosynaptic responses originating from individual canal and/or otolith nerves (Fig. 7, A, 2, and 3, B, 2, and 3, C3) were used to identify these neurons as particular types of 2° VN.

Antidromic conduction velocities of vestibular projection neurons

The conduction velocities of 2° VSP, 2° VOR, and 2° VOS neurons were not correlated with a particular afferent labyrinthine input. Therefore all data were pooled independent of the origin of the afferent labyrinthine input. The conduction velocities of 2° VSP neurons were very heterogeneous and ranged between 0.9 and 27.3 m/s (Fig. 8A). The multimodal distribution of these conduction velocities suggested subpopulations of 2° VSP neurons with conduction velocities of ~3.5, 12.5, and 18 m/s, respectively (Fig. 8A). The conduction velocities of 2° VOR neurons were much lower, more homogeneous and ranged between 0.7 and 8.1 m/s (Fig. 8B). In 2° VOS neurons, the conduction velocities of ascending and of descending axonal branches within a given neuron differed slightly but significantly (P ≤ 0.001). The conduction velocity for the descending axonal branch ranged from 1.0 to 9.6 m/s (Fig. 8C) and was comparable to that of the 2° VSP neurons with the lowest conduction velocities. The conduction velocity for the ascending branch ranged from 1.0 to 6.5 m/s (Fig. 8C) and was similar to that of 2° VOR neurons.

Projection of second-order vestibular neurons mediating combined UT plus canal signals

In the first set of experiments, 2° VN had been identified by monosynaptic inputs from the UT nerve and/or from a canal nerve (Fig. 9A; data adopted from Fig. 2A). In about half of these 2° VN (n = 133), the axonal projection pattern was investigated by stimulation of the spinal cord or of the midbrain in the vicinity of the oculomotor nuclei. Antidromic action potentials were evoked, however, only in 58 of the 133 2° VN (44%). For the other 2° VN (n = 75; 56%), the axonal projections remained unknown (Fig. 9B). The presence of an antidromic action potential was used to classify these neurons as 2° VSP neurons (n = 27; Fig. 9C), as 2° VOR neurons (n = 21; Fig. 9D) or as 2° VOS neurons (n = 10; not shown).
The distribution of afferent EPSPs originating from the UT nerve, from a canal nerve, or from the UT as well as a canal nerve in 2°VN identified by one of these inputs in the first set of experiments (Fig. 9A) was similar to that in 2°VN with unknown projections (Fig. 9B). Likewise, the distribution of afferent EPSPs originating from a canal nerve and/or the UT nerve (e.g., Fig. 7A, 2 and 3) was similar in 2°VSP and 2°VOR neurons (Fig. 9, C and D). In both groups of projection neurons with monosynaptic UT + canal nerve EPSPs, the UT nerve-evoked EPSP comprised either the major (25%) or the minor input (75%). In 2°VOS neurons, the afferent EPSP originated in most neurons (8 of 10 neurons) from a canal nerve (e.g., Fig. 7C) while the remaining two 2°VOS neurons received an afferent UT nerve as well as a canal EPSP. Afferent UT nerve inputs or afferent UT + canal nerve inputs were more frequently observed in the population of 2°VSP neurons (16 of 27 neurons in Fig. 9C) and 2°VOR neurons (13 of 21 neurons in Fig. 9D) than in 2°VN with unknown projections (only 25 of 75 neurons in Fig. 9B). 2°VN mediating afferent UT signals (e.g., 2°UT and 2°UT + canal neuron) projected about equally with an ascending axon to the midbrain (23%) or with a descending axon to the spinal cord (29%). Only few of them had ascending as well as descending projections (3%). The projections of the remaining neurons with an afferent UT nerve input (45%) was unknown.

