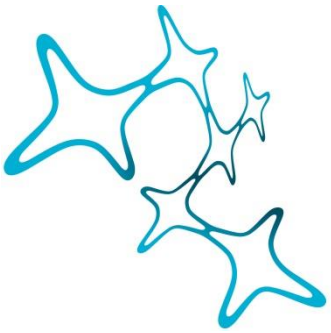
SAME SAME BUT DIFFERENT: PLASTICITY OF A ‘CONSERVED’ REFLEX

Michael Josef Stefan Forsthofer



Graduate School of Systemic Neurosciences

LMU Munich

Dissertation at the

Graduate School of Systemic Neurosciences

Ludwig-Maximilians-Universität München

March, 2023

Supervisor

Prof. Dr. Hans Straka, Prof. Dr. Ruben Portugues

Faculty of Biology

Ludwig-Maximilians-Universität München

First Reviewer: Prof. Dr. Ruben Portugues

Second Reviewer: Prof. Dr. Anja Horn-Bochtler

Third Reviewer: Prof. Dr. Uwe Ilg

Date of Submission: 31.03.2023

Date of Defense: 23.10.2023

I dedicate this thesis to my mentor, Prof. Dr. Hans Straka.

Acknowledgments

I can't believe it's (almost) over. It's going to be a challenge to convey how sad, yet also excited I am about closing up this chapter, and moving on from both the work and the people that contributed to who I became these past four years.

First off, Hans. There's so many things left unsaid. So many things I have to thank you for, discuss with you, joke about or just show to you because I'm proud of them. Or just that I hope you knew that i'm happy I ended up joining your lab. No matter how much you cared about science and good writing, I've always had the impression you cared just as much about conveying that to me and helping me become the scientist I am by helping me figure out my strengths, weaknesses, and passions in the work I did. I will also never forget the memorable ways in which you taught these lessons, be it indirectly through elaborate metaphors highlighting the impracticality of an endeavor or practical lessons in disassembling a 20-year-old amplifier, or directly by stating cold hard truths, such as that mice and frogs are different species. I'm incredibly grateful that I got to call you my supervisor, and I wish you would have had a chance to read this thesis. I'll miss you, your crazy ideas, and your unwavering support. Antje, if you're reading this: thank you, too, for coming by and following Patty's and my progress. It helped, feeling like there is still some connection to Hans' life.

I would also like to thank Ruben, who stepped up as my new supervisor, and helped me finish my thesis work, a manuscript, and supported me in my postdoc applications. Further I want to thank Anja Horn-Bochtler and Uwe Ilg for reviewing this thesis, and Lars Kunz and Andreas Zwergal as part of my examination committee. A big thanks also to Francois and Mathieu, for stepping up and giving helpful feedback on my writing.

Along with my supervisors and mentors, I'd also like to thank the entire Straka lab for all the support throughout the years. Clayton, you're one of the smartest people I've ever met, and sometimes you felt more like my second supervisor than a colleague. I'm glad I could repay it by helping you through the thicket of German bureaucracy, choice of sampling rates, or picking the right brand of mold removers. Patty, you're one of the most intimidating people I've ever met, and I'm grateful you're on my side. You've changed so much in your time here, and your confidence and attention to detail in combination with your kindness made you a joy to work with, and a massive help in these last few months. Now it's your turn.

While it took me a while to socialize outside of the Straka lab, it's been worth it. Pablo, Valentin, Pepe, thanks for all the conversations over beer, gin and coffee. You've created an environment here that makes it hard to move on.

Boris and Tobi, you two are the reason i became a scientist. Tobi, you showed me how fun science can be and which exotic topics there are. Without you, I would not have decided to do

a PhD. Boris, you showed me the potential of what I can achieve, to be confident in my work and to stay ambitious. And along that way, you've always been there for questions or support.

Big thanks is also due to the bouldering crew, Lisa, Sandra, Joel, Ash, Joanna and Uwe and all the others, those thursday nights with fake rocks and pizza were a much needed disconnect from work.

Thanks also go to my Family. Jadwiga, Josef, somehow, you've set me on this – not always straightforward – path, from a kid fishing for cucumbers in the backyard to here. Andi, I'll never forget fishing for dragonfly larva, chasing grasshoppers, exploring the natural history museum or comparing our favorite spider species in a book in a language that we didn't speak back then. No wonder I ended up as a biologist.

Finally, Orsi. When I first met you on that parking lot, I had no idea how much you'd mean to me by now. It's hard to put into words how much you've helped me get through the last few months, especially December; either by helping me be productive and discussing ideas, or by telling me to calm down. My trips to visit you gave me things to be excited about and stay motivated. Looking forward to the next chapter, see you in the UK.

Abstract

Transformation of sensory percepts into motor output form a core element of how any animal interacts with their environment. While some such sensorimotor transformations can be very elaborate and depend on the lifestyle of a species, others serve basic functions and are ubiquitous across vertebrates. Among the latter ones are gaze-stabilizing reflexes, which serve to maintain stable vision during head motion through compensatory eye movements. Despite this conservation throughout evolution, these reflexive behaviors must remain plastic depending on context or past experience to maintain functionality after e.g. impairments of motor or sensory systems through compensation, or to changes in the environment through adaptation. In this thesis, I employ tadpoles of the frog *Xenopus laevis* to investigate how neuronal circuits contribute to either adaptive or compensatory plasticity on otherwise conserved gaze-stabilizing reflexes.

My first study centers on the role of bilateral visual pathways in the development of the optokinetic reflex (OKR). In early embryos, I unilaterally remove the precursor of the eye, the optic vesicle. Tadpoles that develop under such monocular conditions display pathfinding errors of retinal ganglion cells at the optic chiasm. Tadpoles with near normal contralateral projections functionally compensate for the loss of one eye and show consistent responses to both leftward and rightward moving stimuli. In animals with an induced aberrant ipsilateral projection, compensation is increasingly impaired with more pathfinding errors. Combined, this study shows that binocular eyes are required for appropriate visual circuit formation, and that resulting anatomical aberrations impose limitations on compensatory plasticity.

In my second study I focus on the role of the cerebellum in plasticity. Combinations of prolonged, repetitive stimulation with lesions of the cerebellum revealed adaptive plasticity of the OKR, where initially very variable OKR responses converge towards a homeostatic motor output by selective increase and decrease of response magnitude. The cerebellum is specifically associated only with response increases, and only starts to exert this influence well after initial OKR onset. This study therefore shows that multiple brain areas differentially contribute to plasticity of eye movements, leading to heterogenous appearance of different modes of plasticity throughout development.

Combined, these studies contribute to the understanding of development and purpose of plasticity in *Xenopus* OKR. Multiple brain areas are involved with plasticity, and their formation depends on canonical, bilateral visual input. Once functional, plasticity mechanisms serve to maintain homeostasis of the OKR response in response to both adaptation and compensation.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AOS	Accessory Optic System
CF	Climbing Fiber
IO	Inferior Olive
K^+	Potassium Ion
N	Nasal
N-T	Naso-Temporal
nBOR	Nucleus of the Basal Optic Root
nLM	Nucleus Lentiformis Mesencephali
OKR	Optokinetic Reflex
PC	Purkinje Cell
PF	Parallel Fiber
RGC	Retinal Ganglion Cell
T	Temporal
T-N	Temporo-Nasal
VOR	Vestibulo-Ocular Reflex

CHAPTER I:

INTRODUCTION

Plasticity

'Life finds a way'. This quote by the fictional character Dr. Ian Malcolm (Jurassic Park, 1993) summarizes in few words that living beings can and will adapt to their environment for survival. In the long run and in context of this quote, such changes or adaptations refer to inter-generational variability of animals. They may make an organism behaviorally or physiologically better suited to survive within their specific environment, driving evolution and resulting in the variety of organisms we encounter in the world (Darwin, 1859). However, such changes do not just occur between generations and species, but also occurs on a much shorter scale within individuals to adapt even to short-term environmental changes (Van Buskirk et al., 2012). In animals, which travel and actively change their environment, rapid changes of their responses to the environment, or plasticity, are especially critical (Ghalambor et al., 2007; West-Eberhard, 2003).

While definitions of behavior vary, at a very basic level a behavior can be viewed as the generation of a motor output in context of a sensory percept of the environment, or in short, sensorimotor transformation (Calhoun & Hady, 2021; Dewey, 1896). To allow for dynamic interaction with changing environments, different behaviors are executed at varying intensity in response to different stimuli, e.g. fish swim at different velocities depending on the speed of water flow, in order to maintain position (Lupandin, 2005). Throughout life or depending on context, however, the same stimulus may not always warrant the same response, either due to changing needs of an animal or injury of sensory organs and thus leads to a changed perception of a physically unchanged stimulus (Anderson, 2016; Siju et al., 2021). For instance, impairment of the inner ear, which serves to perceive orientation of the body in space and helps maintain balance, leads to a changed perception of the corresponding physical parameters and may result in motor output that compensates for a body motion that is not actually occurring (Burt & Flohr, 1991). In such instances, plasticity allows for adaptation of behavior as long as feedback mechanisms are in place to allow assessment of appropriateness of behavior (Burt & Flohr, 1991; Curthoys, 2000; Dieringer, 1995). In this thesis, I aimed to investigate how brain circuits contribute to different forms of plasticity. First, I wanted to test whether circuit formation itself can contribute to compensatory plasticity following early embryonic sensory manipulations. Subsequently, I wanted to investigate how plasticity appears during physiological maturation of circuits, and whether different brain areas

help mediate different modes of plasticity. I chose the frog *Xenopus laevis* to undertake these studies, as embryos and larvae of this species are readily accessible for circuit manipulation and can be reduced to *in-vitro* experimental models allowing for highly controlled stimulus settings (Straka & Simmers, 2012). Furthermore, as a behavioral readout to quantify plasticity I required an otherwise reliably inducible and well characterized behavior, ideally ubiquitously performed by most vertebrates for comparative approaches. Gaze stabilizing reflexes are both stereotyped within species and exist in some form in virtually all vertebrates, while still showing plasticity (Balleine, 2019; Carew et al., 1981; Hardcastle & Krapp, 2016).

Gaze-stabilizing reflexes

For an animal's survival, it is essential to detect an approaching predator, a passing car on the street or escaping prey. Detection of location and motion of objects therefore is a key feature of sensory systems with spatial perception, such as the visual system. However, as animals themselves locomote, so do the eyes, leading to perception of motion and creating ambiguity: Am I moving, or is it the world around me (Straka & Chagnaud, 2017)? Hence, it is essential for the visual system to disambiguate between self- and external motion. Despite the singular aim of gaze stabilization to maintain a stable eye position, a multitude of both sensory and motor systems are involved in this task (Horn & Straka, 2021; Land, 2019; Straka & Dieringer, 2004; Walls, 1962).

Vestibular-based motion detection

Primarily poised to detect self-motion is the vestibular system, as it perceives translational and rotational movements in space, as well as orientation relative to gravity (Benson, 1990; Day & Fitzpatrick, 2005). Detection of different types of motion is achieved by embedding sensory epithelia into morphologically different surrounding structures, creating two classes of vestibular end-organs located in the vertebrate inner ear: Macular organs and semicircular canals (Platt & Straka, 2020; Rabbitt et al., 2004; Steinhausen, 1986). In both cases, hair cells form the sensory element, consisting of a stair-like arrangement of apically interconnected cilia with a kinocilium at the end. Cilia protrude into K^+-rich endolymph and possess mechanosensitive K^+-channels. Deflection of the kinocilium will shear the cilia, thereby opening or closing K^+-channels depending on deflection towards or away from the kinocilium, and increase or decrease K^+-flow into the cell respectively (Engström et al., 1962; Hudspeth & Jacobs, 1979). As a result, an increase or decrease of the baseline firing rate is induced in the postsynaptic vestibular afferent, which signals to central processing nuclei a forward or backward motion in the activation plane of a respective hair cell (Precht, 1978; Van Egmond et al., 1949). The characteristics of the kinocilium, such as length, flexibility but also

ion channel composition of the hair cell dictate the sensitivity of the cell for temporal characteristics of a particular motion, e.g. frequency or velocity (Baird, 1992; Lewis & Li, 1975). Arranging types of frequency-tuned hair cells in different orientations then allow detection of direction and velocity of motion (Straka et al., 2009). As mentioned before, to discriminate between linear (translational) and angular (rotational) motion, sensory epithelia are embedded in either macular organs or semicircular canals. Macular organs follow a different design, consisting of the sensory epithelium with an apically attached inertial mass of otoconia or a single otolith (Popper et al., 2005). During translation or tilt of the head, hair cells move relative to the inertial otoconia and lead to shearing of the cilia (tilt, Fig. 1A), (Clément & Reschke, 2008). Through combinations of cells in diverse orientations and different alignment of macular organs (Fig. 1B) they help detect a head translation or tilt in a particular direction.

The second type of vestibular end-organ are the semicircular canals, which detect angular acceleration and allow for disambiguation between rotational and tilt motions through integration with macular organ signals (Angelaki & Cullen, 2008). The canals consist of tubes in a semi-circular shape (Fig. 1C) filled with K^+-rich endolymph, including a thickening called the ampulla at their base containing the hair cell epithelia. Cilia of the hair cells protrude into the gelatinous cupula which spans the entire ampulla. Rotational motion of the inner ear displaces the canal including the hair cells relative to the inert endolymph, deflecting the cilia in opposite direction of the rotational movement and causing increase or decrease of K^+-influx depending on direction (Chang et al., 2004; Simpson & Graf, 1981; Steinhausen, 1986). As with macular organs, combination of three canals in a complementary orientation – two perpendicular vertical canals and a horizontal one – allows detection of motion in all rotational directions (de Burlet, 1934; Jørgensen et al., 1998; Platt & Straka, 2020; Simpson & Graf, 1981).

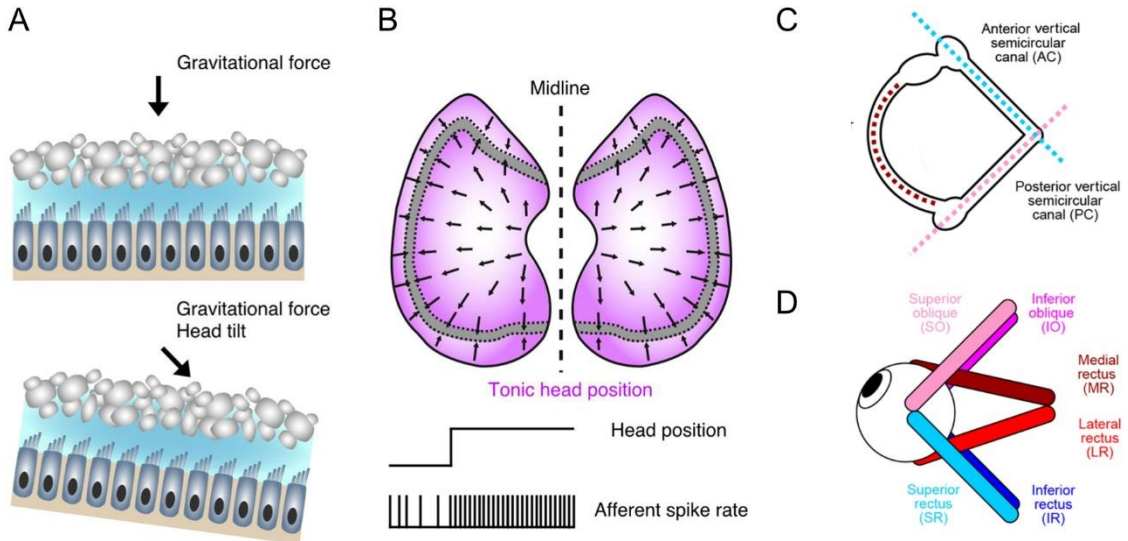


Fig. 1: Structure and function of vestibular end- organs. A) Shearing of hair cells in response to head tilt. B) Distribution of directionally selective hair cell populations across the left and right utricle (top) and firing pattern of a vestibular afferent fiber in response to an adequate change in head position. A), B) adapted from (Gordy & Straka, 2021). C) Semicircular canal orientation. D) Orientation of eye muscles, color coded with the corresponding canals of the labyrinth in the inner ear (dorsal view). C), D) adapted from (Branoner et al., 2016)

In the vestibulo-ocular reflex (VOR), the inner ear is functionally connected to eye muscles to move the eye. The goal is to detect head motion, which causes movement of the eye and therefore a blurred image and move the eye in the opposite direction to stabilize gaze and ensure a sharp image on the retina. Anatomically, it is facilitated by connections from vestibular afferents onto the vestibular nuclei, which connect to the ipsi- or contralateral ocular motor nuclei in an inhibitory or excitatory fashion to finally drive antagonistic pairs of eye muscles (Straka & Dieringer, 2004; Szentágothai, 1950). Detection of angular and linear motion is thus directly employed to produce compensatory eyes movements, and this tight link is reflected in both anatomical and physiological features. (Straka et al., 2009; Walls, 1962). First, sensitivity planes of the semicircular canals are aligned with the pulling directions of the extraocular muscles (Branoner et al., 2016). Second, the vestibular nuclei, the primary recipients of vestibular afferents, are topographically organized as a motor, rather than a sensory map as e.g. is the case for the visual system, which further demonstrates the importance of vestibular signals for gaze stabilization (Straka et al., 2014; Udin & Fawcett, 1988). Finally, temporal characteristics of motion detection conveyed by different cilia morphologies are also maintained. Afferent fibers show more phasic or tonic firing neurons and are thus more geared to convey information about fast motion or onset thereof, versus slow and ongoing motion. A similar differentiation is found in eye muscles which consist of fast twitch fibers and slower pulling ones, which, in concert, drive different kinds of eye motion (Horn & Straka, 2021; Hudspeth & Corey, 1977; Lisberger et al., 1983; Spencer & Porter, 1988; Straka et al., 2009). These characteristics allow the inner ear to faithfully drive compensatory eye movements and maintain a stable gaze (Büttner-Ennever & Büttner, 1992; Graf et al., 1997).

