Dissertation

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Studies of the evolution of visual and visuo-motor structures in vertebrates

by

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I certify herewith that the dissertation at hand was completed and written independently and without outside assistance. The “Guidelines for Good Scientific Practise” according to § 9, Sec. 3 were adhered to. This work has never been submitted in this or similar form at this or any other domestic or foreign institution of higher learning as a dissertation.

Bochum, 1.7.08

Olivia Andrea Masseck
Dedicated to my grandparents Albert & Liebtraut Lehmann
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<tr>
<td>A II</td>
<td>Area II</td>
</tr>
<tr>
<td>APT</td>
<td>Area pretectalis</td>
</tr>
<tr>
<td>AON</td>
<td>Accessory optic nucleus</td>
</tr>
<tr>
<td>AOS</td>
<td>accessory optic system</td>
</tr>
<tr>
<td>AOTMD</td>
<td>area optica tegmenti mesencephali dorsalis</td>
</tr>
<tr>
<td>CC</td>
<td>Corpus cerebelli</td>
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<tr>
<td>CgL</td>
<td>corpus geniculatum laterale</td>
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<td>Cho</td>
<td>chiasma opticum</td>
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<tr>
<td>CPN</td>
<td>central pretectal nucleus</td>
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<tr>
<td>DI</td>
<td>directional index</td>
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<tr>
<td>DS</td>
<td>direction-selective ganglion cell</td>
</tr>
<tr>
<td>DTN</td>
<td>dorsal terminal nucleus</td>
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<tr>
<td>EOG</td>
<td>electrooculography</td>
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<tr>
<td>FL</td>
<td>facial lobe</td>
</tr>
<tr>
<td>G</td>
<td>gain</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutric acid</td>
</tr>
<tr>
<td>Gp</td>
<td>nucleus geniculatus pretectalis,</td>
</tr>
<tr>
<td>gr</td>
<td>granular layer of the cerebellum</td>
</tr>
<tr>
<td>hOKR</td>
<td>horizontal optokinetic reflex</td>
</tr>
<tr>
<td>HRP</td>
<td>horse radish peroxidase</td>
</tr>
<tr>
<td>IO</td>
<td>inferior olive</td>
</tr>
<tr>
<td>Pret</td>
<td>nucleus pretectalis</td>
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<tr>
<td>LARP</td>
<td>left anterior right posterior axis</td>
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<td>LLF</td>
<td>lateral longitudinal fasciculus</td>
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<td>lateral rectus muscle</td>
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<td>MTN</td>
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<tr>
<td>MW</td>
<td>molecular weight</td>
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<td>nBOR</td>
<td>nucleus of the basal optic root</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>NC</td>
<td>nucleus corticalis</td>
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<td>NDL</td>
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<tr>
<td>NDM</td>
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<td>NO</td>
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<td>NOCPd</td>
<td>nucleus opticus commissurae posterioris pars dorsalis</td>
</tr>
<tr>
<td>NOCPv</td>
<td>nucleus opticus commissurae posterioris pars ventricularis</td>
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<td>NODPT</td>
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<td>NOPC</td>
<td>nucleus optic pretectalis centralis</td>
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<td>NOT</td>
<td>nucleus of the optic tract</td>
</tr>
<tr>
<td>NOTMAT</td>
<td>nucleus opticus dorsomedialis anterior thalami</td>
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<tr>
<td>NOTMV</td>
<td>nucleus opticus tegmenti mesencephali ventralis</td>
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<td>NPH</td>
<td>nucleus praepositus hypoglossi</td>
</tr>
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<td>NRTP</td>
<td>nucleus reticularis tegmenti pons</td>
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<tr>
<td>NT</td>
<td>naso-temporal</td>
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<tr>
<td>NTTOM</td>
<td>nucleus thalamicus tractus optici marginalis</td>
</tr>
<tr>
<td>NVL</td>
<td>nucleus ventralis lateralis</td>
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<tr>
<td>OKAN</td>
<td>optokinetic after nystagmus</td>
</tr>
<tr>
<td>OKN</td>
<td>Optokinetic nystagmus</td>
</tr>
<tr>
<td>OKR</td>
<td>optokinetic reflex</td>
</tr>
<tr>
<td>PB</td>
<td>phosphate buffer</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffer saline</td>
</tr>
<tr>
<td>PD</td>
<td>preferred direction</td>
</tr>
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<td>PITCH</td>
<td>transverse axis</td>
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<td>PRN</td>
<td>post rotatory nystagmus</td>
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<tr>
<td>RALP</td>
<td>right anterior left posterior axis</td>
</tr>
<tr>
<td>RD</td>
<td>Tetramethyl rhodaminexetrane</td>
</tr>
<tr>
<td>RF</td>
<td>reticular fomation</td>
</tr>
<tr>
<td>ROLL</td>
<td>longitudinal axis</td>
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<tr>
<td>SCN</td>
<td>nucleus suprachiasmaticus</td>
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<td>TeO</td>
<td>Tectum opticum</td>
</tr>
<tr>
<td>Th</td>
<td>thalamus</td>
</tr>
<tr>
<td>Thdl</td>
<td>thalamus dorsalis pars lateralis</td>
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<tr>
<td>Thvl</td>
<td>thalamus ventralis pars lateralis</td>
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<tr>
<td>TN</td>
<td>temporo-nasal</td>
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<td>TR</td>
<td>tuberal region</td>
</tr>
<tr>
<td>TW</td>
<td>tuning width</td>
</tr>
<tr>
<td>VA</td>
<td>vertical axis neurons</td>
</tr>
<tr>
<td>VL</td>
<td>vagal lobe</td>
</tr>
<tr>
<td>VN</td>
<td>vestibular nuclei</td>
</tr>
<tr>
<td>VOR</td>
<td>vestibulo-ocular reflex</td>
</tr>
<tr>
<td>VPI</td>
<td>Velocity-to-position-integrator</td>
</tr>
<tr>
<td>VTRZ</td>
<td>Visual tegmental relay zone</td>
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<tr>
<td>YAW</td>
<td>vertical axis</td>
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<tr>
<td>III</td>
<td>nucleus oculomotorius</td>
</tr>
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<td>IV</td>
<td>nucleus trochlearis</td>
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<tr>
<td>VI</td>
<td>nucleus abducens</td>
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1. General introduction

1.1 Visual structures in fish

Fish represent the “superclass” of vertebrates with the richest variety and number of species. Most of them possess specific adaptations in their visual system, like specialisations of the optics or the retina, even the occurrence of visual related nuclei varies from species to species. So far only the goldfish (*Carassius auratus auratus* L.) and the zebrafish (*Danio rerio* L.) have been examined closer.

Although the occurrence of visual nuclei is highly variable, all fish have completely crossed optic nerves and retinofugal targets are always located in the diencephalon and the mesencephalon (Smeets 1981), where main parts of retinal input terminate in the pretectum and tectum opticum. The pretectum is located just in front of the rostral margin of the midbrain tectum and comprises both diencephalic and mesencephalic components (Nieuwenhuys 1998).

1.1.2 Chondrichtyans (i.e. *Scyliorhinus canicula*)

*Scyliorhinus canicula* is a good model organism to investigate the evolution of visuo-motor structures, as it belongs to the order of Carcharhiniformes, which comprises most common of all living sharks. Within this order *S. canicula* is one of the more primitive representatives of ground-dwelling nocturnal sharks and can be found in the Northeast Atlantic and in the Mediterranean Sea.

Bozzano and Collin (2000) showed that the small spotted dogfish (*S. canicula*, our model animal) owns a horizontal visual streak, with a ganglion cell densities of 1,500 - 2000 cell per mm$^2$ and a temporal area with the highest density of 2396 cells per mm$^2$.

Further descriptions of shark retinae features are missing in the literature.

For chondrichtyans two studies described in detail retinorecipient areas. Different tracing approaches in these studies have lead to different results. Smeets (1981) named six retinofugal nuclei in *S. canicula*: the nucleus suprachiasmaticus (NSC), thalamus dorsalis pars lateralis (Thdl), thalamus ventralis pars lateralis (Thvl), corpus geniculatum laterale (Cgl), nucleus pretectalis (Pret) and the Tectum opticum (TeO).

Whereas Reperant et al. (1986) revealed eleven retinofugal areas, namely the nucleus suprachiasmaticus (SCN) in the hypothalamus, the nucleus opticus dorsolateralis anterior thalami (NODLAT), Nucleus opticus dorsomedialis anterior thalami
1. General introduction

(NODMAT), nucleus thalamicus tractus optici marginalis (NTTOM) also named as Cgl, nucleus opticus ventralis thalami (NOVT), nucleus opticus dorsalis posterior thalami (NODPT) in the thalamus, the nucleus optic pretectalis centralis (NOPC), nucleus opticus commissurae posterioris pars dorsalis (NOCPd), nucleus opticus commissurae posterioris pars ventricularis (NOCPv) in the pretectum, the optic tectum and the area optica tegmenti mesencephali dorsalis (AOTMD) and the nucleus opticus tegmenti mesencephali ventralis (NOTMV) in the mesencephalic tegmentum. Own intraocular tracer injections resembled results by Smeets (1981); therefore Smeets nomenclature is used throughout this thesis.
1.1.3 Osteichthyes (**Carassius auratus auratus**)

*Carassius auratus auratus* is a member of the Cypriniformes and has become the traditional model organisms in fish research. The “modern” companion is besides the zebrafish. We selected the goldfish for its brain size as experimental animal for our research.

Four morphological distinct types of ganglion cells (Fig. 1.2) are described for the goldfish retina (Hitchcock and Easter, 1996). Not only on a morphological basis can retinal ganglion cells be classified, but also on a physiological level: comparable to the cat’s retina three distinct types exist, X-, Y and W cells (Bilotta and Abramov, 1989). Receptive fields of X-cells are tonic concentric, receptive fields of Y-cells are phasic concentric and W-cells can be either tonic or phasic in their response properties. Indeed W-cells can have various kinds of response characteristics, like contrast sensitivity, colour specificity, direction-selectivity and orientation-selectivity. W-ganglion cells provide input to the accessory optic system (see 2.1.2).

So far a correlation of morphological and physiological classification is missing for the goldfish, whereas in other vertebrates physiological properties and morphology are consistent with each other (Boycott and Wässle, 1974). α ganglion cells have the largest

![Figure 1.2: Four morphological types of ganglion cells in the goldfish retina adapted from Hitchcock and Easter 1996. O.D. and arrow head indicate the position of the optic disc relative to the displayed ganglion cells](image-url)
somata, far-reaching dendrites and thick axons, $\beta$ cells posses small, fine dendritic trees and medium-sized somata and $\gamma$ ganglion cells own the smallest somata and thinnest axons of all types and have far-reaching dendrites. $\alpha$ cells correspond to the physiological class of Y-cells, $\beta$ cells to X-cells and $\gamma$ cells are related to W-cells. (Boycott and Wässle, 1974)

The goldfish retina has a higher ganglion cell density in dorsalmost parts of the retina (5404 cells/mm$^2$) compared to more nasal parts (4696 cells/mm$^2$). Hence Mednick and Springer (1988) concluded a temporal located area centralis in the goldfish retina.

The goldfish brain, as in all other vertebrate brains, can be divided into five distinct brain regions, telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon.

The tectum opticum and pretectum belong to the mesencephalon, whereas the metencephalon comprises the cerebellum and the myelencephalon the hindbrain. A specific characteristic of the cyprinid brain (e.g. goldfish brain) are the vagal lobes, where gustatory afferents terminate (see Fig. 1.3).

A variety of anatomical and histological studies on teleost fish were done in the past decades. Nevertheless, a consistent nomenclature is not available. As already stated by Vanegas and Ito (1983) “The teleostean thalamus and pretectum constitute a veritable tower of Babylon”. Generally nine nuclei and the tectum opticum (TeO) are described as retinofugal areas, however the description of their occurrence and specificity differs from species to species.

In goldfish, the suprachiasmatic nucleus (SCN) and the tuberal region (TR) of the hypothalamus are directly innervated by the retina. Furthermore three diencephalic nuclei receive retinofugal fibers, the nucleus dorsolateralis thalami (NDL), the nucleus dorsomedialis thalami (NDM) and the nucleus ventralis lateralis (NVL). In addition four retinorecipient targets are situated in the pretectum, the nucleus lateralis geniculatus (LGN), the area pretectalis (APT), the nucleus pretectalis (Pret) and the nucleus corticalis (NC). Our goldfish brain nomenclature refers to Peter and Gill (1975).
1.2. Visual structures in reptiles (i.e. Gekkonidae)

Geckos are small lizards occurring in tropical and subtropical regions. Gekkonidae have an interesting history of development, as primarily nocturnal geckos developed from primarily diurnal geckos with pure cone retinas (Walls, 1934, 1942). Hence they have rods which are actually modified cones (Röll, 2000). Some of the primarily nocturnal genera underwent another developing step and became diurnal again and transmuted their visual cells back to cones. For nocturnal genera foveae have never been shown. In contrast diurnal geckos posses a centrally located fovea (Underwood, 1951; Tansley, 1964; Röll, 2000).

In our study five different gecko genera were used. Diurnal foveate geckos belonging to following species Lygodactylus capensis, L. bradfieldi, L. chobiensis, L. arnoulthi and Phelsuma madagascariensis and following nocturnal afoveate geckos Lepidodactylus lugubris, Gecko gecko and Eublepharis macularis. All utilised diurnal geckos had centrally located fovea, where foveae of the genus P. madagascariensis are less specialized and shallower as in the other used genera where the foveal pit has a concaviclicate shape.

A large part optic nerve fibers cross to the contralateral side, only a small amount of fibers stays ipsilateral. Retinofugal fibers terminate in following nuclei in the thalamus dorsal and ventral parts of the lateral geniculate nucleus and ventral parts of the ventrolateral nucleus are innervated. At the pretectum the nucleus geniculatus pretectalis, the nucleus lentiformis mesencephali and nucleus posterodorsalis are termination sites of retinofugal fibers. Furthermore the tectum opticum and the nucleus of the basal optic root in the ventral tegmentum receive direct retinal projections. Ipsilateral the thalamic lateral geniculate nucleus, all prepectal nuclei and the tectum opticum receive sparse retinal fibers (Nortcutt and Butler, 1974).
1.3. Gaze stabilizing mechanisms

To maintain stable gaze during ego and environmental movements two mechanisms exist, the optokinetic reflex (OKR) and the vestibulo-ocular reflex (VOR). The OKR is mediated through direction-selective retinal ganglion cells, whereas the VOR is conveyed by hair cells in the semicircular canals. Under natural conditions both reflexes are coactivated. Anyhow, the working range of the OKR and VOR are quite different: direction-selective cells mediating the OKR are more sensitive to slow field motion, which makes them an ideal complement to the semicircular canal system, which is only an effective speed detector for high accelerations. Even more, the OKR can monitor constant velocities, whereas the semicircular canals system fails to do so. (i.e. Soodak and Simpson 1988).

These two reflexes are so essential that all vertebrates own them (e.g.: fish: Beck et al., 2004; Easter et al., 1974; amphibians: Fite, 1985; Chochran et al., 1985, Dieringer and Precht, 1982; reptiles: Ariel, 1997; Fite et al. 1979; Tauber and Atkin, 1968; birds: Gioanni et al., 1981; Wallmann, 1985; mammals: ter Braak 1936, Hess et al., 1985; Hanke et al., 2008; Kato et al., 1986; Klauer et al. 1990,).

Not only are these two reflexes highly conserved among vertebrates but also the arrangement of extraocular muscles is consistent. All vertebrates have six extraocular eye muscles, four rectus muscles (superior, inferior, medial, and lateral) and two oblique muscles (superior and inferior). They build up three pairs, which act in an antagonistic fashion: medial rectus and lateral rectus, inferior and superior recti and superior and inferior oblique.

Comparing lateral-eyed with frontal-eyed animals it is remarkable that the interocular angle changes with the eye position in the head. Thus same body movements (e.g. ROLL) require different compensatory eye movements. With head tilt to the right side in frontal-eyed animals the resulting eye movements are intorsion (right eye) and extorsion (left eye), whereas in lateral-eyed animals the executed eye movement will be vertical (elevation in the right eye and depression in the left eye) (Fig.1.5). These quite different compensatory eye movements are realized by identical neuronal mechanism, as the spatial relationship between semicircular canals and eye muscles remains similar, independent from the enclosed interocular axis (Ezure and Graf, 1984; Simpson and Graf, 1985). Insertion points of eye muscles in frontal-eyed animals change in a way
1. General introduction

that their pulling direction is still aligned with the semicircular canal they are best excited by (Graf and Simpson, 1981) (Fig. 1.6).

Neuronal connectivity of the vestibulo ocular reflex arc thus remains the same in all vertebrates. Always the horizontal canal acts excitatory on the ipsilateral medial rectus and the contralateral lateral rectus, the anterior semicircular canal is excitatory connected to the ipsilateral superior rectus and to the contralateral inferior oblique, whereas the posterior vertical canal excites the ipsilateral superior oblique and the contralateral inferior rectus.

Figure 1.5: Comparison of eye movements in lateral and frontal-eyed animals during head tilt. A head movement right-side down is executed. In both animals the anterior and posterior right vertical canals are excited and thus lead to a contraction of the ipsilateral superior rectus, the contralateral inferior oblique, the ipsilateral superior oblique and the contralateral inferior rectus. Thin dashed lines indicate axes of eye rotation. Muscles are shown as thick dashed lines. Adapted from Simpson and Graf, 1985.
Figure 1.6: Spatial relationship between vertical semicircular canals and extraocular muscles in man and rabbit. Lines of action of the superior rectus and oblique remain parallel to that semicircular canal they are maximally excited by. Adapted from Graf and Simpson, 1981.
1.3.1 The Optokinetic reflex (OKR)

1.3.1.1 General description

Research has mainly focused on mammals, where a system of subcortical and cortical structures is involved in gaze stabilization. In vertebrates, other than mammals only subcortical mechanisms are responsible for image stabilization on the retina. The OKR assures image stabilization as follows, the eyes/head or even the whole body move in the same direction and with roughly the same velocity as the visual stimulus. These so-called pursuit movements are interrupted by resetting saccades in the other direction, if the stimulus is long lasting. Alternating of smooth pursuit and saccades is called optokinetic nystagmus (OKN). Quality of OKN is expressed by its gain.

\[ \text{gain} = \frac{\text{eye velocity}[\text{deg/s}]}{\text{stimulus velocity}[\text{deg/s}]} \]

A value of 1 indicates perfect compensation of retinal slip. Gain is influenced by stimulus velocity, contrast and frequency. Although all vertebrates exhibit OKN its occurrence varies considerably among them. E.g. the velocity profile of OKN is different in different species (Fig. 1.7A). Mammals are capable to compensate velocities at a higher extent, whereas amphibians fail to do so. Not only velocity profiles differ from species to species also amplitudes of eye movements, beating fields and the interplay of eye and head movements during OKN vary (Fig. 1.7B).

Figure 1.7: A Velocity profile of OKN. Adapted from Dieringer (1986), B Occurrence of OKN in different vertebrates. Adapted from Huang (2008)

In reptiles 80% of gaze stabilization is realised by head movements (Dieringer et al., 1983), whereas in mammals gaze stabilization is mostly done via eye movements.
The OKR system can best be described as a closed loop system, where the input (actual retinal slip velocity) is continually compared to the output (eye velocity) of the system, to minimize actual retinal slip. The optokinetic response is composed of two components, the fast and slow one. The fast component is marked by a rapid initial acceleration movement of the eye, and its smooth eye velocity reaches only up to 40-80% of the steady state velocity, which is mediated by the slow component (Collewijn, 1985; Waespe and Henn, 1985). The fast component of OKR is thought to be related to the smooth pursuit system and the flocculus. In lateral-eyed vertebrates, like goldfish, birds, and rats the fast component of OKR is almost entirely missing, as their smooth pursuit system is only poorly developed (Büttner et al., 1983; Hess et al., 1985; Marsh and Baker, 1977). The slow system of OKR builds up in velocity over time and charges a velocity integrator, which is responsible for the optokinetic afternystagmus (OKAN) in the dark (Cohen et al., 1977). Slow phases and saccades of the OKAN I are in the same direction as the OKN, the OKAN I decays linearly (e.g. goldfish: Marsh and Baker, 1997, rabbit: Collewijn et al., 1980) or exponentially (monkey: Cohen et al., 1977) over time depending on the investigated species. Often OKAN I is followed by a second OKAN II which is beating on the opposite side. (e.g.: Maioli, 1988). Interestingly, the same velocity integrator which is responsible for OKAN I, is coupled to the vestibular system, as labyrinthectomies result in loss of OKAN I (Cohen, 1974). It seems that visual and vestibular motion information is stored in a common neuronal integrator (See chapter 1.3.1.2.).

One striking feature of OKN is an asymmetry between opposite directions during monocular stimulation. In most species temporo-nasal (TN) motion (e.g.: Butterflyfish: Fritsches and Marschall, 2002; frog: Katte and Hoffmann, 1980, Lazar, 1973; pigeon: Fite et al. 1979; chicken: Wallmann and Velez, 1985, Bonaventure et al., 1992; rabbit: Collewijn, 1975; rat: Hess et al., 1985)) and upward motion (e.g.: chicken: Wallman and Velez, 1985; cats: Grasse and Cyander, 1988) elicits rather higher responses to the stimulus. The only known exception is the pike, where naso-temporal motion elicits higher responses then temporal-nasal stimulation (Klar PhD-thesis, 2005). It is eye catching, that mainly lateral-eyed animals possess strong asymmetries. Forward locomotion produces naso-temporal optic flow; therefore asymmetry is thought to facilitate a suppression of optokinetic drive. Optic flow produced during forward locomotion can now be used as cue for self motion (Nakayama, 1985).
In addition, different adaptations of the optics, the retina or cortical involvement are proposed to build up symmetry. The “fovea theory” developed by Tauber and Atkin (1968) states that foveation is a prerequisite of symmetrical OKN. Anyhow generality of this theory is doubtful, as in frontal-eyed mammals also afoveate species (e.g.: ferret, cat) show symmetry. Fukuda and Tokita (1957) suggested in their “decussation theory” the decussation pattern of retinofugal fibers as key determinant for symmetry, the more fibers stay ipsilateral the more symmetric OKN gets. Some authors even tried to correlate different lifestyles with optomotor reflexes (Dieringer et al., 1992; Fritsches and Marshall, 2002).

In frontal-eyed mammals symmetry is mediated by binocular backprojections from the visual cortex to subcortical structure which are involved into OKN. E.g. during infancy (1-7 weeks) in kittens OKN is asymmetric (van Hof-van Duin, 1978) and becomes symmetric not until retino-geniculo-cortico-pretectal loops have build and thus binocular input to subcortical structures is present (Distler and Hoffmann, 1992; Tusa et al., 1989).

1.3.1.2 Neuronal substrate of OKR

Direction-selective ganglions can be found in each vertebrate retina, from fish up to mammals, they respond with tonic firing during stimulation in their preferred direction and they are inhibited by movements in the opposite or null direction. Three types of direction-selective ganglion cells (DS) exist, ON DS and ON-OFF DS, and OFF DS. OFF DS are not involved in the generation of OKR, as OFF DS do neither project to the AOS nor to the NOT (Emran et al., 2007; Kim et al. 2008, Knapp et al., 1988).