**Projection of second-order vestibular neurons mediating combined LA plus canal signals**

In the second set of experiments, 2°VN were identified by monosynaptic inputs from the LA nerve and/or from a canal nerve (Fig. 10A; data adopted from Fig. 4A). In a large number of these 2°VN (n = 196), the axonal projection pattern was investigated by stimulation of the spinal cord or of the midbrain in the vicinity of the oculomotor nuclei. Antidromic action potentials were evoked in 101 of 196 (52%) of these 2°VN. In the other 2°VN (n = 95; 48%), the axonal projections remained unknown (Fig. 10B). The presence of an antidromic action potential was used to classify these neurons as 2°VSP neurons (n = 63; Fig. 10C) as 2°VOR neurons (n = 32; Fig. 10D) or as 2°VOS neurons (n = 6; not shown).

The distribution of afferent EPSPs originating from the LA nerve, from a canal nerve or from the LA as well as a canal nerve (e.g., Fig. 7A, 2 and 3) was similar to that in 2°VN with unknown projections (compare A and B in Fig. 10) or to that in 2°VSP neurons (Fig. 10C). In 2°VSP neurons with monosynaptic LA + canal nerve EPSPs (n = 25 in Fig. 10C), the LA nerve-evoked EPSP comprised either the major (32%) or the minor input (68%). In 2°VOR neurons, the afferent EPSP originated either from a canal nerve (n = 27 in Fig. 10D; 84%) or from the LA as well as a canal nerve (n = 5 in Fig. 10D; 16%). Most importantly, no 2°VOR neurons was encountered that received an afferent EPSP from the LA nerve only. Moreover, in 2°VOR neurons with a LA + canal EPSP, the LA nerve-evoked EPSP always comprised the minor input. Most 2°VOS neurons (5 of 6 neurons; 83%) received the afferent EPSP from a canal nerve, whereas in one 2°VOS neuron (17%) the afferent EPSP originated from the AC nerve (major input) and in addition from the LA nerve (minor input).

Thus different from 2°VN mediating UT nerve signals in about equal proportions to midbrain and spinal cord, 2°VN with mono-
synaptic LA nerve inputs mediated their signals predominantly to the spinal cord (40%). Only few 2°VN with an afferent LA nerve input had an ascending projection to the midbrain (5%) or an ascending as well as a descending projection (1%). These results suggest that the contribution of LA signals to maculo-ocular reflexes is small. The projections of the remaining neurons with an afferent LA nerve input (54%) was unknown.

**Projection of second-order vestibular neurons mediating combined SA plus canal signals**

In a third set of experiments, 2°VN had been identified by monosynaptic inputs from the SA nerve and/or from a canal nerve (Fig. 11A; data adopted from Fig. 5E). In about half of these 2°VN (n = 78), the ascending and/or descending axonal projection pattern was investigated as in the other two sets of experiments. Antidromic action potentials were evoked, however, only in half of these 2°VN (39 of 78 neurons; Fig. 11, C and D). In the other half, the axonal projections remained unknown (Fig. 11B). The presence of an antidromic action potential was again used to classify these neurons as 2°VSP neurons (n = 25; Fig. 11C) or as 2°VOR neurons (n = 14; Fig. 11D).

The overall distribution of afferent EPSPs originating from the SA and/or from a canal nerve in 2°VN identified by one of these inputs (Fig. 11A) was more or less similar to that in 2°VN with unknown projections (Fig. 11B) and was characterized by the limited convergence of SA and canal nerve-evoked afferent responses. The afferent input to these 2°VN originated in most neurons from a canal nerve, in fewer neurons from the SA nerve and in very few neurons from the SA as well as a canal nerve (Fig. 11, A and B). In contrast, in the majority of 2°VSP neurons the afferent EPSP originated from a canal nerve and in only two neurons from the SA nerve (Fig. 11C). In all 2°VOR neurons the afferent EPSP originated from a canal nerve (Fig. 11D). Comparison of the distribution of the origin of afferent EPSPs in different vestibular neurons showed that the input pattern of 2°VSP and 2°VOR neurons (Fig. 11, C and D) differed from that of 2°VN with unknown projections shown in Fig. 11, A and B. While a considerable number of the latter neurons received afferent SA signals (see 2°SA neurons in Fig. 11, A and B), the afferent input to 2°VSP and 2°VOR was predominated by canal signals (Fig. 11, C and D). Thus the projection pattern of 2°VN that received an afferent SA nerve input consisted of few neurons with a descending projection to the spinal cord (11%), while the projection of the majority of 2°VN with an afferent SA nerve input (89%) remained unknown. Thus comparison of the projections of 2°VN that received an afferent otolith input indicated that vertically oriented otolith organs seem to contribute only little to the maculo-ocular reflex.

