Question of Reference Frames: Visual Direction-Selective Neurons in the Accessory Optic System of Goldfish

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Masseck OA, Hoffmann KP. Question of reference frames: Visual direction-selective neurons in the accessory optic system of goldfish. J Neurophysiol 102: 2781–2789, 2009. First published August 26, 2009; doi:10.1152/jn.00415.2009. We investigated if visual direction-selective neurons in the pretectal area (APT) of goldfish (Carassius auratus auratus) preferred visual stimuli resulting from rotations around axes corresponding to the best responsive axes of the semicircular canals (optic flow that is consistent to a maximal activation of the horizontal canal pair (yaw), to a maximal activation of the right anterior/left posterior semicircular canal pair (RALP), and to a maximal activation of the left anterior/right posterior semicircular canal pair (LARP)). Our sample of neurons recorded in the left pretectum had two preferred axes of rotation: first, rotation around the yaw axis and second, rotation around the RALP axis. Both axes of rotation correspond to best responsive axes of the semicircular canals. For this reason, coding in a reference frame defined by the vestibular system or the pulling direction of the eye muscles is suggested. In our population of recorded APT neurons, we did not find segregation of different preferred axes of rotation into different anatomical structures. Furthermore in all axes no bias for clockwise or counterclockwise rotations was obvious. This is particularly noteworthy for the yaw axis because preference for temporally biased nasal and naso-temporal rotations was found at the same recording side. Hence we conclude that in fish the accessory optic system may consist of one nucleus on each side of the midbrain only, the APT. Segregation into different nuclei coding for different axes and different senses of rotation probably first developed in amphibians.

INTRODUCTION

In general, two major gaze stabilization mechanisms exist in all vertebrates, the vestibuloocular reflex (VOR) and the optokinetic reflex (OKR). The two mechanisms complement each other in their response characteristics and usually are coactivated as self motion mostly results in activation of both sensory systems, i.e., head/body movements sensed by the semicircular canals are accompanied by image motion encoded by the retina (retinal slip). Thus the question arises about how the information from these different sensory modalities is combined into one appropriate sensory-motor signal to facilitate neuronal processing for gaze stabilization (Graf et al. 1988; Hengstenberg 1998; Wallman and Velez 1985; Wylie et al. 1998).

The discharge of extraocular motoneurons correlates with eye position and eye velocity, indicating that they code in a reference frame defined by the pulling directions of the extraocular muscles (Pastor et al. 1991). In most animals, the spatial organization of the extraocular muscle planes and semicircular canals are nearly aligned (Ezure and Graf 1984) independent of the position of the eyes (lateral vs. frontal eyed animals) (Graf and Simpson 1981). Thus the vestibular system and the motoneurons of the oculomotor nuclei already share one internal frame of reference. Simpson and co-workers described the relationship between the preferred rotational axes of the semicircular canals and the spatial organization of visual direction-selective neurons in the accessory optic system (AOS) that together with the pretectal nucleus of the optic tract comprise the subcortical neuronal substrate of OKR in mammals (Simpson 1984; Simpson et al. 1979, 1988). Recordings from the medial terminal nucleus (MTN) of the AOS and the visual tectal relay zone (VTRZ) of rabbits revealed neurons with monocular and binocular bipartite receptive fields, preferring opposing vertical stimulus movements in the left and right parts of the receptive field, making them ideal detectors for head rotations around the best responsive axes of the vertical semicircular canals. This observation led to the hypothesis that the AOS complies with the reference frame of the semicircular canals or the extraocular muscles (Simpson et al. 1988).

Wylie and Frost (1996) investigated response properties of direction-selective neurons in the AOS and nucleus lentiformis mesencephali (LM) of pigeons and revealed that preferred directions are rather aligned with the pulling directions of the extraocular muscles than with the best responsive axes of the vestibular system. In pigeons, the pulling direction of the lateral rectus muscle is oblique compared with that of the medial rectus muscle. Interestingly, this arrangement is mirrored in the horizontal preferred directions of AOS neurons, which are separated by an angle of 162° instead of 180°. In Wylie and Frost’s view, this peculiarity shows that the optokinetic system is organized with respect to extraocular muscles coordinates rather than to the vestibular system (Wylie and Frost 1996).