In the rabbit retina ON and ON-OFF DS exhibit quite different velocity and direction preferences profiles (Oyster, 1968; Oyster et al., 1972). ON DS are movement detectors for slow field motion, whereas ON-OFF DS are sensitive to higher velocities (Fig. 1.8A). ON-OFF DS show a four lobe distribution of preferred directions (Fig. 1.8B), the preferred directions of ON-OFF DS were thought to correlate with image motion resulting from contraction of the four recti muscles, and thus could provide information about retinal slip (Oyster, 1968). Anyway this assumption was proven to be wrong. In studies were ON pathways of the retina are selectively blocked optokinetic reaction are cancelled (Ariel, 1991). Though at least ON DS are responsible for retinal slip.
information in the AOS (Ariel et al., 1988; Hoffmann and Stone; 1985; Oyster 1972), where in all vertebrates the neuronal substrate of OKR can be found. Mora-Ferrer et al. (2005) investigated pharmacological properties of motion vision in goldfish, where blocking of nACh-receptors and GABA-receptors lead to impairment of the optomotor response. Thus they confirmed that like in all other vertebrates cholinergic and GABAergic mechanisms play an important role in the development of retinal direction-selectivity.

Furthermore direction-selective cells in the AOS resemble the speed sensitivity and direction preference of the ON-DS (Knapp et al., 1988, Soodak and Simpson, 1988). The three-lobed distribution of preferred direction and the fact that preferred directions are not collinear with null-directions (Soodak and Simpson, 1988), led to suppose that neurons in the AOS rather code for rotational motion than for pure linear motion. These features would enable the AOS to monitor selfmotion in a reference frame similar to the semicircular canals.

The AOS is characterized by its major input from the contralateral retina and its contribution to gaze stabilization. Anatomically the AOS of mammals is comprised of three mesencephalic nuclei: the dorsal terminal nucleus (DTN), the medial terminal nucleus (MTN) and the lateral terminal nucleus (LTN) (Simpson, 1984). All three nuclei have in common, that neurons belonging to them respond best to large moving stimuli and have large receptive fields. A special feature of all three nuclei is their specificity to particular directions, as the DTN responds best to horizontal ipsiversive motion, the LTN preferentially to downward movements and the MTN mostly to upward directed stimuli (e.g.: Cooper and Magnin, 1986; Grasse and Cynader, 1982). In
addition neurons in the nucleus of the optic tract (NOT), a prefrontal structure, behave like those in the DTN with a strictly ipsiversive motion preference (e.g.: Collewijn 1975; Grasse and Cynader 1984; Hoffmann and Schoppmann, 1976). Interestingly, neurons in the NOT not only resemble response properties similar to ON-DS even more neurons with higher speed preferences mirroring the responses of ON-OFF DS can be found. Thus some authors suggest that also ON-OFF DS projects directly to the AOS (Collewijn, 1975), but this could never be proven. Not only the responsiveness to wholefield movements, but also the anatomical connectivity of those nuclei makes them suitable candidates for stabilize gaze during ego or external motion. In mammals all nuclei of the AOS and the NOT project directly to the ipsilateral inferior olive (Giolli et al., 1984, 1985; Hoffmann et al., 1976; Maekawa and Simpson, 1973), which in turn projects via climbing fibers to the cerebellum. These projections are arranged in such a way that direct projections from the AOS innervate rostral parts of the dorsal cap, whereas terminals from the NOT innervate more caudal parts (Giolli et al., 2005). All these direct projections arising from the AOS and NOT to the IO are of non-GABAergic nature (Horn and Hoffmann, 1987; Schmidt et al. 1998).

In addition efferent fibers from the AOS and NOT to deep brainstem nuclei exist, like to the nucleus praepositus hypoglossi (NPH) and to the nucleus reticularis tegmenti pontis (NRTP) (Magnin et al. 1989; Cazin et al., 1984). These brainstem nuclei provide input to the extraocular motor neurons (e.g.: Robinson et al., 1994), which accomplish eye movements (Fig. 9). To support the vestibular system with additional visual information for stabilize gaze also the vestibular nuclei receive direct input from the NOT (Watanabe et al., 1993) and from all other AOS nuclei (Giolli et al. 1984; Giolli et al., 1988). Also a indirect pathways from the AOS to the vestibular nuclei via the NPH is described (Büttner-Ennever et al., 1996).

Another important feature of the AOS and NOT are the interconnections in between the AOS and between the AOS and NOT by which all AOS nuclei are reciprocally connected with the NOT (van der Togt et al., 1991; Simpson et al., 1988b) and with themselves (Simpson et al., 1988b). Each nucleus of the AOS and NOT is not only interconnected with other AOS nuclei or the NOT, but also with its counterpart on the other brain side via the posterior comissure. These interconnections are almost exclusively GABAergic (van der Togt et al. 1991) and are thought to fine tune direction specific responses (Giolli et al., 2005; Schmidt et al., 1994).
In non mammalian species the AOS is less complicated as in mammals; it consists only of one nucleus, the nucleus of the basal optic root (nBOR), which is located in the midbrain tegmentum (McKenna and Wallman, 1985a; Simpson, 1984). The nBOR codes for retinal slip in all directions of motion except for horizontal ipsiversive movements (Fan et al., 1995; Gruberg and Grasse, 1984; Zhang et al. 1999), which are coded by the nucleus lentiformis mesencephali (LM) the homolog pretectal structure of the NOT (McKenna and Wallman 1985; Winterson and Brauth, 1985b). In birds, reptiles and amphibians the AOS projects directly to the inferior olive and to the vestibulocerebellum (Brecha et al., 1980). In contrast to mammals, non-mammalian vertebrates posses direct connection from the AOS and LM to the oculomotor nuclei (birds: Brecha et al, 1980; McKenna and Wallmann, 1985a, amphibians: Montgomery et al. 1981) (Fig. 1.9). As in mammals the LM and the nBOR are extensively interconnected (Brecha et. al., 1980; Wylie et al., 2007).

In teleost fish, a pretectal structure, namely the area pretectalis (APT) is thought to be responsible for image stabilization (Klar and Hoffmann, 2002). This nucleus contains neurons which code for all directions of motion. Thus it seems that in teleost fish only one nucleus undertakes the function of the AOS. Probably this arrangement resembles the original state in evolution.
Figure 1.9: Schematic overview of known efferent projections of the AOS and related nuclei in different vertebrate classes. DTN dorsal terminal nucleus; IO inferior olive; LTN lateral terminal nucleus; MTN medial terminal nucleus; nBOR nucleus of the basal optic root; LM nucleus lentiformis mesencephali; NOT nucleus of the optic tract; NPH nucleus praepositus hypoglossi; NRTP nucleus recticularis tegmentis pons; III nucleus oculomotorius; IV nucleus trochlearis; VI nucleus abducens.
1.3.2 The vestibulo-ocular reflex (VOR)

1.3.2.1 In general

The VOR is, as the OKR, active almost all the time, and ensures unblurred vision during self-motion. In contrast to the OKR, the VOR is an open loop system, which is on itself not able to detect errors (Ito et al., 1974). The VOR is only effective during brief and rapid head movements and fails to compensate for sustained or slow head movements. The VOR can be further divided in two sensory subsystems, the otoliths which perceive linear acceleration and the vestibular haircells detecting angular acceleration (Angelaki et al., 1995). All following descriptions of the VOR system will refer to the angular VOR:

This gaze stabilizing reflex is made up of smooth eye movements in the opposite direction as the actual head movement, they are interrupted by resetting saccades in the direction of head movement to maintain accurate vision during continuous rotation of the head; as for the OKR this pattern is called nystagmus. VOR is mainly working during head rotations of 0.1-7 Hz (Delgado-Garcia, 2000), below 0.1 Hz gain of VOR decreases continuously and the OKR will assume control. Baarsma and Collewijn (1974) described the interplay of OKR and VOR as a linear interaction:

\[ G_h = G_o (1 - G_v) + G_v \]

with \( G_h \) representing the total gain, \( G_o \) the optokinetic gain and \( G_v \) the vestibular gain.

Necessity of visual feedback for high VOR gains should therefore not be underestimated, as several studies revealed the contribution of visual feedback to the VOR and vice versa (Fig. 1.10). In the absence of visual feedback (i.e. rotation in the dark) VOR gains are significantly lower as with visual feedback (e.g.: Baarsma and Collewijn, 1974; Marsh and Baker, 1997). Even more also OKN gain in bilateral labyrinthectomized goldfish is significantly lower as in intact goldfish for high stimulus velocities (Dieringer et al., 1992).

Not only gain is an objective measurement of compensation quality, also the phase (eye position vs. stimulus) can describe the goodness of the reflex (e.g. Barnes, 1993). For low stimulus frequencies (<0.1 Hz) a significant phase lead can be observed, whereas at higher frequencies phases are near zero. Under dark conditions phase lead is amplified (Fig. 1.10A). It resembles the typical low frequency filtering of the VOR, as the
semicircular canals are unable to detect slow motion accurately (Schairer and Bennet, 1986).

Figure 1.10: A Gain and phase of VOR without visual feedback for different frequencies and amplitudes. B As in A but with visual feedback. Adapted from Baarsma and Collwijn, 1973

Also the VOR posses an afternystagmus, called post-rotatory nystagmus (PRN), the PRN is produced, if an animal is rotated for some time in the dark and then rotation stops suddenly. The slow phase of the PRN is in the same direction as the former stimulus movement and its time course resembles the OKAN. Under normal condition OKAN and PRN cancel each other out and no afternystagmus at all occurs (Collewijn, 1981; Rapahan, 1977). Hence OKAN and PRN share the same characteristics; a common velocity storage mechanism is suggested (Cohen et al., 1977). Further evidence of a common velocity integrator comes from labyrinthectomies, which cancel out OKAN completely (Collewijn, 1976b)
1.3.2.2. Neuronal substrate of VOR

In all vertebrates the neuronal circuitry of VOR consists of a three-neuron-arc (e.g.: Szentagothai, 1950, see Fig. 1.11). Semicircular canals work as a push-pull pair, each of the three semicircular canals has a counterpart on the other body side; if one canal is excited during rotation its counterpart is inhibited. The challenge of the whole system is to alter head acceleration ($\dot{\phi}$) into eye velocity ($\dot{\varepsilon}$) and eye position ($\int \dot{\varepsilon}$). The first integration step is done via the cupula, where acceleration is transferred into velocity information (e.g.: Precht, 1979). During head rotations displacement of the cupula (and therewith deflection of the haircells) leads to excitation or inhibition of vestibular nerve fibers, whose spiking activity is proportional to head velocity ($\dot{\phi}$) and head acceleration ($\ddot{\phi}$). The vestibular nuclei now receive this information and relay it directly or indirectly via interneurons to the motoneurons of the eye muscles (N III, N IV, and N VI). The vestibular neurons themselves behave like a bandpass filter, with in a range of 0.02 Hz up to 5 Hz. (Schmid et al., 1979). A second integration step to eye position is done in part by intra- and intervestibular circuits, the nucleus prepositus hypoglossi (NPH) and the interstitial nucleus of Cajal, which get input by vestibular neurons and projects to the motoneurons of the eye muscles (Precht, 1979, Schmid et al., 1979). Motoneurons pass an eye position and velocity signal to the muscle fibers. During saccades in the on-

Figure 1.11: Three neuron arc of horizontal VOR. Information flow during a head movement to the right side. Red lines indicate excitation, blue lines inhibition. LR lateral rectus muscle; MR medial rectus muscle; VN vestibular nuclei; III nucleus oculomotorius; VI nucleus abducens.
direction these neurons burst, whereas during saccades in the off-directions they are silenced. In almost the same manner eye position is coded with a steady discharge, depending on the actual eye position (e.g.: Pastor et al., 1991).

The three-neuron-arc on itself is an open loop system, so adaptive gain changes must be mediated by other pathways, namely indirect ones. The vestibulo-cerebello-vestibular pathway is one of them. Vestibular neurons project via mossy fibers to the flocculus of the cerebellum and excite Purkinje cells which in turn give inhibitory input back to the vestibular nuclei. In addition mossy and climbing fibers provide visual input to the flocculus (Maekawa and Simpson, 1973; Maekawa and Takeda, 1976).

1.4. Reference frames

Gaze stabilization always requires a sensorimotor transformation, from sensory signals into motor commands. Sensory information arising from visual, vestibular and proprioception is used to elicit compensatory eye movements. All three sensory modalities possess their own frame of reference. Thus it seems obvious, that different sensory modalities have to be combined into one reference frame to elicit the appropriate motor commands. Coding in one internal reference frame would facilitate neuronal processing (Hengstenberg 1998; Wallman and Velez 1985; Wylie et al. 1988). It seems favourable to share a reference frame similar to the reference frame of the motor output, i.e. pulling direction of the extraocular muscles. Oculomotor neurons already code eye position and velocity in a reference frame defined by the extraocular muscles. So the question arises in which reference frame upstream structures code. The vestibular system is composed of three pairs of nearly perpendicular to each other situated semicircular canals. The system is composed of three pairs of nearly perpendicular to each other situated semicircular canals. Orthogonality of them is not necessarily required to build up a three dimensional reference frame. As to span a three-dimensional vector space only linear independency is required, although orthogonality would lead to the best signal-to-noise ratio of the system (Robinson, 1982). In general semicircular canal and extraocular muscles planes are lined up with each other, independent from the position of the eyes in the head (lateral-eyed vs. frontal-eyed animals (Ezure and Graf, 1984, Graf and Simpson, 1981). In frontal-eyed animals insertion points of the eye muscles change in a way that this configuration is still
1. General introduction

retained (Fig. 1.12). In the cat extraocular muscles plane and semicircular canals are still in a close spatial relationship, but are more distinct then in the rabbit.

In all vertebrates the horizontal semicircular canal plane is aligned with ipsilateral horizontal recti muscles, the anterior semicircular canal plane is aligned with the vertical recti muscles and the posterior semicircular canal is aligned with the oblique muscles (see Fig. 1.12).

Simpson and co-workers already dealt with the question of reference frames in the visual system and investigated the spatial organization of direction-selective neurons in the medial terminal nucleus (MTN) and in the visual tegmental relay zone (VTRZ) (Simpson et al., 1988) of rabbits. Some of the recorded neurons, independent from a monocular or binocular input had large bipartite receptive fields. In which the receptive field was divided into two parts, one preferring the opposite direction as the other. In MTN this special feature seems to occur to a lesser extent (15%) as in the VTRZ (81%). Neurons with a bipartite receptive field structure would be ideal detectors of rotational ego motion (Simpson et al. 1988). Thereon Simpson and co-workers took a closer look at the preferred axes of rotation for these neurons. As a population neurons preferred rotational axes in close spatial relationship to the vertical semicircular canals (45°

Figure 1.12: Spatial orientation of semicircular canals and extraocular muscle planes in rabbit and cat. Adapted from Simpson and Graf, 1981.
azimuth and 135° azimuth) and are thus thought to code in reference frame similar to the reference frame of the vestibular system. As semicircular canals and eye muscles planes are aligned no further sensory-motor transformation would be needed. Anyway a definite answer regarding coding in an eye muscle reference frame opposed to coding in vestibular coordinates is still missing. Since eye muscles pulling direction (i.e. muscle planes) and semicircular canals planes are not disparate enough to conclude which frame of reference is realized.

Not only the AOS was examined but also subsequent structures involved in gaze stabilization, like the vestibulocerebellum (Graf et al., 1988; Kano et al., 1990; Wylie et al., 1988; Wylie et al., 1993a, 1993b).

Complex spike activity of purkinje cells in the vestibulocerebellum showed direction-selectivity to rotational as well as to translational optic flow. At this processing level most neurons had already binocular receptive fields (91%). Wylie et al. (1993a) classified them into four response groups:

1. Descent neurons preferring upward motion in both eyes (upward neurons)
2. Ascent neurons preferring downward motion in both eyes (downward neurons)
3. Roll neurons preferring upward motion in the ipsilateral eye and downward motion in the contralateral eye.
4. Yaw neurons preferring forward motion in the ipsilateral eye and backward motion in the contralateral eye.

Obviously ascent (downward neurons) and descent (upward neurons) neurons rather code for translational than rotational optic flow. Visual input to the vestibulocerebellum arises from the AOS via climbing fibers from the inferior olive and binocular receptive field are generated by a simultaneous input from the ipsilateral and contralateral situated AOS, where receptive field are still monocular organized.

Also in mammals rotational optic flow is coded by complex spike activity of purkinje cells located in the vestibulocerebellum. Graf et al. (1988) and Kano et al.(1990) analysed them in regard to their spatial organization. As in the vestibulocerebellum of pigeons best visual responsive axes seems to be aligned with the orientation of the vestibular system. This spatial arrangement of direction-selectivity supports coding in a vestibular reference frame, as it is suggested by Graf (1988) and Kano (1988).
A consequent study by Wylie and Frost (1996) put coding in vestibular coordinates into question: Recordings were made in the nucleus of the basal optic root (nBOR) and in the nucleus lentiformis mesencephali (LM); were neurons are sensitive to translational as well as to rotational optic flow. Their major finding is related to neurons coding horizontal optic flow. LM units prefer mostly temporo-nasal (forward) motion, whereas in the nBOR so called backward (naso-temporal) coding neurons were found. The averaged preferred direction of the population is 87° for temporo-nasal neurons (where 90° would represent temporo-nasal motion and -90° corresponds to naso-temporal motion) and -111° for naso-temporal units (Fig. 1.12A). Not only spatial organization of preferred directions of AOS and LM neurons were considered but also neurons sensitive to horizontal optic flow (VA neurons) in the flocculus were taken into account (Fig. 1.12B).

Altogether the mean for temporo-nasal neurons is 91° and 111° for naso-temporal neurons. In addition orientation of the medial and lateral recti muscles planes were obtained (Fig. 1.12C).

It is eye catching that obtained values for preferred directions and orientation of the horizontal recti muscles agree. The non-parallel orientation of the horizontal recti (they are only 152° apart instead of 180°) is reflected by the preferred directions of the AOS LM and flocculus neurons, hence Wylie and Frost (1996) concluded a reference frame similar to the eye muscle orientation rather than a vestibular one. Not only Wylie and colleagues suggest a reference frame defined by the pulling directions of the eye muscles, also Maiolli concluded from electrophysiological data, obtained in the cat, that coding in eye muscle pulling directions seems more favourable. Calculation of preferred direction in the cat LTN revealed a strong bias to optic flow fields which are produced by rotation of the head around the best responsive axis of the vertical semicircular canals. Anyway a closer look at the preferred directions, without considering the sense of rotation, revealed a bimodal distribution, with peaks at 20° and 60°. Both peaks correspond well to the pulling directions of the vertical and oblique recti, which are in fact (29° for the vertical recti and 57° for the oblique ones, see figure 1.12).

In this context results by Simpson and co-workers should be carefully reconsidered. The obvious problem in the rabbit is the close alignment of semicircular canals and extraocular muscles planes. Thus a definitive answer of the reference frame of the AOS is still missing.
Figure 1.12: Direction preferences of neurons in the nucleus of the basal optic root (nBOR), nucleus lentiformis mesencephali (LM) and flocculus.

A. Polar histogram of direction preferences of LM and nBOR neurons.
B. Polar histogram of direction preferences of LM, nBOR and flocculus neurons.
C. Polar histogram of the orientation of the medial and lateral rectus muscle.
2. Responses to moving visual stimuli in pretectal neurons of the small-spotted dogfish (*Scyliorhinus canicula*)

2.1 Introduction

In teleosts the area pretectalis (APT) contains highly direction-selective neurons and is involved in optokinetic retinal image stabilisation. Neurons in this nucleus respond direction specifically to temporonasal, nasotemporal as well as vertical movements (Klar and Hoffmann, 2002). This is significantly different from tetrapods, in which the pretectum and accessory optic system contains different nuclei coding for different directions of visual motion.

In mammals the accessory optic system (AOS) is comprised of the dorsal terminal nucleus (DTN), the medial terminal nucleus (MTN) and the lateral terminal nucleus (LTN). In addition, neurons in the nucleus of the optic tract (NOT) behave like those in the DTN. These neurons are highly directionally selective and respond over a wide speed range. The direction-selective neurons of the NOT and the DTN have a strictly ipsiversive motion preference (e.g. Collewijn 1976; Hoffmann and Schopmann 1976; Grasse and Cyander 1984). In MTN and LTN vertical motion is represented (e.g. Grasse and Cyander 1984; Grasse and Cyander 1982).

In amphibians, reptiles and birds the nucleus lentiformis mesencephali (LM) is the visuomotor interface of the horizontal optokinetic nystagmus (frog: Katte and Hoffmann 1980; Fite 1985; turtle: Fan et al. 1995; bird: Fite et al. 1979; Fu et al. 1998; Winterson and Brauth 1985). Neurons in the LM code predominantly for ipsiversive motion, some are selective for contraversive and vertical motion (frog: Katte and Hoffmann 1980; turtle: Fan et al. 1995; pigeon: Winterson and Brauth 1985). The nucleus of the basal optic root (nBOR), a major nucleus of the AOS in tetrapods other than mammals, processes information about retinal slip for all directions of motion except horizontal ipsiversive (Dieringer et al. 1982; Fan et al. 1995; Gruberg E.R. and Grasse K.L. 1984; Zhang et al. 1999) which is represented by the LM.

It has been suggested that the AOS and its downstream targets transform visual motion signals from retinal coordinates into vestibular coordinates (Graf et al. 1988; Simpson et al. 1988; Wylie and Frost 1993). A vestibular reference frame is characterized by an alignment of the preferred directions with the response axes of semicircular canals (Graf...
et al. 1988), i.e.: the neuronal population should show a bias for rotation around axes which correspond to semicircular canal axes. Transformation of the visual motion information into a vestibular reference frame would facilitate combining visual and vestibular information in the computation of self movements and stabilizing gaze (Hengstenberg 1998; Walman and Velez 1985, Wylie et al. 1998).

In contrast to other vertebrate groups, little is known about the visual input to gaze stabilisation in chondrichtyans. A possible input, the retinorecipient corpus geniculatum laterale (Cgl), is comprised of diencephalic and pretectal parts and despite its name, has no evident homology with the mammalian corpus geniculatum laterale. Furthermore the oculomotor organisation of chondrichthyans seems to be different from all other vertebrates investigated so far, in that the motoneurons of the medial rectus muscle are located contralaterally to their innervated muscle (elasmobranches: Graf and Brunken 1984). Also in lampreys the oculomotor organization is equally different from that of other vertebrates (for example, the horizontal semicircular canals are lacking, [Simpson and Graf, 1985]). Here motoneurons of the medial rectus muscle are innervated ipsilaterally. Thus, the underlying circuits of optokinetic control in sharks may differ from other species studied so far.