Vestibular basis of eye movements

The eyes of most vertebrates are moved by three antagonistic pairs of eye muscles (Fig. 1D). Like the semicircular canals, each pair is oriented orthogonally to each other to cover all possible directions of motion (Simpson & Graf, 1981). The superior and inferior recti pull the eye up and down, and are innervated through the oculomotor nerve by midbrain motoneurons in the oculomotor nucleus (Matesz & Székely, 1977). The medial and lateral rectus pull the eye towards the nasal and temporal direction, respectively (Fig. 1D). While the

medial rectus has its motor neurons also located in the oculomotor nucleus, the lateral rectus is innervated by motor neurons in the abducens nucleus through the abducens nerve (Cochran et al., 1984). Finally, the superior and inferior oblique muscles rotate the eye in the orbit, with the dorsal side rotating towards or away from nasal, respectively. These rotational movements are especially critical in lateral-eyed and non-terrestrial animals like tadpoles, which frequently perform angular rotations in the vertical plane (Land, 2015). The inferior rectus is once again innervated by oculomotor neurons, but the superior oblique muscle through the trochlear nerve (Straka et al., 2014). The motor neurons receive direct input from neurons in the vestibular nucleus except for the medial rectus, where interneurons in the contralateral abducens nucleus relay vestibular information through the oculomotor nucleus (Büttner-Ennever & Akert, 1981; Horn, 2020; Straka & Dieringer, 1991). Within all the previously mentioned nuclei, populations of cells innervating different eye muscles are mostly separated from each other, once again emphasizing the motor-, rather than sensory mapping of vestibular-based gaze stabilization (Branoner et al., 2016). In the VOR, eye movements are generally classified into two different modes: Slow phases constitute the compensatory part of eye movement and are executed in opposite direction of body motion to maintain stable gaze. As the eye is continuously moved in one direction during slow phase movement, it eventually reaches a maximum outward or inward position within the orbit. The second type of vestibulo-ocular eye movements are fast phases, which yoke the eye in the opposite direction to reset it in the orbit, so slow following movements can continue (Horn & Straka, 2021).

While the vestibulo-ocular reflex clearly plays a fundamental role in gaze stabilization, it is just as apparent that it cannot fulfill this role on its own due to multiple shortcomings (Land, 2019). For one, some animals display only limited eye movements, and thus need to shift the motor effector of gaze stabilization onto other motor systems (Bath et al., 1998; Straka & Dieringer, 2004; Wallman & Letelier, 1993). Compensatory movement of the neck, called the vestibulo-collic reflex, is prevalent in birds and visible during head bobbing of walking pigeons, for example (Frost, 1978). Likewise, the whole body can be moved in addition to the eyes and the head, as apparent in frogs (Straka & Dieringer, 2004). These three different motor patterns are not mutually exclusive, and indeed might work jointly in a goal-oriented manner, that is contributions of eyes, neck and body can be freely combined as needed to provide optimal gaze stabilization (Wallman & Letelier, 1993). While body and neck motions complement eye movements of the VOR on a motor level, gaze stabilization also requires complementation at a sensory level, such as the lateral line system in fish and amphibians or proprioception in most vertebrates, to capture all aspects of motion (Cullen & Zobeiri, 2021; Engelmann et al., 2000). Likewise, copies of motor signals, which are predictive of self-motion percepts during

locomotion, are employed in gaze stabilization and can attenuate vestibular evoked gaze stabilization (Chagnaud et al., 2012; Straka et al., 2018).

Up to this point, I have explained how gaze stabilization mechanisms detect motion and translate this into motor commands, which allows animals to be displaced by, for example, water motion, or dynamically move through their environment, without losing visual acuity. What is lacking thus far is the main aspect of this thesis, plasticity of such reflexes. This is because the afore mentioned reflexes lack one critical prerequisite of plasticity: feedback about the appropriateness of motor output, as they all operate in an open-loop configuration, stabilizing the eyes without directly receiving visual feedback. This highlights the special role of the optokinetic reflex (OKR) in gaze stabilization and as a model for a plastic reflex.

Visual motion detection

To fulfill the function of a feedback mechanism in gaze stabilization, the eye must identify motion, discern between self- and environmental motion, and elicit compensatory eye movements. This is achieved by detecting large visual scene motion as opposed to motion of single objects, and like the VOR, the OKR manifests as alternating slow following and fast resetting motion (Distler & Hoffmann, 2011; Ilg, 1997; Szekely & Szentagothai, 1956). A popular example of a stimulus eliciting the OKR in humans is a train moving through the visual field of the observer, which induces following eye motions in the same direction as the train. Under artificial experimental conditions, the reflex can be elicited by light dark transitions, or more commonly by motion of a large field visual pattern (often vertically oriented black and white stripes) (Distler & Hoffmann, 2011; Kist & Portugues, 2019). That this reflex is geared towards self-motion detection is supported by two observations. First, moving trains and optokinetic drums are, at the very least, unlikely to have played a role in the evolution of visual behaviors in early aquatic vertebrates. A much more likely source of large field visual motion is the displacement of the head, and therefore the eyes, relative to the environment – self-motion. Second, and tying back to the moving train stimulus, is the phenomenon of vection. It describes the illusion of self-motion in response to large field visual stimulus motion without experiencing real motion (Dichgans & Brandt, 1978; Hettinger et al., 1990). It can be experienced firsthand when seeing a neighboring train leave the station in the visual periphery, giving the impression of the own train moving despite being stationary. This illusion of motion, created solely by a visual stimulus, provides a powerful example of the role of large field visual motion, and by extent, the optokinetic reflex, serving as a self-motion detector.

In vertebrates, visual motion detection already happens on the retina (Wässle, 2004). Initially, the visual scene is perceived by phototransduction in photoreceptor cells – rods, cones, or both – in the outer layer of the retina, leading to the perception of an image of the

visual world where each photoreceptor corresponds to one position of the visual field (Tomita, 1970; Yau, 1994). To detect motion, the positional change of an object needs to be extracted, thus necessitating the comparison of at least two detectors in the visual field, as well as their activation sequence in time to identify the direction of motion. Multiple models have been proposed as to how this is achieved, and interestingly, implementations of both have been found throughout the animal kingdom (Barlow & Levick, 1965; Egelhaaf et al., 1989; Hassenstein & Reichardt, 1956; Matsumoto et al., 2019; Mauss et al., 2017). In accordance with the versatile approach of how motion is detected in nature, the question of where in the retina this detection happens also does not have a simple answer. Following light transduction, visual input across photoreceptors is integrated by horizontal cells, transmitted to bipolar cells which, like horizontal cells, receive input from multiple photoreceptors. The bipolar cells synapse onto retinal ganglion cells that form the exclusive output of the retina and are also horizontally connected by amacrine cells (Fig. 2A) (Haverkamp & Wässle, 2000). Motion detection seems to happen at all these different stages of horizontal integration past the photoreceptors. Already at the level of bipolar cells, responses specific to motion have been found – albeit preferably to radial motion of a small object, whereas amacrine cells respond more commonly to more types of directional motion (Ding et al., 2016; Kim et al., 2014; Strauss et al., 2022). Most importantly, at the final point of retinal processing and directly connected to visual centers in the brain, retinal ganglion cells (RGCs) play the main role in retinal motion detection after receiving bipolar cell input, with specific directionally sensitive ganglion cells showing clear diversification for different velocities and direction of motion (Baden et al., 2016; Mauss et al., 2017). In addition to mammals from which the majority of retinal understanding is derived, motion-directive ganglion cells have also been found in frogs (Grüsser et al., 1968; Lettvin et al., 1959). Most relevant for this thesis is the fact that specific populations of these direction selective RGCs prefer slow visual motion, and are, directly wired, in some cases exclusively, to the accessory optic system (AOS) in mammals, which conveys function of the OKR and links these RGCs directly to gaze-stabilization (Britto, 1983; Buhl & Peichl, 1986; Cruz-Martín et al., 2014; Dhande et al., 2013; Oyster et al., 1980). These nuclei are found in the di- and mesencephalon, and while the majority of visual fibers target the tectum, are associated with gaze stabilization across most vertebrates (Lázár, 1972; Roeser & Baier, 2003; Sperry, 1956).

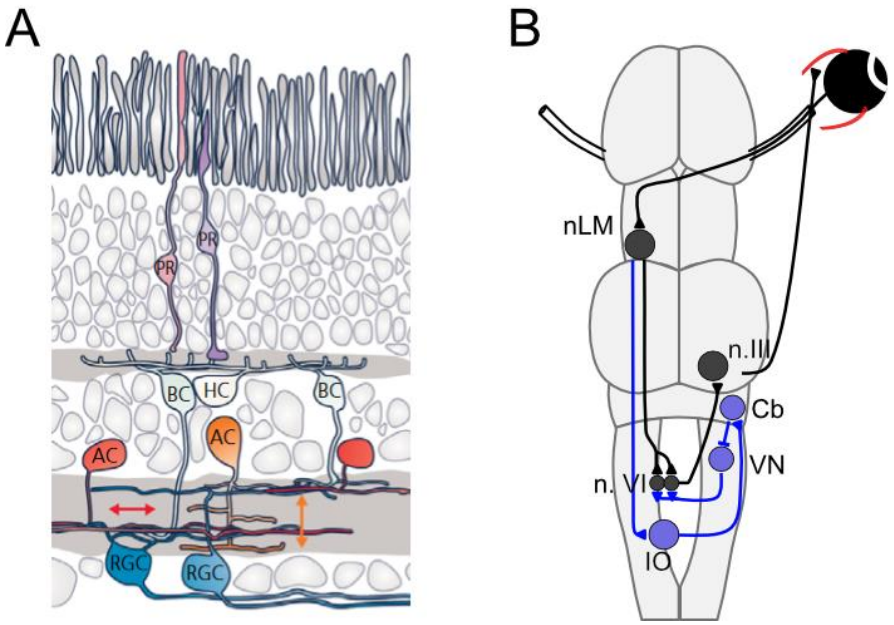


Fig. 2: Peripheral and central components of large-field motion detection. A) Cell types in the mammalian retina. B) Direct (black) and indirect (blue) circuits of the horizontal optokinetic reflex. A) adapted from (Franke & Baden, 2017)

The accessory optic system

The accessory optic system is associated with optokinetic behavior in most vertebrates but shows a considerable amount of variety in its organization between vertebrate groups (Bechterew, 1883; Giolli et al., 2006; Gudden, 1870; Hayhow, 1959; Simpson, 1984). In mammals, the accessory optic system consists of three subnuclei, the dorsal, lateral and terminal nuclei, which show a functional dependence on the nucleus of the optic tract in the pretectum as well as cortical inputs (Giolli et al., 2006; Masseck & Hoffmann, 2009). In birds and amphibians, a simpler structure is found: the nucleus of the basal optic root (nBOR). It forms the functional homologue to the dorsal, lateral and terminal nuclei, and the equivalent of the nucleus of the optic tract, the nucleus lateralis mesencephali (nLM) becomes critically involved in optokinetic behavior as elaborated later on (Fite et al., 1979; Lázár, 1972; Masseck & Hoffmann, 2009; Montgomery et al., 1985; Wylie & Frost, 1990). Finally, in fish, even the delineation of nBOR and nLM becomes blurred and the standing hypothesis is a single nucleus in the pretectum, the dorsal accessory nucleus (or arborization field 5 in larval fish), facilitating optokinetic behavior (Burrill & Easter Jr, 1994; Klar & Hoffmann, 2002). While data from mammals, birds and amphibians provide insight on anatomy and single-/ multi-cell physiology, more recently popularized model species such as zebrafish contribute to the understanding on population-level coding of neurons in these brain areas (Fite, 1969; Fite et al., 1979; Kubo et al., 2014; Matsuda & Kubo, 2021; Simpson, 1984; Simpson et al., 1988).

While focusing on the accessory optic system in amphibians, I will nonetheless give an overview of functional principles in this system across vertebrates to gather a more thorough conceptual understanding of the variable neuronal underpinnings of conserved optokinetic behaviors.

In larval frogs, RGCs exclusively cross at the optic chiasm, and target only the contralateral brain hemisphere (Guillery, 1995; Holt, 1989; Nakagawa et al., 2000). The accessory optic nuclei receive input from RGCs that are located centrally in the retina, which, in zebrafish, is aligned with horizontal gaze and represents the part of the visual field most responsive to optokinetic stimuli (Dehmelt et al., 2021; Fite, 1985). They further show preference for slow stimuli in line with their presynaptic RGCs, and have large receptive fields as would be expected for optic flow detectors (Fite, 1969; Wang et al., 2019). Despite the majority of projections targeting the tectum, ablation of this structure in zebrafish revealed that the tectum is indeed not necessary to facilitate optokinetic behavior itself, and rather is involved in adaptation and habituation of the OKR (Roeser & Baier, 2003). Instead, the dorsal accessory nucleus, the equivalent of tadpole nLM and nBOR, is essential for optokinetic eye movement (Wu et al., 2020). Neuron populations within this nucleus respond differently to different motion directions of a stimulus, a selectivity that becomes distributed between the nLM and the nBOR in frogs with the emergence of multiple nuclei (Cochran et al., 1984; Kubo et al., 2014; Lázár, 1983; Portugues et al., 2014). Neurons in the nBOR preferentially respond to upward, downward and temporal (i.e. rightward motion for the right eye) motion, and are each suppressed by motion in the non-preferred direction. Neurons with a nasal motion preference are found in the pretectal nLM instead, and despite their anatomical separation, both nuclei are strongly interconnected through predominantly inhibitory connections (Brecha et al., 1980; Cochran et al., 1984; Li & Fite, 2001). Distribution of direction preferences across the tegmental accessory optic nuclei and the nBOR (or the nucleus of the optic tract in mammals) appears conserved across vertebrates, and in the more distributed nuclei of mammals, direction preferences appear to be in alignment with the geometric orientation of the semicircular canals in the inner ear, once again corroborating the collaborative nature of OKR and VOR (Simpson et al., 1988; Soodak & Simpson, 1988; Walley, 1967). Directional tuning of neurons in these sensory nuclei are reflected in behavior: lesions of the nBOR, but not the nLM, selectively impair vertical gaze stabilization (Lázár, 1983). While in this study, head, rather than eye motion served as the behavioral readout, it nonetheless shows the nucleus-specific sensory direction preference and its direct link to motor performance. Another question arising from this distribution into different nuclei, and especially pooling three motion directions (up, down, temporal) into the nBOR as opposed to the clear nasal preference of the nLM, is if isolation of nasal motion is likewise reflected in behavior. In binocular animals, no

strong preference of e.g. nasal motion would be expected over temporal motion, as the left and right complements of the nLM and nBOR are strongly interconnected through commissures that serve binocular integration (Kubo et al., 2014; Portugues et al., 2014). Most animals show strong conjugation of optokinetic eye motions with equal motion components in either horizontal direction, even though less so in fish (Masseck & Hoffmann, 2009; Matsuda & Kubo, 2021). Therefore, to investigate the behavioral impact of a sensory preference of one nucleus, it is necessary to stimulate only one eye to drive activity in only one nLM but not its contralateral counterpart. Such experiments confirmed a nasal motion preference in the OKR under monocular conditions in frogs, but also in rabbits and fish, which suggests that in these animals, binocular eyes are essential for a symmetric OKR, i.e. equal response to leftward and rightward motion (Masseck & Hoffmann, 2009; Portugues et al., 2014; Yücel et al., 1990). On the other hand, humans, cats and macaques do not show such monocular asymmetries. Based on data acquired from recordings in the cortex of macaques and behavioral changes observed in OKR performance of developing cats, one hypothesis implicates cortical circuits in establishing a symmetric OKR even under monocular conditions by increasing the temporal motion component to complement the nasal one of the pretectum (Distler & Hoffmann, 1992; Hoffmann et al., 2002). However, cortical dependencies are not the sole mechanism by which bidirectionally symmetric responses can be achieved, and a reduced OKR asymmetry, and a lack of nasal motion preference in the nLM, have been found in owls and hummingbirds (Gaede et al., 2017; Wagner et al., 2022; Wagner et al., 2021). While cortical involvement in mammals thus explains the acquisition of monocularly symmetric OKR in primates, clearly the pretectum and the AOS themselves can also contribute to plasticity in OKR symmetry. Despite the conserved nature of the OKR from a behavioral standpoint, it is very clear that the underlying circuitry facilitating it is indeed very plastic between species. Further, it highlights the importance of bilateral visual input for appropriate execution of visuomotor behaviors. Key for this bilateral interaction are correct transmission of bilateral visual percepts from the eye to the brain, and subsequently, intra-hemispheric commissural connections to integrate these percepts.