Former studies (Finger and Karten 1978) suggest that two pretectal nuclei represent the AOS in fish. By contrast, electrophysiological, histological, and lesion experiments from our laboratory indicate that only one of these nuclei, the pretectal area (APT) constitutes the AOS of bony fish (Klar and Hoffmann 2002). For chondrichyans (S. canicula), visual responses of pretectal neurons in a vestibular coordinate system could not be proven (Masseck and Hoffmann 2008). Thus the main objective of the present study was to investigate the evolution of the spatial reference frame of visual direction-selective neurons in the pretectal area of bony fish.

METHODS

Data from 23 goldfish were included in the present study. Animal size varied between 5 and 15 cm in length and included animals of both sexes. All experiments were approved by the local authorities (Regierungspräsidium Arnsberg, animal experiment approval num-
ber: 50.8735.1 Nr. 100/3) and carried out in accordance with the Deutsche Tierschutzgesetz of 12 April 2001, the European Communities Council Directive of 24 November 1986 (S6 609 EEC) and National Institutes of Health guidelines for care and use of animals for experimental procedures.

Before surgery animals were initially anesthetized by immersion in a bath containing 0.1% 3-aminobenzoic acid ethyl ester (MS222). Anesthesia was supplemented locally with 2.5% lidocaine, before a craniotomy was performed to allow access to the left tectum opticum and pretectum. Immediately after surgery the animals were immobilized with Flaxedil (0.5–1 mg) and transferred to a transparent recording hemisphere (Ø 70 cm), where they were artificially ventilated with cooled water (19°C). Single units were recorded with glass-coated tungsten microelectrodes or glass micropipettes (impedance 1–2.5 MΩ) in the left pretectum.

**Experimental setup**

The fish were fixed in a holding device inserted in a water filled opaque semicircular tank (Ø 70 cm). Animals were tilted 45° right side down. With this approach, the right eye was completely immersed and its visual field could be stimulated homogenously under water (Fig. 1). The wholefield visual stimulus consisted of random light dots (dot size: 2–4°, luminance: 2 cd/m²) projected into the hemisphere by a planetarium 15 cm in diameter, centered 10 cm above the fish’s head, and also tilted 45° to the right to be aligned with the yaw axis of the fish’s head. In addition, the planetarium was suspended above the goldfish head with gimbals such that the axis of rotation could be positioned to any orientation in three-dimensional space. Stimulus speed was kept constant at 10°/s, as former experiments from our laboratory have shown that 10°/s is optimal for direction-selective neurons in the APT of the trout (Klar and Hoffmann 2002). At the horizon for the right eye, a mean temporal frequency (dots had variable separations) of ~1 dot/s was reached at a velocity of 10°/s. Once a direction-selective neuron was isolated the receptive field size in the contralateral hemisphere was determined using a handheld lamp producing single spots of light (diameter: 4–10°). Mainly the contralateral (right) eye was stimulated. The ipsilateral eye was situated above the water surface and therefore out of focus. In addition, only a small portion of its visual field was stimulated by our planetarium (see Fig. 1). Stimulation of the left eye directly with the hand lamp never yielded a visual response. Translational optic flow was not used for stimulation of direction-selective neurons.

**Visual stimuli**

Visual stimulation with the random dot pattern (see preceding text) was achieved by rotating the planetarium around seven rotational axes in the midsagittal and horizontal plane. Four rotational axes were tested in both the midsagittal and horizontal plane. From this it follows that the roll axis was tested twice (Fig. 2).

**Stimulus axes lying in the midsagittal plane**

Four rotational axes separated by an angle of 45° in the midsagittal plane were used (Fig. 2A).

1) Planetarium rotation around the yaw axis (vertical axis of the fish) resulted in horizontal movements from temporal to nasal (TN) and nasal to temporal (NT) for the right eye (Fig. 2C). 2) Planetarium rotation around the roll axis (longitudinal axis of the fish) resulted in vertical movements from ventral to dorsal (roll up) and dorsal to ventral (roll down) in the right central visual field (Fig. 2C). 3) Planetarium rotation around the oblique down axis produced either temporal-down to nasal-up (N-up) or nasal-up to temporal-down (T-down) optic flow in the right central visual field (Fig. 2C). 4) Planetarium rotation around the oblique up axis resulted in nasal-down to temporal-up (T-up) or temporal-up to nasal-down (N-down) optic flow in the right eye (Fig. 2C) (for further details, see Masseck and Hoffmann 2008).