Scyliorhinus canicula is one of the more primitive members of the Galeomorpha, which represents 73% of all living sharks (Reperant et al. 1986). Thus, this species represents a good model for studying the evolution of visuo-motor pathways. Previous studies presumed that the retinofugal system of S. canicula resembles that of actinopterygians (Smeets 1981), in that as in osteichthyes, the optic nerves are completely crossed. Two retinorecipient nuclei in the pretectum were described in S. canicula, the corpus geniculatum laterale (Cgl) and the nucleus pretectalis (Pret) (Smeets 1981; Reperant et al. 1986). However, a structure corresponding to the nucleus of the basal optic root (nBOR), a key component of the accessory optic system in tetrapods, does not seem to be present in S. canicula (Smeets et al. 1983). The aim of this study was to investigate the pretectum of the small spotted dogfish (Scyliorhinus canicula) with electrophysiological and histological methods to locate the visuo-motor interface coding retinal slip subserving the optokinetic reflex in chondrichthyans. In particular, we ask whether the pretectum of chondrichthyans shows evidence of a transformation from a retinal into a vestibular reference frame.
2.2. Materials and Methods

Data from fourteen *S. canicula* provided by the Observatoire Oceanologique de Banyuls, the Biologische Anstalt Helgoland and the Aquazoo-Löbbecke Museum were included in the present study. Animal were at least half a year old, between 10cm - 50cm in length and included animals of both sexes. All experiments were approved by the local authorities (Regierungspräsidium Arnsberg) 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 NIH guidelines for care and use of animals for experimental procedures.

Animals were anesthetized during surgery in a bath containing 0.1% MS222. After additional local anaesthesia with 2.5% lidocaine, a craniotomy was performed to allow access to the left tectum opticum and pretectum. After surgery the animals were immobilized with pancuroniumbromide (0.6mg/kg) and transferred to a transparent recording hemisphere (diameter 70cm), where they were artificially ventilated with cooled sea water (14°C). Single unit recordings with glass-coated tungsten microelectrodes or glass micropipettes (impedance 1-2.5 MΩ) were made in the left pretectum. Receptive field sizes were qualitatively tested with single dots (diameter 4°-10°) produced by a hand lamp. For quantitative investigation of the responses to movement the visual stimulus consisted of random light dots projected into the hemisphere by a planetarium projector centered above the fish’s head. The planetarium, consisting of a spherical shell (diameter 15 cm) with small holes in it attached to a computer controlled motor. A lamp inside the shell produced an optokinetic stimulus, covering the whole visual field of the right eye with dots sized from 2°-4° in diameter and 1 cd/m² in luminance on the translucent hemisphere (for further information see Simpson et al. 1988). The following stimulus movements were presented in the whole visual field of the right eye.

2.2.1. Linear motion stimuli (testing for a retinal reference frame)

Four axes of linear motion were used to clarify, whether a bias for horizontal or vertical movements exists amongst neurons in the pretectum of chondrichthyans: 1. Horizontal movements from temporal to nasal (0°) and nasal to temporal (180°), 2. Vertical movements from ventral to dorsal (90°) and from dorsal to ventral (270°), 3. Oblique movement from temporo-ventral to naso-dorsal (45°) and naso-dorsal to temporo-
ventral (225°), 4. Oblique movements from naso-ventral to temporo-dorsal (135°) and from temporo-dorsal to naso-ventral (315°). All eight stimulus directions produce near linear movements on the fish’s central retina. We call this the retinal reference frame, as all linear stimuli correspond to straight movements on the central retina (Fig. 1 A).

2.2.2. Rotational stimuli (testing for a vestibular reference frame)
In addition, four axes of rotational axes in the horizontal plane were tested (Fig. 1 B, Fig. 2.1 C) to find out whether the strongest responses were elicited by rotations around axes of the vertical semicircular canals. Around every axis the planetarium turned in clockwise (CW) and counterclockwise (CCW) directions leading to image motion on the right retina like that during the following body movements: 1. ROLL (planetarium rotation around the longitudinal axis of the fish), body rotation around this axis leads to upwards motion (ROLL up) or downwards motion in the central retina (ROLL down). 2. LARP (planetarium rotation around the left anterior right posterior axis of the fish). Rotation around this axis leads to a visual stimulus, which corresponds to a maximal activation of either the right anterior vertical semicircular canal (left ear up, LARP up) or the left posterior vertical semicircular canal (left ear down, LARP down). 3. PITCH (planetarium rotation around the transverse axis of the fish): nose down (PITCH up) or nose up (PITCH down) 4. RALP (planetarium rotation around the right anterior left posterior). Visual stimuli resulting from a rotation around the RALP-axis correspond to a maximal activation of either the right posterior vertical semicircular canal (left ear up, RALP up) or the left anterior vertical canal (left ear down, RALP down). The angles

Figure 2.1: A Illustration of stimulus directions seen by the central 60° of the retina during linear stimulation. B Illustration of stimulus axes used in the horizontal plane, i.e. axes of rotation of the planetarium to test for a vestibular reference frame. LARP left anterior right posterior axis of the fish. How clockwise and counterclockwise rotation around the LARP axis appears on the right central retina is shown in Fig. 1C and in the supplementary movies, PITCH transverse axis, RALP right anterior left posterior axis, ROLL longitudinal axis. C Stimulus movements seen by the central retina during stimulation around LARP, RALP, ROLL and PITCH axes.
selected for the LARP and RALP axes are based on vestibular canal orientation (own dissection) and physiological studies on other species (e.g. Simpson and Graf 1985, Simpson et al. 1988). Stimulus speed was kept constant at 10°/s. Each trial consisted of a stationary phase (0-2000ms), a rotation in CW direction (2000ms-5000ms), another stationary phase (5000ms-7000ms) and a rotation in CCW direction (7000ms-10000ms).

2.2.3. Data Analysis

Action potentials were converted to TTL pulses by a window discriminator. In some experiments data acquisition involved storing TTL pulses on the audiotrack of a videotape for manual off-line analysis with a counter. In the remaining experiments preamplified signals were acquired with CORTEX (NIMH, Laboratory of Neurophysiology. Version 5.96), and off-line analysis was performed with a customized Matlab (version 7.0.1) program.

To test for direction selectivity the weighted preferred direction vector was calculated as following:

First the rectangular coordinates of the mean vector are calculated, where 8 angles $\alpha_i$ are given (i.e.: $\alpha_1 = 0^\circ, \alpha_2 = 45^\circ, ..., $ corresponding to the sampled stimulus directions), $m_i$ represents mean activity in the corresponding angle $\alpha_i$ and $n = \sum_{i=1}^{8} m_i$.

\[
x = \frac{\sum_{i=1}^{8} m_i \cdot \cos \alpha_i}{n}, \quad y = \frac{\sum_{i=1}^{8} m_i \cdot \sin \alpha_i}{n}
\]

from which we get $r = \sqrt{x^2 + y^2}$.

Where $r$ is the length of the mean vector. The value of the mean angle $\theta$ is now determined by the angle having the following cosine and sin:

\[
\theta = \cos^{-1} \left( \frac{x}{r} \right), \quad \theta = \sin^{-1} \left( \frac{y}{r} \right)
\]

The four neighbouring directions of the weighted preferred direction vector were compared to the four opposite directions with a t-test or a rank sum test. Only neurons with a p-value below 0.01 were taken as direction-selective. Null direction is taken as the direction with the lowest response. Weighted preferred direction vectors have the advantage that all responses, even in the null direction, are taken into account to estimate preferred direction and tuning width. The length of the mean vector ($r$) was
taken as the tuning width index (TWI), with values near 1 indicating no dispersion of the mean values (i.e. all except one direction have null activity).

To test for axis-selective cells a multi comparison test (one way analysis of variance, ANOVA) was applied, activity of the preferred axis had to be significantly different from all other directions. To look for unimodal, bimodal or uniform distribution of the whole population a Rayleigh test was used.

2.2.4. Histological procedures

At the end of the experiments electrolytic lesions (10s, 10μA, anodic and cathodic) were made to identify the recording sites. In some experiments Tetramethylrhodamine dextran (MW 3000, anionic, lysine fixable, Molecular Probes, administered in 0.3 M PBS) was applied iontophoretically (positive current pulses 7s on/ 3s off, 10μA for 30 min) via the recording pipette to verify the recording side. The fish were deeply anaesthetized and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in 0.1M 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. Brains were removed and stored overnight in the same fixative at 4°C. The next day the brains were cryoprotected with 30% sucrose in 0.1M PB for another 24 h. The brains were then embedded in chicken albumin (Sigma) and 30μm sections were cut in a frontal plane on a cryostat. Two series were collected, the first was stained with cresylviolet to reveal cytoarchitecture, the second was stained with a combination of a myelin stain (Gallyas) and cresyl violet or according to Klüver-Barrera (Romeis and Böck 1989). Brain regions were named following the nomenclature of Smeets and coworkers (1983).

To evaluate retinofugal fiber courses one animal got an in vitro Di-I application onto the optic nerve disc. The whole head of the animal was stored for 3 months at room temperature in 4% paraformaldehyde in 0.1M phosphate buffer (PB, pH 7.4). Afterwards the brain was cut frontally with a vibratome in 100μm slices. Tissue sections were examined using a fluorescence microscope (Zeiss Axiophot). Fibers were reconstructed using a fluorescence microscope (Zeiss Axiophot) coupled to a computer.
based reconstruction system (Neurolucida) equipped with a digital camera (Zeiss Axiophot).

2.3 Results

2.3.1. Linear stimuli on the central retina

Altogether 130 visual neurons in the pretectum of fourteen sharks were recorded during whole field visual stimulation with a stimulus velocity of 10°/s. The five reconstructed microlesions and the two rhodamine injections were all located in caudal parts of the pretectum within the so called corpus geniculatum laterale (Fig. 2.2 A, B). All of these lesions and rhodamine injections were located in the prepectal parts of the Cgl.

Forty-five (35%) of the recorded neurons were significantly direction-selective (p<=0.01), ten (8%) were axis selective (p<=0.01) and seventy-five (58%) were sensitive to motion, but neither direction nor axis selective during whole field stimulation with a stimulus velocity of 10°/s. Fig. 3 shows an example of each neuronal class: a direction-selective neuron (Fig. 2.3 A), an axis selective neuron (Fig. 2.3 B) and a motion selective neuron (Fig. 2.3 C). None of the direction and axis selective neurons
showed a clear inhibition to motion in the null-direction; instead, responses were nearly all above spontaneous activity (Fig. 2.3 and Fig. 2.4).

Figure 2.3: Polar plots of cells which were measured with linear stimuli. Radials represent direction of stimulus motion, dash-dotted circles represent activity in spikes per second, grey circle represents mean spontaneous activity in spikes per second A direction-selective neuron. B axis sensitive neuron. C motion sensitive neuron.

Figure 2.4: Peristimulus time histograms and raster plots of a direction-selective neuron in the left Cgl of S. canicula, as tested through the right eye with a linear stimulus moving at a velocity of 10°/s. Black line represents spike density function, which is based on a Gaussian filtering of the spike train. 0ms-2000ms stationary phase, 2000ms-5000ms linear movement in direction of the assigned angle. Angles give direction of the linear movement seen be the central retina (see Fig. 2.1A)
The responses of a typical linear direction-selective neuron are shown in Fig. 2.4. The neuron responds to each direction of motion with a transient response at movement onset and tonic firing above the rate of spontaneous activity (15 Imp/s). No clear inhibition in the null direction (180°, mean activity, 16 Imp/s) occurs. The neuron ceased firing only during stationary phases of the stimulus.

Most of the direction-selective neurons tested had broad tuning curves with large receptive field, spanning nearly the whole lower horizontal visual field of the right eye. (Azimuth: 20°-150°, Elevation: -20°-45°).

Weighted preferred directions of the 45 direction-selective neurons were uniformly distributed, i.e. there was no bias for horizontal movements from temporal to nasal as found in the pretectum or dorsal terminal nucleus of tetrapods (Fig. 2.5 A).

Additionally seventeen neurons were recorded with stimulus velocities of 5°/s and 20°/s, as well as 10°/s. (Table 2.1). In general, direction-selective responses occurred more frequently at lower velocities, whereas axis sensitive neurons behave complementary. The percentage of motion selective cells is stable over all tested stimulus velocities.
2. Visual responses in *S. canicula*

### Table 2.1 Percentage of direction, axis and motion selective neurons for different velocities

<table>
<thead>
<tr>
<th></th>
<th>Direction-selective</th>
<th>Axis selective</th>
<th>Motion selective</th>
</tr>
</thead>
<tbody>
<tr>
<td>5°/s</td>
<td>30%</td>
<td>17%</td>
<td>53%</td>
</tr>
<tr>
<td>10°/s</td>
<td>18%</td>
<td>35%</td>
<td>47%</td>
</tr>
<tr>
<td>20°/s</td>
<td>12%</td>
<td>35%</td>
<td>53%</td>
</tr>
</tbody>
</table>

Table 2.1: Each column represents one neuron class, each row a certain velocity. The table shows the percentage of neurons which were found at the given stimulus velocity. Altogether seventeen neurons were recorded with 5°/s, 10°/s and 20°/s.

### 2.3.2. Axis of rotation in the horizontal plane

Fifty-eight neurons in four animals were recorded during whole field stimulation with a stimulus velocity of 10°/s, created by the planetarium rotating around axes in the horizontal plane. Thirty-six of them were significantly direction-selective (62%), three were axis selective (5%) and nineteen were motion sensitive (33%). Examples of each neuron class are shown in Fig. 2.6. A characteristic peri-stimulus time histograms (PSTH) of a direction-selective neuron recorded with axes in the horizontal plane is depicted in Fig. 2.7. As we found with linear motion stimulation, the responses in each axis and direction were above the spontaneous activity (15 Imp/s). Almost all direction-selective neurons recorded with the rotational stimuli showed no inhibition to motion in the null direction.

![Figure 2.6: Polarplots of cells which were measured with rotational stimuli. A direction-selective neuron. B axis sensitive neuron. C motion sensitive neuron. Legend see Fig. 2.3.](image)
Thirty-seven neurons were recorded with both linear and rotational motion. Twenty (54%) of them were direction-selective stimulated by the planetarium rotating in the horizontal plane and eighteen (48%) of them were direction-selective for linear stimuli. There was no difference in the tuning width of direction-selective neurons for linear and rotational stimulus movement, i.e. the tuning width was not narrower for the horizontal axes nor was the length of the weighted preferred direction vectors significantly different (t-test, p=0.345). If we consider the distribution of weighted preferred directions, a very similar picture as for the linear stimuli appears (Fig. 2.5 B). The preferred directions in our sample of pretectal neurons can be uniformly assigned to the axes in the horizontal plane, i.e. a unimodal or bimodal distribution can be rejected (p=0.001). If a transformation into a vestibular reference frame would take place our sample of Cgl neurons should show a uni- or bimodal distribution superimposed to the RALP or LARP axes, as it has been shown for the rabbit (Graf et al. 1988). Hence it seems unlikely that transformation and coding of preferred directions occurs strictly in vestibular coordinates.

A clustering of preferred directions appears, as in each penetration a bias for a particular axis of rotation and direction is visible (see different shaded symbols in Fig. 2.5B), although, our histology failed to show a clear segregation of preferred stimulus directions among different recording sites. Possibly a segregation on a smaller scale as in mammals is present.
Figure 2.7: Peristimulus time histograms and raster plots of a direction-selective neuron in the left Cgl of *S. canicula*, as tested through the right eye with a rotational stimulus. Black line represents spike density function, which is based on a Gaussian filtering of the spike train. 0ms-2000ms and 5000ms-7000ms stationary phase, 2000ms-5000ms rotation in CW direction and 7000ms-10000ms rotation in counterclockwise (CCW) direction. **PITCH DOWN**: clockwise rotation of the planetarium around the interaural axis of the fish; **PITCH UP**: counterclockwise rotation of the planetarium around the interaural axis of the fish direction. Rotation around the PITCH axis result in circular motion on the central retina (see Fig. 2.1C); **LARP DOWN**: CW rotation of the planetarium around the left anterior to right posterior axis of the fish; **LARP UP**: same axis as LARP CW, but rotation in CCW direction; **ROLL DOWN** rotation of the planetarium around the longitudinal axis of the fish in CW direction; **ROLL UP**: rotation in CCW direction. Stimulation around the ROLL axis results in vertical (up-down, down-up) movements on the central retina (see Fig. 2.1C); **RALP DOWN** planetarium rotation around the right anterior to left posterior axis of the fish in CW direction; **RALP UP**: rotation in CCW direction.
2.3.3 Efferent projections

Only in one animal efferent projections could be evaluated. Fibers from the Cgl pass via the tractus tecto-bulbaris to the midline and follow their way lateron in the fasciculus longitudinalis medialis, where terminals to motoneurons of the nucleus oculomotorius and nucleus trochlearis branch up. Another fiber bundle establishes terminals with the cerebellum. (Fig. 2.8)

Figure 2.8: A Terminals to the nucleus oculomotorius. B Terminals to the nucleus trochlearis. C Fibers reaching the corpus cerebelli, Scalebar represents 50µm respectively.
2.4. Discussion

2.4.1. Visual responses to moving stimuli

We recorded direction-selective neurons in the corpus geniculatum laterale in the pretectum of the small-spotted dogfish. All direction-selective neurons responded best to large slowly moving (5°/s, 10°/s) random dot stimuli and had large receptive fields. These uniform response characteristics in our recordings lead us to suppose that the Cgl in chondrichthyans may represent a part of their AOS. In addition the Cgl receives direct retinal input (Smeets 1981, Reperant 1986) and projects directly to the nucleus oculomotorius and to the cerebellum (O. A. Masseck, unpublished observations), which underlines its function in eye movement control. Also the presence of motion sensitive neurons resembles the functional characteristics like in the NOT in mammals (Schoppmann and Hoffmann 1979) or LM in pigeons (Fu et al. 1998). Ibbotson and Mark (1994) suggested that motion sensitive neurons might prevent ocular following responses during saccades. Also our third neuron class (axis selective neurons) has been described in the LM of pigeons (Fu et al. 1998). Therefore, our data suggest that the anatomical nomenclature of the caudal part of the Cgl, where direction-selective neurons are located, should be reconsidered. Although no definitive anatomical or morphological data support the homology of Cgl to the LM, we propose to rename it LM (nucleus lentiformis mesencephali) because of its functional similarities to the LM of amphibians, reptiles and birds.

2.4.2. Lack of inhibition

In our sample of direction-selective neurons, suppression of spike activity below the spontaneous level was not observed, even during stimuli moving in the null direction. What might be responsible for the lack of suppression in the null direction? One possibility is that the neuronal connectivity is different, i.e. the separation of the input from retinal ganglion cells with different preferred directions is not as strict as in other vertebrates, so that input from a small percentage of the afferent ganglion cells might not be direction-selective or might be excitatory in the null direction of the pretectal direction-selective neurons. Alternatively direction-selective retinal ganglion cells
might lack inhibition in the null direction. Direction selectivity in retinal ganglion cells is mediated by GABAergic mechanisms (Caldwell, Daw and Wyatt, 1978), and a series of experiments by Bonaventure and Jardon (Bonaventure et al. 1983, Bonaventure et al. 1991, Jardon et al. 1991) on monocular OKN in frog and chicken showed that intravitreal eye injections of GABA agonists and antagonist could modulate OKN gain and even alter the asymmetry of monocular OKN. So it is possible that the underlying GABAergic or cholinergic mechanisms involved in directional selectivity might not be as specific as in mammals.

Inhibition in the null direction is not necessarily required to stabilize gaze during self-movements. In a push-pull system it is the activity difference which counts. For example, temporo-nasal stimulus movement seen by the right eye leads to strong activation of neurons with TN preferred direction in the left Cgl, whereas neurons with NT preferred direction are activated much more weakly in their null direction. The activity of TN preferring neurons may, in turn, be relayed to motoneurons in the nucleus oculomotorius initiating a contraction of the right medial rectus muscle. Conversely, the NT preferring neurons might lead to a much weaker contraction of the right lateral rectus muscle. Overall, this would lead to an eye movement to the left being executed. A direct activation of abducens motoneurons via the Cgl is also conceivable, as Chochran et al. (1984) assumed a direct linkage of the pretectum to the oculomotor and abducens nuclei in the frog and unpublished data from our lab (Gürke, 2004) showed direct projections from the area pretectalis (APT) to the oculomotor and abducens nuclei in the trout.

2.4.3. Distribution of preferred directions

In contrast to some tetrapods, no segregation of coding retinal slip during self-motion around different axes into distinct nuclei seems to occur in chondrichthyans, perhaps because a structure homologous to the nucleus of the basal optic root is missing in S. canicula (Smeets et al. 1983). This uniform distribution of preferred directions in fish seems to represent the primitive condition. This result fits well to the parcellation theory suggested by Ebbesson (1980), which states that “..., nervous systems become more complex, not by one system invading another, but by a process of parcellation...”. It
seems likely that during evolution one nucleus for encoding retinal slip splits up into a more complex system were different directions of retinal slip are encoded in different nuclei. Our findings additionally support the existence of a monocularly organized oculomotor system, as described for some fish (goldfish: Easter et al. 1974; sandlance and pipefish: Fritsches and Marshall 2002) and the chameleon (Gioanni et al. 1993). However further behavioural studies of the optokinetic system in chondrichtyans are needed to verify or refute a monocular organization.

2.4.4. Vestibular reference frame

Strict coding in vestibular coordinates was not found in the Cgl, i.e. neurons recorded with both linear and rotational stimuli showed no significant bias for the rotational stimuli, although half of the tested axes correspond to semicircular canal axes. So far a transformation of reference frames has been shown in mammals (Simpson et al. 1988) and birds (Wylie et al. 1998; Wylie and Frost 1999), it is questionable whether neurons in the LM and nBOR of other tetrapods show a transformation from a visual into a vestibular reference frame already in the LM and nBOR. Further studies are needed to clarify this question. It is also possible that transformation to a vestibular reference frame occurs later in the processing of visual inputs to the vestibular nuclei (Graf et al. 1988; Leonard et al. 1988; Wylie et al. 1993).
3. Connectivity of the pretectal area in goldfish (*Carassius auratus auratus*)

3.1. Introduction

Two mechanisms act hand in hand to stabilize gaze: the vestibulo-ocular reflex (VOR) and the optokinetic reflex (OKR). The VOR ensures image stabilization during eye-, head- and body rotations by eye movements directed opposite to the ego motion and receives its input from the semicircular canals. On the other hand the OKR counteracts retinal slip via input from direction selective ganglion cells and initiates eye and/or head movements in the same direction as the stimulus. In all vertebrates tested so far, the neuronal substrate of OKR lies within the pretectum and the accessory optic system (AOS) (Fite, 1985, 1979; Grasse and Cyander, 1982, 1984; Hoffmann and Schoppmann, 1981; Katte and Hoffmann 1980; Simpson, 1984; Winterson and Brauth, 1985). In fish, different from all other vertebrates, only one nucleus relays the visual information for gaze stabilization. In chondrichtyans the so called corpus geniculatum laterale (Masseck and Hoffmann, 2008a) contains the direction selective neurons characteristic for the AOS, whereas in the rainbow trout they are found in the pretectal area (Klar and Hoffmann, 2002). In tetrapodes, other than mammals, segregation of preferred directions into different nuclei occurs. Now a pretectal nucleus, namely the nucleus lentiformis mesencephali (LM) codes horizontal ipsiversive retinal slip (McKenna and Wallman, 1985) and the nucleus of the basal optic root (nBOR), is sensitive to all other directions of motion (Fan et al., 1995; Gruberg and Grasse, 1984; Zhang et al. 1999). Both nuclei complement each other in their properties and build up the neuronal substrate for gaze stabilisation relying on visual information.