Functional interhemispheric connectivity in the pretectum

Anatomical studies suggest that, while the nasal motion is facilitated through retinal activation of the nLM, the weak temporal component relies on double inhibition through pretectal commissures. Moreover, retino-pretectal projections activated by temporal motion are inhibitory (Hoffmann & Schoppmann, 1975). Data from zebrafish suggest that a subset of commissures originating from their target neurons are likewise inhibitory (Portugues et al., 2014). Detection of temporal motion of the eye inhibits spontaneous activity in the contralateral nBOR, alleviating their inhibitory effect on ipsilateral premotor neurons, thereby driving eye

motion (Burgess et al., 2009; Portugues et al., 2014). In line with this hypothesis, both genetic or acute surgical elimination of posterior pretectal commissures impair temporally directed optokinetic eye motion (Burgess et al., 2009; Wang et al., 2019). Such lesion studies also provide an explanation for the need to detect temporal and nasal motion through different pathways: Neurons that under binocular stimulation specifically respond to different combinations of temporal and nasal motion in the left and right eye decrease their activity in absence of pretectal commissures. E.g. temporal motion for both eyes would occur during forward motion, whereas, a nasal motion on one eye with a temporal motion on the other one would indicate rotational stimulus movement. Splitting of nasal and temporal pathways and later differential convergence through commissures thus may serve to identify different types of visual motion (Kubo et al., 2014; Wang et al., 2019).

Studies in frog suggest the involvement of both GABA and Acetylcholine in commissural pathways. Activation of muscarinic and inhibition of nicotinic acetylcholine receptors both had the same effect of eliminating directional preference, and suggest the ability of this neurotransmitter to both increase and decrease OKR directional preference (Jardon & Bonaventure, 1992a, 1992b; Yücel et al., 1990). GABA, on the other hand, contributes to maintaining low responsiveness to temporal motion (Li & Fite, 2001). In frogs with one sutured eye, the remaining eye eventually compensates for the loss of the contralateral eye by generating symmetric leftward and rightward responses. This effect can be reversed, i.e. the temporal component can be impaired back to near baseline levels, by injection of GABA into the pretectum (Jardon & Bonaventure, 1992a). Perhaps more interestingly in context of this dissertation, however, is the fact that these experiments demonstrate a clear case of plasticity of the OKR: the afore mentioned impairment of temporal OKR constitutes a clear behavioral impairment, which the brain can apparently compensate for. Further, one possible site of this plasticity lies directly in the pretectum, potentially linked to bilateral integration, which raises the question how bilaterality contributes to establishment of these circuits.

Development of retinotectal circuits

Perhaps the most essential step to ensure bilateral visual input is correctly connecting the eye to the brain during development. In *Xenopus,* Retinal ganglion cells start differentiating in the optic vesicle of *Xenopus* embryos at approximately stage 26, approximately 29 hours post fertilization (Cima & Grant, 1980; Nieuwkoop & Faber, 1994). Their axons then start forming growth cones that migrate towards the forming chiasma, which they reach at stage 32 40 hours post fertilization, and where they exclusively cross the midline to the contralateral side (Holt, 1989). The molecular cues involved in axonal pathfinding are manifold. Initially, *Vax1* plays a role in formation of the chiasm, where then *netrin*1 acts as a chemoattractant in

Xenopus guiding axons to the midline through DCC receptors (Shewan et al., 2002). Once arriving at the chiasm, axons can either cross to the contralateral hemisphere, or remain on the ipsilateral one. In animals with ipsilateral projections, crossing axons originate in the temporal retina where the visual field of the left and right eye overlap, and binocular integration is most critical (Guillery, 1995). Cues from both the RGCs themselves according to their origin on the retina, as well as cues at the chiasm originating from neurons and glia, play a role in appropriate RGC pathfinding through the chiasm (Guillery, 1995; Jeffery, 2001; Wizenmann et al., 1993). *Netrin*1, originally serving as an attractant to the midline, starts being perceived as a repellant by neurons reaching the chiasm and thus now promotes crossing (Höpker et al., 1999). Also associated with contralateral RGCs are Nr-CAMs, which are associated with contralaterally projecting axons (Williams et al., 2006). More interesting is the gene *islet2.* It is active in contralaterally projecting RGCs; however, it is required to facilitate ipsilateral crossing of RGC axons (Herrera & Garcia-Frigola, 2008; Vigouroux et al., 2021). These ipsilateral axons are specified by zic2 expression, which is increased following islet2 knockout and manifests as an increase of the ipsilateral projection (García-Frigola et al., 2008). This molecular interaction therefore poses a potential interaction between axons from the left and the right eye, where islet2 expression in contralateral fibers is necessary to maintain the contralateral projection through inhibition of zic2 expression (Pak et al., 2004). Islet2 and zic2 expression themselves depend on the transcription factors FoxD1 and FoxG1, which are associated with nasal and temporal patterning of the retina (Yuasa et al., 1996). The effector at the end of this transcription factor to gene t molecule cascade is ephrin B. It's receptor EphB is expressed by ipsilaterally projecting axons that are repelled by ephrin B (Herrera et al., 2003; Williams et al., 2003), and like zik2, it is not present in e.g. zebrafish, which do not possess ipsilateral RGC projections (Vigouroux et al., 2021). In *Xenopus,* the larva of which do not possess an ipsilateral fraction of RGCs, it is only expressed during metamorphosis when ipsilateral projections start to form. However, application of ephrin B at the optic chiasm in tadpoles is sufficient to induce an early ipsilateral projection (Nakagawa et al., 2000). In addition to showing the role of ephrin B for axonal pathfinding, this also highlights the importance of timing of protein expression during development.

Combining both criticality of timing and binocular interaction during visual circuit formation, removal of one eye early during embryo development has different effects on pathfinding of axons from the remaining eye in mammals. Enucleations performed before axons reached the chiasm resulted in a decrease of the ipsilateral projection, whereas enucleations in embryos past initial crossings at the chiasm maintain normal crossing patterns in both rats and ferrets, which also highlights the importance of binocular interaction in addition to timing (Chan & Guillery, 1993; Godement et al., 1987). Similar studies, but performed at a

different embryonic stages and with different methods, however, yielded different results, and highlight once again the importance of timing, validation, but also the variability one can expect from such highly invasive, developmental studies (Sretavan & Reichardt, 1993). The exact mechanism by which the ipsilateral fraction was reduced remains elusive (Chan & Guillery, 1993). Curiously, in frogs, these results could not be reproduced and monocular embryonic eye removals had no consequence on retinotectal pathfinding (Kennard, 1981). However, these experiments were performed at developmental stage 30, in which RGCs have already started to differentiate and innervate the optic nerve. As mammalian experiments proved timing of removals to be critical, it is unclear whether earlier eye removals may impair RGC pathfinding (Taylor, 1987; Wilson et al., 1988).

Importantly, none of these studies show the functional output or plasticity of circuits showing retinotectal pathfinding errors following monocular development. In other transplant studies, placing the eye of frogs on the tail in absence of normal eyes, the displaced third eye was able to functionally connect to the brain and mediate visual behavior (Blackiston & Levin, 2013). Likewise, displaced ears were also able to be functionally connected to vestibular circuits, whereas one-eared tadpoles showed the capacity to functionally compensate for loss of one ear, showcasing impressive plasticity during embryonic development (Gordy & Straka, 2022; Gordy et al., 2018). In studies on adult frogs, impairments on visual behavior following sensory manipulation were compensated for few days after the procedure, principally showing plasticity in response to loss of one eye in appropriately formed circuits (Jardon & Bonaventure, 1992a). Considering these plastic compensations in adults and embryos, it may well be that ‘aberrant’ pathfinding may contribute to plastic changes in OKR under monocular conditions, either by compensation or even further impairment. Further, correlation of different anatomical pathways, resulting from embryonic manipulations allow directly correlating the impact induced anatomical plasticity has on behavioral output, knowing that an otherwise unimpaired adult circuit is indeed capable of compensation. Therefore, investigation of such embryonically induced aberrant circuits, and how they affect behavior and plasticity thereof, was the first topic of my thesis, and indeed revealed an impairment of plasticity that was directly correlated with the appearance of aberrant, ipsilateral projections. Having shown that anatomical changes in visual pathways impair, rather than enhance, plasticity, I next focused on which mechanisms in the adult brain contribute to facilitating OKR plasticity.

Cellular basis of cerebellar plasticity

In addition to the pretectal – ocular motor nucleus circuit, many other brain areas are involved in the OKR (Eberhorn et al., 2006; Giolli et al., 2006; Macé et al., 2018; Portugues et al., 2014). In context of plasticity, a focal one is the cerebellum (Ito, 1982). Both the nLM and the nBOR connect to the cerebelllum indirectly through fibers running dorsally or ventrally in

the medial longitudinal fascicle to the ipsilateral inferior olive (IO) (Blanks et al., 1995; Horn & Hoffmann, 1987; Maekawa & Takeda, 1979). From the IO, visual signals reach purkinje cells (PCs) through so called climbing fibers (CF), which convey information about image slip on the retina and therefore an error signal during imperfect gaze stabilization (Ito, 1982, 2013; Lisberger et al., 1984; Miki et al., 2018). The second main input to PCs stems from granule cell parallel fibers (PFs) which constitute the main driver of PC baseline activity (Knogler et al., 2019; Matsui et al., 2014). These are located in the granular layer of the cerebellum , receive input from motor and sensory areas and convey information about eye and head motion (Ekerot & Jörntell, 2008; Huang et al., 2013). While climbing fibers wire strongly in a 1:1 ratio to the dendritic tree of a single purkinje cell and reliably trigger a so-called complex spike upon firing, the main driving force of PC baseline activity derives from context-dependent PF inputs, many of which synapse onto a single PC (Knogler et al., 2019; Lisberger & Fuchs, 1978; Llinás & Sugimori, 1980). PCs, according to a simple model of the cerebellum, serve as coincidence detectors and are thought to detect co-occurrence of erroneous or incomplete gaze stabilization through CFs, and a specific motor pattern through PFs. Such coincidence of CF and PF input induces long-term depression of the PF-PC synapse. As a result, synaptic efficacy and thus PC firing in response to the error-associated context is reduced, thereby altering motor output with the aim of minimizing the error signal (Albus, 1971; Ito, 2006; Knogler et al., 2017; Lac et al., 1995; Marr, 1969). To maintain homeostasis of this synapse, long-term depression needs to be reversible, which is achieved through long-term potentiation at both the pre- and postsynapse of the PF-PC synapse, and in combination long-term potentiation and long-term depression serve to tune and maintain PC activity and cerebellar modulation of motor performance (Gittis & du Lac, 2006; Lev-Ram et al., 2002; Pastor et al., 1997; Pham et al., 2020). Furthermore, PC somata lie in the purkinje layer of the cerebellum dorsal to the granule layer, and extend their single, branched out dendrite into the dorsal molecular layer where they form synapses with PF and CF (Eccles et al., 1966; Precht & Llinás, 1969). Their axon connects to either deep cerebellar nuclei, or in case of the OKR and VOR, directly to the vestibular nuclei, and acts inhibitory (Magherini et al., 1975; ten Donkelaar et al., 1981). Decrease of PC firing due to error-induced long-term depression reduces inhibition onto vestibular nucleus neurons, which in turn drive ocular motor neurons, thereby increasing eye movements. Due to this modulatory nature, reduced or absent activity of the cerebellar PCs through experimental ablation of the cerebellum does not impair gaze stabilization *per se.* Instead, removed inhibition from PCs onto vestibular nuclei increases VOR amplitudes, while optokinetic performance initially is mostly unimpaired (Michnovicz & Bennett, 1987). Similarly, genetic inactivation of granule cells, which drive PC activity, initially does not have a major effect on gaze stabilization and rather impairs adaptation and learning of the OKR and VOR (Faulstich et al., 2004; Galliano et al., 2013; Miki et al., 2018).

Behavioral effects of cerebellar plasticity

To induce meaningful adaptation of visual behavior, visual feedback about the appropriateness of visuo-motor transformation is essential. This feedback is conveyed by inferior olivary climbing fibers, which are critical to changes in both the VOR and OKR that aim to reduce retinal image slip. A behavioral example that highlights the extent of such plasticity is elimination of the VOR: Normally, as the head turns to the right, the visual scene is displaced to the left relative to the retina; thus, a rightward turn of the head is compensated for by leftward motion of the eyes. In experimental settings, a rightward turn of the head can be accompanied by an identical motion of the visual scene, in which case no eye motion would have to be performed to maintain gaze and execution of the VOR would induce, rather than eliminate retinal image motion. With prolonged visuo-vestibular stimulation where the visual scene moves with the eyes, the VOR can be strongly reduced in its performance, and this reduction persists for a short time even after removal of the matching visual stimulus (Collewijn, 1989; Collewijn & Grootendorst, 1979). Visual input is therefore essential for plasticity of the VOR not just in context of visual signals, and reinforces the collaborative nature of the OKR onto the VOR (Ahrens et al., 2012). However, both the VOR and the OKR show plasticity also in isolation. This is an intriguing concept in the VOR, as what would be the guiding input to modulate performance of behavior if there is no feedback? It appears that in this case, plasticity serves to maintain homeostasis. Isolated, prolonged stimulation of the VOR in tadpoles results in attenuation of extraocular motor neuron activity for strong stimuli and initially high firing rates, whereas weak stimuli with initially low firing rates show increased activity after half an hour of repetitive stimulation. Therefore, near maximal or minimal responses to strong and weak vestibular stimuli are adapted to intermediate activity, allowing further increases or decreases of eye motion in response to additional changes (Dietrich & Straka, 2016). Curiously, in one-eared frogs, compensation for the loss of one ear also appeared to favor homeostasis over full compensation (Gordy & Straka, 2022). On the contrary, visual plasticity is more feedback driven: as the VOR and OKR work complementarily, neither of them fully compensates for head motion (gain of one), and rather a combination of both is needed for full compensation. Instead, in isolation, both show gains far below one and thus imperfect compensation. However, in many species, prolonged optokinetic stimulation under head-fixed conditions leads to an increase of OKR gain. This appears to be the case across many species from fish (Beck et al., 2004; Marsh & Baker, 1997) to mouse (Faulstich et al., 2004; Pham et al., 2020) and is mediated by inferior olivary projections to the cerebellum. In addition to response magnitude changes, timing is also subject to cerebellar plasticity. Here, more variability exists in the mode of plasticity: Goldfish

and carp can adapt their optokinetic response in terms of timing, anticipating directional changes in a repetitive pattern, while zebrafish and medaka are unable to acquire a predictive OKR (Miki et al., 2020). In case of predictive OKR learning, such plasticity may depend on brainstem velocity integrators, which are present in some species of fish, but not others (Miki et al., 2020). This, for one, highlights that despite the central role of the Cerebellum in learning, many more nuclei are involved in plasticity. Further, there seem to be large interspecies differences of plasticity even within groups of vertebrates. While in some cases, minimizing retinal image slip by enhancing compensatory eye motion is observed, in other cases homeostasis is preferred over perfect compensation.

Overall, despite interspecies differences in anatomical and functional properties of the cerebellum as well as the apparent aim of plastic changes, its involvement in vertebrate gaze-stabilization plasticity is just as ubiquitous in as gaze stabilization itself. This combination of an anatomically well defined, direct OKR pathway with equally well-understood development, and the superimposed plasticity-associated cerebellar loop makes the optokinetic reflex in frog an attractive model for plasticity. In this thesis, I employed both advantages to investigate different aspects of visual plasticity in *Xenopus*. Specifically, I had two main questions: In chapter II, I focused on plasticity in the core circuit, and quantified the extent of anatomical and behavioral plasticity during embryonic development of visual circuits. As *Xenopus* show a large extent of anatomical plasticity during embryonic development in both the visual and vestibular system in response to sensory organ manipulation, I wanted to investigate the necessity of bilateral visual input to properly establish functional visual circuits (Constantine-Paton & Law, 1978; Gordy & Straka, 2022; Ruthazer & Cline, 2004). In Chapter III, I characterized plasticity in the OKR mediated by the cerebellum of tadpoles as well as the developmental onset thereof. As the VOR often shows homeostatic, rather than fully compensatory plasticity in this species, I tested whether this was also the case for the OKR (Dietrich & Straka, 2016; Gordy & Straka, 2022; Soupiadou et al., 2020). Finally in Chapter IV, I will elaborate the key contributions of my research to the understanding of the OKR plasticity, and the apparent goal of it in tadpoles in comparison to other organisms.