**Stimulus axes lying in the horizontal plane**

In addition, four rotational axes in the horizontal plane were tested to examine the responses to rotations around axes corresponding to maximal activation of the vertical semicircular canals (Fig. 2B). Rotation of the planetarium produced image motion on the right central retina as follows.

1) Roll planetarium rotation around the longitudinal axis of the fish produced optic flow either up (roll up) or down (roll down; Fig. 2D). 2) Left anterior/right posterior semicircular canal pair (LARP) planetarium rotation produced either optic flow (LARP up) consistent with a head movement maximally activating the left anterior semicircular canal or optic flow (LARP down) consistent with maximal activation of the right posterior semicircular canal (Fig. 2, B and D–F). 3) Pitch planetarium rotation around the interaural axis of the fish’s head produced either upward-directed optic flow in the frontal part of the central visual field (pitch up) or downward-directed optic flow in the frontal part of the central visual field (pitch down)). 4) Right anterior/ left posterior semicircular canal pair (RALP) planetarium rotation produced either optic flow (RALP up), consistent with maximal activation of the right anterior semicircular canal or (RALP down) consistent with maximal activation of the left posterior semicircular canal (Fig. 2, B and D–F).

Each trial consisted of a stationary phase (0–2,000 ms), a rotation in one direction (2,000–5,000 ms), another stationary phase (5,000–7,000 ms), and a rotation in the opposite direction (7,000–10,000 ms).

**Data analysis**

Action potentials were converted to TTL pulses by a window discriminator. Preamplified signals were acquired with CORTEX (NIMH, Laboratory of Neurophysiology, Version 5.96), and off-line analysis was performed with a customized Matlab program (version 7.0.1).

First of all for analysis of axis preference a directional index (DI) was calculated for each axis as follows.
DI = \frac{\text{max} - \text{min}}{\text{max}}

where max represents the maximal mean spike activity for a particular axis of rotation (i.e., yaw, roll, etc.) and min refers to the minimum mean spike activity for the same axis. All cells with a DI > 0.3 for at least one axis were taken into account for further calculations. The analysis was performed for all seven recorded axes of rotation, and the axis with the highest DI was considered to be the best responsive axis, independent of whether the rotation leading to the stronger response was clockwise or counter-clockwise. To test for significant differences between an equal distribution of preferred rotational axes against a distribution in favor of the vestibular system, a \(\chi^2\) test was used. In a further analysis also the sense of rotation (clockwise or counterclockwise) was considered. A fourfold test was used to determine if a bias for the sense of rotation or axes was present.

Afterward, in a second analysis, all recorded visual neurons were analyzed for their preferred and null-directions in each of the used stimulus planes, i.e., midsagittal and horizontal. A two-harmonic Fourier expansion was fitted to the data according to the following formula

\[ a_0 + a_1 \cos(x \cdot w) + b_1 \sin(x \cdot w) \]

where \(a_0, b_0, \ldots, a_n, b_n\) represent Fourier coefficients and \(0 \leq x \leq 2\pi\). Neurons with data fits with \(R^2 < 0.9\) were excluded from our further analysis. The null and preferred directions were taken as the absolute minimum and maximum of the function, respectively; the minimum and maximum were calculated using the first and second derivatives (Fig. 3) (see also Maioli and Ohgaki 1993).