Anatomically the AOS of mammals consists of three nuclei: the dorsal terminal nucleus (DTN), the medial terminal nucleus (MTN) and the lateral terminal nucleus (LTN) (Simpson, 1984). A special feature of all three nuclei is their responsiveness to particular directions, as the DTN responds best to horizontal ipsiversive motion, the LTN preferentially to upward and downward movements and the MTN mostly to downward directed stimuli (e.g.: Grasse and Cynader, 1982; Schmidt and van der Togt, 1998). In addition neurons in the nucleus of the optic tract (NOT), a pretectal structure, behave like those in the DTN with a strictly ipsiversive motion preference (e.g.
The first who addressed the question of the AOS in fish were Finger and Karten (1978). They identified retinofugal projections to the following structures in the goldfish: Nucleus suprachiasmaticus, tectum opticum (TeO), dorsomedial optic nucleus, P1 and P2. In addition, two of the retinal targets are labelled retrogradely from the corpus cerebelli, P1 and P2. Based on these afferent and efferent projections, Finger and Karten proposed the P1 and the P2 as suitable candidate substrates to be the telost equivalent of the AOS in other vertebrates. In spite of these findings definite functional and anatomical support for this notion are missing until now. As stated already by Vanegas and Ito (1983) “The teleostean thalamus and pretectum constitute a veritable tower of Babylon”. P1 of (Finger and Karten, 1978) has also been called area pretectalis (APT) by Peter and Gill (1975); area pretectalis pars dorsalis by Uchiyama et al. (1988) and central pretectal nucleus by Wullimann and Northcutt (1988) and Northcutt (1988).

In general, it may be said that the efferent connections of the AOS are relatively conserved in vertebrates. The inferior olive and brainstem nuclei are always innervated by fibers arising from the AOS (Brecha et al., 1980; Giolli et al., 1984, 1985; Maekawa and Simpson, 1973). In addition, the vestibular nuclei receive direct input from retinal slip neurons of the AOS (Bangma and Donkelaar, 1983; Brecha et al. 1980). Non-mammalian vertebrates possess direct connection from the AOS to the cerebellum and oculomotor nuclei (birds: Brecha et al, 1980; McKenna and Wallmann, 1985, amphibians: Montgomery et al., 1981) whereas the mammalian AOS projects via the nucleus praepositus hypoglossi (NPH) and the vestibular nuclei indirectly to the oculomotor nuclei (Cannon et al 1983; Cazin et al., 1984). All these nuclei are therefore possible target sites also of the fish AOS.

*Carassius auratus auratus* (goldfish) were chosen as experimental animal, as they represent one of the model organisms to investigate oculomotor functions in fish. Despite this, only behavioural data are available regarding the OKR in goldfish (Easter 1972, 1974, Beck et al. 2004), whereas the sensory neuronal substrate of OKR remains unknown. During visual whole field stimulation the goldfish’s optokinetic eye movements are similar to those seen in other vertebrates (Easter 1972; Dieringer et al.1992, Marsh 2007). Even gains of 0.8 or higher are reached during binocular stimulation with low stimulus velocities (<4°/s) (Dieringer et al., 1992). Coupling
between saccades of both eyes appear during OKR, whereas at low stimulus velocities a slight asymmetry between the slopes of pursuit movement in both eyes is visible.

It is also known that goldfish possess an optokinetic afternystagmus (OKAN), an indicator for a velocity storage network (Pastor et al., 1994, Marsh and Baker, 2007). Pastor et al. (1994) further identified the neuronal substrate of the velocity storage. Two separate brainstem nuclei, one for position integration (area I) and one for velocity integration (area II) were found in the hindbrain of goldfish. Area I and II are thought to be the functional equivalent of the mammalian nucleus praepositus hypoglossi (NPH) (Beck et al., 2006; Pastor et al., 1994) in fish. Thus also area I and II are suitable candidates for a direct projection from the AOS.

Our aim was to identify the visuomotor interface of OKR in teleosts and to elucidate its connectivity, leading to a comprehensive overview of the AOS in teleost fish.

3.2. Materials and Methods

Data from five goldfish were included in the present study. Animal size varied between 5cm -15cm in length and included animals of both sexes. All experiments were approved by the local authorities (Regierungspräsidium Arnsberg) 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 NIH guidelines for care and use of animals for experimental procedures.

Animals were first anesthetized before surgery by immersion in a bath containing 0.1% MS222. Anesthesia was further supplemented locally with 2.5% lidocaine, before a craniotomy was performed to allow access to the left tectum opticum and pretectum. Immediately following surgery the animals were immobilized with Flaxedil (0.5-1 mg) and transferred to a transparent recording hemisphere (diameter 70cm), where they were artificially ventilated with cooled water (14°C). Single unit recordings with glass-coated tungsten microelectrodes or glass micropipettes (impedance 1-2.5 MΩ) were made in the left pretectum. Receptive field sizes were qualitatively tested with single spots of light (diameter 4°-10°) produced by a hand lamp. For quantitative investigation of the responses to movement the visual stimulus consisted of random light dots projected into the hemisphere by a planetarium projector centred above the fish’s head (For additional information see Masseck and Hoffmann, 2008). Wholefield visual stimuli of the
planetarium were used to identify direction-selective neurons in the APT. Stimulus speed was kept constant at 10°/s.

3.2.1. Data Analysis

Action potentials were first converted to TTL pulses by a window discriminator. Preamplified signals were then acquired with CORTEX (NIMH, Laboratory of Neurophysiology. Version 5.96), and off-line analysis was performed with a customized Matlab (version 7.0.1) program. As a measure for direction selectivity we fitted a custom gaussian to the tuning curve. Tuning width (TW) was calculated as half of the binwidth at half of the maximum. The direction tuning index (TI) was then derived from the following equation:

\[
TI = 1 - \left(\frac{TW}{(360° - TW)}\right).
\]

All cells with a TI between zero and one were considered as direction-selective. Sharp tuning is indicated by values close to one, whereas broadly tuned cells are characterized by values close to zero. To test for axis-selective cells a multi comparison test (one way analysis of variance, ANOVA) was applied, where activity of the preferred axis had to be significant different from all other directions.

3.2.2. Histological procedures

After identification of direction-selective neurons Tetramethylrhodamine dextran (MW 3000, anionic, lysine fixable, Molecular Probes, administered in 0.3 M PBS) was injected iontophoretically (positive current pulses 7s on/ 3s off, 10µA for 30 min) via the recording pipette to verify the recording site and reveal antero- and reterograde projections.

After a survival time of three to five days, all animal subjects were euthanized by an overdose of MS-222. Fish were perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in 0.1M 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. Fixated brain tissue were then removed and stored overnight in the same fixative at 4°C. The next day the brains were cryoprotected with 30% sucrose in 0.1M PB for another 24 h. Brains were then embedded in chicken albumin (Sigma) and 30µm sections were cut in the frontal plane on a cryostat. Two series were collected, the first was used for fluorescence; the second was stained either with cresyl violet only or with a combination of a myelin stain (Gallyas) and cresyl violet, or according to Klüver-Barrera (Romeis and Böck 1989).

### 3.2.3. Anatomical reconstruction

Tissue sections were examined using a fluorescence microscope (Zeiss Axiophot) and under brightfield illumination. Fibers were reconstructed using a fluorescence microscope (Zeiss Axiophot) coupled to a computer based reconstruction system (Neurolucida) equipped with a digital camera (Zeiss Axiophot). Images were subsequently processed for contrast and brightness by using either Adobe Photoshop 7.0.1. or Corel Photo Paint 13. Brain regions were named following the nomenclature of Billard and Peter, 1982 (rainbow trout) and Peter and Gill, 1975 (goldfish).
3.3. Results

3.3.1. Electrophysiology

At the beginning of our studies penetrations perpendicular to the tectal surface were made within the whole pretectum to pinpoint places, where direction selective neurons are located. It turned out that direction-selective cells were always present at a particular location and depth. Penetrations carried out at positions with tectal receptive fields located between 80°-115° Azimuth and 0°-40° Elevation yielded frequently to direction-selective neurons in the pretectum. Furthermore direction-selective neurons were always located in a depth of 1800-2600µm underneath the tectal surface. Later, tracer applications confirmed that all penetrations were located within the pretectal area (APT, Gill and Peter, 1975)

Altogether thirty direction-selective neurons from twenty-three goldfish were recorded. All direction-selective neurons enhanced firing during stimulation in their preferred direction and ceased firing during stimulation in the non-preferred direction (Fig.3.1). Moreover all direction-selective neurons had large receptive fields situated in the contralateral visual field.

Considering the whole sample of direction-selective neurons no bias for horizontal or vertical stimuli was existent (Rayleigh-test, p-value= 0.24). This equal distribution of preferred directions is in accordance with former findings in the trout (Klar and Hoffmann, 2002).

Not only direction-selective neurons were found, also a large sample (thirty) of recorded neurons was sensitive to motion (i.e.: moving stimuli lead to enhanced firing, independent from the presented direction, whereas during presentation of stationary stimuli neurons were only spontaneously active).
Figure 3.1: Peristimulus time histograms and raster plots of a direction-selective neuron in the left APT of *C. auratus* recorded with a recording pipette. Stimulus velocity 10°/s. x-axis time in ms, y-axis Imp/s. Black line represents spike density function, which is based on a Gaussian filtering of the spike train. Grey lines indicate start and end of the stimulus movement. 0ms-2000ms stationary phase, 2000ms-5000ms horizontal movement in naso-temporal direction (180°), 5000ms-7000ms another stationary phase, 7000-10000m movement in temporo-nasal direction (0°).
3.3.1. Projections of the APT in Carassius auratus auratus

In five out of seventeen goldfish the application of RD into the APT after recording of direction-selective responses was successful (Fig. 3.2 B). Retrogradely labelled ganglion cells were not localised to a particular region, but instead were distributed evenly across the entire contralateral retina. No retrogradely labelled cells could be found in the ipsilateral eye. Terminals labelled after APT injections were found in a number of different areas. Ipsilateral to the injected APT fibers run lateral via the anterior tractus mesencephalocerebellaris caudal to the midbrain and reached their first termination field, the nucleus oculomotorius (NIII) (Fig. 3.2 B) and nucleus trochlearis (NIV). From here fibers continued their way to the hindbrain via the medial longitudinal fasciculus (MLF) and the lateral longitudinal fasciculus (LLF). Some fibers passed from the LLF to the anterior and posterior tractus mesencephalocerebellaris and projected to the valvula cerebelli (Fig. 3.2 C) and the corpus cerebelli. Residual fibers continued to the hindbrain in the MLF, LLF and tractus tecto-bulbaris (TTB). At the hindbrain level, the vestibular nuclei (VN) (Fig. 3.2 D) and the reticular formation were termination sites of APT. At more caudal levels of the hindbrain the nucleus abducens (N VI) was innervated by fibers coming from the tractus tecto-bulbaris. Farthermost caudal area II and the inferior olive receive ipsilateral fibers from the APT. Contralateral projections were sparsely developed. Few fibers from the APT passed via the comissura posterior to the contralateral side and innervated different target areas, such as the N III, N IV, N VI, valvula cerebelli, corpus cerebelli, reticular formation and the vestibular nuclei. In summary, a general overview across all animals (Table 3.1) revealed that contralateral termination sites are non-uniformly distributed and not always present in all animals. Injections into the APT sometimes led to labelling of fibers in the tractus opticus continuing to the optic tectum. These projections were not mentioned specifically, as we were not able to exclude the possibility that terminals in the tectum opticum could have arisen from retinal fibers of passage. Furthermore also the ipsi- and contralateral torus longitudinalis were labelled after most of the APT injections in goldfish (Table 3.1).
3.3.2. Control experiments

In addition, three goldfish inadvertently received RD injections in areas located near the APT which allowed us to exclude aberrant efferent connections of the pretectum from the specific APT projections. One injection was made into the nucleus rotundus (Peter and Gill, 1975), which is also called nucleus pretectalis superficialis pars magnocellularis by Wullimann and Northcutt (1988), another one was located in the nucleus ventromedialis thalami and the last one was situated in the tectum opticum. None of these areas had connections to the described termination sites of the APT. The only overlap with the APT projections was seen in the torus longitudinalis after the tectum opticum injection.

Figure 3.2: A-D. Photomicrographs of frontal sections after injection of RD into the APT. A Injection site of RD into the APT in goldfish. Scalebar represents 100µm. B Anterogradely marked terminals at the level of the ipsilateral N III. Scalebar represents 50µm. C Anterogradely marked terminals at the level of the valvula cerebelli. Scalebar represents 50µm. D Terminals at the level of the ipsilateral vestibular nuclei. Scalebar represents 50µm. E Photomicrograph of a retrogradely labelled ganglion cell in the contralateral retina after RD injection into the APT. Scalebar 20µm. Position of each cut-out is indicated in figure 3.3.
Table 3.1: Efferent projections of the APT in goldfish.

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Each row represents a specific animal. Red shading corresponds to ipsilateral projections and light green represent contralateral projections. x terminals in the marked area; --- no terminals. III nucleus oculomotorius; IV nucleus trochlearis; VI nucleus abducens; A II area II; CC corpus cerebelli; IO inferior olive; RF reticular formation; TL torus longitudinalis; VC valvula cerebelli; VN vestibular nuclei.
3. Connectivity of the APT in goldfish

3.4. Discussion

This study clearly identifies the APT as the AOS of teleost fish. The afferent and efferent connectivity of the APT are remarkably similar to that in tetrapods. As in all other vertebrates, input to the AOS is derived primarily from the contralateral retina. All recorded neurons were also physiologically similar to AOS units, possessing large receptive fields and direction-selective responses to large slowly moving random-dot stimuli. Furthermore, direct connections to the oculomotor nuclei, the inferior olive, cerebellum, vestibular nuclei and area II underpin homology of the APT to the AOS in tetrapods.

Marking of the CPN, which was also supposed to be part of AOS of fish, was not observed at all. However, further functional and anatomical studies are needed to clarify if the CPN plays a role in gaze stabilization.

3.4.1. Retrograde labelled ganglion cells

As with other vertebrate classes, visual input to the AOS and pretectum arises primarily from direction-selective ganglion cells in the contralateral retina. Retrogradely marked ganglion cells were distributed over the entire retina and were not accumulated to a specialized region like in some other vertebrates.

After HRP injection into the nucleus of the basal optic root in frogs and pigeons displaced ganglion cells were labelled over the entire retina, with emphasis on the periphery (Cook and Podugolnikova, 2001; Karten et al., 1977). As in birds, visual projections to the AOS of the chameleon are also exclusively derived from displaced retinal ganglion cells evenly distributed across the contralateral retina (Bellintani-Guardia and Ott, 2002).

In mammals, input to the AOS arises mainly from specialized regions of the retina if present. Injecting the medial terminal nucleus (MTN) of rabbits with HRP, Oyster and Simpson (1980) observed that labelled ganglion cell were localized within the horizontal streak of the contralateral retina; in the same manner ganglion cells of the area centralis and of the horizontal streak in cats were also marked more abundantly than the retinal periphery following HRP injection into the MTN (Farmer and Rodieck, 1982).
3.4.2. The AOS of fish

A major concern of former anatomical studies of the pretectum of fish was the connectivity of the corpus cerebelli and therewith also the identification of pretectal areas which might be involved in oculomotor functions. Unfortunately, the existence and occurrence of pretectal nuclei varies considerably among teleost species. Uchiyama et al. (1988) revealed in *Navodon modestus* ipsilateral efferent connections from the dorsal pretectal area to the corpus cerebelli (CC) and to the tectum opticum. Furthermore ventral parts of the pretectal area exhibit ipsilateral projections to the CC, the nucleus oculomotorius (NIII) and the nucleus abducens (NVI). Uchimaya et al. (1988) concluded homology of the ventral pretectal area to the nucleus pretectalis, which correspond to P2 of Finger and Karten and homology of the dorsal pretectal area to APT, which corresponds to P1 of Finger and Karten. Due to the fact that we found projections from the APT to the CC, NIII and NVI, ventral and dorsal parts of the pretectal area of *N. modestus* might both correspond to the APT. Also Ito et al. (1982) confirmed a direct projection from the APT in goldfish to the CC. Furthermore Wullimann and Northcutt (1988) described a direct projection from the APT, to the corpus cerebelli and to the valvula cerebelli in goldfish, termed in their study as central pretectal nucleus (CPN). Xue et al (2008) investigated the connectivity of the inferior olive in goldfish and found that the accessory optic nucleus (AON) is the source of pretectal input to the inferior olive. Considering the histology of the AON of Xue et al. (2008), it seems that dorsal parts of the AON correspond to caudal parts of the APT of Peter and Gill (1975) and P1 of Finger and Karten (1978) and ventral parts of the AON correspond to the pretectal nucleus of Peter and Gill (1975), which is equivalent to P2 of Finger and Karten (1978).

Summarized our experiments confirm former results, that the APT is a source for input to the CC and the IO. Moreover results obtained by Uchiyama et al. (1988) regarding dorsal and ventral parts of the pretectal area in *N. modestus* point to the existence of direct projection from the pretectal area to the NIII and NVI, although it is hard to conclude homology of the APT in goldfish and the pretectal area of *N. modestus*, as both species are not closely related and the occurrence of visual related nuclei differs considerably among them.
3.4.3. Efferent connections of the APT

Ipsilateral projections

As in amphibians, reptiles and birds the AOS in teleost fish projects directly to the oculomotor nuclei (III, IV and VI). In frog the III and IV are connected with the nBOR (Montgomery et al. 1981) and in addition the VI receives direct input from the LM (Chochran et al., 1984). After HRP injections into the nBOR of pigeons the III and IV were labelled bilaterally (Brecha et al., 1980). In our case, contralateral projections to the oculomotor nuclei were not as well established as the ipsilateral projections, but in the trout a bilateral innervation of the oculomotor complex was clearly visible (Gürke, diploma thesis, 2004). All fish showed strong ipsilateral projections to the cerebellum and the valvula cerebelli (Fig. 3.2). In addition to efferent connections to the oculomotor nuclei, a direct link to the cerebellum is a common feature of the AOS in tetrapods, except in mammals (Brecha et al., 1980; Finger and Karten, 1978). In most of our cases, the inferior olive (IO) also received ipsilateral input from the APT like from the AOS in all other vertebrates (e.g.: Giolli et al., 1984; Walberg et al., 1981; McKenna and Wallman, 1985; Wylie et al., 2007). In addition, terminals to the reticular formation, the vestibular nuclei and the area II were present. Direct or at least indirect projections from the AOS to the vestibular nuclei via the cerebellum, inferior olive or the reticular formation, have been verified for most species (e.g.: Brecha et al., 1980, Bangma and Ten Donkelaar, 1983; Magnin et al., 1989).

In mammals, direct projections from the NOT to the nucleus prepositus hypoglossi (NPH) are well established (Magnin et al., 1989). The NPH is a velocity-to-position integrator and is needed to produce horizontal eye position commands for the oculomotor nuclei. The functional analogue in fish is the area I located in inferior parts of the reticular formation (Pastor et al., 1994). For this reason we can neither confirm a direct projection to area I nor refute it.

In our experiments, a connection between bilateral APTs was never seen, although in tetrapods connectivity among nuclei of the AOS is well established (e.g.: Giolli et al., 1984; Prochnow et al., 2007). In birds the nucleus lentiformis mesencephali and the nucleus of the basal optic root (nBOR) are interconnected with each other and furthermore also interconnections of the nBOR with its counterpart on the other brain side are described (Brecha et al., 1980; Wylie et al. 2007). Also mammals posses these
extensive interconnection between nuclei of the AOS (Blanks et al., 1995; Simpson, 1988; van der Togt and Schmidt, 1994) and furthermore nuclei of the AOS are interconnected with the nucleus of the optic tract (NOT) (Simpson 1988, Blanks et al. 2000). In all of our application also the terminals to the ipsialertal located tectum opticum were existent, as discussed earlier we can not exclude that this projection is due to labelling of fibers from the nearby optic tract. But a study by Uchiyama et al. (1988) revealed efferent fibers from the dorsal pretectal area of *N. modestus*, the presumptive homolog to the APT in goldfish, to the tectum opticum. Also for mammals direct projections from the pretectum to the superior colliculus (homolog to the tectum opticum) are reported (Holstege and Collewijn, 1982) and are of GABAergic nature (Born and Schmidt, 2007). It seems likely that also in goldfish a direct pretectal-tectal connection is evident.

In goldfish, the torus longitudinalis also seems to be a target area of the APT. Our result is supported by a study of Ito et al. (2003) which revealed direct projections from the pretectal area in the carp to the torus longitudinals. Main input to the torus arises from the tectum opticum and exhibits burst proportional to saccade amplitudes (Ito et al., 2003; Northmore, 1984). It is suggested that the torus provides corollary discharge to the tectum opticum to distinguish between retinal slip resulting from saccades and slip produced by body or external world movements.

**Contralateral projections**

Contrallateral projections were always only sparsely developed and inconsistent over different animals. One reason for this irregularity might be that tracer applications were made into different parts of the APT over different animals. As a study by Wylie et al. (2007) nicely demonstrated that individual neurons of the nBOR have only one single target. Hence different application sites could lead to different projection sites.
3. Connectivity of the APT in goldfish

3.4.4. Functional implications

Our recordings of direction-selective neurons in the APT showed no bias for vertical or horizontal directed movements. Therefore the direct projections from the APT to all oculomotor nuclei could initiate eye movements to reduce retinal slip in all directions. It is conceivable that neurons would innervate oculomotor nuclei corresponding to their preferred direction (i.e.: neurons which prefer ipsiversive horizontal movements would project to the N VI to activate the lateral rectus muscle of the ipsilateral eye). This connectivity pattern is present in all other non-mammalian tetrapods, and seems to be a universal wiring plan of the vertebrate nervous system. Chochrane et al. (1984) suggested: “… these direct connections act to initiate ocular movements and accelerate the eye, whereas more indirect pathways may act to maintain eye position”. Indirect pathways to maintain stable gaze might be the following in teleost fish:

Direct and indirect projections via the IO to the cerebellum could build up a feedback loop to correct inappropriate eye movements and to calibrate the VOR, as in other vertebrates (e.g.: Ito et al., 1984; Lisberger, 1988).

Vestibular neurons modulate their discharge due to visual input (Dichgans et al 1973; Allum et al., 1979). Additional visual input allows a way to compensate for the disability of the vestibular system to detect low frequency body movements and prolonged constant velocity rotations. Projections arising from direction-selective neurons in the APT are suitable candidates for the visual input to the vestibular nuclei.

To maintain stable gaze an eye velocity storage mechanism is needed. Pastor et al. (1994) were able to characterize area II in the goldfish hindbrain as a velocity integrator. Neurons in area II are sensitive to visual and vestibular stimulation and exhibit discharge in proportion to eye and head velocity (Beck et al., 2006). Our direct projection coming from the APT could provide the appropriate visual input to area II.