**DISCUSSION**

The convergence pattern of monosynaptic afferent canal and otolith signals onto 2°VN was determined by separate stimulation of individual vestibular nerve branches. About half of the 2°VN received convergent afferent nerve inputs from one semicircular canal and from one otolith organ. However, particular combinations of canal-otolith afferent signals (i.e., HC and UT or vertical canal and LA inputs) were encountered more frequently than other combinations (i.e., canal-SA or otolith-otolith inputs). The projection pattern of 2°VN was investigated by antidromic identification from the midbrain and/or from the spinal cord. 2°VN mediating afferent UT and/or canal nerve signals had ascending and/or descending projections. 2°VN mediating afferent SA or LA nerve signals had descending but no or only very few ascending projections.

**Convergence pattern of afferent otolith and canal signals in frog 2°VN**

Afferent signals from individual semicircular canals remained largely separate at the level of 2°VN because most 2°canal neurons recorded in this study (87%) received an afferent canal input from only one canal nerve. The remaining 2°canal neurons received their canal inputs mostly from two and, in a few exceptions, from all three canal nerves. This convergence pattern is compatible with results from earlier studies in the same species (Holler and Straka 2001; Straka et al. 1997). Afferent signals from individual otolith organs remained also largely separate at the level of 2°VN (91%)
because most 2° otolith neurons received an afferent otolith input from only one otolith nerve. Among the few 2°VN with an afferent input from two otolith organs, the combination of SA inputs and inputs from one of the other two otolith organs was particularly rare (see Fig. 6, B and C).

Convergence of afferent signals from one canal and from one otolith organ, however, was seen in a larger number of 2°VN. This convergence was not random but distinct combinations of afferent canal and otolith signals were more numerous than other combinations. In 2° LA neurons, the convergent canal input originated mainly from one of the two vertical canal nerves (Fig. 12, A and B) and in 2° UT neurons the convergent canal input originated mainly from the HC nerve (Fig. 12C). Afferent SA nerve signals, however, remained largely separate and did not converge with afferent canal nerve signals (Fig. 12D). The relatively few 2° VN with an afferent input exclusively from the HC nerve, encountered in the first set of experiments (utricle-canal convergence) is explained by a pronounced convergence between afferent UT and HC nerve signals. Likewise, the relatively few 2° VN with an afferent input exclusively from the AC nerve, which were encountered in the second set of experiments (lagena-canal convergence), is explained by the pronounced convergence of afferent LA and vertical canal nerve signals. Consistent with this explanation are equally large numbers of 2° HC, 2° AC, and 2° PC neurons, which were encountered in the third set of experiments (saccule-canal convergence), because of the absence of a convergence between afferent SA and canal nerve signals.

About 30% of 2° VN received afferent signals from one canal and one otolith (utricle or lagena) nerve. This percentage is most likely an underestimation because in each of the two sets of experiments, only one of the two possible canal-otolith combinations was tested. A more realistic estimation of the percentage of 2° VN with convergent afferent canal and otolith inputs may be that >50% of the 2° VN receive convergent afferent otolith and canal signals. Interestingly, the synaptic strength of converging canal and otolith inputs differed systematically. EPSPs in a given 2° VN evoked by afferent canal or otolith nerve stimulation differed in amplitude and were classified as major or minor inputs as in an earlier study on the convergence of afferent canal nerve inputs in 2° VN (Straka et al. 1997). The major input originated from a canal nerve in ~75% and from an otolith nerve only in ~25% of the identified 2° VN. This predominance of the canal input was similar for 2° canal + UT as for 2° canal + LA neurons.