Afterward, preferred directions and null directions of the whole population were plotted in a polar diagram for further analysis. For all cells where the Fourier fit failed, a multicomparison test (1-way ANOVA) was applied to test for axis selectivity. To judge a neuron as axis selective, activity of the preferred axis had to be significantly different from all other axes. All neurons that could not be classified as either direction- or axis-selective were called motion sensitive. Spontaneous activity was calculated by averaging activities from all

FIG. 2. A: illustration of stimulus axes in the midsagittal plane as indicated by the black circle. Yaw axis, vertical axis of the fish; roll, longitudinal axis of the fish; oblique up and oblique down lie intermediate between the roll and yaw axes. B: illustrations of stimulus axes lying in the horizontal plane, i.e., axes of rotation used to test for a vertical semicircular canal reference frame as indicated by the black circle. LARP: planetarium rotation around the LARP axis produced optic flow that is consistent with a maximal activation of either the left anterior or right posterior semicircular canal. Pitch, transverse axis of the fish head; RALP: planetarium rotation around the RALP axis produced optic flow that is consistent with a maximal activation of either the right anterior or left posterior semicircular canal. Roll, longitudinal axis of the fish. C: illustration of optic flow seen by the central 60° of the retina during stimulation with axes lying in the midsagittal plane. Rotation around the yaw axis resulting in horizontal movements from temporal to nasal (0°) and nasal to temporal (180°). Rotation around the roll axis resulting in vertical movements from ventral to dorsal (90°) and dorsal to ventral (270°). Rotation around the oblique up axis producing either temporal-down to nasal-up (45°) or nasal-up to temporal-down (225°). Rotation around the oblique down axis producing either temporal-down to nasal-up (45°) or nasal-up to temporal-down (225°). D: illustration of optic flow seen by the central 60° of the retina during stimulation with axes lying in the horizontal plane. E: frontal view of the goldfish with the orientation of the LARP and RALP canal planes in space. Indicated rotation would excite the left anterior canal maximally, LARP up, planetarium rotation would be the corresponding visual stimulus. F: top view of the goldfish head with LARP and RALP canal planes.

FIG. 3. Example of a 2-harmonic Fourier expansion fitted to the discrete firing rates. x axis, location of the stimulus axis lying in the horizontal plane. An azimuth angle of 0° corresponds to roll up, 45° to RALP up, 90° to pitch up, 135° to LARP up, 180° to roll down, 225° to RALP down, 270° to pitch down, 315° to LARP down. y axis, imp/s. Black dots, real data points; black crosses, calculated preferred and null-direction; gray line, mean spontaneous activity. Preferred direction of this direction-selective neuron is 45° and its null direction is 205°.
presentations of the stationary pattern. The results were tested for significant differences with a rank sum test and plotted in a bar plot.

**Histology**

After identification of direction-selective neurons with glass-coated tungsten microelectrodes, these were replaced by glass pipettes filled with either tetramethylrhodamine dextran (RD; MW 3000, anionic, lysine fixable, Molecular Probes, administered in 0.3 M PBS) or biotinylated dextran amine (BDA; MW 3000 Molecular Probes, administered in 0.05M PBS, pH 7.4). After reconfirmation of direction-selective neurons, tracers were iontophoretically injected (RD: positive current pulses 7 s on/3 s off, 10 µA for 30 min, BDA: positive current pulses 2 s on/2 s off, 10 µA 30 min) via the recording pipette to verify the recording site and reveal afferent and efferent projections.

After closure of the craniotomy and recovery, the fish survived for 1–4 days and were killed with an overdose of MS-222. Fish were perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) containing 10% sucrose. To avoid blood coagulation, 0.1 ml heparin was injected into the ventricle immediately before the perfusion. The fixed brain tissue was then removed and stored overnight in the same fixative at 4°C. For processing of the RD-injected animals, brains were cryoprotected with 30% sucrose in 0.1 M PB for another 24 h. Brains were then embedded in chicken albumin (Sigma), and 30-µm sections were cut on a cryostat. Always two series were collected whereby one was always used for RD fluorescence visualization and the other one was stained with cresyl violet. For BDA visualization, brains were immersed in 0.1 M PB (pH 7.4) containing 20% sucrose for 24 h, embedded in 5% agarose containing 20% sucrose and then frozen in n-Hexane. Serial sections were cut at 60 µm on a cryostat. Section were mounted on slides, dried, soaked in 0.1 M PB containing 0.5% Triton X-100 (pH 7.4) for 10 min. After washing twice in 0.1 M PB for 10 min, sections were treated with 3% H₂O₂ in 10% methanol to block endogenous peroxidase activity. Sections were rinsed two times in 0.1 M PB and then incubated for 2 h in an avidin-biotin complex solution (ABC elite kit); after washing two times in 0.1 M PB and one time in 0.05 M tri-(hydroxymethyl)-aminomethan (pH 7.1), sections were reacted for 8–20 min in 3,3’-diaminobenzidine solution containing 0.4% nickel-ammonium sulfate and 0.015% H₂O₂. Afterward sections were rinsed three times in 0.05 M Tris and one time in distilled water. Finally sections were counterstained with cresyl violet, dehydrated, and cover-slipped.