In both goldfish and trout the reticular formation obtains terminals from the APT. In general, the reticular formation of fish is involved in saccadic eye movements (Luque et al., 2006). Thus signals arising from the APT could contribute to resetting saccades occurring during OKN. Pastor et al. (1994) described a nucleus termed area I as a velocity to position integrator for horizontal eye movements. We were not able to clearly distinguish area I neurons from neurons of the reticular formation, as area I lies straight above the IO in inferior parts of the reticular formation. Anyway, direction-selective input from the APT to the velocity to position integrator seems plausible.
Sensory input coming from the visual system is needed to establish feedback mechanisms in this integrator. This and the homology of area I and II to the nucleus praepositus hypoglossi (NPH) of mammals, which receives direct input from the AOS (Magnin et al. 1989) support our assumption that area I is a projection site of the APT. Identified motion sensitive neurons in the APT might provide input to the tectum opticum and the torus longitudinalis, which are both involved in the generation, execution and fine tuning of saccades (Luque et al. 2005). If these motion sensitive neurons in the goldfish APT correspond to jerk neurons in the pretectum of mammals (Schweigart and Hoffmann, 1992; Schmidt, 1996) is still outstanding and further electrophysiological studies need to be done.
Figure 3.3: Left side panel: Neurolucida reconstruction of frontal sections from *C. auratus*. Reconstruction sequence from rostral to caudal. Triangles mark termination sites. Right side panel: Photomicrographs of corresponding Nissl stained sections. Insets refer to photomicrographs in Fig. 3.1 taken from the specified region.

A II area II; APT area pretectalis; CC corpus cerebelli; FL facial lobe; IO inferior olive; gr granular layer of CC; LLF lateral longitudinal fasciculus; MLF medial longitudinal fasciculus; NP nucleus pretectalis; PC posterior commissure; RF reticular formation; TeO tectum opticum; TL torus longitudinalis; TMCa tractus mesencephalicerebellaris anterior; TMCp mesencephalicerebellaris posterior; TTB tractus tecto-bulbaris Val valvula cerebelli; VL vagal lobes; VN vestibular nuclei; III nucleus oculomotorius; IV nucleus trochlearis; VI nucleus abducens;
4. Do visual direction-selective neurons of the area pretectalis of goldfish share a common reference frame with the vestibular system?

4.1. Introduction
To stabilize gaze a sensorimotor integration is needed. Reference frames of sensory input and motor commands differ considerably, as sensory input coordinate systems are based on spatiotemporal activity, whereas motor commands are aligned with the pulling directions of their muscles. Different sensory representations of ego motion have to be combined into one reference frame to elicit the appropriate eye movement. Such a unique internal representation of ego motion would facilitate neuronal processing for gaze stabilisation (Hengstenberg 1998; Wallman and Velez 1985; Wylie et al. 1988). In the simplest case only one sensory modality (e.g. visual or vestibular) has to be transformed to a motor output. Also proprioception (e.g.: of the neck) plays an important role in gaze stabilization, but for simplification we will only refer to visual and vestibular integration.
Extraocular motor neurons discharge already correlates with eye position and eye velocity, at least they code in a reference frame defined by the pulling directions of the extraocular muscles (Pastor et al., 1991). It seems favourable to retain this internal reference frame for all sensorimotor transformations. Anyway, two more reference frames come into question:

1. A vestibular reference frame based on the orthogonal organisation of the semicircular canals, which build up a three-dimensional coordinate system.
2. A retinal reference frame coding in a two-dimensional coordinate system

In most animals, like in the rabbit, spatial organization of the semicircular canals and the extraocular muscles planes are lined up (Ezure and Graf, 1984). Whereas in other animals canal planes and muscle planes differ considerably (e.g. cat, Maioli). Anyway, in general the spatial reference frame of the extraocular muscles and the semicircular canals are very similar, independent from the position of the eyes (lateral vs. frontal eyed animals) (Graf 1981). I.e. the pulling direction of a specific extraocular muscle is aligned with the semicircular canal it is maximally excited by. In frontal eyed animal’s insertion points of extraocular muscles changes in a way that their pulling direction is
still aligned with the semicircular canal they are best excited by (Graf and Simpson, 1981).

The vestibulo-ocular reflex (VOR) and the optokinetic reflex (OKR) are intrinsically tied to each other. In daily life gaze stabilization is a result of input from both sensory systems, as head/body movements are always combined with retinal slip on the retina. Vestibular and visual information merge in the vestibular nuclei, where second order vestibular neurons are sensitive to vestibular as well as to visual stimulation (Allum et al., 1976; Dichgans et al., 1973; Waespe and Henn, 1977). Not only in vestibular neurons visual and vestibular information are combined, also at the vestibulocerebellum information from both sensory systems is processed (Brecha et al., 1980; Maekawa and Simpson, 1972, 1973, Wylie et al., 1993).

Simpson and co-workers dealt already with the spatial organization of direction-selective neurons in the AOS (Simpson et al., 1988). Recordings from the medial terminal nucleus (MTN) and the visual tegmental relay zone (VTRZ) of rabbits revealed neurons with monocular and binocular bipartite receptive fields, preferring opposing stimulus movements, making them to ideal detectors of ego motion. Preferred rotation axes of these neurons lie in close spatial relation to the best response axis of the vertical semicircular canals. This led to the assumption that the AOS inherits a reference frame similar to the semicircular canals (Simpson et al., 1988).

Other studies revealed neurons in the vestibulocerebellum coding for rotational and translational optic flow (Wylie, 1993; Simpson, 1988). Rotational sensitive neurons in the vestibulocerebellum of rabbits are best excited by flow fields resulting from head rotation of maximally activating the semicircular canals and thus seem to be organized in vestibular coordinates (Graf et al. 1988). In pigeons visual climbing fiber input to the purkinje cells are thought to share one reference frame with the vestibular system (Wylie and Frost, 1993). But a closer look at the eye muscles pulling directions and the averaged preferred direction of neurons in the nucleus lentiformis mesencephali of pigeons accomplished that the AOS of pigeons is organized in an eye muscle reference frame rather than in a vestibular one (Wylie and Frost, 1996).

The area pretectalis of bony fish represents their accessory optic system (Klar PhD-thesis, 2005, Masseck et al., for revision). In contrary to tetrapodes the AOS of fish consists of only one nucleus, where all directions of motion are represented (Klar and Hoffmann 2002; Masseck and Hoffmann, 2008a). Whereas anatomical connectivity is remarkable similar to the AOS of tetrapodes, except mammals (Masseck et al., in
For chondrichtyans (*S. canicula*) a conversion of coordinate systems could not be shown for pretectal structures (Masseck and Hoffmann, 2008a). Thus the main objective of our study was to investigate the spatial reference frame of direction-selective neurons of the APT in a bony fish.

### 4.2. Materials and Methods

#### 4.2.1. Visual Stimuli

1. **Testing for a retinal reference frame**

   A random dot pattern projected by a planetarium projector was used to map the preferred directions of stimulus movements existing amongst neurons in the pretectum of goldfish. Eight stimulus directions were presented: 1. Horizontal movements from temporal to nasal (0°) and nasal to temporal (180°) (YAW), 2. Vertical movements from ventral to dorsal (90°) and from dorsal to ventral (270°) (ROLL), 3. Oblique movement from temporo-ventral to naso-dorsal (45°) and naso-dorsal to temporo-ventral (225°), 4. Oblique movements from naso-ventral to temporo-dorsal (135°) and from temporo-dorsal to naso-ventral (315°). (For further information see chapter 2.2 or Masseck and Hoffmann, 2008a). All stimulus movements resulted from rotation around axes lying in the sagittal plane.

2. **Testing for a vertical semicircular canal reference frame**

   In addition, four rotational axes in the horizontal plane were tested to find out whether the strongest responses were elicited by rotations around axes of the vertical semicircular canals. Around every axis the planetarium turned in clockwise (CW) and counterclockwise (CCW) directions leading to image motion on the right retina like that during the following body movements: 1. ROLL (planetarium rotation around the longitudinal axis of the fish), 2. LARP (planetarium rotation around the left anterior right posterior axis of the fish). 3. PITCH (planetarium rotation around the transverse axis of the fish): 4. RALP (planetarium rotation around the right anterior left posterior). [Stimuli are described in detail in: Masseck and Hoffmann, 2008a]. Stimulus speed was kept constant at 10°/s. Each trial consisted of a stationary phase (0-2000ms), a rotation in CW direction (2000ms-5000ms), another stationary phase (5000ms-7000ms) and a rotation in CCW direction (7000ms-10000ms).
4.2.2. 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 (version 7.0.1) program. To test for direction selectivity a customized gaussian fitting was used (Fig. 4.1). Before a gaussian curve was fitted to the data, obtained data were smoothed by an algorhytm (see Fig.4.1). Tuning width (TW) was calculated as half of the binwidth at half of the maximum. From the TW the tuning index (TI) was calculated as follows:

$$TI = 1 - \left( \frac{TW}{360^\circ - TW} \right). \quad (\text{See Hoffmann et al. 2002})$$

All cells with a TI between zero and one were considered as direction-selective. Sharp tuning is indicated by values close to one, whereas broadly tuned cells are characterized by values close to zero (Fig. 4.2 A, B). To test for axis-selective cells a multi
comparison test (one way analysis of variance, ANOVA) was applied, where activity of the preferred axis had to be significant different from all other directions.

Spontaneous activity was calculated by averaging activities from all presentations of the stationary pattern. As null direction the axis with minimal activity was taken. Obtained results were tested for significant differences with a rank sum test and plotted in a scatterplot.

To look in detail for axis preference and therefore coding in a vestibular vs. retinal reference frame a directional index (DI) was calculated as following:

$$\text{DI} = \frac{\text{max} - \text{min}}{\text{max}};$$

where max represents the maximal mean spike activity in a particular axis of rotation (i.e. yaw, roll, etc.) and min refers to the minimal mean spike activity in the same axis. All cells with a DI over 0.3 where taken into account for further calculations. Analysis was done for all recorded axes and the axis with the highest DI was considered to be the best responsive axis, independent from the direction of rotation (i.e. CW vs. CCW). To test for significant differences between an equal distribution of preferred axes against a distribution in favour of the vestibular system a $\chi^2$-test was used. In further analysis also the direction of rotation was considered. A fourfold test is used to figure out if a bias for directions or axes is present. $\chi^2$ is calculated by:

$$\chi^2 = \frac{n(a^2d - c^2b)^2}{(a + c)(b + d)(a + b)(c + d)},$$

in which a, b, c, d, and n are determined by following table:
4. Reference frame APT

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<th>Direction</th>
<th>Opposite direction</th>
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<td>Obtained results</td>
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<tr>
<td>Results for an equal distribution</td>
<td>c</td>
<td>d</td>
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<tr>
<td>( \sum )</td>
<td>a + c</td>
<td>b + d</td>
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Depending on the obtained \( \chi^2 \) value \( p \) is calculated:

\[
p = \frac{1}{2} \times 10^{3.84}.
\]

4.2.3. Data comparison with other studies

To calculate, like Maioli and Ohgaki (1993), the preferred and null-directions of a direction-selective neuron a two-harmonic Fourier expansion was fitted to the obtained data. General formula of a two-harmonic Fourier fit:

\[
a_0 + a_1 \cos(x*w) + b_1 \sin(x*w) + a_2 \cos(2*x*w) + b_2 \sin(2*x*w)
\]

Minimum and maximum of the function were taken as null and preferred direction (Fig.4.3), whereas minimum and maximum of the function were calculated via the first and second derivative.

Figure 4.3: Example of a Fourier fit. X-axis angle of rotation in degrees, y-axis Imp/s. Black points correspond to real data points, green dots represent calculated preferred and null-direction. Black line indicates mean spontaneous activity. Preferred direction of this direction-selective cell is 52 degrees, and calculated null direction is 171 degrees.
Afterwards, preferred directions and null directions of the whole population were plotted in a polar diagram for further analysis.

### 4.3. Results

#### 4.3.1. Retinal reference frame

All in all sixty-one area pretectalis neurons from twenty-three goldfish were recorded with visual wholefield stimulation. Thirty of them were significantly direction-selective, one was axis selective and the remaining thirty were motion sensitive. All neurons had large receptive fields located in the contralateral visual field.

A characteristic peristimulus time histogram (PSTH) of a direction-selective neuron is shown in Fig. 4.4. Preferred direction of the recorded neuron is 214 degrees with a TI of 0.78. The resulting tuning curve is shown as a polar plot in Fig. 4.5A. Mean activity in null-direction is 2 Imp/s, whereas spontaneous activity is on average 5.2 Imp/s. All recorded direction-selective neurons exhibited inhibition in null-direction (Fig. 4.6A) (p<= 0.001). Considering all preferred directions no bias for any movement is obvious (Fig. 4.8A), preferred directions are uniformly distributed (Rayleigh-test, p-value= 0.24). No recorded neuron had its preferred direction around vertical down (270°); however this might be due to our sampling size.

![Figure 4.4: Peristimulus time histograms and raster plots of a direction-selective neuron in the left APT of *C. auratus*, as tested through the right eye with a retinal stimulus moving at a velocity of 10°/s. Black line represents spike density function, which is based on a Gaussian filtering of the spike train. 0ms-2000ms stationary phase, 2000ms-5000ms stimulus movement in direction of the assigned angle.](image-url)
Figure 4.5: Polar plots of direction-selective neurons, dash-dotted circles represent activity in spikes per second, grey circle represents mean spontaneous activity in spikes per second. A Neuron recorded with a retinal reference frame stimulus. B Neuron recorded with axes lying in the horizontal plane. Abbreviations see figure 4.4.

Figure 4.6: Scatter plot comparison of spontaneous and activity in null-direction. X-axis: activity in null-direction [Imp/s]. Y-axis: Spontaneous activity [Imp/s]. A Scatterplot for neurons which are direction-selective with the retinal reference frame stimulus. B Scatterplot of direction-selective neurons recorded with axes lying in the horizontal plane.

Figure 4.7: A Each arrow represents the weighted preferred direction of one direction-selective neuron recorded for rotation around axes in the sagittal plane. B Polar plot of weighted preferred directions for rotation around axes in the horizontal plane. Legend see figure 4.4.
4.3.2. Vertical semicircular canal reference frame

Also sixty one neurons were recorded with stimulus axes lying in the horizontal plane. Eighteen of them were significantly direction-selective and all remaining forty-three were motion sensitive. A typical example of a direction-selective neuron is depicted in Fig. 4.8 (the same neuron was also direction-selective to the first stimulus series Fig. 4.3). Its preferred direction lies between LARP DOWN and PITCH DOWN (257°) and the TI is 0.83 (Fig. 4.5B). As in the first stimulus series inhibition in null-direction occurs. Nearly all neurons showed inhibition in null-direction (p<= 0.001) (Fig. 4.6B).

The preferred directions of the direction-selective population are uniformly distributed in the horizontal plane (Rayleigh-test, p>0.9). On closer inspection preferred direction vectors fall into two opposing quadrants (I and III). The mean angle of preferred directions in the first quadrant (between ROLL UP and PITCH UP) is 19 degrees, whereas in the third quadrant (between ROLL DOWN and PITCH DOWN) the mean angle is 229 degrees. To define the best responsive axis of the whole population, all mean preferred direction vectors are plotted modulo 180 degrees (Fig. 4.9) and again the mean population vector is calculated. The angle of the population vector is 45° and its length is 0.788. Our sample of direction-selective neurons is thus bimodally oriented along the LARP axis. To confirm these result a Rayleigh test is applied and proves significance (p<0.001). Thus as a population the left APT would be maximally excited by flow fields produced by head and/or body rotations around the axis of the contralateral anterior vertical canal and the ipsilateral posterior vertical canal – the LARP axis.
Figure 4.8: Same neuron as in figure 4.2 stimulated with axes lying in the horizontal plane. PITCH DOWN: clockwise rotation of the planetarium around the interaural axis of the fish; PITCH UP counterclockwise rotation of the planetarium around the interaural axis of the fish direction. LARP DOWN: CW rotation of the planetarium around the left anterior to right posterior axis of the fish; LARP UP: same axis as LARP CW, but rotation in CCW direction; ROLL DOWN rotation of the planetarium around the longitudinal axis of the fish in CW direction; ROLL UP: rotation in CCW direction. RALP DOWN planetarium rotation around the right anterior to left posterior axis of the fish in CW direction; RALP UP: rotation in CCW direction.
4.3.3. Axes preferences

Fifty cells out of sixty-one recorded had a directional index above 0.3 at least in one axis. Twenty-one cells (42%) had YAW as preferred axis, fourteen (28%) preferred the LARP axis, 2 (4%) preferred the RALP axis, seven (14%) preferred ROLL and the remaining 3 axes (PITCH, 45°-225°, 135-315°) were represented by two cells (2%) each (Fig.4.9 A). Comparing the cells with their best response in one of the three semicircular canal axes (YAW, LARP, RALP) opposed to cells preferring axes which do not correspond to the vestibular system (ROLL, PITCH, 45°-225° and 135°-315°) a proportion of thirty-seven to thirteen is present. This is significantly different from an equal distribution of preferred axes ($\chi^2$-test, $p <= 0.001$). Also within axes corresponding to semicircular canal axes a non uniform distribution is visible. Neurons preferring rotations around the YAW axis are most frequently, followed by neurons preferring rotations around the LARP axis.

To look in detail for preferred axis also the direction of rotation is taken into account (Fig. 4.10B). In the YAW axis eleven neurons preferred motion from nasal to temporal (NT) opposed to ten neurons preferring motion from temporal to nasal (TN). No bias for one of the horizontal directions is existent (fourfold-test, $p=0.82$). In the LARP axis eight neurons code for LARP down for the contralateral eye, whereas six neurons prefer upward directed motion; each direction is equally represented (fourfold-test, $p=0.59$). Five neurons preferred ROLL down, in contrast to two neurons preferring ROLL up, again both directions do not differ in their occurrence (fourfold-test, $p=0.25$). In all other axes the amount of direction-selective neurons is too small to run a fourfold-test. Anyway no obvious bias for one of the recorded rotations in a particular axis is visible.
Figure 4.9: In grey averaged preferred direction vectors of figure 4.5 B plotted modulo 180 degrees. Black arrow represents the mean preferred direction of the whole population.

Figure 4.10: A Bar plot of direction-selective neurons preferring a particular stimulus axis. Y-axis: number of neurons. X-axis: stimulus axis. YAW: Rotation around the vertical axis of the fish corresponds to a horizontal movement from nasal to temporal and vice versa; LARP rotation around the left anterior right posterior axis of the fish; RALP planetarium rotation around the right anterior left posterior axis. PITCH planetarium rotation around the transverse axis of the fish; ROLL planetarium rotation around the longitudinal axis of the fish; 45°-225° around an axis producing oblique movement from temporo-ventral to naso-dorsal (45°) and naso-dorsal to temporo-ventral (225°); 135°-315° around an axis producing oblique movements from naso-ventral to temporo-dorsal (135°) and from temporo-dorsal to naso-ventral (315°). B Bar plot of direction-selective neurons preferring a particular direction presented to the contralateral eye. NT horizontal movements from nasal to temporal; TN horizontal movement from temporal to nasal; LD LARP down; LU: LARP up; RD RALP down; RU RALP up; OD ROLL down; OU ROLL UP; PD PITCH down; PU PITCH UP, 45° oblique movement from temporo-ventral to naso-dorsal; 225° oblique movement from naso-dorsal to temporo-ventral; 135° oblique movements from naso-ventral to temporo-dorsal; 315° oblique movement from temporo-dorsal to naso-ventral (315°). Axes corresponding to semicircular canal axis are plotted in black, whereas all other axes are grey.
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4.3.4. Comparison with data obtained by other investigators

To compare our data directly with other studies dealing with the question of reference frames, some of our data were in addition evaluated with methods used by other authors. Maioli and Ohgaki used a two-harmonic Fourier expansion to calculate the inhibitory and excitatory directional preferences of direction-selective cells recorded in the LTN. A comparison of our data and data from Maioli and Ohgaki are shown in Fig. 4.11. Even with a Fourier fit of our data the overall orientation of preferred directions are the same as with the gaussian fitting (for comparison see figure 4.7 B). A direct comparison of preferred directions of cat LTN neurons and goldfish APT neurons shows that neurons in both structures share similar preferred direction responses.

Figure 4.11: Comparison of data obtained in the LTN of cat and APT in goldfish. A Polar plot of preferred directions in the cat LTN, full lines correspond to preferred direction. Adapted from Maioli and Ohgaki, 1993 B Polar plot of preferred directions in the APT of goldfish. C Polar plot of null directions in the cat LTN, broken lines correspond to null-direction. Adapted from Maioli and Ohgaki, 1993. D Polar plot of null-directions in the APT of goldfish. 0° correspond to ROLL up, 90° PITCH up, 180° ROLL down, 270° PITCH down.
A comparison of null-directions reveals differences between cat and goldfish. In the cat null-directions are more clustered around the LARP-axis, whereas in goldfish an almost equal distribution of null-directions occurs.

For further analysis Maioli and Ohgaki plotted preferred directions modulo 180 and obtained a bimodal distribution with one peak around 20° and another around 60°. These bimodal peaks correspond well with the orientation of the pulling direction of the vertical recti (29°) and vertical oblique (57°) muscles in the cat. Due to this they concluded that sensory information in the LTN is coded in an extraocular muscles frame. If our data are plotted modulo 180 a unimodal distribution around a 47° is present (Fig. 4.12).

An explanation for these differences is a quite different spatial organization of vertical semicircular canals and eye muscles in the goldfish (Fig. 4.13). In contrast to the cat, extraocular muscles planes in the goldfish enclose an angle of 52° for both vertical recti and vertical oblique with the midsagittal plane. And also angels formed with the semicircular canals are different with 43° for the anterior semicircular canal and 44° for the posterior vertical semicircular canal opposed to 41° for the anterior canal and 52° for the posterior canal in the cat. Hence pulling directions of the extraocular muscles and semicircular canal are more aligned in the goldfish and an preferred population angle of 47° is inbetween angles enclosed with the semicircular canals and extraocular muscles in the goldfish.

Preferred and null direction of direction-selective neurons in the cat LTN are close to collinear oriented (Grasse and Cynader, 1984). Although other authors like Simpson and co-workers found a non collinear arrangement in the rabbit (DTN, MTN and LTN) and in the pigeon nBOR (Morgan and Frost, 1981). In our sample of direction-selective neurons preferred and null directions were collinear oriented, i.e. minimal and maximal.
Figure 4.14: Distribution of angular separation between preferred and null-direction vectors. Two major subgroups of vectors were apparent in figure 4.11B, showing a LARP upward and LARP downward component, respectively. A All preferred directions have been aligned to 45° (red arrow). Grey dashed arrows indicate the angular difference between preferred and null directions. Black dashed arrow represents the mean angular difference of all null direction vectors. B As in A but now the LARP down component has been plotted. C Direct comparison of the mean angular difference between preferred and null direction (173°) for all LARP up preferring neurons. D Direct comparison of the mean angular difference between preferred and null direction (181°) for all LARP down preferring neurons.