CHAPTER II:

BILATERAL RETINOTECTAL MISWIRING IMPAIRS BEHAVIORAL COMPENSATION IN EMBRYONIC ONE-EYED *XENOPUS LAEVIS*

Publication 1

Michael Forsthofer[1,2#*], Clayton Gordy[1,2#], Meghna Kolluri[1], Hans Straka[1]

[#]These Authors contributed equally

[1]Faculty of Biology, Ludwig-Maximilians-University Munich, Planegg, Germany

[2]Graduate School of Systemic Neurosciences, Ludwig-Maximilians-University Munich, Großhaderner Str. 2, 82152 Planegg, Germany

[*]**Correspondence:** Michael.forsthofer@tum.de (MF)

Contribution of Authors:

HS and CG conceptualized, and HS, CG and MF designed experiments for the study. CG, MF and MK performed embryonic extirpations and quantified successful removals. CG and MK performed immunohistochemistry. MF and CG acquired behavioral data, and MF performed and imaged dye tracings. MF and CG established analysis scripts, and MF analyzed data. MF and CG created figures, and CG, MF and HS edited the manuscript. HS acquired funding and supervised the project.

My contributions to this manuscript:

I contributed to design of experiments and analysis and contributed to coding of analysis scripts. I performed the majority of embryonic extirpations and subsequent assessment of successful eye removals. I performed anatomical dye tracings, microscopy and analysis thereof. I acquired the majority of behavioral data, and performed analysis thereof. I created initial versions of figure 1 panel b) and c), as well as all other main and supplemental figures, and contributed to revisions on all figures. I wrote the first version of the manuscript, and contributed to the revision process.

The following manuscript is unpublished.

Bilateral retinofugal pathfinding impairments limit behavioral compensation in near-congenital one-eyed *Xenopus laevis*

Michael Forsthofer[1,2#*], Clayton Gordy[1,2#], Meghna Kolluri[1], Hans Straka[1‡]

[#]These Authors contributed equally

[‡]Deceased December 2022

Affiliations:

[1]Faculty of Biology, Ludwig-Maximilians-University Munich, Planegg, Germany

[2]Graduate School of Systemic Neurosciences, Ludwig-Maximilians-University Munich, Großhaderner Str. 2, 82152 Planegg, Germany

[*]**Corresponding author:** Michael Forsthofer, Faculty of Biology, Ludwig-Maximilians-University Munich, Großhaderner Str. 2, 82152, Planegg, Germany,

Tel: +49 89 218074315

Email: michael.forsthofer@tum.de

KEYWORDS: optokinetic reflex, OKR, Xenopus laevis, development; plasticity, eye extirpation, retinotectal

ABBREVIATIONS

Le, leftward; N, nasal; RGC, retinal ganglion cell; Ri, rightward; T, temporal; OKR, optokinetic reflex

ABSTRACT

To generate a coherent visual percept, information from both eyes must be appropriately transmitted into the brain, where binocular integration forms the substrate for visuomotor behaviors. To establish the anatomical substrate for binocular integration in mammals, the presence of bilateral eyes and interaction of both optic nerves during retinotectal development play a key role. Such interactions are less understood in amphibian models. Further, the extent to which embryonic monocularly derived visual circuits can convey

visuomotor behaviors is unknown. In this study, we assessed the retinotectal anatomy and visuomotor performance of embryonically generated one-eyed tadpoles. In one-eyed animals, the axons of retinal ganglion cells from the singular remaining eye exhibited striking irregularities in their central projections in the brain, generating a non-canonical ipsilateral retinotectal projection, indicative of impaired pathfinding abilities. We further show that these novel projections are correlated with an impairment of behavioral compensation for the loss of one eye.

INTRODUCTION

The bilaterality of sensory systems is crucial for detailed and environmentally relevant processing. Such processing aids in the extraction of behaviorally important sensory features. In the visual system for example, vision with two eyes, or stereopsis, contributes to distance estimation (Poggio & Poggio, 1984) or motion detection (Van Hof-van Duin & Mohn, 1986). In order to accomplish this, anatomical pathways conveying separate information from the left and right eye must be integrated in the central nervous system to form a coherent visual percept (McLaughlin & O'Leary, 2005).

This combination of bilateral information can occur already during transmission of sensory information into the brain. In some animals, such as e.g., mice, this combination happens at the level of retinal ganglion cell (RGC) projections: axons project into the brain either ipsi- or contra-laterally depending on where they arise from in the retina. In other animals, such as zebrafish or larval *Xenopus* tadpoles, all axons cross to the contralateral side (for review, see Petros et al., 2008). In this latter case, binocular information must still converge, which happens anatomically via interhemispheric commissures, to form a binocular visual percept and facilitate binocular-dependent behaviors (Gambrill et al., 2018; Gebhardt et al., 2019; Grant & Keating, 1989; Keating & Feldman, 1975).

Following loss or obstruction of one eye in mature organisms, these established circuits display an impressive capacity to compensate for loss in input, on both a functional and behavioral level, which permits to certain extents recovery of visual behaviors (Dews & Wiesel, 1970; Prusky et al., 2006; Rose et al., 2016; Steeves et al., 2008). In frogs, for example, acute monocular deprivation normally leads to direction-selective impairment of the optokinetic reflex (OKR). However, within 8 days this impairment is remedied, and the OKR responds equally to all directions even under monocular stimulation conditions (Jardon & Bonaventure, 1992a). To enable this plasticity in mature stages, circuits must be correctly assembled during embryonic development (Huang et al., 2006). Such canonical assembly occurs with contribution from both eyes, with genetic and molecular factors playing a key role

for pathfinding of bilateral RGCs, and neural activity serving to fine tune morphological arborizations and other aspects of neuronal connectivity (Holt, 1989; Hua et al., 2005; Jeffery & Erskine, 2005; Niell & Smith, 2005; Oster et al., 2004). Genetically induced deviations in canonical chiasmatic crossing have a direct impact on visual behaviors, such as the complete ipsilateral RGC projections in the Zebrafish *belladonna* mutant that cause a left-right inversion of eye-following motion in the optokinetic reflex (Huang et al., 2006; Karlstrom et al., 1996).

In contrast to the large body of literature on the genetic basis of the establishment of retinotectal connections and their specific functions, less is known about the impact of having bilateral eyes, and thus interaction of bilateral visual pathways, in the shaping of embryonic visual circuit formation and resulting visuomotor behaviors. In animals where each eye possesses RGC projections to the ipsi- and contralateral brain hemispheres, such as ferrets and mice, early embryonic ablation of an optic vesicle and subsequent one-eyed embryonic development results in reduced ipsilateral projections from the remaining eye (Chan et al., 1999; Taylor & Guillery, 1995). This suggests that binocular RGC axons are involved in the chiasmatic-crossing decision, specifically in contributing to the ipsilateral projection of the contralateral eye.

In animals with exclusively contralateral RGC projections, similar experiments yielded less unanimous results. In chick embryos, early optic vesicle removal induces an ipsilateral fraction of RGC projections from the unablated eye. However, these are lost again within few days of development before any visual behavior in this species, and do not allow conclusions about anatomical or functional impact on post-embryonic behavior (Thanos et al., 1984). Unilateral embryonic eye removals in *Xenopus* on the other hand show no sign of an aberrant ipsilateral projection following metamorphosis (Taylor & Gaze, 1990). However, optic vesicle removals were performed after onset of RGC differentiation, and mammalian studies showed a large variability in phenotypes depending on timing of optic vesicle removal (Chan & Guillery, 1993). Additionally, neither study assessed function of circuitry formed under embryonic monocular conditions, leaving a potential beneficial or detrimental role of embryonic plasticity open. While a detrimental effect poses the more likely outcome based on previous genetic studies on pathfinding errors (Huang et al., 2006), the complete absence of visual input to half of the circuit, rather than miswiring to both, may allow for stronger functional connections of the remaining eye, and to functional plasticity (Straznicky et al., 1980). Indeed, *Xenopus* embryos show a large degree of plasticity during embryonic development, allowing for anatomical integration of e.g. a third eye into midbrain circuits (Constantine-Paton & Law, 1978). More recent studies reveal that supernumerary or displaced eyes and ears can be anatomically and functionally connected to their central targets and facilitate behavior to

certain extents and under certain conditions (Blackiston & Levin, 2013; Cline, 1991; Elliott & Fritzsch, 2018; Gordy et al., 2018).

In this study we therefore revisit embryonic visual circuit formation in one-eyed *Xenopus laevis*, to answer the question whether visual circuits can develop appropriately in absence of one eye during embryogenesis, and whether this developmental plasticity conveys a compensatory or detrimental effect on visuomotor behavior. In contrast to previous studies in this species, we performed embryonic enucleations before differentiation of RGCs in the optic vesicle. Following development into late larval stages, where we expect exclusively contralateral RGC projections in two-eyed animals, we assessed the anatomy of retinotectal projections. Here, we identify a novel ipsilateral RGC projection and quantify eye motion elicited by the optokinetic reflex, to assess the extent of functional plasticity this monocular visual circuit allows. The results here reveal that embryonically generated one-eyed *Xenopus* tadpoles with aberrant RGC projections exhibit more impaired visuomotor behaviors, highlighting a critical limitation of visuomotor plasticity in retinotectal development.

RESULTS

Generation of one-eyed tadpoles

One-eyed tadpoles were generated by removal of the left optic vesicle in early embryonic stages, similar to embryonic removal of the otic vesicle in previous studies (Fig. 1a, Stages 25-27)(Gordy & Straka, 2022; Nieuwkoop & Faber, 1994). Removals were validated one week post -surgery at stage 46 by visual inspection (Fig. 1b, top, Suppl. fig. 1b) in comparison with unmanipulated controls (Fig. 1b, bottom, Suppl. fig. 1c). To further visualize the observed absence of gross anatomical optic structures, immunohistochemical labelling of cranial nerves (acetylated tubulin, green) and extraocular musculature (myosin VI, red) was performed. On the unmanipulated side in these animals (Fig. 1a, right inset), and in unmanipulated controls (Suppl fig. 1b, c), the extraocular muscles insert themselves in a stereotyped fashion around the eye and are innervated by their respective extraocular motoneurons (Fig. 1a, right inset), consistent with features for *Xenopus laevis* (Branoner & Straka, 2018). In embryonic one-eyed animals however, such an organizational scheme was not observed (Fig. 1a, left inset), given the absence of an eye in this region. Lacking individual eye muscles to innervate, the suspected extraocular motor neurons in the optic-area of these animals, identified based on their exit sites from the skull, failed to innervate in canonical fashion (Suppl fig. 1b, arrowheads). This immunohistochemical data validated the absence of an eye in these embryonically manipulated animals. (Schönenberger et al., 1983). One-eyed

animals were then further reared until stages 52-54 (Nieuwkoop & Faber, 1994), where visuomotor behaviors and central retinotectal circuits are readily profiled (Knorr et al., 2017), in order to anatomically and behaviorally asses the effects of embryonic removal of the optic vesicle. *In-vitro* preparations (Fig. 1a, right, Suppl. fig. 1 d, e) of control and one-eyed animals were generated and subsequently subjected to functional assessment paradigms which have been previously shown to induce optokinetic eye movements (Fig. 1c; (Soupiadou et al., 2020)).

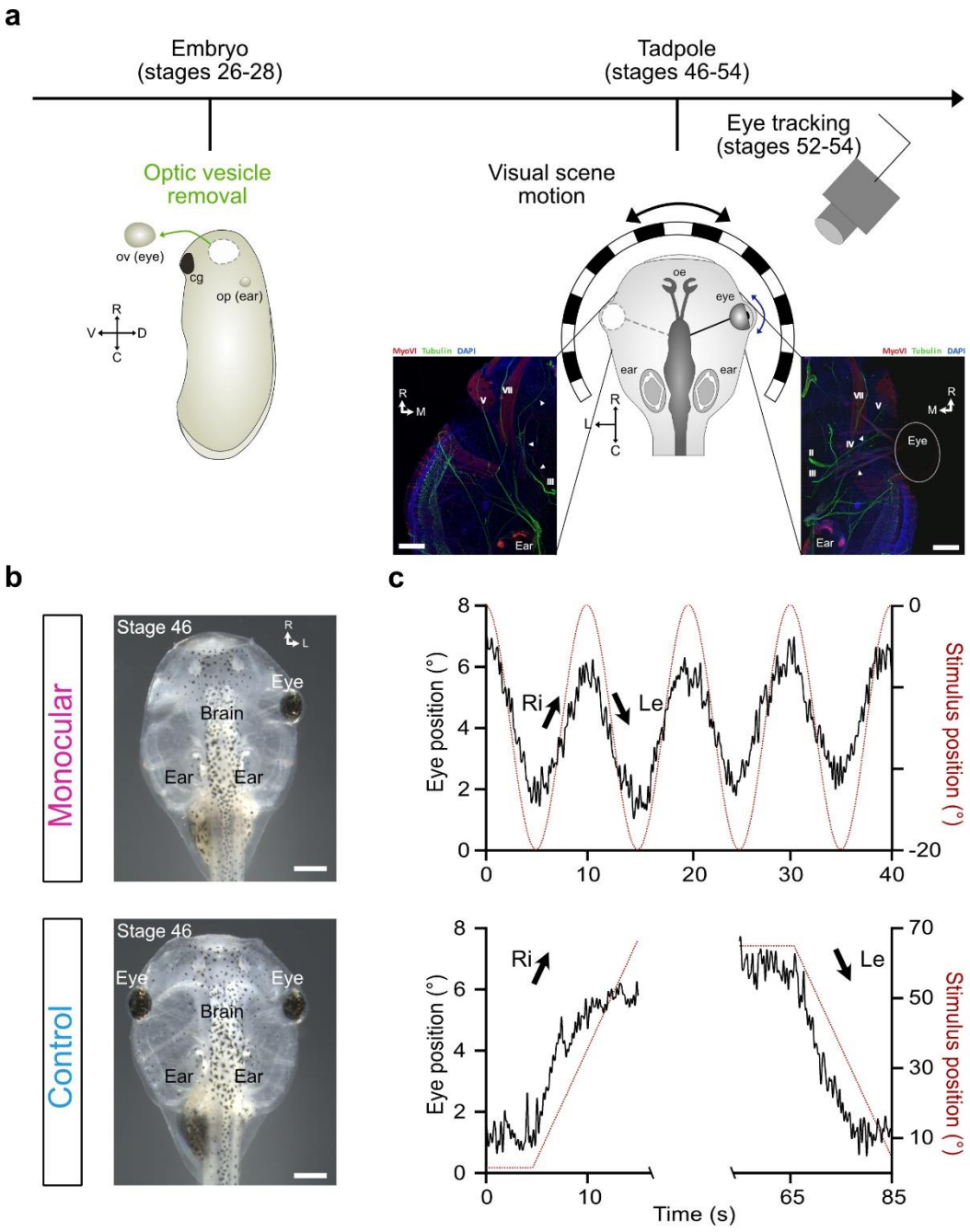


Fig. 1: Methodology of eye removals and behavioral tracking. a) Schematic of the experimental timeline, consisting of embryonic removal of the left optic vesicle (left) and behavioral tracking in a visual stimulation paradigm following maturation (right). Insets show immunohistochemical staining of the eye

region in a one-eyed (left) and a control (right) tadpole at stage 46. Arrows indicate the trajectory of the oculomotor nerve. Scalebars = 200 µm b) Representative images of one-week old one-eyed (top) tadpoles in comparison to controls (bottom). Scalebar = 0.5 mm c) Representative traces of eye motion in response to sinusoidal motion stimulation (top) or constant velocity unidirectional motion (bottom). Eye motion depicted in black, stimulus motion in red.