To obtain data about the spatial relationship of the semicircular canals and the extraocular muscles, two formalin fixed goldfish heads were dissected. Skin and bone above the eyeballs were carefully removed to get access to the superior rectus and superior oblique muscles. Additionally skin and bone above the labyrinth organ were removed to uncover the position of the vertical canals. Afterward a picture of the goldfish head was taken and the enclosed angles between the midline, the extraocular muscles and the vertical semicircular canals were measured in an image editing program.

**RESULTS**

A total of 61 neurons were recorded during rotation of the planetarium around axes lying in both the midsagittal and horizontal planes. All neurons had large receptive fields located in the contralateral (right) visual field. Receptive fields included large parts of the contralateral visual field (−20° azimuth to 160° azimuth; approximately −75° elevation to +45° elevation). With our approach, bipartite receptive fields could not be observed, as receptive fields were stimulated with the projected planetarium image covering the entire receptive field. Using single light dots to investigate receptive field size, we never observed a bipartite receptive field. During the presentation of the stationary stimulus, all direction-selective neurons were spontaneously active, whereas during stimulation in their preferred direction they tonically enhanced their firing rate as long as the stimulus moved. In contrast, stimulation in the null-direction led to tonic suppression of activity. A transient movement onset burst was only recognized in one third of the direction-selective neurons.

Iontophoretic tracer applications after successful recordings of direction-selective responses confirmed that all recorded neurons where located within the pretectal area (Fig. 4).

**Do preferred rotational axes of APT neurons correspond to the best responsive axes of the semicircular canals?**

Responses following stimulation in each of the seven rotational axes lying in the midsagittal and horizontal plane were analyzed. Three of the axis (yaw, LARP, and RALP) correspond to the best responsive axes of the semicircular canals. A directional index was calculated for each stimulation axis (see METHODS), and the axis with the highest directional index was considered to be the best responsive one.

Fifty cells of the 61 recorded had a directional index >0.3 for at least one of the axes tested. Twenty-one cells (42%) preferred rotations around the yaw axis, 14 (28%) around the RALP axis, 2 (4%) around the LARP axis, 7 (14%) around the roll axis, and the remaining three axes (pitch, oblique up, oblique down) were each represented by 2 direction-selective cells (4%) (Fig. 5).

A nonuniform distribution of preferred axes was evident. Neurons with visual direction selective responses to rotations around the yaw axis are the most frequent (n = 21; 42%).
Directional tuning in the horizontal plane

Stimulated by the planetarium rotating around axes in the horizontal plane 23 neurons of our sample of 61 recorded neurons were direction-selective when analyzed with a two-harmonic Fourier expansion; all remaining were motion sensitive, i.e., none was axis selective. Sixteen of the 23 neurons were direction selective also for stimulation axes lying in the midsagittal plane.

An example of a direction-selective neuron is depicted in Fig. 6A (the same neuron was also direction-selective for stimulus axes lying in the midsagittal plane, Fig. 6B). This neuron’s preferred optic flow direction was between RALP up and roll up (42° azimuth). This characteristic simply means that rotations around the roll and RALP axes producing upward-directed optic flow in the contralateral central visual field were preferred by this neuron. For all 23 direction-selective neurons, activity in the null-direction was always suppressed below spontaneous activity (P ≤ 0.001). In some cases, direction-selectivity in the horizontal plane was seen only as suppression of activity.

The distribution of the 23 preferred rotational axes measured with rotations around axes in the horizontal plane is depicted in Fig. 7A, where at first glance a bimodal distribution around the RALP axis is evident. On closer inspection, preferred direction vectors fall into two opposing quadrants clustered around the RALP axis, i.e., a rotation around the RALP axis was always preferred. The mean angle of preferred directions in the first quadrant (I) is 34° azimuth, whereas in the third quadrant (III) the mean angle is 229° azimuth (Fig. 7A).