Figure 4.13: Spatial relationship between vertical semicircular canals and extraocular muscles. Left side: Denoted angels are mean values over two animals. Right side: Photomicrograph of taxidermy of vertical superior extraocular muscles and vertical semicircular canals. AC anterior canal, PC posterior canal, SO superior oblique, SR superior rectus.
responses did almost lie in one rotational axis. Further support for collinearity in the goldfish comes from inspection of data plotted corresponding to the difference between preferred and null-direction (Fig. 4.14). On average preferred and null-direction are separated by an angle of 173° for LARP up preferring neurons and 181° for LARP down preferring neurons. Hence collinearity of preferred and null-directions follows (Fig.4.14).
4.4. Discussion

4.4.1. General properties of direction-selective neurons

All in all sixty-one, at least motion sensitive, neurons in the left APT of goldfish were recorded. All neurons shared large receptive fields in the contralateral visual field and were most sensitive to large moving stimuli. Thirty-three percent of the direction-selective neurons exhibited phasic bursts to movement onset. Spike responses to preferred direction stimulation lead to a sustained tonic firing as long as the movement persisted, whereas stimulation in null-direction leads to inhibition below spontaneous activity. All neurons were only excited by movements in the contralateral visual field, whereas stimulation of the ipsilateral eye did not lead to modulation of the firing rate. Thus direction-selective neurons share the same properties as direction-selective neurons belonging to the AOS of tetrapodes (Collewijn, 1975; Grasse and Cyander, 1982; Hoffmann and Schoppmann, 1981; Katte and Hoffmann, 1980; Winterson and Brauth; 1985). The APT of fish is involved in gaze stabilization (Klar PhD-thesis, 2005) and is the homolog structure to the AOS of tetrapodes (Masseck et al., for revision). In sharks and trout no bias for horizontal or vertical directions in the APT is present (Masseck and Hoffmann, 2008a; Klar PhD-thesis, 2005), our results confirm this layout also for the goldfish APT.

4.4.2. Inhibition in null-direction

In our sample of direction-selective neurons inhibition in null-direction is almost always present. This result was unexpected, as in chondrichtyans (S. canicula) and rainbow trout (trout: Klar PhD-thesis, 2005; shark: Masseck and Hoffmann, 2008a) no inhibition in null-direction occurs. However, our data are highly significant and resemble a more tetrapode like behaviour. In most tetrapodes direction-selective neurons exhibit inhibition in null-direction (Hoffmann and Distler, 1989; Hoffmann and Schoppmann, 1975; Ibbotson et al. 1994, Katte and Hoffmann, 1980; McKenna and Wallman, 1985). In retinal direction-selective ON-center ganglion cells, which provide input to the AOS, inhibition is mediated by GABAergic mechanism (Caldwell et al., 1978; Bonaventure et
4. Reference frame APT

al. 1983, 1992; Jardon et al., 1992). Thus inhibition in null-direction could already be existent at retinal levels and is only relayed to the APT.

Terminals from the retina which reach the APT are GABA-immunonegative (Nunes Cardozo and Van der Want, 1990) and excitatory at least in the rat (Schmidt, 1991); only synaptic input from intrinsic axonal and dendritic origin are GABAergic (Van der Want et al., 1992). Direction-selective cells receive GABAergic input from local pretectal circuits which modulates their tonical firing independent from their preferred or non-preferred direction (Schmidt et al., 1994). Hence inhibition could also be mediated by mechanism other then retinal one.

As in goldfish no interconnection of both area in the two hemispheres is reported (Masseck et al., for revision), inhibition in null-direction, which is not convey by the retina must evolve within the APT itself via GABAergic interconnections. Anyway further investigations are needed to clarify the origin of suppression.

4.4.3. Retinal reference frame vs. vestibular reference frame

Our study revealed two neuronal groups one preferring rotation around the YAW-axis and the other one being most sensitive to rotation around the LARP-axis. These results are consistent with results obtained in rabbits and pigeons. In pigeons neurons in the flocculus of the vestibulocerebellum share similar properties; Wylie et al. (1993) divided them into two classes: one group responded best to rotation about a vertical axis (YAW-axis) and the other one responded best to rotation around the H-135°/45°-axis (LARP axis). Response properties of purkinje cells in the rabbit are in agreement with this division, where Graf et al. (1988) and Simpson et al. (1988) revealed neurons coding for rotational and translational optic flow. Again these neurons had their best responsive axis in the vertical and H-135°/45°-axes. Furthermore already in pretectal structures like the MTN and VTRZ neurons preferred movements corresponding to the 45°/135° axis in the horizontal plane (Simpson et al. 1988). In contrast to tetrapodes in chondrichtyans no such preference could be found at pretectal structures (Masseck and Hoffmann, 2008a).

Neurons of the flocculus and vestibulocerebellum in rabbits and pigeons share similar receptive field properties, most of the recorded neurons have binocular bipartite receptive fields, with one part of the receptive field preferring a direction opposite to the
one preferred in the other part. Neurons with these receptive field properties are suitable candidates to detect rotational flow fields. Even in the MTN and VRTZ such bipartite receptive fields can be found (Simpson et al., 1988). These data are in contrast to our result, where neurons were only excited by flow fields in the contralateral eye. This difference can be explained by the layout of the AOS in fish, where no interconnections between both area is present and visual input is only mediated by the contralateral retina. Furthermore binocular input from other areas is missing (Masseck et al., for revision), whereas in mammals binocular backprojections from the visual cortex reach the AOS (Distler and Hoffmann, 1992; Tusa et al., 1989). So per se only neurons with contralateral receptive fields can be found. Another striking difference is the receptive field structure itself, bipartite receptive fields were never observed in our study.

Another main objective of our study was to figure out the internal reference frame in which AOS neurons code ego motion. On the basis of our data a retinal reference frame can be excluded. Since as a population the APT is maximally excited by optic flow fields produces by head or body rotations around the best responsive axis of the contralateral anterior vertical canal, the ipsilateral posterior vertical canal and the horizontal canals. It seems favourable to assume a three dimensional vestibular reference frame based on the orientation of the YAW and LARP axes in space.

In goldfish extraocular muscles planes and semicircular canal planes are aligned (Graf, 1981; Ezure and Grad, 1984; own dissections). The ipsilateral posterior vertical canal lies in one plane with the ipsilateral oblique muscles and the contralateral vertical recti muscles, while the ipsilateral anterior vertical canal is aligned with the ipsilateral vertical recti muscles and the contralateral oblique muscles (Fig. 4.13); moreover the horizontal semicircular canal is in one plane with horizontal recti muscles. As the vestibular and extraocular muscles reference frame are in such close spatial relationship a definite answer in which frame of reference the AOS is coding is still missing. Extraocular motor neurons code already in a reference frame defined by the pulling direction of the muscles they innervate, thus is seems favourable, that already the AOS inherits a head fixed extraocular muscle coordinate system, aligned with the semicircular canals orientation.
5. The optokinetic reaction in foveate and afoveate geckos

5.1. Introduction

In all vertebrates the optokinetic reaction (OKR) ensures a stable image of the environment on the retina during ego and external motion. During OKR the eyes, head or even the whole body rotate at nearly the same velocity and in the same direction as the retinal stimulus. If stimulation is long lasting these pursuit movements are interrupted by resetting saccades in the opposite direction. Monocular horizontal OKR (hOKR) varies in different vertebrates: Some species have a largely symmetrical monocular hOKR where motion in temporo-nasal (TN) and naso-temporal (NT) direction elicits largely equal responses, e.g.: rainbow trout (Klar and Hoffmann, 2002), chameleon (Tauber and Atkin, 1967, Gioanni, Bennis and Sansonetti, 1993); ferret (Hein, Courjon, Flandrin and Arzi, 1990); cat (e.g.: Wood, Spear, Braun, 1973; Markner and Hoffmann, 1985; Distler and Hoffmann, 1992), monkey (Kato, Hasegawa, Igarashi, Koike and Kawasaki, 1986) and human (e.g.: van de Berg and Collewijn, 1988). In other species, e.g. Butterflyfish (Fritsches and Marshall, 2002), frog (Katte and Hoffmann, 1980, Lazar, 1973), pigeon (Fite, Reiner, and Hunt, 1979), chicken (Wallman and Velez, 1985; Bonaventure, Kim, Jardon, and Yucel,. 1992), rabbit (Collewijn, 1975), rat (Hess, Precht, Reber and Cazin, 1985) and mouse (Grüsser-Cornehls and Böhml, 1988), monocular hOKR is asymmetrical, i.e. motion in temporo-nasal direction elicits a larger response than in the opposite direction. Several hypotheses to explain this diversity have been put forward. The “fovea theory” proposed by Tauber and Atkin (1968) proposes that foveate animals perform a symmetrical monocular hOKR. The “decussation theory “proposed by Fukuda and Tokita (1957) suggests the decussation pattern of retinal axons as the key determinant for a symmetrical monocular hOKR: the larger the amount of ipsilaterally projecting retinofugal fibers the more symmetrical the monocular hOKR should be. Other authors tried to correlate different lifestyles with the characteristics of optomotor reflexes (e.g. Dieringer, Reichenberger and Graf, 1992; Fritsches and Marshall, 2002). Generally lateral-eyed mammals without a fovea show asymmetric monocular hOKR (e.g. rat, mouse, rabbit) whereas frontal-eyed mammals show symmetrical hOKR independent of the presence of a fovea (e.g. ferret, cat, monkey, human). Because in
mammals the existence of a fovea or an area centralis is coupled with an increased proportion of uncrossed fibers the above hypotheses cannot be separately tested. In all vertebrates tested so far, the neuronal substrate for the hOKR involves pretectal structures and structures of the accessory optic system. In sharks the corpus geniculatum laterale and in rainbow trout the area pretectalis (APT) contain direction-selective neurons, which code for all directions of motion (shark: Masseck and Hoffmann, 2008a; rainbow trout: Klar and Hoffmann, 2002). Thus in contrast to the decussation theory many fish with their completely crossed optic nerves perform a nearly symmetrical monocular hOKR. In amphibians, reptiles and birds the nucleus lentiformis mesencephali (LM) has been identified as the visuomotor interface for OKR (frog: Katte and Hoffmann, 1980; Fite, 1985; turtle: Fan, Weber, Pickard, Faber and Ariel, 1995; bird: Fite et al 1979, Winterson and Brauth, 1985). Neurons in the LM code predominantly for temporo-nasal motion; however also neurons which code for other directions than ipsiversive (i.e.: neurons of the left LM code for leftwards movements, whereas neurons of the right LM code for rightward motion) can be found.

In mammals neurons of the nucleus of the optic tract and the dorsal terminal nucleus (NOT-DTN) code for ipsiversive horizontal stimulus movements, whereas neurons in the medial and lateral terminal nucleus (MTN and LTN) code for vertical directions. Binocular projections from the visual cortex to the NOT-DTN are responsible for a symmetrical monocular hOKR (ferret: Klauer, Sengpiel and Hoffmann, 1990; cat: Wood et al. 1973; monkey: Zee et al., 1987) in mammals. As such corticopretectal projections are absent in fish, amphibians, reptiles and birds, therefore the question of the cause for a monocular symmetry in non-mammals arises. In addition some differences between foveal vision (like prey tracking) and gaze stabilization exist. Diurnal geckos use foveal vision mainly for binocular prey fixation and not for gaze stabilization. They can direct their highly movable eyes forward to reach binocular vision (Röll, 2001). Furthermore foveal tracking cannot be performed separately in the two eyes, e.g.: chameleons were not able to follow two prey items independently with their two eyes (Kirmse, 1988, Ott 2001). In contrast hOKR can be executed independently in the two eyes (Kirmse, 1988, Ott 2001). A study by Bellintani and Ott (2002) revealed that displaced ganglion cells projecting to the nBOR in the foveate chameleon are evenly distributed throughout the entire retina and have no retinotopic organization. As well as in the chameleon in afoveate chicken ganglion cells from the entire retina project to the nBOR (Reiner et al. 1979). Thus a
foveal dominance in generating OKR or even in a symmetric monocular OKR seems unlikely.

To date the optokinetic system has been studied in only a few reptiles (gecko: Tauber and Atkin, 1968; turtle: Fite et al., 1979; Ariel, 1997; chameleon: Gioanni et al., 1993, Ott, 2001). For our investigation geckos were chosen for the following reasons.

Geckos are small lizards which live in tropical and subtropical regions. Most of them (ca. 75%) are nocturnal. Nocturnal geckos developed from primarily diurnal lizards with pure cone retinas (Walls, 1934, 1942). The rod-like photoreceptors of nocturnal geckos are actually modified cones (Tansley, 1964, Röll, 2000). However, some genera became tertiarily diurnal again and transmuted their visual cells back to cones. The retinas of primarily diurnal lizards are usually characterized by centrally located foveae either convexiclivate or more concaviclivate or shallow (Röll, 2001). In geckos, foveae could only be demonstrated in diurnal representatives (Underwood, 1951, Tansley, 1964, Röll, 2001). Here, foveation reaches its highest development in the genera *Gonatodes, Lygodactylus* and *Sphaerodactylus* with concaviclivate foveae, whereas in species of the genus *Phelsuma* the foveae are shallow and less specialized. Eyes of both the strictly nocturnal geckos (e.g. of the genera *Coleonyx, Gekko, Paroedura, Uroplatus*) and the diurno-nocturnal species (e.g. *Lepidodactylus*) completely lack foveae (Röll, 2001, Tansley, 1964, Underwood, 1951, 1970). However, nocturnal geckos exploit binocular vision to enhance visual sensitivity (Röll, 2001). Diurnal gekkonid species have retained binocular vision from their nocturnal ancestors and have developed foveae which are consequently located not in the central but in the temporal region of the retina (Röll, 2001). Species of the genus *Lygodactylus* possess a binocular visual field of approximately 30 degrees (unpublished observations).

We measured optokinetic head movements under binocular and monocular conditions in diurnal foveate geckos and nocturnal afoveate geckos to determine if the presence of a fovea is a prerequisite for symmetrical monocular hOKR in this monophyletic group of reptiles.
5.2. Materials and Methods

5.2.1. Animals

Five different gecko genera were studied. Diurnal foveate geckos belonged to the species *Lygodactylus capensis* (n=1), *L. bradfieldi* (n=1), *L. chobiensis* (n=1) *L. arnoultii* (n=2) (~ 40-50 mm) and *Phelsuma madagascariensis* (n=3) (25 cm). Nocturnal afoveate species were *Lepidodactylus lugubris* (n=5) (50 mm), *Gekko gecko* (n=3) (35 cm) and *Eublepharis macularius* (n=4) (25-30 cm). All experiments were approved by the local authorities (Regierungspräsidium Arnsberg) 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 NIH guidelines for care and use of animals for experimental procedures.

All animals were kept in a terrarium at a twelve hour light cycle and fed twice a week with house crickets. Drinking water enriched with calcium, phosphate and vitamins was available ad libitum. All species of *Lygodactylus* and *Lepidodactylus* were housed individually, whereas *Phelsuma, Eublepharis* and *Gekko* were kept in groups.

5.2.2. Optokinetic measurements and analysis

Binocular and monocular measurements were performed using an optokinetic drum covered with a black and white Julesz-random dot pattern and moving in clockwise (CW) and counterclockwise (CCW) direction. Animals were placed in a Petri dish in the center of the optokinetic drum (small geckos: ø 30 cm, h 29 cm; others: ø 70 cm, h=64 cm). For *Lepidodactylus lugubris* and *Lygodactylus spp.* stimulus velocities of 20 °/s and 50 °/s were used. All other geckos were measured at 20 °/s, 30°/s and 40 °/s. For the small geckos each dot of the random dot pattern was 1.9° x 1.9° visual angle, whereas for all other geckos dots had a size of 1.6° x 1.6° visual angle.

For monocular measurements a black cap was reversibly attached on the right or the left eye. Usually the left eye was stimulated during monocular viewing, however the right eye was always monocularly tested for control. Head movements were video taped. One session consisted of a 30s-60 s long lasting stimulation, in each session up to 10
consecutive pursuit movements were calculated. For analysis the angle between consecutive head orientations during the pursuit movements was calculated by a frame by frame (frame-rate of the video:50 Hz) analysis of the video data (Fig. 5.1). The angle was measured between the start position of the slow phase eye-movement and its end position (before a resetting saccade starts).

The gain for each value was calculated as the head pursuit movement angle divided by the duration of movement and stimulus velocity. All in all fifty smooth pursuit head movements of *L. capensis*, *L. bradfieldi*, *L. chobiensis*, *L. arnouldi* and *L. lugubris* are included in the data analysis, whereas for *P. madagascariensis*, *G. gecko* and *E. macularius* hundred binocular gain values are used to calculate the median. The median of the gains was calculated and plotted in a boxplot diagram. To test for significant differences a t-test was used for normally distributed data; otherwise a Mann-Whitney Rank Sum test was used (Sigma-Stat).

### 5.2.3. EOG recordings

In addition EOG recordings were made in *P. madagascariensis*, *E. macularis* and *G. gecko*. Therefore animals were either restrained with tape or in a plexiglas tube. Subcutaneous electrodes were placed laterally at the orbitae. Signals were amplified and recorded with eyemove. For each animal slopes of five consecutive smooth pursuit movements were calculated for each stimulus. And compared with a rank sum test for significant differences. The same stimuli as for the head OKN measurements were used in a randomized manner.
5.2.4. Tracer studies

Under halothane anesthesia (2-2.5%), *P. madagascariensis* (n=3) and *G. gecko* (n=3) received intraocular injections of tetramethylrhodamine dextran (RD) (MW 3000, anionic, lysine fixable, Molecular Probes, 10% in destilled water) and horseradish peroxidase (HRP) (Roche) (20% in destilled water containing 1% dimethyl sulfoxide). The injected volumina depended on the eye size (*P. madagascariensis* 5 µl, *G. gecko* 10 µl). After removal of the fused eye lids (“brille”) injections were made with a 10-µl Hamilton syringe. After a survival time of ten days the animals were euthanized by an overdose of halothane and perfused transcardially with 0.9% saline containing 0.1% procaine hydrochloride followed by 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) with 5.76% sucrose. To avoid blood coagulation 0.1 ml heparine was injected into the ventricle immediately before the perfusion.

Brains were removed and stored overnight in the same fixative at 4°C. The next day the brains were cryoprotected with 30% sucrose in 0.1M phosphate buffer (pH 7.4) overnight. The brains were then embedded in chicken albumin (Sigma) and cut frontally at 30µm on a cryostat. Three series were collected: the first was stained for cytoarchitecture, the second series was defatted in xylene and coverslipped with Depex. In the third series peroxidase was visualized using tetramethylbenzidine as a chromogen.

5.2.5. Anatomical reconstruction

Tissue sections were examinend using a fluorescence microscope (Zeiss Axiophot) or under dark- and brightfield illumination, respectively. Retinofugal fibers were reconstructed on a fluorescent microscope (Zeiss Axiophot) coupled to a computer based reconstruction system (Neurolucida) equipped with a digital camera (Zeiss Axiophot). Images were processed for contrast and brightness by using either Adobe Photoshop 7.0.1. or Corel Photo Paint 11. Brain regions were named following the nomenclature of Nieuwenhuys and coworkers [1998].
5.3.6. Retinae

Both eyes were removed after the perfusion and fixed in 4% paraformaldehyde in 0.1M phosphate buffer (PB, pH 7.4) with 5.76% Sucrose over night. Until the next day the eyes were stored in 0.1 M phosphate buffer until preparation. Cornea, lens, vitreous and conus were removed. If possible also the pigment layer was excluded. The whole retina was mounted on a gelatinised slide (vitreous side up). After the wholemount was dried the ganglion cell layer was stainend with 0.5% cresyl-violet (P. madagascariensis) or with NeuroTrace Fluorescent Nissl (Molecular Probes)(G.gecko). The wholemounts were mounted with Glycerin in 0.1M PB(2:1).

For further analysis Neurolucida was used. After plotting the contour of each retina at a magnification of 200x a 50µmx50µm grid was laid over the retina and at intervals of 1000µm the number of ganglion cells was counted at a magnification of 400x.. With the help of NeuroExplorer and a custom made matlab program iso-density contour maps were constructed. The total number of ganglion cells was calculated using the equation of Ito and Murakami 1984.

5.3. Results optokinetic measurements

Altogether 12 nocturnal and 8 diurnal geckos were measured. All geckos responded to the start of the stimulus with a smooth pursuit movement, independent from binocular or monocular measurements and independent of whether they possess a fovea or not.

5.3.1. Afoveate animals

Lepidodactylus lugubris

The optokinetic reactions were measured in 5 individuals of the nocturnal afoveate L. lugubris. In the binocular condition there was no significant difference between CW and CCW stimulation at 20°/s or 50°/s (p=0.798 and p=0.452) (Fig. 5.2A). However, the gain was significantly lower at 50°/s than at 20°/s (20°/s gain 1.0; 50°/s: gain=0.9, p<=0.003). In TN direction again at 20°/s the median of the gain was significantly
higher than at 50°/s (20°/s: gain=1.1; 50°/s: gain=0.6, p<=0.001). During monocular viewing, no OKR could be elicited by stimulation in NT direction (Fig. 5.2B).

**Eublepharis macularius**

The data of 4 nocturnal afoveate E. macularius are presented in Fig. 5.2C, Fig. 5.2D. During binocular stimulation the median of the gain of OKR declined with increasing stimulus velocity (20°/s: CW 0.9; CCW 0.9; 30°/s: CW 0.8, CCW 0.9; 40°/s: CW 0.8, CCW 0.7) (Fig. 5.2C). Only at the highest stimulus velocity a significant difference in the response to the two stimulus directions (p=0.004) existed. Gain was generally lower during monocular viewing at TN stimulation as in the binocular condition. At 30°/s and 40°/s the gain declined significantly (20°/s: 0.7, 30°/s: 0.6, 40°/s: 0.4, p=0.025) during monocular stimulation. No OKR could be elicited at all in NT direction (Fig. 5.2D).

**Gekko gecko**

A total of 3 individuals of the nocturnal afoveate species G. gecko was tested. During binocular viewing these animals showed equal OKR in CW and CCW direction at all velocities (Fig. 5.2E). Again, gain clearly declined with increasing stimulus velocity (20°/s:0.8, 30°/s: 0.7, 40°/s: 0.6). This decrease was stronger than in the other species investigated. Also in this species, monocular gain was lower than binocular gain (20°/s: 0.7, 30°/s: 0.7, 40°/s: 0.5, p<=0.026). Monocularly, OKR was asymmetric (Fig. 5.2F), i.e. no OKR could be elicited by stimulation in the NT direction.