OKR performance in bidirectional stimulation

In control animals with two bilateral eyes (hereafter, “Binocular”), visuomotor behaviors were profiled following visual stimulation. For this, we presented a sinusoidal horizontally moving black-white striped pattern, which readily induced reflexive eye movements that followed the motion cyclically (Fig. 1a, c, fig. 2a). In binocularly stimulated controls, eye motions were conjugated (Suppl. fig. 2a, b, slope = 0.92 ± 0.01, mean ± SD) and showed a gain of 0.28 ± 0.11 (Fig. 2a, b, left, eye motion amplitude/stimulus motion amplitude) in line with previous studies in this species (Gordy & Straka, 2022). To better understand the individual contributions of each eye to these bilateral visuomotor behaviors, we next wanted to investigate OKR performance following acute loss of visual input on one side. This was induced by selective lesioning of the left optic nerve (hereafter, “Lesioned”) close to the brain as previously described for the statoacoustic nerve in such preparations(Soupiadou et al., 2020), which induced an acute monocular condition. Such a condition allowed us to assess the extent to which mature and entrained visuomotor circuits are affected by sudden loss of input from one side. In lesioned animals, the OKR gain decreased to 0.18 ± 0.05 (Fig. 2a, b, p=0.041, Kruskal-Wallis test with Dunn’s multiple comparison), indicating an impairment after acute unilateral eye loss. Coverage of the left visual field instead of surgical intervention yielded the same effect of a decrease of the OKR gain from 0.34 ± 0.06 to 0.21 ± 0.04 (Fig. 2b, inset, p=0.015, Wilcoxon signed rank test). As impairments from surgical lesions and restrictions of the visual field yielded similar results, the OKR impairment likely originated from visual signal loss rather than detrimental effects of the surgical procedure itself.

With reference values for OKR performance in binocular and left eye lesioned tadpoles, we next investigated whether embryonically one-eyed tadpoles (hereafter, ‘Monocular’) were able to compensate for impairment of the OKR in monocular stimulation conditions. They displayed eye movements in response to sinusoidal stimuli at a gain of 0.22 ± 0.09, which puts them in between binocular and lesioned tadpole, with no significant difference to either group ($p = 0.36$ and $p>0.99$, respectively, Kruskal-Wallis test with Dunn’s multiple comparison). Accordingly, the OKR in these animals was not strongly impaired

anymore during exclusive right eye stimulation, and suggests compensation for the lack of one eye.

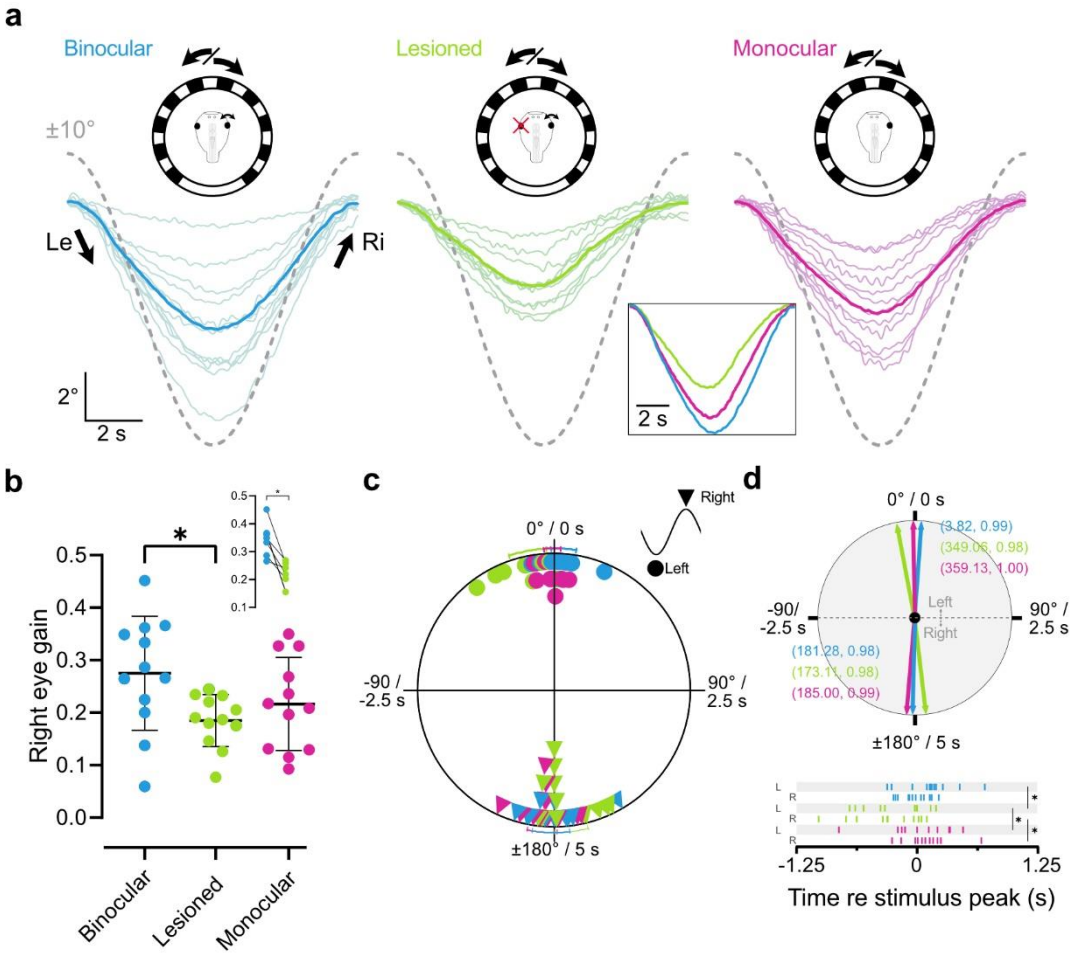


Fig. 2: Responses of the right eye to sinusoidal motion stimulation. a) Eye motion traces (colored lines) of the right eye of binocular, left eye lesioned and monocular tadpoles in response to sinusoidal stimulus motion (dotted lines). Thin lines represent average responses per animal, thick lines in plots and in the inset represent the average response across paradigms. b) Gain values (eye motion amplitude/stimulus amplitude) for the binocular, lesioned and monocular paradigms. c) Individual phase values of the eye following motion respective stimulus motion in degrees and seconds. Top: 0 represents the peak at the leftward to rightward direction change, 180° the reverse. D) Top: Mean vectors (length, strength) of the data plotted in c) bottom: timing values are given in relation to the stimulus motion peak corresponding to the leftward or rightward eye motion peak. Data re-used from panel c. L: left to right peak, R: right to left peak.

Optic nerve lesions also changed the timing of the OKR response peak from a mostly in-phase response for both leftward and rightward motion (Fig. 2c, d, 3.8 ± 8.0°, 1.3° ± 11.7°) to a phase lead in the leftward (Fig 2c, top, d, -11.0 ± 11.8°, p=0.002, Watson-Williams F-test) but not the rightward direction (-7.9±10.8°, p = 0.1, Watson-Williams F-test, Fig. 2c, bottom,

d), and indicate that not just response strength, but also timing of OKR is impaired upon acute loss of one eye. Response latencies of one-eyed animals were clearly improved from lesioned controls in both leftward and rightward direction (Fig. 2d -0.97 ± 5.3°, p = 0.017, 5 ± 9.5°, p = 0.012, Watson-Williams F-test) and were similar to binocular animals (Fig. 2d, p = 0.117, p = 0.103, Watson-Williams F-test).

Overall, sinusoidal OKR stimulation reveals that tadpole OKR requires binocular visual input to maintain strength and timing of the response, and loss of one eye impairs both factors. Tadpoles which only developed with one eye, however, were able to compensate for these impairments.

Direction-specific impairment of OKR

As our manipulation induced an asymmetry on the sensory side of the OK, we next investigated if the impairment also affected the OKR in a direction specific, and therefore asymmetric manner. In many lateral eyed animals, each eye in isolation preferentially responds to motion in the nasal (N) direction (Masseck & Hoffmann, 2009). We therefore tested whether the amplitude impairment observed in sinusoidal stimuation is caused by a reduction of the temporal (T) motion component specifically, i.e. rightward (Ri) movement in case of the right eye. For this, we measured eye velocity in the first two seconds during unidirectional motion stimulation at a constant velocity. In binocular control tadpoles, the right eye responded more strongly to temporal motion at a gain of 0.39 ± 0.12 (eye velocity/stimulus velocity) versus 0.24 ± 0.07 for nasal motion (Fig. 3a, b, p<0.001, two-way ANOVA, Bonferroni's multiple comparison). Therefore, under binocular viewing conditions, the eye responds well in both motion directions with a preference for temporal motion, or temporal asymmetry of the horizontal OKR.

Optic nerve transection of one eye selectively impaired OKR of the remaining eye in the temporal direction. Under such monocular viewing conditions, gain of the right eye in the temporal direction was reduced to 0.10 ± 0.05 and therefore lower than in binocular tadpoles (Fig. 3b, middle, p<0.001, two-way ANOVA with Bonferroni's multiple comparison), while nasal motion was unaffected at a gain of 0.19 ± 0.05. Therefore, under monocular conditions, the remaining eye shows a direction preference for nasal stimulus motion, or a nasal asymmetry, (Fig. 3a middle, p<0.001, two-way ANOVA, Bonferroni's multiple comparison, Fig. 3c, middle, 0.48 ± 0.2°, p<0.001, Kruskal-Wallis test with Dunn's multiple comparison), in line with other lateral-eyed animals (Masseck & Hoffmann, 2009), and confirms that acutely induced asymmetry in the visual system imposes an asymmetry on visuo-motor transformation.

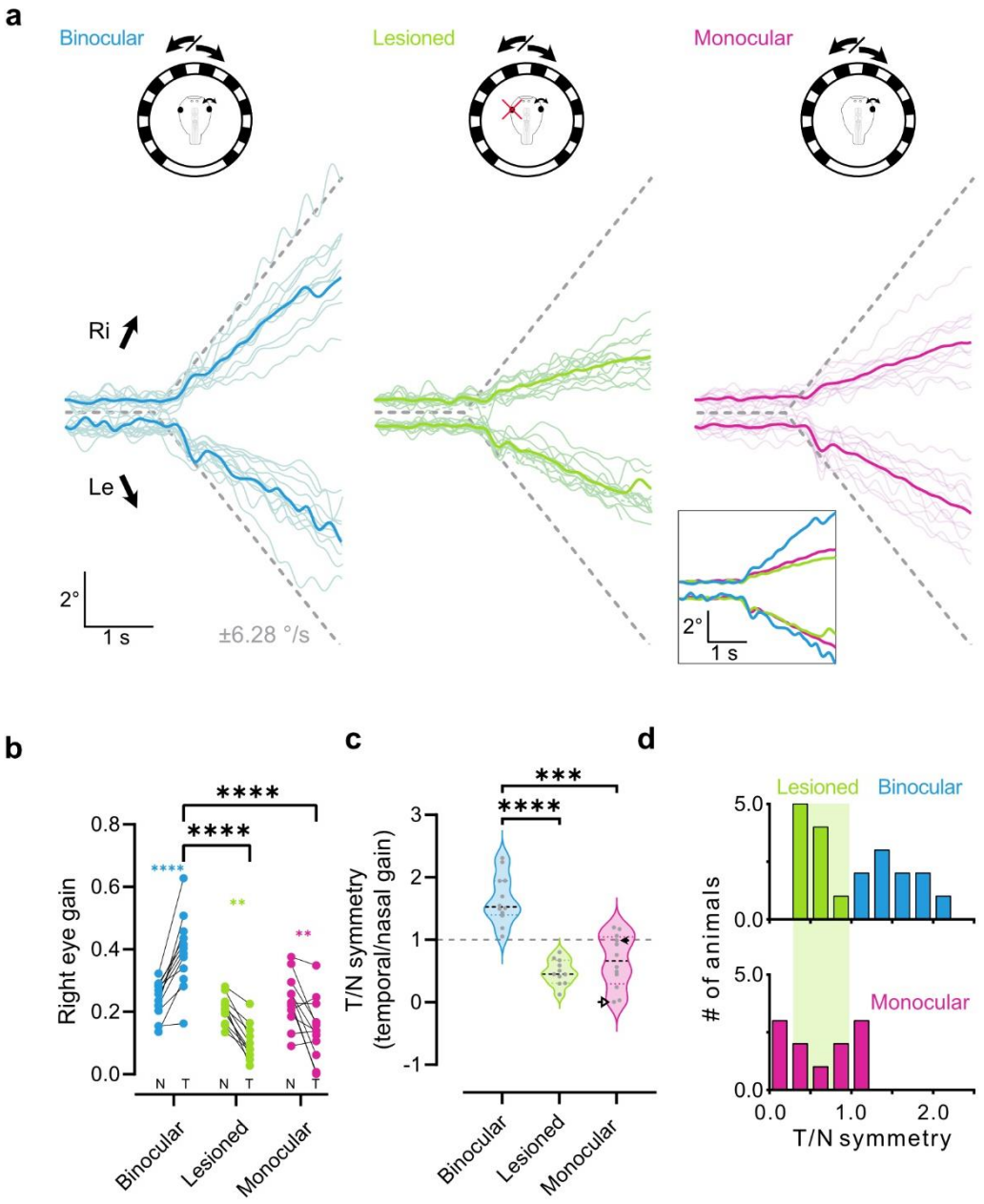


Fig. 3: Responses of the right eye to unidirectional motion stimulation. a) Motion traces (colored lines) of the right eye of Binocular, left eye lesioned and monocular tadpoles in response to unidirectional stimulus motion (dotted lines). Thin lines represent average responses per animal, thick lines in plots and in the inset represent the average response across paradigms. b) Gain values (eye velocity/stimulus velocity) within the first two seconds of motion onset for the three paradigms. Connected dots represent leftward (Le) and rightward (Ri) motion of the same eye. c) Symmetry indices (rightward gain/leftward gain) for the three paradigms. Values >1 indicate stronger motion towards the right, <1 motion towards the left. 1 (grey dotted line) indicates equal motion to the left and the right and a symmetric response. Arrowheads indicate low and high representative datapoints corresponding to anatomical values in Fig. 4e) and comparisons of anatomy and function in Fig. 4f) d) Histograms of the distribution of symmetries in binocular and lesioned (top) versus monocular (bottom) animals. Data taken from panel c). Bin width on the x-axis is 0.25.

Having established a direction-specific motor impairment of unilateral loss of visual input in post-embryonic tadpoles, we next tested whether tadpoles that were reared from early embryo stages with only one eye compensated for such a sensory asymmetry. Despite the compensation shown by monocular tadpoles in sinusoidal conditions, temporal eye motion at a gain of 0.14 ± 0.1 was still impaired compared to binocular animals ($p < 0.001$, Bonferroni's multiple comparisons test), andcomparable to animals with an acute loss of one optic nerve (Fig. 3a, b, right). Motion in the nasal direction was unaffected at 0.24 ± 0.08 ($p = 0.58$, Fig. 2b, right), and as a result, the OKR was asymmetric towards the nasal direction (Fig. 3c, right, 0.65 ± 0.42, $p=0.0007$, Kruskal-Wallis test, Dunn's multiple comparison).

However, within the cohort of monocular tadpoles, left-right asymmetries were more heterogeneous in comparison to lesioned tadpoles. Indeed, the symmetry indices (temporal gain / nasal gain) of monocular tadpoles were not normally distributed (Fig. 3d, Kolmogorov-Smirnow test, $p=0.0038$. Data taken from c.), while both binocular and lesioned tadpoles were. This was likely caused by a subgroup of monocular tadpoles with a an equal leftward and rightward, and therefore symmetric, OKR response (Fig. 3c, grey line, Fig. 3d, bottom). Such behavioral variability following embryonic unilateral sensory deprivation has been previously reported (Gordy and Straka, 2022), and we next investigated whether this functional variability had anatomical correlates following monocular embryonic development.

Binocular motion preference

Following the identification of a directional preference of the right eye even in binocular stimulation, we tested whether this represents a biological effect. First, comparison of eye conjugation of the left and right eye revealed that temporal preference for the right eye (Suppl. fig. 2a, red, slope = 0.69 ± 0.005) was mirrored for the left eye (Fig. 3d, blue, slope = 1.12 ± 0.005). This effect was specific to unidirectional motion, as the overall conjugation during left-right alternating sinusoidal motion (Suppl. fig. 2a, black, slope = 0.92 ± 0.06) was intermediate to the unidirectional paradigms. Further, the leftward and rightward component of sinusoidal stimulation were equally conjugated (Fig. 2c, leftward s = 0.095 ± 0.01, slope = 0.93 ± 0.01). We conclude that in *Xenopus* tadpoles under binocular visual input, each eye preferentially responds to motion in the temporal direction, a preference that is abolished during prolonged, multidirectional motion where eyes move conjugately.

As the conjugation plots suggest, the temporal motion across both eyes is comparable, as is nasal one. To estimate the contribution of the lost, contralateral eye to motion in the leftward direction, we therefore calculated the sum of leftward and rightward gain of the remaining eye of lesioned animals to approximate a contribution of nasal sensitivity of the left eye to temporal motion of the right eye, as would be during whole field visual motion to the

right. The resulting sum was comparable to temporal gain values under binocular stimulation (Suppl. fig. 3c, grey, 0.31 ± 0.08 p<0.08, two-way ANOVA, Bonferroni's multiple comparison. Data taken from Fig. 3b) and reached similar symmetry values (1.64 ± 0.54, p>0.99, Kruskal-Wallis test with Dunn's multiple comparison, Data taken from Fig. 3c). While this requires experimental validation, summation of temporal gain of the ipsilateral eye with the nasal gain of the contralateral eye is therefore theoretically sufficient to explain the temporal preference in binocular motion.