In summary, 2° VN in frog typically receive converging afferent inputs from one canal and from one otolith nerve. Particular combinations were by far more frequently observed than other combinations. The prevalence of particular combinations of canal and otolith signals might be correlated with the fact that these combinations are more frequently coactivated during natural head movements than other combinations. In addition, afferent canal and otolith signals must be combined to create a reference frame that allows a determination of the plane of head rotation with respect to gravity. Rohregger and Dieringer (2002) have shown that the response vectors of ocular motor nerves for angular acceleration and the direction of linear acceleration for UT best responses in extraocular motor nerves are aligned in their spatial orientation. On the other hand, the absent convergence of afferent SA and canal signals is compatible with the assumption that the saccule of frogs is an organ sensitive for auditory or substrate vibration but not a classical vestibular organ (see DISCUSSION in the following text).

Convergence pattern of afferent otolith and canal signals in 2° VN of other vertebrates

A very similar separation of afferent canal signals at the level of 2° VN is also reported in pigeon (Wilson and Felpel
Convergence of Canal and Otolith Afferent Signals

The percentage of convergence between afferent PC and SA signals (Kushiro et al. 2000) was in part similar and in part different to that in frog. The convergence of afferent otolith signals (UT and SA; Zhang et al. 2001) compared with the pronounced convergence of these signals in frog (see Fig. 2B). In contrast to our data, the convergence pattern of afferent canal and otolith signals in cat was not as pronounced and spatially not as distinct as in frog. Some of these differences might be related to differences in the recorded population of 2° VN. In our study, recorded neurons were equally distributed throughout the vestibular nuclear complex as in former studies (see Straka et al. 1997, 2000). Recordings in the cat, however, were obtained predominantly in the lateral and descending vestibular nucleus and only to a minor degree in the superior and medial vestibular nucleus (Kushiro et al. 2000; Sato et al. 2000; Zakir et al. 2000; Zhang et al. 2001). This restriction could have biased the convergence pattern described for the cat, assuming that 2° VN in the SVN and the MVN receive a different combination of afferent canal and otolith signals.

Another difference in the organization of the ipsilateral afferent canal/otolith or otolith/otolith convergence is the presence of a reciprocal inhibition in a subpopulation of 2° VN in the cat (Kushiro et al. 2000; Sato et al. 2000; Zakir et al. 2000; Zhang et al. 2001) and the absence of such an input pattern in frog. However, this absence might be only apparent, assuming that an inhibition is superimposed by a simultaneous excitation in frog. Thus masking an IPSP. In fact, Straka et al. (1997) have shown that a heteronymous inhibition originating from one or both ipsilateral semicircular canals is in fact present in a given 2° canal neuron in frog. This inhibition, however, became only apparent after blocking the inhibition with a GABAergic antagonist (bicuculline) or a glycine antagonist (strychnine). Thus the difference between cat and frog in this aspect might be the presence of a larger number of excitatory inputs and/or more efficient excitatory inputs in frog and the absence or a minor contribution of excitatory inputs from the equivalent canal or otolith nerve branches in cat (Kushiro et al. 2000; Sato et al. 2000; Zakir et al. 2000; Zhang et al. 2001). A similar conclusion was drawn for commissural canal inputs in frog and cat 2° canal neurons. In addition to the commissural canal inhibition that originates from the contralateral coplanar canal in both frog (Holler and Straka 2001) and cat (Kasahara and Uchino 1974), an additional commissural excitation from one or both noncoplanar contralateral semicircular canals is present in frog but not in cat.

In spite of the same spatial organization of the labyrinthine sensory organs in the head, quantitative differences in the convergence pattern were expected between cat and frog, for instance, in VOR neurons because of differences in the lines of sight and in the pulling directions of extraocular muscles between cat and frog. In fact, quantitative species-specific differences in the convergence of inputs from different canals to abducens motoneurons were shown for closely related water and grass frogs (Pantle and Dieringer 1998). These differences corresponded to differences in the direction of the optic axes in both species. Such a species-specific coordinate transformation takes place in part at the level of 2° VN (see in the preceding...
text) and in part at the level of extraocular motoneurons (Graf et al. 1993).