**5.3.2. Foveate animals**

**Lygodactylus spp.**

Altogether 5 individuals of the diurnal foveate genus *Lygodactylus spp.* were analysed. During binocular viewing, the gain was close to one at 20°/s and 50°/s during CW and CCW stimulation. At 20°/s the response to CCW stimulation was significantly higher (p<0.002; median of the gain= 1.3). As a gain higher than 1 is physiologically not plausible this result may be due to the fact that the geckos were not fixed, but could freely move inside the petri dish. Therefore the relative distance of the gecko to the drum wall influenced the perceived pattern velocity.
During monocular viewing all individuals of the genus *Lygodactylus* showed a complete loss of OKR in NT direction (Fig. 5.3B). During NT stimulation the geckos sometimes followed the stimulus by running, but never showed a regular head nystagmus. During the TN stimulation the gain was comparable to the binocular conditions with a median gain of 1 during 20°/s stimulation and a median gain of 0.9 during a stimulation at 50°/s.
Figure 5.2: Boxplot diagrams of binocular and monocular measurements of the gain of OKR in afoveate species. The 5% and 95% percentiles are displayed as dots, boxplots show the range from the 25% percentile to the 75% percentile. A) Binocular condition L. lugubris, n=50, B) Monocular condition L. lugubris, n=50, C) Binocular condition E. macularius, n=100, D) Monocular condition E. macularius, n=100, E) Binocular condition G. gecko, n=100, F) Monocular condition G. gecko, n=100. The median and the 5%, 25%, 50%, 75% and 95% percentiles are shown. CW clockwise stimulation, CCW counterclockwise stimulation, NT naso-temporal stimulation, TN temporo-nasal stimulation.
Phelsuma madagascariensis

The optokinetic reaction was measured also in 3 diurnal foveate P. madagascariensis (Fig. 5.3C). Binocularly, this species showed a robust bidirectional OKR at all velocities (gain at 20°/s. CW 0.9, CCW 0.9; 30°/s CW 0.9, CCW 0.9; 40°/s CW 0.9, CCW 0.8). P. madagascariensis was the only species investigated in the present study that showed an optokinetic reaction during monocular stimulation in NT, albeit significantly weaker than in TN direction (20°/s: TN 0.9, NT 0.6, 30°/s, p<=0.001 and 40°/s TN 0.8, NT 0.5, p<=0.001). A head nystagmus in NT direction could not be elicited in each session thus resulting in fewer measurements in NT than in TN direction. Only in 30% of the sessions during monocular stimulation in NT direction a hOKR could be elicited at all, clearly the NT component is not as reliable as the TN component. Thus gain was calculated only from the periods showing OKR.

Thus summarizing our data we could show that under binocular viewing conditions foveate as well as afoveate geckos show largely symmetrical bidirectional optokinetic reactions in horizontal directions with a gain close to 1. In all but one species (P. madagascariensis) gain of hOKR decreases with increasing stimulus velocity over a range of 20°/s to 50°/s.

Under monocular viewing conditions, two trends were recognizable in our data. Generally, monocular gain in TN direction was lower than binocular gain. A complete loss of head nystagmus in NT direction was observed in all afoveate geckos tested (L. lugubris, G. gecko, E. macularius) as well as in the foveate genus Lygodactylus. By contrast, our second foveate species P. madagascariensis displayed a clear hOKR in NT direction albeit at significantly lower gain than in TN direction.
Figure 5.3: Boxplot diagrams of binocular and monocular measurements of the gain of OKR in foveate species. A) Binocular condition *Lygodactylus* spp., n=50 B) Monocular condition *Lygodactylus* spp., n=50, C) Binocular condition *P. madagascariensis*, n=100, D) Monocular condition *P. madagascariensis*, TN: n=100, NT: n=30. Conventions as in Fig. 5.1. Stars indicate significant differences.
5.3.3. EOG measurements

In general eye movements to an optokinetic stimulus were only seen in 20% of the trials. As described in the literature most compensation of retinal slip seems to be dome via head movements instead of eye movements. At least in *G. gecko* all attempts to elicit eye movements were in vain. Thus only data from *P. madagascariensis* and *E. macularis* are included here.

In all binocular measurements no significant difference is existent between CW and CCW stimulation as well as for *P. madagascariensis* and *E. macularis.*

*E. macularis* showed monocular only eye movements to TN stimuli, whereas for NT stimulation eye movements could never be observed. In *P. madagascariensis* also eye movements to NT stimuli could be observed, but always to a lesser extent as in TN direction. For each stimulus velocity reactions were significant different for TN and NT direction (p<=0.001)

![Figure 5.4: Example of EOG recordings from P.madagascariensis. X-axis time in s. Y-axis mV.](image)

A horizontal eye position during binocular stimulation in CCW direction. B horizontal eye position during monocular stimulation TN direction. C horizontal eye position during monocular stimulation NTdirection

5.3.4. Results tracer studies

5.3.4.1. Intraocular eye injections with HRP

Four geckos (two *G. gecko* and two *P. madagascariensis*) received HRP injections into the left eye. In both species no ipsilateral projections were visible either in the tectum opticum (TeO) or in diencephalic or pretectal nuclei. Contralaterally all other
diencephalic and pretectal projection areas described in the literature were labelled. In the chiasm all fibers crossed to the contralateral side (Fig. 5.5.A). Posterior to the chiasm, the optic tract ascended to the thalamic structures. The densest projection zone in the thalamus was located in the nucleus geniculatus lateralis pars dorsalis (Gld) and pars ventralis (Glv) (Fig. 5.5 B).

In the pretectal area three retinorecipient nuclei, the nucleus geniculatus pretectalis (Gp), the nucleus posterodorsalis (Pd) and the nucleus lentiformis mesencephali (LM) were labelled. The LM as the main target for retinofugal fibers in the pretectum was clearly visible in all geckos (Fig. 5.5C). Laterally and ventrally to the LM the Gp was stained. More dorsally and medially close to the commissura posterior the Pd could be identified. In the tectum opticum, retinal terminals were labelled uniformly over the whole extent of the structure in G. gecko. In P. madagascariensis the labelling was denser in the medial and lateral parts. This suggests that tracer predominantly reached more ventral parts of the retina. At the ventrolateral border of the rostral mesencephalic tegmentum the nucleus of the basal optic root (nBor) was labelled (Fig. 5.5E).

In one animal (P. madagascariensis) the nucleus nervi oculomotorii (III) was stained retrogradely, probably due to leakage of thetracer from the eye bulb and subsequent uptake by extraocular muscles. In all three parts, the dorsal, intermediate and ventral part large and medium-sized neurons were labelled (Fig. 5.5F).

5.3.4.2. Intraocular eye injections with Tetramethylrhodamine dextran (RD)

Three geckos (two G. gecko, one P. madagascariensis) received intraocular eye injections with RD. With this tracer the course of labelled retinal fibers was easier to identify than with HRP. Anyway the revealed retinofugal projections zones were identical to those revealed in the HRP study (Fig. 5.6)
Figure 5.5: Photomicrographs of anterogradly (A,B,C,D,E) labeled fibers and retrogradly (F) labeled neurons after intraocular injections of HRP into the right eye of either G. gecko (A) or P. Madagascariensis (B,C,D,E,F). A Darkfield photomicrograph of the Chiasma opticum, scalebar 100µm. B Brightfield photomicrograph Diencephalon scalebar 200µm, C Brightfield photomicrograph Preptectum and Tectum opticum scalebar 200µm, D Brightfield photomicrograph LM scalebar 100µm E Brightfield photomicrograph Tectum opticum and rostral mesencephalic tegmentum scalebar 200µm, F Brightfield photomicrograph somatomotor nucleus scalebar 100µm. Gld Nucleus geniculatus lateralis pars dorsalis, Glv Nucleus geniculatus lateralis pars ventralis, Gp Nucleus geniculatus pretectalis, Pd Nucleus posterodorsalis, LM Nucleus lentiformis mesencephali, NBOr Nucleus of the basal optic root, TeO Tectum opticum, III Nervi oculomotorii (III).

Figure 5.6: Photomicroscopy of anterogradly labeled fibers after injection of RD into the left eye of P. madagascariensis. A Photomicrograph of preptectal area and tectum opticum. B Photomicrograph of the same section at a higher magnification., Scalebar 100µm. Gp Nucleus geniculatus pretectalis, Glv Nucleus geniculatus lateralis pars ventralis, LM Nucleus lentiformis mesencephali, TeO Tectum opticum.
5.3.5. Results retinae

**P. madagascariensis**

Retinal wholemounts were used to examine the distribution of ganglion cells. Three retinae of two *P. madagascariensis* were examined. For *P. madagascariensis* it is well known that they possess a temporal fovea. Our isodensity contour maps reveal a temporal fovea (Fig. 5.7C). This result is consistent with actual literature, where a temporal fovea for *P. madagascariensis* is verified.

**G. gecko**

Iso-density contour maps and cell density analysis have been done in two retinae of one nocturnal gecko. The area of the whole retina (in average 163,45 mm$^2$) is up to four times higher as the retinae of *P. madagascariensis* (43,5 mm$^2$). Whereas the total amount of GC is nearly the same (around 522*10$^3$ GC). The topography of GC is non-uniform and changes across the retina. A clear GC gradient from the outside to the midline and to temporal regions can be seen (Fig. 5.7A, B) In the periphery the ganglion cell density lies in a range of 400-3000 per mm$^2$. In contrast in more temporal regions the cell density varies from 6000 up to 8500 GC per mm$^2$. Thus a maximum ratio of 1:21.25 GC from the periphery to temporal regions is reached. These GC peak in the temporal retina can be named a temporal area, as no foveal dip is visible. The area is expanded more in ventrally than dorsally. However a clear horizontal visual streak is missing.
Figure 5.7: A, B Isodensity contour maps of two retinas from *G. gecko*, C Isodensity contour map of one retina from *P. madagascariensis*. Colour map indicates cell density. D dorsal, N nasal, T temporal, V ventral. Scalebars represent 1mm.
5.4. Discussion

5.4.1. Optokinetic measurements

Geckos generally stabilize gaze more by head (80%) than by eye movements (Dieringer, Cochran and Precht, 1983). Therefore in our study we concentrated on head movements. All individuals of all species tested showed a robust hOKR during binocular viewing. Monocularly all individuals were asymmetric in their response, i.e. only TN stimulation reliably elicited hOKR whereas NT stimulation yielded no response at all (Lygodactylus, L. lugubris, G. gecko, E. macularius) or a rather weak response in some of the sessions (P. madagascariensis). This monocular asymmetry was independent of lifestyle (diurnal vs. nocturnal) or retinal specialization (foveate vs. afoveate). Especially the findings in the foveate diurnal Phelsuma and Lygodactylus disagree with earlier results of Tauber and Atkin (1968), who claimed that monocular symmetry is related to foveation. It is unlikely that these different results are caused by the different stimulus parameters used by Tauber and Atkin (vertical black and white grating) and in the present study (Julesz random dot pattern). As hOKR could readily be elicited under binocular viewing conditions. Also, during monocular viewing a rapid switch between NT and TN led to an immediate beginning of an optokinetic response in TN direction whereas in NT direction the hOKR was totally abolished (Lygodactylus, L. lugubris, G. gecko, E. macularius) or diminished (P. madagascariensis). All tested species could be judged as asymmetric, although P. madagascariensis showed a weak NT component. In a second preliminary approach, eye movements were recorded in P. madagascariensis and G. gecko using electrooculography (EOG). In head restrained animals an optokinetic reaction could only be elicited in about 20% of the sessions. Nevertheless, our data suggest a slight contribution of eye movements to gaze stabilization in geckos. Both species tested showed optokinetic eye movements during binocular and monocular stimulation. Monocular asymmetry persisted in P. madagascariensis and E. macularius (p<=0.001), whereas in binocular measurements no differences for CW and CCW stimulation can be seen.

In lateral eyed animals (like geckos) monocular asymmetry facilitates a suppression of optokinetic drive during forward locomotion, as optic flow during forward locomotion corresponds to stimulation in naso-temporal direction.
5.4.2 Anatomy

Our tracer studies revealed no ipsilateral fibers at all neither in foveate nor in afoveate animals. This is in contrast to results obtained by Nothcutt and Butler (1974), who found ipsilateral projections to all retinorecipient areas. Our approach might be not sensitive enough to reveal such sparse projections. Anyway a contribution of ipsilateral fibers to the hOKR is doubtful (see 5.4.2.).

For *P. madagascariensis* nissl stained retinal wholemounts revealed a temporal located fovea as it is already described in the literature (Underwood, 1951, Tansley, 1964, Röll, 2001). Therewith our method is reliable to identify specialized regions in retinæ. Retinæ of *G. gecko* have so far not been investigated. Our finding of a temporal area in *G. gecko* was unexpected, as so far no retinal specialization for nocturnal geckos is shown. Anyway a temporal area gives the animals the advantage of high resolving power in the binocular visual field. Gekkonids (also nocturnal geckos) in contrast to other reptiles, like bearded dragons, fixate prey binocularly and thus a temporal area is beneficial for their prey capture.

5.4.3. Neuronal substrate

A study by Northcutt and Butler (1974) was using a degeneration method and silver staining revealed sparse ipsilateral fiber projections to all main retinorecipient areas in *G. gecko*. In turtle, only an ipsilateral projection to the nucleus posterodorsalis, a retinofugal area, which has no known function in OKN control was revealed using HRP (Fan et al. 1995). Anyway, functionality of ipsilateral fibers for the optokinetic reaction is in question, as at least in frogs an ipsilateral retinal projection does not contribute to it. After sagittal section of the optic chiasm no optokinetic reaction can be elicited (Dieringer and Precht, 1982). Furthermore even *G. gecko* possess ipsilateral retinofugal fibers (Northcutt and Butler, 1974) and is monocularly not able to generate hOKR in NT-direction.

Various electrophysiological and lesion studies suggest that the nucleus lentiformis mesencephali (LM) of tetrapodes other than mammals,, the area pretectalis (APT) in trout and the corpus geniculatum laterale in sharks are involved as visuomotor interface in the generation of slow following eye and head movements during hOKR (shark:
5. OKR in geckos

Masseck and Hoffmann, 2008a; trout: Klar and Hoffmann, 2002; frog: Katte and Hoffmann, 1980; Dieringer and Precht, 1982; turtle: Fite et al., 1979, Fan et al., 1995). In frog 15% of the LM neurons code for contraversive motion (i.e.: 15% of the neurons in the right LM code for leftward motion and vice versa for the right LM) (Katte and Hoffmann, 1980). In the mammalian NOT-DTN a strict preference for ipsiversive stimulus movement has been shown in all species investigated so far (e.g: rat: Precht and Strata, 1980; rabbit: Collewijn, 1975; cat: Hoffmann and Schoppmann, 1981; ferret: Klauer et al., 1990; opossum: Volchan, Rocha-Miranda, Picanco-Diniz, Zinsmeisser, Bernardes and Franca, 1989; monkey: Hoffmann, Distler, Erikson and Marder, 1988, Mustari and Fuchs, 1990).

In addition, the nucleus of the basal optic root (nBOR), a major component of the accessory optic system, is involved in gaze stabilization in amphibians, reptiles and birds. Neurons in the nBOR code for all but ipsiversive direction of motion (Dieringer and Precht, 1982, Fan et al., 1995). Together neurons in the LM and nBOR represent all directions of motion. The LM and nBOR are interconnected reciprocally with each other (turtle: Fan et al., 1995). In pigeons electrical stimulation of the LM modulates the firing rate in the nBOR (Nogueira and Britto, 1991) and vice versa. However, the nBOR only modulates hOKR gain and is not responsible for eliciting a horizontal optokinetic reaction (turtle: Fite, 1979). An involvement of telencephalic or tectal structures in the optokinetic reaction of non-mammalian vertebrates seems unlikely (Hertzler and Hayes, 1967, Hobbelen and Collewijn, 1971, Lazar, 1972).

Thus, a NT component of monocular hOKR could be generated by a cooperation of the LM and nBOR. Alternatively, even the LM alone could generate a weak NT component, as in pigeons and frogs the LM is also activated by NT stimulation (Winterson and Brauth, 1985).
5.4.4. Conclusion

Monocular symmetric hOKR in geckos is neither related to retinal specializations nor to lifestyle. So our data do not support the generality of the fovea theory of Tauber and Atkin. The decussation theory can not be verified nor refuted by our data, however it is questionable if sparse ipsilateral fiber connections as described for reptiles could generate a naso-temporal monocular hOKR at all.

Among non-mammalian vertebrates only some fish and reptiles (e.g.: *Chamaeleo melleri*) display a symmetric monocular hOKR, possibly due to a different anatomical organisation (trout: Klar and Hoffmann, 2002, chameleon: Tauber and Atkin, 1967, Gioanni et al, 1993).

Further behavioural and electrophysiological experiments are needed to quantify the symmetry of monocular hOKR in various species to clarify interspecies differences of the underlying neuronal circuits.
6. General Discussion

6.1 The AOS of fish

Karten and Finger (1978) designated two pretectal nuclei in teleost fish as the homolog to the AOS of tetrapodes, namely P1 (APT) and P2 (CPN). Aside from their work no study yielded to a comprehensive picture of the AOS in fish. Also the AOS of sharks was treated stepmotherly.

Our studies, in combination with studies by Klar and Hoffmann (2002), clearly identify the AOS in teleost fish and chondrichtyans.

In chondrichtyans (S. canicula) the corpus geniculatum laterale (Cgl) contains direction-selective neurons with properties (large receptive field, best excitation by large moving textured pattern, direction-selectivity) similar to features of neurons in the AOS of tetrapodes. Also its anatomical connectivity points to its involvement in gaze stabilization. Not only the cerebellum, but also oculomotor nuclei are target areas of efferent projections from the Cgl.

In teleost fish properties of neurons in the area pretectalis (APT) resembles those of AOS neurons of tetrapods. Not only physiological data provide evidence that the APT represents at least a part of the AOS in teleost, also its afferent and efferent connectivity is remarkable similar to the AOS of tetrapodes. Moreover, Klar showed in his PhD thesis (2005) that lesions of the APT in trout lead to severe deficits in the hOKN. My proposed efferent connectivity of the AOS in fish is shown in figure 6.1. In contrast to tetrapodes only one nucleus undertakes the function of the AOS in fish, the APT in bony fish and the Cgl in sharks, respectively. Due to its function and location I suggest renaming the Cgl of sharks into LM the assumed homolog structure of tetrapodes.

Both nuclei contain neurons which are sensitive to all directions of motion, which is in contrast to tetrapodes where segregation of different directions is already realized in amphibians. Direction-selective information is now passed from the APT/Cgl to ipsilateral located oculomotor nuclei, the cerebellum, the valvula cerebelli, the vestibular nuclei, the reticular formation, area II and to the inferior olive, whereas projections to area I could not definitively be proven by our results. Also terminals to the tectum opticum and torus longitudinalis where evident in our tracings. Contralateral fiber courses were also observed, but always to smaller extent than to ipsilateral target areas. Not only projection patterns where more sparse, also their appearance was
Figure 6.1: Efferent connectivity of the AOS in fish. Leftside panel corresponds to areas located ipsilateral to the left APT, rightside panel marks area located contralateral to the left APT. Dashed dotted lines indicate unsecured connections. A I area I, A II area II, APT area pretectalis, CC corpus cerebellum, IO inferior olive, RF reticular formation, TeO tectum opticum, TL torus longitudinalis, VN vestibular nuclei, III nucleus oculomotorius, IV nucleus trochlearis, VI nucleus abducens
inconsistent overdifferent animals. Thus it is in question if such a small amount of contralateral fibers is really able to drive the OKR alone. All of our results point to a monocular organization of the AOS in fish. The appearance of horizontal as well as vertical coding cells supports the hypothesis of a monocular organized system. Furthermore, direct projections from the APT to all oculomotor nuclei emphasize a monocular organization. Former studies by Easter (1974) revealed unyoked saccades during optokinetic stimulation in the goldfish and Harris (1965) stated the same for *Squalus acanthias* (a shark). In addition, Easter (1971) and Mensh (2004) observed that after saccades eye position in both eyes does not necessarily correspond. Fritsches and Marshall (2002) observed asynchronous optokinetic movements in the pipefish and the sand lance. Furthermore, stimulation with a split drum revealed independent compensation in both eyes (Fritsches and Marshall, 2002). It seems that different levels of linkage between both eyes exist. During monocular stimulation the pipefish shows only linking of the slow phase, whereas in the sand lance no linking at all occurs (Fig. 6.2). In addition, an unpublished study by Debowy and Baker on goldfish revealed a monocular organization of the velocity to position integrator (AI and AII). Both behavioural and electrophysiological observations point to a monocular organization of the oculomotor system in fish. This hypothesis is additionally strengthened by fish with highly independent saccadic and fixating eye movements, like the sand lance.

![Figure 6.2.](image)

**Figure 6.2.:** Monocular stimulation of the right eye, left eye occluded. **A** horizontal eye trace of a pipefish showed linked optokinesis in both eyes, but only slow phases are linked. **B** horizontal eye trace of a sand lance. Only the stimulated eye shows optokinesis. Adapted from Fritsches and Marshall, 2002.
Based on its efferent connectivity I propose the following model of the slow phase of
OKR in goldfish (Fig. 6.3.):
During head turns (for simplifications possible body movements will be neglected) the
environment moves on the retina ($\dot{s}$), where a first transformation into retinal slip
velocity takes place ($\dot{r}$). This retinal slip velocity information is in turn projected to the
APT and transformed into an extraocular muscles reference frame. Afterwards retinal
slip velocity is conveyed via direct pathways to the oculomotor nuclei, where it is
finally coded in terms of appropriate eye velocity and eye position ($\dot{e} + \int \dot{e}$), this motor
command ($m$) elicits now a compensatory eye movement. This direct pathway is
responsible for the fast component of OKR, whereas more indirect pathways via a
velocity-to-position integrator build up the slow phase of OKR.
The oculomotor system is a closed loop system where new emerging retinal slip is
composed of the difference between actual stimulus and eye velocity ($\dot{r} = \dot{s} - \dot{e}$), thus
the system itself detects error or inaccuracies of the resulting eye movement.
Simultaneously head acceleration ($\ddot{\phi}$) is assimilated by the semicircular canals, where a
first integration from head acceleration to head velocity ($\dot{\phi}$) is done via the cupula, then
via the 8th nerve a head velocity signal is passed to the vestibular nuclei, where the
corresponding eye velocity is calculated and is passed on directly to the oculomotor
nuclei. At the vestibular nuclei a sign conversion of the resulting eye movement has to
be done, as eye movements during VOR are always opposit to the turning direction of
the head.
In addition indirect pathways modulate and calibrate the OKR and VOR:
After turning of the head eyes should remain in an eccentric position. To achieve this
and prevent the eyes from drifting back to the orbit center an indirect pathway via the
velocity to position integrator (VPI) is needed. Information about head velocity, eye
velocity and retinal slip velocity reaches the VPI (in goldfish the horizontal VPI consists
of AI and AII in the hindbrain) via the semicircular canals, the vestibular nuclei and the
APT. Now the VPI integrates incoming signals into an eye position and eye velocity
signal which is afterwards directly transferred to motoneurons of the oculomotor nuclei.
For calibration of eye movements indirect feedback pathways via the vestibulo
cerebellum exist. Visual information reaches the vestibulo cerebellum by two pathways,
first via a direct mossy fiber projection from the APT and second via climbing fibers
arising from the inferior olive, which receives excitatory input from the APT. In the
inferior olive already information from both eyes is merged and further processed. Vestibular input to the vestibulo cerebellum arises from the semicircular canals and the vestibular nuclei. The vestibulo cerebellum in turn is now able to detect an actual mismatch ($-\mu$) between sensory inputs and motor outputs and calibrate eye movements via the vestibular nuclei.
Figure 6.3: Model of the slow phase generation in goldfish. Solid lines represent direct pathways, dashed lines indirect ones. Green arrow indicates the pathway by which the fast phase of the slow phase is generated, red arrow indicates the pathway by which the slow component of the slow phase is generated. APT area pretectalis, VC vestibulo cerebellum, VPI velocity-to-position integrator, VN vestibular nuclei.
6. General discussion

6.1.2. Evolutionary viewpoints on the AOS

Sharks appeared first in the Silur era around 430 million years ago and are one of the earliest known yawed fish. It seems that during millions of years of evolution their basic appearance changed only little. Their origin is still a mystery: Some scientists consider sharks as the primitive form of yawed fish (Long, 1995).