RGC projections

As the OKR in *Xenopus* is mediated by the pretectum (Cochran et al., 1984; Kubo et al., 2014), a direct target of retinofugal fibers, we directly investigated the connectivity of the eye to this area in binocular and monocular tadpoles. Applications of dextran-conjugated dyes into the eyes of *in-vitro* preparations (Fig. 4a, insets) revealed differences in RGC axon pathfinding between one-eyed and control tadpoles. Control tadpoles showed canonical, exclusively contralateral RGC projections, reaching targets in the pretectum optic tectum (Fig. 4a, c) and thalamus (Suppl. fig 2f)(Beazley, 1975; Ruthazer et al., 2003). In monocular tadpoles, a subset of RGC axons did not cross to the contralateral side, and instead innervated ipsilateral thalamic, pretectal and tectal targets (Fig. 4b, d). We quantified the number of labelled processes in the anterior midbrain across the medio-lateral aspect of the brain (Fig. 4c, d) for multiple animals, to approximate the amount of erroneously non-crossing RGCs (Fig. 4e). While in both cases, most projections terminated in the contralateral anterior tectum (Binocular: 98.74 ± 0.4%, Mean ± SD, Monocular: 83 ± 21.6%, Mean ± SD), only monocular animals showed a fraction of RGC projections to the ipsilateral anterior tectum (Fig. 4e, f, 16.82 ± 21.62%, mean ± SD, p = 0.03, two-tailed Mann-Whitney test, Data in 4f, taken from 4e). The number of ipsilateral projections was very variable within the monocular versus the binocular group (Fig. 4e, f). Overall, we found that RGC pathfinding in one-eyed tadpoles is mainly impaired at the optic chiasm, with subsequent pathfinding to the ipsilateral or contralateral pretectum and tectum being largely intact. Further, the extent of chiasmatic pathfinding errors is highly variable between individuals, and reminiscent of the behavioral variability found within monocular tadpoles.

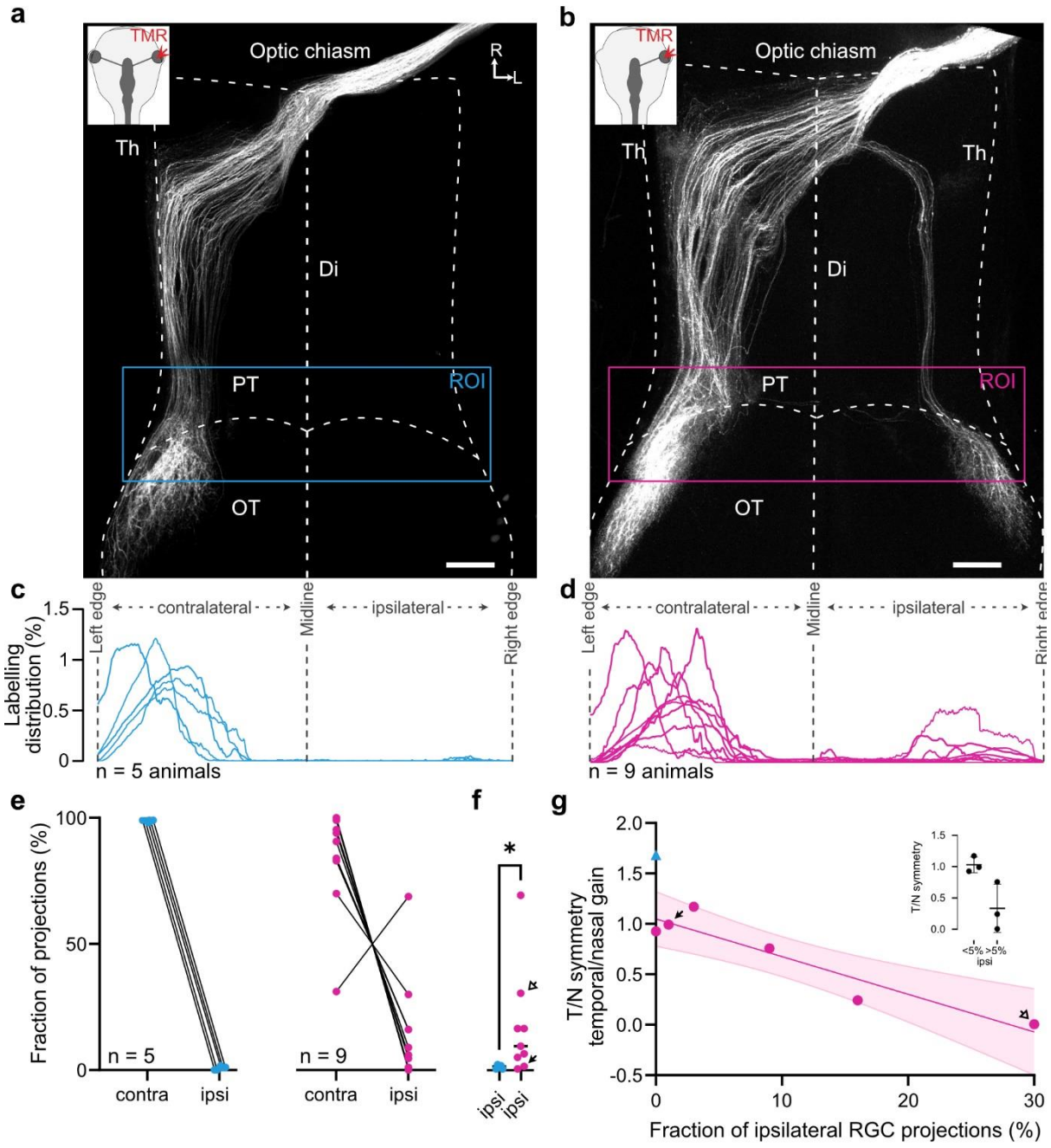


Fig. 4: RGC projections in control and one-eyed tadpoles. Representative confocal z-stacks of cleared whole brains of control (a) and one-eyed (b) tadpoles with RGC axons labelled. White background insets indicate dye application site. Scalebar = 200 µm c), d) Histograms of the amount of above threshold labelling across the medio-lateral axis of the brain at the anterior tectum. e) Cumulative sum of sections of the histograms corresponding to the left (contra) and right (ipsi) tectal hemisphere in control (left) and one-eyed (right) animals, and statistical comparison of the amount of ipsilateral signal (right). Arrows indicate corresponding to two representative datapoints for low and high ipsilateral projection values as found in panel f). f) correlation of the fraction of ipsilateral RGC projections and symmetry of OKR. Negative slope indicates a negative correlation between the amount of ipsilateral RGC projections and OKR symmetry. R2 = 0.884, fit: y = -3.734y + 1.049. Blue triangle represents the

average value of binocular animals. Inset shows comparison of symmetry values between two groups of animals, grouped based on an anatomical threshold. Th Thalamus, PT Pretectum, OT Optic tectum, Di Diencephalon, OT Optic Tectum, R rostral, L lateral, ROI Region Of Interest

We therefore correlated anatomical and behavioural data in animals in which dye tracings following behavioural assessment were successful (n=6), by plotting the fraction of ipsilateral projections *versus* the symmetry of the OKR response. Linear fits through this relation revealed a significant negative correlation (Fig. 4g, red, $r^2 = 0.88$, $p = 0.005$ compared to a slope of 0. Representative high (filled arrow) and low (empty arrow) marked through Figs. 3c, 4f), indicating that an increased amount of erroneous ipsilateral RGC projections had a detrimental effect on functional compensation of the OKR. As nasal motion of these animals is unimpaired (Fig. 3b) and does not correlate with ipsilateral fibers (Suppl. fig. 2e, blue) whereas the nasal direction does (Suppl. fig. 2e, red), it is likely that miswiring-caused impairments are either selective for temporal motion, or can be compensated for in the nasal direction. Accordingly, animals with mostly or exclusively canonical contralateral RGC projections partially compensated for the temporal OKR impairment following loss of one. While this is not a full restoration to the temporally asymmetric of binocular tadpoles, it provides evidence for compensation of the temporal impairment observed in acute left-eye lesioned tadpoles as observed in adult frogs (Jardon & Bonaventure, 1992b).

Overall, our data show that embryonic development of the visual system under monocular conditions causes aberrations in retinotectal pathfinding of the remaining eye. These anatomical aberrations directly correlate with functional impairment of OKR symmetry and an impairment of compensatory plasticity to a symmetric OKR shown by monocular tadpoles with little pathfinding errors.

DISCUSSION

The results of this paper demonstrate the requirement of bilateral eyes for appropriate establishment of contralaterally projecting RGCs in *Xenopus* tadpoles. They further show the detrimental effect of monocularity-derived retino-pretectal pathfinding errors on compensatory plasticity of behavior.

OKR circuit development

Our results reported on the influence of binocularity on complete RGC axon decussation at the optic chiasm during embryonic development of *Xenopus*. While induction of ipsilateral retinotectal fibers has been demonstrated in this species previously, such results

were observed in adult animals and resulted from regenerative, rather than embryonic, axonal growth (Gaze & Straznicky, 1980; Glastonbury & Straznicky, 1978). Due to different molecular environments in embryonic *versus* adult tissue (Jeffery, 2001), this regenerative effect does not necessarily translate to embryonic axonal pathfinding (Gaze & Straznicky, 1980). In fact, studies on embryonic axonal pathfinding in *Xenopus* were thus far unable to show a role of binocular interaction to establish the exclusive contralateral retinofugal projection during embryo development (Taylor & Gaze, 1990). The discrepancy of our data with this study can be explained by two differences in study design: First, extirpations in our study are performed earlier in embryonic development at stages 26-28, as opposed to stage 30 in previous studies. Since RGCs already start differentiating at stage 28 with their axons being detectable in the optic stalk (Cima & Grant, 1980), our removals at earlier stages of optic development may be more effective at preventing induction of an environment at the optic chiasm or in the optic stalk that facilitates contralateral, or prevents ipsilateral guidance. Second, as anatomical data in the earlier study was gathered in adult frogs, it is possible that the ipsilateral fibers we observe in larval stages disappear during metamorphosis. Indeed, a transient, non-canonical ipsilateral RGC innervation in the absence of a contralateral eye has been shown previously in chick embryos (Thanos et al., 1984); however, aberrant projections are lost again during embryonic development in this species, before potential manifestation of behavioral relevance of this pathway. The role of the contralateral eye in RGC pathfinding is further corroborated by studies in mice and ferrets. These studies, however, show an opposite effect to ours, where rather than inducing a non-canonical ipsilateral projection, a canonical ipsilateral projection is reduced (Chan et al., 1999; Taylor & Guillery, 1995). Overall, while the importance of bilateral eyes for facilitation of appropriate decussation at the optic chiasm has been known, our study is the first to show this in *Xenopus*, while also highlighting an interspecies difference in the role of the contralateral eye as an inhibitor, rather than a promoter of ipsilateral RGC guidance.

Our study suggests the existence of mechanisms in chiasmatic pathfinding that remain thus far unidentified. Molecularly, ephrin B is implicated in the formation of ipsilaterally crossing fibers in this species (Nakagawa et al., 2000). In fact, adult *Xenopus* display ipsilaterally projecting RGC axons, which are established during metamorphosis and can be induced pre-metamorphically in the presence of ephrin B (Hoskins & Grobstein, 1985; Nakagawa et al., 2000). However, these axons target the thalamus rather than the tectum, and our results suggest that other mechanisms are relevant for axonal crossing in this species, either of a chemical or mechanical nature (Koser et al., 2016; Murcia-Belmonte & Erskine, 2019). Even though we cannot verify whether individual fibers target the ipsilateral equivalent of their

canonical contralateral target area, overall pathfinding to pretectal and tectal areas appears intact (Dingwell et al., 2000).

Directionality in Xenopus OKR

Anatomical deviations from canonical, all-contralateral RGC fibers are negatively correlated with functional compensation for monocularity in the OKR. In our tadpoles, compensation manifests as an improvement of the nasal motion direction in some embryonically one-eyed tadpoles. Curiously, we also observe an asymmetry under binocular conditions in the temporal direction. In Zebrafish, such asymmetries are associated with higher spatial frequency of stimulus patterns than employed in our study, suggesting a different spatial frequency preference in *Xenopus (Qian et al., 2005)*. The stronger temporal component could originate from a summation of the small, temporal component of the respective eye with the stronger, nasal component of the contralateral eye during initial motion detection, based on the symmetry and gain values we obtain by summing the temporal and nasal gain under monocular stimulation (Suppl. fig. 2c, d, grey dots). This may be mediated via intratectal and pretectal commissural connections, as in Zebrafish (Portugues et al., 2014).

Preference for nasal motion in monocular stimulation is expected and fits data from other species (Masseck & Hoffmann, 2009; Naumann et al., 2016; Portugues et al., 2014). In our *Xenopus* tadpoles, acute loss of one eye leads to an impaired OKR in the remaining eye as expected. When such acute condition responses were compared to embryonically generated one-eyed animals, we found that behavioral compensation was possible, but correlated with retinotectal connectivity phenotypes. Behavioral compensation for loss of one eye was observed predominantly in animals with near-canonical, contralateral retinofugal pathways, and less in animals with ipsilateral retino-pretectal fibers. Compensation, however, does not restore OKR back to binocular levels with a temporal motion preference, but rather increases temporal motion to be equivalent with the nasal component, to facilitate symmetric OKR responses. Functionally, this could have two possible reasons: 1) Reaching maximum temporal motion requires bilateral eyes, and plasticity mechanisms are insufficient to compensate for the loss of the entire visual input from one. 2) While each eye shows asymmetric OKR for unidirectional motion in binocular animals, the left and right eye cooperate to ensure an equal leftward and rightward response across the entire visual system, comprised of bilateral, complementary eyes. In monocular animals with control-like projections of the remaining eye, the single eye represents the entire visual system, and the only way to facilitate a symmetric OKR response is a symmetric response of this singular eye. Therefore, symmetry of the overall system, rather than a maximized motor response, may be desirable. Multiple

studies back up the second hypothesis. First, embryonically generated one-eared animals compensate in a similar fashion in vestibular processing: At the cost of the normally preferred ipsiversive direction, the normally weak contraversive motion detection is increased to facilitate bidirectional motion sensing (Gordy & Straka, 2022). Second, multiple studies suggest that balance and homeostasis are common themes in tadpole plasticity, rather than full compensation to achieve a maximum motor response. In both VOR and OKR, training stimuli induce motor output homeostasis rather than stronger compensation (Dietrich & Straka, 2016; Forsthofer & Straka, 2022). Theoretical studies corroborate this finding, and suggest that consistency may be preferred, and full compensation may not be necessary to minimize retinal image slip under naturalistic conditions (Glasauer & Straka, 2022).

Role of ipsilateral RGC fibers

As only animals with no major ipsilateral projection show OKR compensation, we conclude that, while these connections are likely functional, they impair function rather than contributing to compensation, providing a possible alternative explanation for the impairment for the normally preferred motion direction in the vestibular system of embryonically one-eared tadpoles (Gordy & Straka, 2022). The detrimental effect of ipsilateral retinotectal connections may stem from functional miswiring, as RGCs selective for one motion direction wire to brain areas geared to process motion in the opposite direction. In *Xenopus* experiments placing a supernumerary eye or ear onto the head or even the tail, sensory afferents wire appropriately to their sensory nuclei, in large part due to genetic factors (Blackiston & Levin, 2013; Constantine-Paton & Law, 1978; Elliott & Fritzsch, 2018). Asides from erroneous crossing, the ipsi- or contralateral canonical pretectal and tectal targets of RGCs are correctly innervated, and our study conforms with the notion that retinotectal and pretectal wiring is based majorly on genetic and molecular factors (Fraser & Hunt, 1980; Mumm et al., 2006; Nevin et al., 2010; Sperry, 1956). Within target nuclei, in addition to genetic factors, activity of axons plays a major role to establish map formation and appropriate connectivity to postsynaptic neurons (Katz & Shatz, 1996). In experiments in zebrafish and *Xenopus,* ipsilateral connections from the eye to the tectum were induced through ablation of one tectal hemisphere. In both cases, these rerouted neurons targeted their appropriate topographical targets within the tectum, and in case of zebrafish, even wired to the same directionally selective neurons as their ipsilateral counterparts along the rostro-caudal axis (Ramdya & Engert, 2008; Ruthazer et al., 2003). In absence of an appropriately wired eye like in the present study, such direction appropriate wiring is likely to be detrimental: temporally selective RGCs of the right eye respond to rightward stimulus motion. Appropriately wired fibers to contralateral temporally selective pretectal neurons in the left pretectum will then initiate a rightward OKR (Kubo et al., 2014; Wu et al., 2020). However, if contacted, ipsilateral pretectal cells in the right pretectum initiate

a leftward OKR in response to temporal stimulation, which would normally come from a leftward motion detected by the left eye. These competing signals may impair restoration of symmetric OKR. Multiple studies show similar behavioral effects of erroneous RGC projections in zebrafish mutants, where completely inversed retinotectal projections lead to an inversion of OKR (Huang et al., 2006; Karlstrom et al., 1996). Likewise, inversely connecting the left and the right eye with their ipsilateral tectum in adult frogs through surgery achieves inversion of the OKR (Sperry, 1945) in adult frogs. It is hypothesized that temporal motion is conveyed by pretectal commissures (Burgess et al., 2009; Portugues et al., 2014) while nasal motion stems from direct motor neuron activation (Cochran et al., 1984). Behavioral impairments following retino-pretectal miswiring in our study therefore may go hand in hand with errors in commissural signalling, or wrongly crossing fibers originating predominantly from temporal direction selective RGCs.