To calculate the best responsive axis of the whole population with a modified Rayleigh test for circular bimodal data, all preferred direction vectors are plotted modulo 180°. The purpose of this calculation was to consider only the axis of rotation and to disregard the sense of rotation. Vectors from the third quadrant (III) were mirrored at the origin and the mean population vector was calculated again. The resulting angle of the mean population vector is now 42° azimuth. A modified Rayleigh test for bimodal distributions confirms a significant axial orientation of the preferred directions around the RALP axis (P < 0.001). Thus with axes of rotation in the horizontal plane neurons in the left APT would be maximally excited by optic flow fields produced by head and/or body rotations around the RALP axis (Fig. 2), which maximally excite the contralateral (right) anterior semicircular canal or the ipsilateral (left) posterior semicircular canal.

Directional tuning in the midsagittal plane

The responses of the same 61 neurons but now tested with the stimulus axes lying in the midsagittal plane were again analyzed with a two-harmonic Fourier expansion. Thirty of the 61 were direction selective, one was axis selective, and the remaining were motion sensitive. About half (n = 16) of these 30 direction-selective neurons were also direction selective when analyzed with stimulus axes lying in the horizontal plane.

A peristimulus time histogram (PSTH) and raster plot of such a direction-selective neuron is shown in Fig. 6B. As shown in Fig. 6B, this neuron preferred optic flow directions that had temporor-nasal and upward movement components.
in the right central visual field. Its computed preferred direction is 18° elevation, which is close to a rotation around the roll up (0° elevation) axis with temporal upward-directed optic flow in the right central visual field. Mean activity in the null-direction was 1 imp/s, in the preferred direction it was 23 imp/s, and average spontaneous activity was 8 imp/s. All recorded visual direction-selective neurons exhibited a suppression of activity in the null-direction ($P < 0.001$). In some cases, direction selectivity was seen only as suppression of activity.

Figure 7B shows the distribution of the preferred directions of the 30 direction-selective neurons during rotations around axes in the midsagittal plane. No bias for any direction nor a
uni- or bimodal distribution was evident (Rayleigh-test, $P$ value = 0.17).

Nine of the 30 neurons preferred rotations around the roll and oblique up and oblique down axes in the midsagittal plane; interestingly all of them were also direction selective for stimulus axes lying in the horizontal plane. Six of the nine had their overall maximal response to rotations around the RALP axis in the horizontal plane. In conclusion, most of the neurons that did not have yaw as their preferred axis of rotation in the midsagittal plane had their maximal response actually for rotations around the RALP axis in the horizontal plane.

The remaining 21 of the 30 neurons showing direction-selective responses during planetarium rotations around axes in the midsagittal plane preferred rotations around the yaw axis. From these 21, 14 were exclusively direction-selective during yaw rotations (which appeared only in the midsagittal plane). The remaining seven were direction selective for both yaw and stimulus axes lying in the horizontal plane. However four out of these seven had their maximal responses actually for rotations around the yaw axis. In conclusion, almost all direction-selective neurons which had yaw as preferred rotational axis had also their maximal response during rotations around the yaw axis.

If we finally consider only the direction-selective neurons, which had their maximal response during rotations with axes in the midsagittal plane a bias for the yaw axis is evident (Fig. 7C; bimodal Rayleigh test, $P < 0.001$).

In summary, 61 neurons were recorded, 23 of them were direction selective when stimulated with axes lying in the horizontal plane; however, 16 of these were direction selective in both stimulus planes used. Of these 16, 9 had as overall preferred axis RALP, 6 yaw, and 1 was best responsive to a non-semicircular canal axis. Whereas the remaining 7 neurons of the 23 had RALP as preferred axis of rotation.

Thirty neurons were direction-selective when stimulated with axes lying in the midsagittal plane, 16 of these were direction selective in both stimulus planes used (see previous paragraph), the remaining 14 were only direction-selective when stimulated with axes lying in the midsagittal plane, 11 of the 14 had YAW as preferred rotational axis, and the other 3 preferred a non-semicircular canal axis. If we now assign each neuron to one particular axis, 16 neurons preferred rotations around the RALP axis, 17 preferred rotations around the yaw axis, and the remaining 4 preferred rotations around a non-semicircular canal axis.