Bony fish (i.e. osteichthyes) appeared around 410 million years ago and developed to the largest group of living fishes. They comprise the teleosts, which represent the largest and advanced group of ray-finned fish. The first teleosts emerged in the Triassic around 220 million years ago (Fig. 6.4). Although it is unlikely that osteichthyes have arisen from chondrichthyes, both groups share one common ancestor. Sharks seem to represent the original form of fish. On neuronal levels this is also mirrored in the properties of direction-selective neurons (see chapter 6.2), whereas in general the connectivity and appearance of the AOS is shared among all fish. A common feature of the AOS in fish seems that only one single nucleus provides retinal slip information to the oculomotor system. In this nucleus all directions of motion are represented equally and no bias for horizontal or vertical movements is present. This arrangement of the AOS is in contradiction to the AOS of tetrapodes other than mammals, where a pretectal nucleus (LM) codes for ipsiversive motion and a tegmental nucleus (nBOR), representing their AOS, codes for all other directions. In mammals further segregation in the AOS occurred: here, the LTN and MTN encode vertical motion (upward and downward motion respectively), and the DTN is most sensitive to ipsiversive stimuli, like the pretectal NOT. It seems that during evolution a higher grade of complexity is reached by a process of parcellation (Ebbesson, 1980).

According to this, already in the nBOR clustering of preferred directions occurs, with its rostral dorsal part coding for upward retinal slip and more ventral parts coding for downward retinal slip (McKenna and Wallmann, 1985). Unfortunately, our attempt to show clustering of preferred direction in the Cgl and APT failed. In mammals there is further evidence that parcellation in the AOS occurred. Considering the three subclasses of mammalia, the prototheria, metatheria and theria it becomes more and more clear in which way the AOS in mammals has evolved. Representatives of protoheria, like platypus and echidna, have only a well differentiated MTN, whereas the LTN and DTN are lacking (Campbell and Hayhow, 1972, 1972). In metatheria like opossums the MTN is still present, but yet LTN and DTN start to develop (Hayhow, 1966). In most, if not in
all, theria all three nuclei are well differentiated (for an overview see Giolli, 2005). Thus during evolution the AOS seems to become more and more complex by a process of parcellation, with the AOS of fish resembling the original state of the oculomotor system in vertebrates.

6.2 Comparison of direction-selective neurons in S. canicula and C. auratus

General properties, as excitability by large moving patterns, receptive field sizes, and direction-selectivity of recorded neurons in the Cgl and APT are very similar. Beyond, both nuclei are located in the pretectum and are affiliated to the AOS. Nevertheless, a closer look reveals significant differences between them.

In general, directional tuning width is narrower in goldfish (Fig. 6.5) with a mean r-value of 0.19 compared to a mean r-value of 0.08 in shark.

Not only tuning width is broader in sharks, but also inhibition in null direction, as described for other vertebrates, is lacking. By contrast, goldfish show clear inhibition in null direction (Fig. 6.6). In S. canicula mean activity in null direction is almost always higher as spontaneous activity. This phenomenon is independent from the stimuli used and occurs in both stimulus conditions (Fig. 6.5 A, B). On the other hand, in goldfish mean activity in null direction is three to six times lower as spontaneous activity (Fig. 6.5.C, D).

Tuning width and inhibition might be traced back to the same origin. Retinal ON-direction-selective ganglion cells convey direction-selective information to the AOS via excitatory synapses (Hoffmann and Stone, 1985). In contrary to ganglion cells neurons of the AOS are spontaneously active and inhibited in null-direction. On one hand inhibition in direction-selective neurons in the APT could be mediated by missing excitatory input from the retina and on the other hand inhibition could evolve within the

Figure 6.5.: Comparison of r-values over all direction-selective cells. x-axis species, y-axis r-value, where one value corresponds to the mean vector lengths, for calculation see 2.2.3. *** indicate a significant differences with a p-value <=0.001.
Evidence for inhibition mediated by non-retinal afferents comes from studies by Van der Togt and Schmidt (1994) in rats, where stimulation of the MTN leads to inhibition of direction-selective neurons in the NOT-DTN. In addition, interconnections between AOS nuclei are almost exclusively GABAergic (van der Togt et al., 1991).

Only one nucleus comprises the AOS of fish, thus inhibition must be mediated by internal GABAergic networks. Up to date a confirmation of this hypothesis is not possible, further experiments on the intrinsic connectivity of the APT and Cgl are needed.

Tuning develops through variable input from ganglion cells converging on one direction-selective AOS neuron. Broader tuning in sharks might thus result from an inadequate separation of direction-selective input from the retina or already at retinal levels where tuning width of direction-selective ganglion cells might be broader. For the
latter, it might be possible that mechanisms which lead to direction-selectivity in ganglion cells of sharks are not as developed as in other vertebrates. Anyway for the behavioural output (i.e. slow eye movements) inhibition in null-direction and a sharp tuning is not a prerequisite, but would only lead to an enhancement of the signal-to-noise ratio.

6.3. Selection of reference frames (S. canicula vs. Carassius auratus)

Another key point of my investigations was to understand where and how different sensory modalities get fused into one internal reference frame and which kind of reference frame is chosen to represent visual motion in fish. In sharks a clear bias for coding in vestibular coordinates in the Cgl was not found. Preferred axes of rotation were uniformly distributed within the horizontal plane and had also no preference for vertical or horizontal stimuli. Opposed to this, preferred directions of neurons in the APT in goldfish had two preferred axes of rotation: One part of the population preferred rotations around a vertical axis (YAW-neurons) and the other part preferred rotations around the LARP-axis. Findings in goldfish are in accordance with the actual literature, where already for pretectal structures coding in a reference frame similar to the vestibular system is described (Simpson et al., 1988). An overall comparison of properties of direction-selective neurons in S. canicula and C. auratus lead to the impression that goldfish have a more advanced oculomotor system. It is likely that in S. canicula transformation from a retinal into a vestibular or eye muscle reference frame occurs later in visual processing (Masseck and Hoffmann, 2008a). Possible sites for a sensorimotor integration are the oculomotor nuclei, the vestibular nuclei, the vestibulocerebellum, the inferior olive or even in a so far undiscovered velocity-to-position integrator in sharks.

Gaze stabilization in sharks is in a lot of ways different from that in goldfish. During swimming the head of shark oscillates from side to side and as a consequence the semicircular canals are permanently stimulated. Furthermore, sharks do not possess a swim bladder: If sharks want to examine something closer, they have to swim in circles around it, as a disruption of swimming would cause the shark to sink. So fixation and gaze stabilization might be realized in a different manner as in goldfish, which own a
stabilizing swim bladder and do not exhibit oscillating head movements during swimming.

Compensatory eye movements during swimming in sharks are neither influenced by the semicircular canals, nor by visual input. Clamping of the head and therewith cancelling sensory signals arising from the semicircular canals lead to no difference in stabilizing eye movements (Harris, 1965). Even visual stimuli seem to have no effects on this eye movement pattern seen during swimming (Harris, 1965). All this points more to an intrinsic feed-forward mechanism, which relays efference copies of rhythmic swimming patterns to nuclei which are involved in gaze stabilization. Combes et al. (2008) were able to proof the existence of such an intrinsic feed-forward mechanism and verified its influence on gaze stabilization in the tadpole.

It is likely that in sharks such mechanism play a more important role in gaze stabilization than visual processing. In daily life sharks do more rely on electroperception and olfaction than on visual cues, and oculomotor behaviour seems to be only poorly developed (own attempts to elicit OKR in sharks, were more or less unsuccessful). Thus, vision is of minor behavioural importance in sharks. This might lead to an at least underdeveloped oculomotor system, with broadly tuned cells and an absent transformation of visual signals into a vestibular reference frame.

In direct comparison, goldfish are more dependent on their visual system than sharks. They exhibit spontaneous scanning behaviour, fixate and have a robust oculomotor performance. All this might lead to a more fine tuned oculomotor system, with highly tuned direction-selective cells and an AOS which codes already a vestibular frame of reference.

In goldfish it is still a matter of debate, whether the reference frame in which the AOS neurons code is a vestibular or extraocular muscle one. In the end this question is redundant, as both reference frames are in such close spatial relationship in the goldfish (they are only a few degrees apart) that segregation between them is impossible. In pigeons, Wylie and colleagues used a very elegant way to prove coding to be in an extraocular muscles frame: Pulling directions of the medial and lateral recti are not collinear in the pigeon, but rather 210° apart from each other (Fig. 6.7). Due to this extraocular muscle arrangement they were able to show that the pigeons AOS codes rather in a reference frame defined by the pulling direction of the extraocular muscles than in a vestibular one (see general introduction 1.4). In goldfish, medial and lateral rectus pulling directions are nearly collinear with each other (Fig. 6.7). This
arrangement is mirrored in the mean vectors of NT and TN preferring cells, which are 181° apart from each other (Fig. 6.8). For this analysis all neurons with preferred directions lying in between 150°-210° and 330-30° were taken into account. Their mean preferred directions (175° and 354°) lie in one plane with the horizontal semicircular canals and the plane defined by the pulling directions of the medial and lateral rectus muscles. By means of this analysis also no separation between a vestibular and an extraocular muscle reference frame can be drawn.

As semicircular canal planes and extraocular muscles planes are in such close spatial relationship, it does not matter if visual information is first conveyed into a vestibular reference frame or in an extraocular one, as both frames of reference are equivalent. An extraocular muscle reference frame is more presumable, as all studies which took a closer look on spatial relationships between the vestibular system, the extraocular muscles and preferred directions revealed coding in an eye muscle related reference frame (Maioli and Ohgaki, 1985; Wylie et al., 1993).
In the literature not only in the internal reference frame of the oculomotor system is addressed, but also sensory motor transformation in general, as all interactions with the outer world require a translation of sensory signals into effector coordinates (Pouget et al., 2000). One main question regarding sensory transformation is at which level a muscle-like reference frame is realized. Obtained results are ambiguous, e.g. in the primary motor cortex (M1) two subpopulations of neurons can be found, one coding in a muscle-like frame of reference the other one coding in an extrinsic reference frame (Kakei et al., 1999, 2001). A study by Yanai et al. 2008 showed that effectively the coordinate frame of the M1 is intermediate and that definite coding in a muscle-like reference frame is first realized by spinal cord interneurons. Hence they concluded corticospinal interactions as the real source of sensory-motor transformation (Yanai et al. 2008).

This example of ongoing research demonstrates that we are far away from understanding how and where a sensory-motor transformation takes place. And that maybe the classical viewpoint, where transformation from sensory to muscle-like reference frames is already completed before motoneurons are reached is antiquated. Rather downstream processes and wiring schemes on motoneurons might finally define the muscle based reference frame (Yanai et al., 2008). This hypothesis may also be true for the oculomotor system.
6.4. Symmetry and asymmetry of OKR in geckos

Our attempt was to answer an old question of oculomotor research: By which mechanism is symmetry of monocular OKR determined? Symmetry was long correlated with retinal specializations (i.e. fovea) and/or with the degree of uncrossed retinal fibers (Tauber and Atkin, 1968; Fukuda and Tokita, 1957).

Our study provides a comprehensive overview of the occurrence of symmetry and asymmetry of monocular horizontal OKR in geckos. In summary retinal specializations can not be correlated with symmetry or asymmetry (Masseck et al., 2008b). *P. madagascariensis* was the only species which generated OKR during NT stimulation, but always with significant lower gains as in TN directions. All other foveate geckos showed when monocularly tested a failure to generate OKR during NT stimulation. This disproves at least for geckos the fovea theory. Admittedly, also for other species like the goldfish or trout the fovea theory is proven to be wrong. These fish exhibit symmetry during monocular stimulation, although they do not posses a fovea. So a fovea is neither a prerequisite, nor a safe indicator for monocular symmetry in non-mammalian species.

In mammals, symmetry is mediated by the extent of binocular backprojections from the visual cortex to the NOT and AOS. The degree of binocular backprojections enhances with a magnification of the binocular visual field, and thus OKR becomes more and more symmetric in animals with large binocular visual fields. Thus in mammals the decussation theorie of Fukuda is more valid than the fovea theory as cats or ferrets show a symmetric OKR during monocular stimulation without having a true fovea.

In other vertebrates the situation is much more ambiguous: most vertebrates have unequal responses to NT and TN stimulation, like butterflyfish, frogs, geckos and pigeons (Marshall and Fritsches, 2002; Katte and Hoffmann, 1980, Lazar, 1973; Masseck et al., 2008b; Fite et al. 1979), whereas some species show a symmetric monocular OKR (e.g.: goldfish, sandlance, trout, chameleons, owls).

Symmetry of monocular OKR is probably related to the layout of both the visual and the oculomotor system. A monocular organization of the oculomotor system (goldfish, trout) or a large binocular visual field (e.g. owls) would facilitate equal responses to both stimulus directions.

Interestingly, neurons coding for NT directions are present in species which have an asymmetric OKR. Thus, it is a matter of further neuronal connectivity, if NT responses are generated: If these neurons are not connected to the motor output of the system,
asymmetry could develop. It might be that neurons which code for NT directions are only used to enhance the signal–to-noise ratio of the oculomotor system. In order to give a definitive answer to this question, single cells properties need to be correlated with single cell connectivity.

Single cell imaging in behaving zebrafish larvae and tadpoles could give a deeper understanding of the intrinsic organization of the oculomotor system.

Different levels of coupling the eyes might also play an important role in the appearance of OKR. Lateral-eyed animals, like the butterflyfish and geckos with strong coupling of both eyes, have rather an asymmetric response, whereas lateral eyed animals with more or less unyoked eye movements tend to have symmetric responses (e.g.: sandlance, bearded dragon). Yoking of eye movements is also related to the layout of the oculomotor system, where a monocular organization would facilitate unyoked eye movements. In conclusion, symmetry in vertebrates, other than mammals, might therewith be correlated with the degree of linkage of both eyes.
Summary

1. Visual responses in *S. canicula*

In chondrichtyes (*S. canicula*) a pretectal area, namely the corpus geniculatum laterale, was found to contain direction-selective neurons with features comparable to neurons in the AOS of other vertebrates. In general neurons could be classified into three categories:

1. motion sensitive
2. axis selective
3. direction-selective

The major part of recorded cells was represented by motion sensitive cells, which responded with tonic firing in all directions during stimulation with moving stimuli. The second group of neurons was direction-selective cells, which reacted (as neurons in the AOS of other vertebrates) with an enhanced firing during stimulation in their preferred direction and smaller responses to all other presented directions. In contrast to direction-selective cells in other species tuning widths were rather broad and cells showed no inhibition in null-direction. The smallest amount of neurons is provided by axis selective cells that preferred rotations around a particular axis.

As neurons of the Cgl share similar properties to neurons in the AOS of other vertebrates and show similar afferent (input from the contralateral retina) and efferent connectivity (direct projections to the oculomotor nuclei and the cerebellum), the Cgl is supposed to be involved in oculomotor function in the shark (Masseck and Hoffmann, 2008a).

Another major finding is related to the distribution of preferred directions within the Cgl, in contrast to the AOS of other vertebrates no segregation for vertical and horizontal directions was found.

In addition we looked for the internal reference frame of the Cgl. For tetrapodes a conversion from a retinal based reference frame into a vestibular or extraocular muscle reference frame, respectively was shown for AOS (Simpson et al. 1988, Wylie et al.1993, 1999). In our sample of direction-selective cells no bias for rotational axes corresponding to semicircular canal axes could be found and thus coding in a vestibular reference frame seems unlikely.

Properties, like broad tuning, no inhibition in null-direction, no segregation of different directions and no coding in a vestibular or eye muscle reference frame let us suppose that the AOS of chondrichtyes could represent the original state in vertebrate evolution (Masseck and Hoffmann, 2008a).
2. The APT of goldfish
As in chondrichtyans a pretectal area named APT in goldfish was found to contain direction-selective neurons which were sensitive to large moving textured patterns. Direction-selective neurons in the APT behave like AOS neurons of other vertebrates, and again all three classes of neurons were found (motion sensitive, axis selective and direction-selective). Characteristics of APT neurons are in close relationship to properties of AOS neurons in other vertebrates: almost all cells were sharply tuned and showed inhibition in null-direction.

As in chondrichtyans no bias for vertical or horizontal directed movements could be found. Hence we conclude that in fish the AOS is comprised only of one nucleus, where all directions of motion are represented equally and segregation into different nuclei coding different directions is realized only with the development of amphibians. Based on this assumption we propose that neuronal systems become more and more complex by a process of parcellation as suggested by Ebbeson (1980) in his parcellation theory.

Iontophoretical applications of rhodaminedextrane into the APT after recordings of direction-selective cells were made to clarify afferent and efferent connectivity of the APT. Afferent fibers arise exclusively from the contralateral retina. Efferent projections by the APT are predominantly ipsiversive. Also contralateral projection sites have been recognized, but always to a smaller extent. In general connectivity of the APT is remarkable similar to the connection of the AOS in tetrapods. All three oculomotor nuclei, the cerebellum, the valvula cerebelli, the vestibular nuclei, area II and the inferior olive are termination sites of the APT. Furthermore ipsilateral terminals to the reticular formation, probably including area I were found.

Contralateral projections were always sparse and irregular arranged over different animals, thus it is in question if these projections contributed functionally to gaze stabilization.

All of our obtained results: strong contralateral termination sites and no bias for horizontal ipsiversive stimuli point to a monocular organization of the oculomotor system in fish, this conclusion is further supported by fish with highly unyoked eye movements (Marshall and Fritsches, 2002) and a monocular organization of the velocity-to-position integrator in goldfish (unpublished result by Debowy and Baker).

Another objective was to characterise the internal reference frame of the APT. Our sample of recorded neurons had two preferred axes of rotation:

1. Rotation around the YAW-axis
2. Rotation around the LARP axis.
Both preferred axes of rotation correspond to semicircular canal axes (i.e. horizontal and anterior vertical). This result is consistent with the actual tetrapode literature, where also a bias for these two axes was found. Hence coding in a reference frame defined by the vestibular system or eye muscles pulling direction is conceivable.

Anyway, a clear statement if a vestibular or extraocular muscle reference frame is favoured is still pending, as semicircular canals and pulling directions of the eye muscles are aligned in the goldfish (Ezure and Graf, 1984; own dissections).

Summarized the AOS of bony fish is composed of the APT, which contains direction-selective neurons coding for all rotation directions with properties similar to AOS neurons of other vertebrates. These response characteristics of APT neurons and their afferent and efferent connectivity led us to suppose that the APT of teleosts is functionally and anatomically equivalent to the AOS in tetrapods.

**Optokinetic reactions in geckos**

Optokinetic reactions of geckos (*P. madagascariensis*, *Lygodactylus* sp., *G. gecko*, *E. macularis* and *L. lugubris*) were examined to figure out if retinal specializations determine the occurrence of symmetrical monocular horizontal optokinetic reactions.

In conclusion neither a fovea nor diurnal or nocturnal lifestyle could be correlated with the degree of symmetry, as all investigated species were asymmetric in their responses (independent from possessing a fovea or not). Based on this for geckos the fovea theory, developed by Tauber and Atkin (1968) is proven to be wrong (Masseck et al., 2008b).

In addition intraocular tracer injections revealed a lack of ipsilateral fibers in all species, although in the literature sparse ipsilateral projections to all main retinorecipient areas are described for *G. gecko* (Northcutt, 1974). As *P. madagascariensis* exhibited reactions to naso-temporal stimulation, albeit much weaker as to temporo-nasal stimulation also a correlation of ipsilateral fiber density and the occurrence of reactions to NT stimulation failed.

Based on our anatomical and physiological results as well as behavioural measurements in other lateral eyed animals we propose the hypothesis for non-mammalian vertebrates that monocular symmetry correlates with the degree of monocular control of each eye.

Animals with highly yoked eye movements (like geckos, butterfly fish) are rather asymmetric, whereas lateral-eyed animals with unyoked eye movements (like the pipefish, goldfish, trout and chameleons) show symmetric responses.
Appendix

Contents of the enclosed CD:
Selfwritten Matlab data analysis programs (Matlabversion 2006a):

<table>
<thead>
<tr>
<th>Program name</th>
<th>function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ax</td>
<td>Calculation of direction-selective indices for each recorded axis</td>
</tr>
<tr>
<td>Doubling_angles</td>
<td>Plots all obtained mean vectors of preferred direction modulo 180 and calculates the axis preferred axis of the population and its r-value</td>
</tr>
<tr>
<td>Fitting_max</td>
<td>Opens a graphical user interface to read recorded data and asks what type of fitting is desired</td>
</tr>
<tr>
<td>Gauss_fit_horizontal_smooth</td>
<td>Fits a gaussian function to data which were recorded with axes lying in the horizontal plane and gives tuningwidth, tuningindex and preferred direction as output (this application is called by Fitting_max)</td>
</tr>
<tr>
<td>Gaussfit-smooth</td>
<td>Fits a gaussian function to data which were recorded with linear stimuli and gives tuningwidth, tuningindex and preferred direction as output (this application is called by Fitting_max)</td>
</tr>
<tr>
<td>Multi_channel_hori_kernel_scatter</td>
<td>Plots a PSTH, a rasterplot and polarplot of data which were recorded with axes lying in the horizontal plane</td>
</tr>
<tr>
<td>Multi_channel_linear_kernel_scatter</td>
<td>Plots a PSTH, a rasterplot and polarplot of data which with linear stimuli</td>
</tr>
<tr>
<td>Rayleightest</td>
<td>Plots optionally the preferred direction or the preferred direction with its tuningindex in a polarplot. In addition a rayleightest or a rao-spacing test is applicable to the data</td>
</tr>
<tr>
<td>isodensity</td>
<td>Plot an isodensity contour maps for retinae</td>
</tr>
</tbody>
</table>
Planetarium control file written in Cortex:

p.tim, p.cnd, p.itm Controls the direction and speed of the planetarium, and saves up to four spike channels simultaneously. Speed is selectable over the cortex surface.

Supplementary movies from the J. Neurophysiol. article:

Linear_stimuli Simulation of how the linear stimuli would appear on the central 60° of the retina. Optical axis of the eye is in the center of stimulus area. Velocity and contrast do not accord with the original stimuli.

RALP_UP Simulation of a counterclockwise rotation around the RALP axis, seen by the central retina. Simulation of how the RALP UP stimulus would appear on the central 60° of the retina. Optical axis of the eye is in the center of stimulus area. Velocity and contrasts do not accord with the original stimuli.

LARP_UP Simulation of a counterclockwise rotation around the LARP axis, seen by the central retina. Simulation of how the LARP UP stimulus would appear on the central 60° of the retina. Optical axis of the eye is in the center of stimulus area. Velocity and contrasts do not accord with the original stimuli.

ROLL_UP Simulation of a counterclockwise rotation around the ROLL axis, seen by the central retina. Simulation of how the ROLL UP stimulus would appear on the central 60° of the retina. Optical axis of the eye is in the center of stimulus area. Velocity and contrasts do not accord with the original stimuli.

PITCH_DOWN Simulation of a counterclockwise rotation around the PITCH axis, seen by the central retina. Simulation of how the PITCH DOWN stimulus would appear on the central 60° of the retina. Optical axis of the eye is in the center of stimulus area. Velocity and contrasts do not accord with the original stimuli.
Enclosed pdf-files:


Bibliography


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