As embryonic changes in connectivity appear to be detrimental, plasticity is therefore likely mediated by canonical adult, rather than embryonic mechanisms. In adult mice for example, functional plasticity in response to impairments in direction perception is following monocular deprivation, (Bauer et al., 2021; Prusky et al., 2006), as it is in frog (Jardon & Bonaventure, 1992b). In the latter case, evidence suggests that the pretectum itself plays a role in maintaining directional symmetry(Jardon & Bonaventure, 1992b). Another likely candidate in *Xenopus* is the cerebellum, which is in general associated with OKR plasticity (Boyden et al., 2004). Specifically the gain up-regulation required for the observed compensation in the temporal direction is linked to the cerebellum in *Xenopus*, which is close to functionally mature at our employed developmental stages (Forsthofer & Straka, 2022). Coupling of embryonic eye removals with developmentally early inactivation of the cerebellum could help identify the cerebellum either as a mediator or initiation site of such plasticity. Other sites are equally probable, particularly the hindbrain vestibular nuclei, which receive visual motion information and aid in consolidation of cerebellar-aided gaze stabilizing plasticity, or the pretectum, where changes in neurotransmitter concentration facilitate or impair OKR symmetry (Jardon & Bonaventure, 1997; Van Alphen & De Zeeuw, 2002; Yücel et al., 1990), and in the latter case, visuo-vestibular mismatch studies could help elucidate vestibular mediated plasticity.

Overall, this study highlights two main aspects relating to plastic compensation of the OKR: In Xenopus, bilateral eyes are essential to reliably develop contralateral retinofugal fibers as previously shown in other species. Moreover, aberrant ipsilateral retino-pretectal connections do not appropriately mediate behavior in absence of another eye. Rather, they are associated with impairments of specifically temporal eye motion, and embryonic pathfinding changes put a limit on optokinetic plasticity.

MATERIAL AND METHODS

Animals

Experiments were conducted on wildtype *Xenopus laevis* embryos and tadpoles obtained from the animal breeding facility of the Biomedical Center at the Ludwig-Maximilians-University (LMU) Munich. Animals at early embryonic stages (stage 4-16) had their jelly coat removed with 2% cysteine, and were incubated in 0.1x Marc's modified Ringer solution (MMR, diluted from a 10x stock solution: 1 M NaCl, 18 mM KCl, 20 mM $CaCl_2$, 10 mM $MgCl_2$, 150 mM HEPES, pH 7.6–7.8) at 17°C under a 12/12 dark/light cycle regime. Animals were then reared until stage 46 either as control group, or with prior performance of embryonic optic vesicle extirpations at stage 26-28 (described below). At stage 46, animals were screened for successful removal of one eye, and either euthanized with 3-aminobenzoic acid ethyl ester methanesulfonate (MS-222; Parmaq Ltd. UK) and fixed in 4% paraformaldehyde (PFA) for immunohistochemistry, or were transferred into de-chlorinated water and further reared in standard tanks at 17-19°C at the animal facility of the Biocenter of the LMU Munich. Animals were kept on a 12/12 dark/light cycle, fed daily with powdered Spirulina (Algova, Germany) suspended in tank water, with a daily 50% water exchange. At stages 53-54, behavioral and anatomical experiments (described below) were performed on the animals in accordance with the "Principles of animal care" publication No. 86–23, revised 1985, of the National Institutes of Health and in accordance with the ARRIVE guidelines and regulations. Permission for the experiments was granted by the legally responsible governmental body of Upper Bavaria (Regierung von Oberbayern) under the license codes ROB-55.2.2532.Vet_03-17-24, ROB-55.2.2532.Vet_02-19-146 and ROB-55.2.2532.Vet_02-22-54. All experiments were performed in accordance with the relevant guidelines and regulations of the LMU Munich.

Optic vesicle extirpation

Extirpation of the optic vesicle on the left side was performed at developmental Stages 26-28 (Fig. 1a) with tungsten needles (0.125 mm, Fine Science Tools, 10130-05). Embryos were transferred into 1x MMR at 22°C and anesthetized with 0.02% Benzocaine (Sigma-Aldrich, E1501; see Gordy and Straka, 2022) prior to the surgical manipulations. The optic vesicle was visually identified, the overlaying ectoderm layer removed and the optic vesicle excised along with the underlying optic stalk. Following this manipulation, animals were left in 1x MMR to promote healing of the excision site and subsequently returned to 0.1x MMR for further rearing until stage 46. At this stage, animals were anesthetized in 0.02% benzocaine for visual inspection and sorted for successful eye removals under a stereo microscope (SteREO Discovery.V20, Axiocam 305 color camera, Carl Zeiss Microscopy GmbH). Successfully extirpated, one-eyed tadpoles were then transferred to the animal facility for

rearing until stage 53-54, when eye motion recordings were executed. Similar removals of sensory organs have been performed successfully, with no further detrimental effect on development (Blackiston & Levin, 2013; Gordy & Straka, 2022; Gordy et al., 2018).

Immunohistochemistry

After sorting of phenotypes at stage 46, prototypic one-eyed tadpoles were prepared for the immunohistochemical analysis of nerve and muscle tissue around the site of the extirpated eye for further verification of a successful removal (Fig. 1B). To this end, animals were deeply anesthetized in 0.05% MS-222 and immersion-fixed in 4% PFA in 0.1x phosphate buffered saline (PBS, 0.3 mM Na_3PO_4, 15 mM NaCl, 0.105 mM K_3PO_4) for 3-6 hours at 4°C. Tadpoles were then transferred into 0.1x PBS, and lower jaws, viscera, tail, dorsal skull cartilage and brain were removed. The residual tissue was then dehydrated in 70% ethanol for 3-12 hours at 36°C, washed 3x in 0.1x PBS, and blocked in 5% normal goat serum in 0.1x PBS with 0.1% Triton X100 at 22-24°C for one hour. Incubation with the primary antibodies against muscle (MyosinVI, 1:400, Proteus Biosciences, 25-6791) and neuronal (acetylated tubulin, 1:800, Sigma-Aldrich, T7451) tissue was performed for 14 hours at 22-24°C. Subsequently, washing and blocking steps were performed as described above, followed by an incubation in secondary fluorescent antibodies (1:500, Alexa Goat anti-Mouse IgG2b, A-21141, Alexa Goat anti-Rabbit IgG, A32733) and DAPI (Thermo Fisher Scientific; 62248, 1:500) in 0.1x PBS with 0.1% Triton X100 for 1 hour at 22-24°C. Tissue was then washed 6 x for 15 minutes each in 0.1x PBS, mounted on microscope slides with spacers on each side, and coverslipped with Aqua Polymount (PolyScience, 18606). Imaging of the tissue was performed on a Leica SP5-2 confocal microscope (center for advanced light microscopy (CALM)).

In vitro preparations

Activation and tracking of eye movements was performed in semi-intact *in vitro* preparations of either control or one-eyed tadpoles at stage 53-54. The generation of such isolated preparations occurred as described previously (Forsthofer and Straka, 2022; Özugur et al., 2022). Animals were deeply anesthetized in 0.05 % MS-222 at 22-24°C for 2-5 minutes and were then transferred into ice-cold Ringer solution (75 mM NaCl, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 2 mM KCl, 0.1 mM $MgCl_2$, and 11 mM glucose, pH 7.4). Following decapitation, the lower jaws, viscera and skin above the skull were removed, the skull opened and the choroid plexus above the IV^{th} ventricle was taken off. Preparations were then allowed to recover in 200 ml Ringer solution for ~2 hours at 17°C before commencing with the eye motion recording session (Gordy and Straka, 2022). Unilateral optic nerve transections were performed directly

before behavioral assessments. The optic nerve was located from dorsally through the opened skull, and cut proximally to its exit point at the diencephalon with scissors under visual control.

Visual motion (optokinetic) stimulation

Optokinetic stimuli consisted of horizontally moving vertical black and white bars (subtending 16° of visual angle each), projected with three orthogonally oriented digital light processing video projectors (Aiptek V60) onto a cylindrical screen covering 275° of the horizontal visual field of the tadpole. Stimulus motion consisted of either sinusoidal left-right oscillations or unidirectional constant velocity stimulus motion either to the left or to the right. Sinusoidal stimuli were presented at different frequencies (0.1 Hz, 0.2 Hz, 0.5 Hz; ±10° magnitude), presented in 2 trials of 15 cycles each, with a 30 second inter-trial interval. Constant velocity visual stimuli consisted of a 30 second stimulus motion at a velocity of 6.25°/s, with 30 seconds in either direction, with alternating starts to the left or to the right, with a stationary pattern for 30 seconds between the two motion directions. Stimulus motion was recorded and synchronized with the Spike2 software and a CED 1401 A/D interface (Cambridge Electronic Design, UK) at 50 Hz.

Eye motion tracking

Preparations were recorded from above with a digital CCD camera (Grasshopper Mono, Point Grey Research Inc., Canada) equipped with a high-pass filtered lens and a zoom objective (Optem Zoom 70XL, Qioptiq Photonics GmbH & Co. KG, Germany) using infrared illumination of the preparation. Eye movements were tracked in real-time with a custom-written program (Gravot et al., 2017, Knorr et al., 2021), measured as deflection angle between the vertical image axis and the long axis of an ellipse fitted around the two eyes which were detected by a manually adjusted darkness threshold. Eye positions were recorded in Spike2 at 30 Hz (Fig. 1c). In both control and one-eyed tadpoles, only data from the right eye was used for analysis.

Retinal ganglion cell projections

Retinal ganglion cell (RGC) projections into the brain of control and one-eyed animals were fluorescently labelled following completion of the eye motion recording protocol with Tetramethylrhodamine, conjugated to 3000 MW Dextran (Invitrogen, D3308). The fluorescent dye was dissolved in ddH_2O before crystallization onto 0.1 mm *minutiae* pins. *In vitro* preparations were mounted right lateral-side up, the cornea was incised and the lens removed. Following a transient removal of the Ringer solution and insertion of a needle with crystallized dye into the eye cup, the cornea was resealed with superglue (Uhu, Germany). Thereafter*, in vitro* preparations were incubated for 14-24 hours at 14°C and, following visual assessment

of labelling quality and extent, immersion-fixed in 4% PFA at 4°C for 24 hours. Subsequently, the brain was extracted, DAPI-stained for 2 hours (1:500) and cleared with the uDISCO-Protocol (Pan et al., 2016), with 2 hours per butanol step and clearing in BABB D-15. Cleared brains were mounted using custom built metal-spacers, sealed with Roti Histokitt II (Carl Roth, T160.1), and imaged on a Leica SP5-2 confocal microscope at an optical section spacing of 2.4 µm.

Data analysis

Eye motion tracking: Eye movement data obtained in spike2 was processed as previously described (Forsthofer and Straka, 2022; Gordy and Straka, 2022). Sinusoidal and unidirectional eye and stimulus motion data was converted into the matlab (Mathworks, USA) file format, resampled at 200 Hz and filtered with a 4 Hz low-pass filter. Sinusoidal eye movements were segmented into individual cycles, sorted for cycles that exclusively contained slow-following eye movements, and averaged. The amplitude and phase of the cyclic responses were measured based on magnitude and temporal occurrence of the response peaks, relative to the stimulus cycle. Eye movements evoked by constant velocity motion stimuli were similarly cut into individual episodes for each direction. The initial 0.75 seconds of each stimulus episode were discarded due to the response latency of motion onset. The following 2 seconds of each stimulus episode was then screened manually for resetting fast-phases. If fast-phases occurred prior to 1.5 second after stimulus onset, the episode was discarded; if fast-phases occurred after 1.5 second following stimulus onset, eye movements were included until fast-phase onset. Eye motion velocity was calculated as the mean derivative of the eye position and averaged across either leftward or rightward episodes per animal. In addition, an average leftward and rightward position trace was generated per animal for presentation purposes.

Neuronal tracing: Image stacks acquired from cleared brains were loaded into FiJi as 8-bit images (Schindelin et al., 2012) and thresholded at an intensity of 3-4 depending on background fluorescence. Subsequently, a region-of-interest (ROI) was manually selected at the rostral boundary of the optic tectum across the whole medio-lateral extent of the brain and an intensity profile was generated for the ROI in each image of the stack. The ROI was limited to the caudal diencephalon and the rostral midbrain to measure intensity specifically in the approximate area of the pretectum. Intensity profiles were then summed up across the z-stack, normalized, and plotted, to generate a plot showcasing the amount of above-threshold labelled neuronal fibers within the ROI across the entire medio-lateral and dorso-ventral aspect of the brain. These plots were then split into left and right hemispherical halves, and the integral of each plot was calculated, approximating the total amount of fibers in the right and left (ipsi-

and contralateral with respect to the right eye, respectively) rostral tectal region. The calculations served as proxy for the fraction of RGC axons projecting into the ipsi- and contralateral pretectum and optic tectum, respectively.

Statistical analysis

Statistical comparisons between independent (one-eyed animals, control animals, left optic nerve-transected animals) for sinusoidal data were performed with a Kruskal-Wallis test for unpaired non-parametric data, followed by a Dunn's multiple comparisons test to find group differences in Prism 9 (GraphPad Software Inc, USA). Statistical comparisons between and within animals (leftward/rightward eye movements for different groups) were tested with a 2-way ANOVA followed by a Bonferroni multiple comparisons test for group differences. The ROUT outlier test in Prism (maximum FDR = 0.1%) was performed to identify outliers in all plots, whith 1 data point removed from all plots in Figure 3 due to being an outlier in T/N asymmetry at a value of 2.36. To calculate motion direction preference for the left and right eye in control animals, preference of rightward/leftward eye movements for either unidirectional or oscillating eye movements, were calculated as the slope obtained by fitting the eye positions of both eyes plotted against each other, with the r^2 indicating the quality of the fit (Python 3.7). Dots that are connected to each other with lines indicate paired data. Circular statistics were performed in Oriana (Version 4.02; Kovach Computing Services, see (Gordy & Straka, 2022). A mean vector was calculated from phase values yielding the mean direction as well as the strength of the vector as an indication of data clustering (scale 0-1). Differences in physe values were identified with a Watson-Williams-F test.

Acknowledgements:

The authors dedicate this paper to the late Prof. Dr. Hans Straka, a valued friend, colleague, and mentor. The authors would also like to thank Prof. Dr. Ruben Portugues for support in finalizing and submitting the manuscript. The authors would like to thank Parthena Schneider-Soupiadis and Dr. Francois Lambert for critical proofreading, and the Rupp lab and Hörmanseder lab for supply of *Xenopus* embryos. We further thank PD Dr. Steffen Dietzel and the center for advanced light microscopy at the LMU for support and access to microscopes.

Author contributions:

HS and CG conceptualized, and HS, CG and MF designed experiments for the study. CG, MF and MK performed embryonic extirpations and quantified successful removals. CG and MK performed immunohistochemistry. MF and CG acquired behavioral data, and MF performed

and imaged dye tracings. MF and CG established analysis scripts, and MF analyzed data. MF wrote the manuscript and created figures, and CG, MF and HS edited the manuscript.

Disclosure:

The authors declare that they have no competing interests.

References

Bauer, J., Weiler, S., Fernholz, M. H., Laubender, D., Scheuss, V., Hübener, M., Bonhoeffer, T., & Rose, T. (2021). Limited functional convergence of eye-specific inputs in the retinogeniculate pathway of the mouse. *Neuron*, *109*(15), 2457-2468. e2412.

Beazley, L. (1975). Factors determining decussation at the optic chiasma by developing retinotectal fibres in Xenopus. *Experimental Brain Research*, *23*, 491-504.

Blackiston, D. J., & Levin, M. (2013). Ectopic eyes outside the head in Xenopus tadpoles provide sensory data for light-mediated learning. *Journal of Experimental Biology*, *216*(6), 1031-1040.

Boyden, E. S., Katoh, A., & Raymond, J. L. (2004). Cerebellum-dependent learning: the role of multiple plasticity mechanisms. *Annu. Rev. Neurosci.*, *27*, 581-609.

Branoner, F., & Straka, H. (2018). Semicircular canal influences on the developmental tuning of the translational vestibulo-ocular reflex. *Frontiers in Neurology*, *9*, 404.