Second-order vestibular neurons with ascending and/or descending axons

More 2°VSP than 2°VOR neurons were encountered even though recordings were equally distributed within the vestibular nuclei. Two reasons might account for this difference. First, a smaller number of extraocular motoneurons than spinal motoneurons might require fewer 2°VN with ascending and more with descending axons. Second, the selection of recorded neurons might have been biased toward 2°VSP neurons due to their larger size in comparison to 2°VOR neurons. Considerably lower average conduction velocities of 2°VOR neurons compared with most 2°VSP neurons (see Fig. 8, A and B) suggest that 2°VOR neurons

FIG. 9. Percentage distribution of afferent utricular and semicircular canal inputs in different groups of 2nd-order vestibular neurons. A–D: percentage distribution of afferent UT, afferent canal and converging afferent UT + canal nerve-evoked EPSPs in 2nd-order vestibular neurons (2°VN; A), in 2°VN with unknown projections (B), in 2nd-order vestibulo-spinal (2°VSP) neurons (C), and in 2nd-order vestibulo-ocular (2°VOR) neurons (D). Data in A were adopted from Fig. 2A. Numbers of neurons are indicated in parentheses.

Distribution of utricular inputs in antidromically identified vestibular neurons

A

2°VN identified by canal or UT input (242)

B

2°VN with unknown projections (75)

C

2°VSP neurons (27)

D

2°VOR neurons (21)

FIG. 10. Percentage distribution of afferent lagenar and semicircular canal inputs in different groups of 2nd-order vestibular neurons. A–D: percentage distribution of afferent LA, afferent canal and converging afferent LA + canal nerve-evoked EPSPs in 2nd-order vestibular neurons (2°VN, A), in 2°VN with unknown projections (B), in 2nd-order vestibulo-spinal (2°VSP) neurons (C), and in 2nd-order vestibulo-ocular (2°VOR) neurons (D). Data in A were adopted from Fig. 4A. Numbers of neurons are indicated in parentheses.
were in fact smaller than $2^\circ$VSP neurons. A similar bias toward more $2^\circ$VSP than $2^\circ$VOR neurons was also present in results obtained in cat (Kushiro et al. 2000; Sato et al. 2000; Zakir et al. 2000; Zhang et al. 2001) and monkey (Boyle et al. 1992).

The broad range of conduction velocities of frog $2^\circ$VSP neurons, indicative for a heterogeneous cell population, is consistent with results obtained in frog by Fanardjian et al. (1999, 2001). The distinct population of $2^\circ$VSP neurons with low conduction velocities (see Fig. 8A) was missing in the latter studies perhaps because of the use of recording electrodes with a much lower resistance by Fanardjian et al. (1999, 2001). Thereby, recordings were biased toward larger neurons with thicker axons. The conduction velocities of these larger neurons were rather similar to those described in this study (see Fig. 8A).

A limited number of neurons was classified as $2^\circ$VOS neurons. The small number of $2^\circ$VOS neurons could have resulted from a failure to evoke an antidromic spike from both the midbrain as well as from the spinal cord or from a small number of $2^\circ$VN with ascending as well as descending axon collaterals. Results from an anatomical study in larval ranid frogs (Straka et al. 2001) are consistent with the latter assumption. Double-labeling studies with fluorescent tracers revealed very few double-labeled neurons that were located in rhombomeres 5–7 (Straka et al. 2001), a hindbrain region where $2^\circ$VOS neurons were also located in this study. However, different to our results in frog, more $2^\circ$VOS neurons compared with $2^\circ$VSP or $2^\circ$VOR neurons were encountered in cat (Kushiro et al. 2000; Sato et al. 2000; Zakir et al. 2000; Zhang et al. 2001) and monkey (Boyle et al. 1992; Büttner-Ennever 1992).
**Functional role of otolith organs in frog and mammals**

The utricle is a classical vestibular organ sensing linear acceleration and gravity in frog (Blanks and Precht 1976; Lewis and Narins 1999) as in mammalian species (see Wilson and Melvill Jones 1979). UT afferents terminate in all major vestibular but not in auditory nuclei (frog: Birinyi et al. 2001; Will et al. 1985; monkey: Bütter-Ennever 1999). Moreover, 2°VN mediating UT signals project to ocular motor and spinal targets (Fig. 12C) and contribute to maculo-ocular as well as to spinal reflexes in frog (Hess and Precht 1984; McNally and Tait 1925; Rohregger and Dieringer 2002; Tait and McNally 1934) as in cat (Ikegami et al. 1994; Kushiro et al. 2000; Uchino et al. 1994, 1996; Wilson et al. 1977; Zakir et al. 2000; Zhang et al. 2001).