In conclusion, a clear bias for rotations around the yaw and RALP axis is evident in our sample of visual direction-selective neurons.

Spatial relationship of the vertical semicircular canals and the extraocular muscles

As in other vertebrates, a strong alignment of the semicircular canal planes and extraocular muscles planes is evident on the two dissected fish heads. The anterior vertical canal is aligned with the ipsilateral vertical recti muscles, while the posterior vertical canal is aligned with the ipsilateral oblique muscles (Fig. 8). The superior oblique and the superior rectus
muscle enclosed on average \((n = 2)\) an angle of 52° with the midline, whereas the anterior semicircular canal plane formed an angle of 44° with the midline and the posterior semicircular canal enclosed an angle of 43° with the midline (Fig. 8). Hence the spatial orientation of the semicircular canal planes and the extraocular muscles planes is strongly aligned in goldfish as well.

**DISCUSSION**

**General properties of direction-selective neurons**

Sixty-one neurons were isolated in the left APT of 23 goldfish. All direction-selective neurons had large receptive fields in the contralateral (right) visual field and were most sensitive to large moving stimuli. Hence direction-selective neurons in goldfish have properties remarkably similar to direction-selective neurons in the AOS of lateral eyed tetrapods (Collewijn 1975; Katte and Hoffmann 1980; Winterson and Brauth 1985).

As direction-selective neurons in the APT of bony fish share the same properties as direction-selective neurons in the AOS of tetrapods and afferent and efferent connections of the APT are remarkably similar to the connectivity of the AOS in tetrapods other than mammals, i.e., direct projections to the oculomotor nuclei, the cerebellum and the inferior olive are evident (unpublished results), we conclude that the APT is indeed the homologue structure to the AOS of tetrapods.

Like in sharks and trout (Klar and Hoffmann 2002; Masseck and Hoffmann 2008), no anatomical separation of neurons preferring stimuli produced by rotation around different axes is present. We suggest that the AOS of fish is only composed of one nucleus where clockwise and counterclockwise rotations around the best responsive axes of the semicircular canals are represented equally (in contrast to the purely anatomical considerations of Finger and Karten 1978). This arrangement of the AOS differs from that of the AOS of tetrapods other than mammals, where a pretectal nucleus (LM) codes mainly for temporono-nasal motion and a tegmental nucleus of the basal optic root (nBOR), representing their AOS, codes for other directions except temporono-nasal. In mammals, further segregation in the AOS occurs: the lateral terminal (LTN) and medial terminal nucleus (MTN) encode vertical motion, and the dorsal terminal nucleus (DTN) is most sensitive to horizontal ipsiversive stimulus movement, like the pretectal nucleus of the optic tract (NOT). It seems that during evolution a higher grade of differentiation in the AOS is reached (Ebbesson 1980).

**Comparison of visual direction-selective neurons in the APT with direction-selective neurons in the AOS of tetrapods**

The majority of neurons recorded in this study fall into two populations, one preferring rotation around the yaw axis and the other preferring rotation around the RALP axis. Our results in the goldfish APT are consistent with results obtained in the AOS of rabbits, cats, and pigeons.

In the rabbit, neurons in the left MTN and VTRZ preferred predominantly movement around the RALP axis in the horizontal plane (Simpson et al. 1988), whereas the other preferred axis (LARP) of the vertical semicircular canal system was less prominently represented.

Some of the recorded neurons in the MTN and VTRZ of rabbits had bipartite receptive fields with a preferred direction in one part of the receptive field opposite to the one preferred in the other part. Neurons with these receptive field properties are suitable candidates to detect rotational flow fields (Simpson et al. 1988). Our stimuli were not designed to reveal bipartite receptive fields in the goldfish. Thus it remains to be elucidated if bipartite receptive fields are also present in the APT of goldfish. However, during the receptive field mapping, we never recognized a bipartite organization. Even in the MTN of rabbits bipartite receptive fields were infrequent (Simpson et al. 1988). Simpson et al. (1988) also reported that the majority of recorded neurons in the VTRZ had binocular receptive fields. In our experimental setup, a homogenous stimulation was only possible for the right visual field, the left eye was not tested routinely; so binocular receptive fields might not have been recognized by our approach. However, binocular receptive fields are quite rare in the AOS in animals with lateral eyes and totally crossed optic nerves (e.g.: Wylie 2000).