A similar clear situation exists for the saccule in mammals where this otolith organ is sensitive to vertical linear acceleration and gravity (Wilson and Melvill Jones 1979). A graviceptational role of the mammalian saccule is indicated by the termination of SA afferent fibers in the vestibular nuclei (see Bütter-Ennever 1999), by the presence of SA signals in particular spinal motoneurons that are involved in postural control (Sato et al. 1997; Uchino et al. 1997; Wilson et al. 1977) and by the presence of powerful sacculo-collic reflexes (Lacour et al. 1987; Xerri et al. 1987). However, in contrast to a contribution of SA signals to postural control, only very few 2°VOR neurons in cat receive afferent SA signals (Kushiro et al. 2000; Sato et al. 2000); this is compatible with the absence of a dysnaptic sacculo-ocular reflex pathway (Isu et al. 2000) and a very weak vertical maculo-ocular reflex (e.g., in rat, Hess and Dieringer 1991).

The situation for the lagena and the saccule of frogs is not as trivial and straightforward given that a vibration-sensitivity (Ashcroft and Hallpike 1934; Cortopassi and Lewis 1996, 1998; Koyama et al. 1982) as well as an equilibrium sensitivity (Caston et al. 1977; Gaëlle and Clemens 1973; Lannou and Cazin 1976) have been reported for both otolith organs. Most likely, both otolith organs play a dual role in frog, although, with a differential weighting of the function in vestibular and acoustic sensation (Cortopassi and Lewis 1996, 1998; Lewis and Narins 1999; Lewis et al. 1982; McCormick 1999). Accordingly, the frog lagena is predominantly a vestibular organ as evidenced from the projection of LA afferent fibers to all vestibular but not to auditory nuclei (Birinyi et al. 2001; Will et al. 1985) and from the pronounced convergence of afferent canal and LA nerve signals in 2°VN. A vestibular role of the lagena is further corroborated by the descending projection of 2°LA neurons (Fig. 12, A and B) and the contribution to postural reflexes (e.g., the righting reflex in frog) (see MacNaughton and McNally 1946) but not to maculo-ocular reflexes (Hess and Precht 1984; Rohregger and Dieringer 2002). Otolith organs with the same name in different vertebrate taxa are considered homologous organs. Accordingly, the major functional role must have changed during evolution (Harada et al. 2001; Lewis and Narins 1999). Thus assuming a functionally equivalent role of the saccule in cat and the lagena in frog, a similar basic organization of maculo-ocular and of canal-ocular reflexes is present in frog and cat including the absence of a three-neuronal pathway from a vertical otolith organ to extraocular motoneurons.

In contrast, the frog saccule is predominantly an acoustic organ sensing substrate vibrations (see Lewis and Narins 1999). Such a classification is corroborated by a termination of SA afferents mainly within the dorsal (auditory) and the SA nucleus (Birinyi et al. 2001; Matesz 1988; Suarez et al. 1985; Will et al. 1985), indicating a close connection of SA signals with the superior olive and the classical midbrain auditory nuclei (Kulik et al. 1994; Matesz and Kulik 1996; Wilczynski 1981). The functional role of the saccule is characterized by an absence of a convergence between afferent SA signals and afferent signals from other labyrinthine organs, the absence of SA signals from vestibulo-ocular projections (Fig. 12D), and the absence of SA signals from maculo-ocular reflexes (Hess and Precht 1984; Rohregger and Dieringer 2002) as well as from postural reflexes (McNally and Tait 1925). This absence of a contribution of SA signals to vestibular reflexes is compatible with a major role of the saccule as a vibration sensitive and not as a classical vestibular organ in frog.

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