Because direction-sensitive neurons in the AOS, the inferior olive (Leonard et al. 1988) and the flocculus (Graf et al. 1988) were best responsive to optic flow fields produced by rotations around the best responsive axis of the semicircular canals, Simpson and co-workers suggested an internal reference frame of these visual direction-selective neurons that is quite similar to the reference frame defined by the geometrical relationship of the three semicircular canal pairs or the extraocular muscles planes (Graf et al. 1988; Simpson et al. 1988).

Wylie and Frost (1996) investigated the spatial arrangement of direction-selective neurons in the nBOR and LM of pigeons and found that their preferred directions rather correspond to the pulling directions of the extraocular muscles than to the vestibular system. For this reason, Wylie and co-workers suggested an internal reference frame of visual rotational-sensitive neurons that complies with the spatial arrangement of the pulling directions of extraocular muscles.

Support for coding in an extraocular muscle reference frame comes also from recordings in the cat LTN where direction-selective neurons within the left LTN had a bimodal distribution of preferred directions with one peak around 20° azimuth and another around 60° azimuth for stimulus axes lying in the horizontal plane (Maioli and Ohgaki 1993). In cat, these bimodal peaks correspond well with the orientation of the pulling direction of the vertical recti (29° azimuth) and vertical oblique (57° azimuth) muscles. Therefore Maioli and Ohgaki (1993) concluded that sensory information in the LTN is coded in an extraocular muscle frame.

In addition to rotational-sensitive neurons in the AOS of pigeons, Wylie and colleagues (1998) found translation-sensitive neurons with binocular receptive fields in the nBOR and in the uvula and nodulus of the vestibulocerebellum. We cannot exclude that also in the AOS of fish translation-sensitive neurons can be found. In fact, some of the recorded neurons might be suboptimally stimulated translational neurons, as optic flow occurring during self-rotation or translation about a particular axis is more or less similar for monocular receptive fields. However, this does not affect our hypothesis because both self-rotation and translation share a semicircular canal based reference frame (Wylie et al. 1998). We assume that in the AOS of fish, binocular responses are rare or even non-existent because interconnections between both pretectal areas are missing and direct visual input is mediated only by the contralateral retina (unpublished results). Therefore binocular
neurons can first be postulated in the inferior olive or the vestibulocerebellum. Only at later processing stages could binocular and bipartite receptive fields be formed to analyze self-motion in three-dimensional space.

Reference frame of the AOS in goldfish

A main objective of our study was to determine the internal reference frame in which AOS neurons code self-motion. A high proportion of the direction-selective neurons in the left APT are maximally excited by optic flow fields produced by head or body rotations around the RALP and yaw axes, which maximally excite the contralateral (right) anterior vertical canal or the ipsilateral (left) posterior vertical canal and the horizontal canals, respectively. If one assumes, like Graf et al. (1988) for the AOS of rabbits, that neurons in the right APT behave mirror symmetrically to those recorded in the left APT, they should be maximally excited by optic flow stimuli produced by head or body rotations around the LARP and yaw axes. This arrangement would lead to a three-dimensional vestibular reference frame based on the orientation of the yaw, RALP, and LARP axes in space. It seems that the AOS and downstream structures are composed in a way that they prefer optic flow that is generated either by rotation around the best responsive axis of the horizontal semicircular canals (yaw axis) or by rotations around an axis that maximally excites or inhibits the vertical canals (LARP or RALP axis). We assume that such an internal reference frame (based on the orthogonal orientation of the semicircular canals) is the most parsimonious solution to encode self-motion for gaze stabilization.

Our own dissections of semicircular canals and extracocular muscles in goldfish revealed a strong alignment of extracocular muscles and semicircular canal planes (Fig. 8) as is the case for a number of lateral- and frontal-eyed animals, including humans (Graf and Simpson 1981). Because the vestibular and extracocular muscle reference frames are in such close spatial relationship in the goldfish, the question in which one of them the AOS is coding cannot be decided on the basis of these data.

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