Burgess, H. A., Johnson, S. L., & Granato, M. (2009). Unidirectional startle responses and disrupted left–right co-ordination of motor behaviors in robo3 mutant zebrafish. *Genes, Brain and Behavior*, *8*(5), 500-511.

Chan, S., & Guillery, R. (1993). Developmental changes produced in the retinofugal pathways of rats and ferrets by early monocular enucleations: the effects of age and the differences between normal and albino animals. *Journal of Neuroscience*, *13*(12), 5277-5293.

Chan, S. O., Chung, K., & Taylor, J. (1999). The effects of early prenatal monocular enucleation on the routing of uncrossed retinofugal axons and the cellular environment at the chiasm of mouse embryos. *European Journal of Neuroscience*, *11*(9), 3225-3235.

Cima, C., & Grant, P. (1980). Ontogeny of the retina and optic nerve of Xenopus laevis: IV. Ultrastructural evidence of early ganglion cell differentiation. *Developmental Biology*, *76*(1), 229-237.

Cline, H. T. (1991). Activity-dependent plasticity in the visual systems of frogs and fish. *Trends in Neurosciences*, *14*(3), 104-111.

Cochran, S., Dieringer, N., & Precht, W. (1984). Basic optokinetic-ocular reflex pathways in the frog. *Journal of Neuroscience*, *4*(1), 43-57.

Constantine-Paton, M., & Law, M. I. (1978). Eye-specific termination bands in tecta of three-eyed frogs. *Science*, *202*(4368), 639-641.

Dews, P., & Wiesel, T. (1970). Consequences of monocular deprivation on visual behaviour in kittens. *The Journal of Physiology*, *206*(2), 437-455.

Dietrich, H., & Straka, H. (2016). Prolonged vestibular stimulation induces homeostatic plasticity of the vestibulo-ocular reflex in larval Xenopus laevis. *European Journal of Neuroscience*, *44*(1), 1787-1796.

Dingwell, K. S., Holt, C. E., & Harris, W. A. (2000). The multiple decisions made by growth cones of RGCs as they navigate from the retina to the tectum in Xenopus embryos. *Journal of Neurobiology*, *44*(2), 246-259.

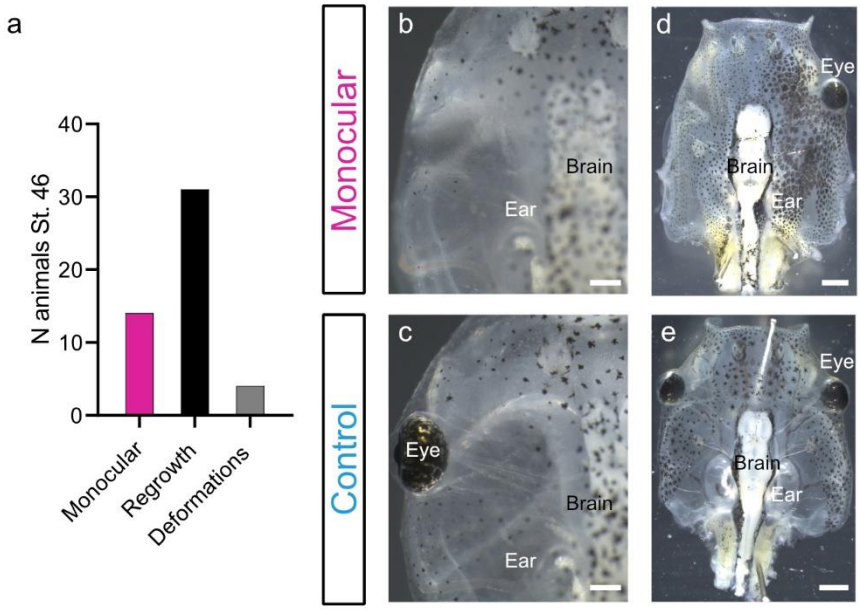
Elliott, K. L., & Fritzsch, B. (2018). Ear transplantations reveal conservation of inner ear afferent pathfinding cues. *Scientific reports*, *8*(1), 13819.

Forsthofer, M., & Straka, H. (2022). Homeostatic plasticity of eye movement performance in Xenopus tadpoles following prolonged visual image motion stimulation. *Journal of Neurology*, *270*(1), 57-70.

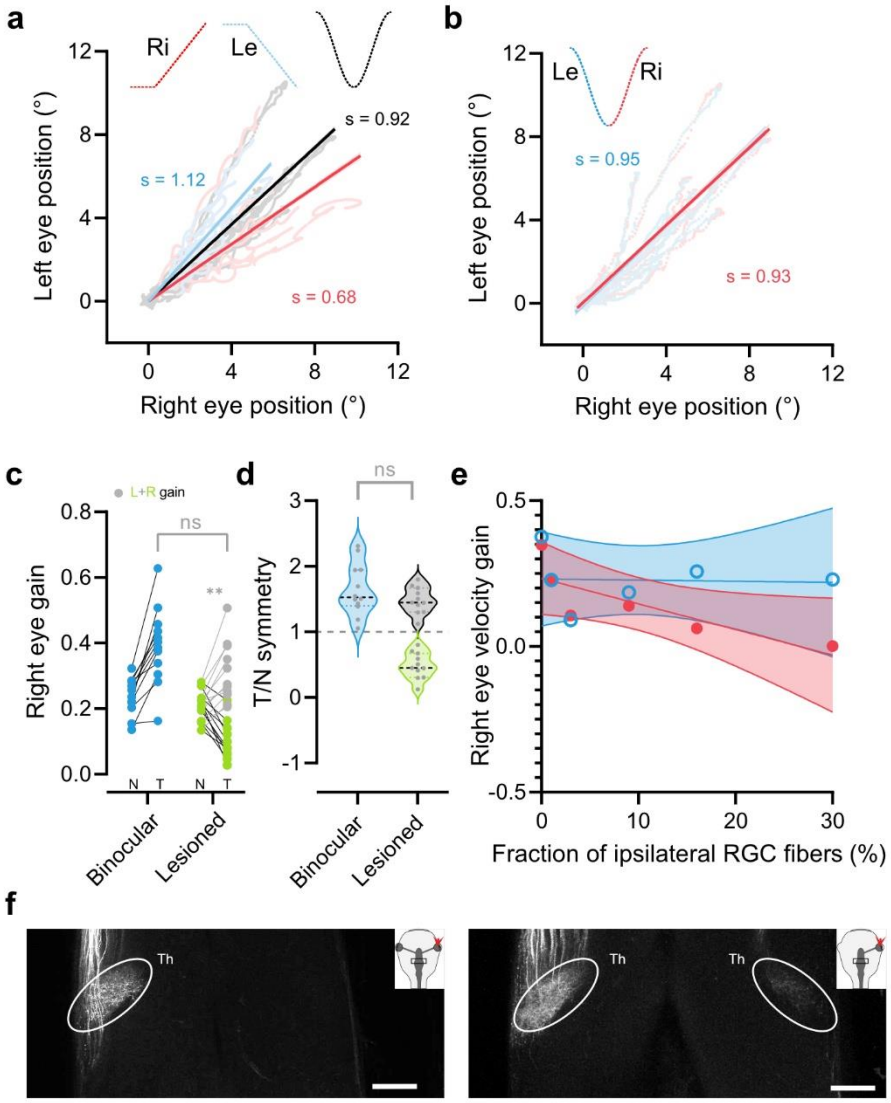
Fraser, S. E., & Hunt, R. K. (1980). Retinotectal specificity: models and experiments in search of a mapping function. *Annual review of neuroscience*, *3*(1), 319-352.
Gambrill, A. C., Faulkner, R. L., & Cline, H. T. (2018). Direct intertectal inputs are an integral component of the bilateral sensorimotor circuit for behavior in Xenopus tadpoles. *Journal of Neurophysiology*, *119*(5), 1947-1961.
Gaze, R., & Straznicky, C. (1980). Regeneration of optic nerve fibres from a compound eye to both tecta in Xenopus: evidence relating to the state of specification of the eye and the tectum.
Gebhardt, C., Auer, T. O., Henriques, P. M., Rajan, G., Duroure, K., Bianco, I. H., & Del Bene, F. (2019). An interhemispheric neural circuit allowing binocular integration in the optic tectum. *Nature communications*, *10*(1), 1-12.
Glasauer, S., & Straka, H. (2022). Low gain values of the vestibulo-ocular reflex can optimize retinal image slip. *Frontiers in Neurology*, *13*.
Glastonbury, J., & Straznicky, K. (1978). Aberrant ipsilateral retinotectal projection following optic nerve section in Xenopus. *Neuroscience letters*, *7*(1), 67-72.
Gordy, C., & Straka, H. (2022). Developmental eye motion plasticity after unilateral embryonic ear removal in Xenopus laevis. *Iscience*, *25*(10), 105165.
Gordy, C., Straka, H., Houston, D. W., Fritzsch, B., & Elliott, K. L. (2018). Transplantation of ears provides insights into inner ear afferent pathfinding properties. *Developmental neurobiology*, *78*(11), 1064-1080.
Grant, S., & Keating, M. (1989). Changing patterns of binocular visual connections in the intertectal system during development of the frog, Xenopus laevis. *Experimental Brain Research*, *75*(1), 99-116.
Holt, C. E. (1989). A single-cell analysis of early retinal ganglion cell differentiation in Xenopus: from soma to axon tip. *Journal of Neuroscience*, *9*(9), 3123-3145.
Hoskins, S., & Grobstein, P. (1985). Development of the ipsilateral retinothalamic projection in the frog Xenopus laevis. II. Ingrowth of optic nerve fibers and production of ipsilaterally projecting retinal ganglion cells. *Journal of Neuroscience*, *5*(4), 920-929.
Hua, J. Y., Smear, M. C., Baier, H., & Smith, S. J. (2005). Regulation of axon growth in vivo by activity-based competition. *Nature*, *434*(7036), 1022-1026.
Huang, Y.-Y., Rinner, O., Hedinger, P., Liu, S.-C., & Neuhauss, S. C. (2006). Oculomotor instabilities in zebrafish mutant belladonna: a behavioral model for congenital nystagmus caused by axonal misrouting. *Journal of Neuroscience*, *26*(39), 9873-9880.
Jardon, B., & Bonaventure, N. (1992a). Plasticity of the frog monocular OKN: involvement of pretectal GABAergic and cholinergic systems. *Neuroscience letters*, *137*(2), 189-192.
Jardon, B., & Bonaventure, N. (1992b). The pretectal cholinergic system is involved through two opposite ways in frog monocular OKN asymmetry. *Experimental Brain Research*, *90*, 72-78.
Jardon, B. L., & Bonaventure, N. (1997). Involvement of NMDA in a plasticity phenomenon observed in the adult frog monocular optokinetic nystagmus. *Vision research*, *37*(11), 1511-1524.
Jeffery, G. (2001). Architecture of the optic chiasm and the mechanisms that sculpt its development. *Physiological Reviews*.
Jeffery, G., & Erskine, L. (2005). Variations in the architecture and development of the vertebrate optic chiasm. *Progress in retinal and eye research*, *24*(6), 721-753.
Karlstrom, R. O., Trowe, T., Klostermann, S., Baier, H., Brand, M., Crawford, A. D., Grunewald, B., Haffter, P., Hoffmann, H., & Meyer, S. U. (1996). Zebrafish mutations affecting retinotectal axon pathfinding. *Development*, *123*(1), 427-438.
Katz, L. C., & Shatz, C. J. (1996). Synaptic activity and the construction of cortical circuits. *Science*, *274*(5290), 1133-1138.
Keating, M., & Feldman, J. D. (1975). Visual deprivation and intertectal neuronal connexions in Xenopus laevis. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, *191*(1105), 467-474.

Knorr, A., Gravot, C., Straka, H., & Glasauer, S. (2017). I spy with my little eye: A simple behavioral assay to test color perception in animal virtual reality setups. *Journal of Vision*, *17*(10), 661-661.
Koser, D. E., Thompson, A. J., Foster, S. K., Dwivedy, A., Pillai, E. K., Sheridan, G. K., Svoboda, H., Viana, M., Costa, L. d. F., & Guck, J. (2016). Mechanosensing is critical for axon growth in the developing brain. *Nature neuroscience*, *19*(12), 1592-1598.
Kubo, F., Hablitzel, B., Dal Maschio, M., Driever, W., Baier, H., & Arrenberg, A. B. (2014). Functional architecture of an optic flow-responsive area that drives horizontal eye movements in zebrafish. *Neuron*, *81*(6), 1344-1359.
Masseck, O. A., & Hoffmann, K. P. (2009). Comparative neurobiology of the optokinetic reflex. *Annals of the New York Academy of Sciences*, *1164*(1), 430-439.
McLaughlin, T., & O'Leary, D. D. (2005). Molecular gradients and development of retinotopic maps. *Annu. Rev. Neurosci.*, *28*, 327-355.
Mumm, J. S., Williams, P. R., Godinho, L., Koerber, A., Pittman, A. J., Roeser, T., Chien, C.-B., Baier, H., & Wong, R. O. (2006). In vivo imaging reveals dendritic targeting of laminated afferents by zebrafish retinal ganglion cells. *Neuron*, *52*(4), 609-621.
Murcia-Belmonte, V., & Erskine, L. (2019). Wiring the binocular visual pathways. *International Journal of Molecular Sciences*, *20*(13), 3282.
Nakagawa, S., Brennan, C., Johnson, K. G., Shewan, D., Harris, W. A., & Holt, C. E. (2000). Ephrin-B regulates the Ipsilateral routing of retinal axons at the optic chiasm. *Neuron*, *25*(3), 599-610.
Naumann, E. A., Fitzgerald, J. E., Dunn, T. W., Rihel, J., Sompolinsky, H., & Engert, F. (2016). From whole-brain data to functional circuit models: the zebrafish optomotor response. *Cell*, *167*(4), 947-960. e920.
Nevin, L. M., Robles, E., Baier, H., & Scott, E. K. (2010). Focusing on optic tectum circuitry through the lens of genetics. *BMC biology*, *8*(1), 1-10.
Niell, C. M., & Smith, S. J. (2005). Functional imaging reveals rapid development of visual response properties in the zebrafish tectum. *Neuron*, *45*(6), 941-951.
Nieuwkoop, P. D., & Faber, J. (1994). Normal table of. *Xenopus laevis*, *252*.
Oster, S., Deiner, M., Birgbauer, E., & Sretavan, D. (2004). Ganglion cell axon pathfinding in the retina and optic nerve. Seminars in cell & developmental biology,
Petros, T. J., Rebsam, A., & Mason, C. A. (2008). Retinal axon growth at the optic chiasm: to cross or not to cross. *Annual review of neuroscience*, *31*(1), 295-315.
Poggio, G. F., & Poggio, T. (1984). The analysis of stereopsis. *Annual review of neuroscience*, *7*(1), 379-412.
Portugues, R., Feierstein, C. E., Engert, F., & Orger, M. B. (2014). Whole-brain activity maps reveal stereotyped, distributed networks for visuomotor behavior. *Neuron*, *81*(6), 1328-1343.
Prusky, G. T., Alam, N. M., & Douglas, R. M. (2006). Enhancement of vision by monocular deprivation in adult mice. *Journal of Neuroscience*, *26*(45), 11554-11561.
Qian, H., Zhu, Y., Ramsey, D. J., Chappell, R. L., Dowling, J. E., & Ripps, H. (2005). Directional asymmetries in the optokinetic response of larval zebrafish (Danio rerio). *Zebrafish*, *2*(3), 189-196.
Ramdya, P., & Engert, F. (2008). Emergence of binocular functional properties in a monocular neural circuit. *Nature neuroscience*, *11*(9), 1083-1090.
Rose, T., Jaepel, J., Hübener, M., & Bonhoeffer, T. (2016). Cell-specific restoration of stimulus preference after monocular deprivation in the visual cortex. *Science*, *352*(6291), 1319-1322.
Ruthazer, E. S., Akerman, C. J., & Cline, H. T. (2003). Control of axon branch dynamics by correlated activity in vivo. *Science*, *301*(5629), 66-70.
Schönenberger, N., Escher, G., & Van der Loos, H. (1983). Axon number in oculomotor nerves in Xenopus: removal of one eye primordium affects both sides. *Neuroscience letters*, *41*(3), 239-245.

Soupiadou, P., Gordy, C., Forsthofer, M., Sanchez-Gonzalez, R., & Straka, H. (2020). Acute consequences of a unilateral VIIIth nerve transection on vestibulo-ocular and optokinetic reflexes in Xenopus laevis tadpoles. *Journal of Neurology*, *267*(1), 62-75.
Sperry, R. W. (1945). Restoration of vision after crossing of optic nerves and after contralateral transplantation of eye. *Journal of Neurophysiology*, *8*(1), 15-28.
Sperry, R. W. (1956). The eye and the brain. *Scientific American*, *194*(5), 48-53.
Steeves, J. K., Gonzalez, E. G., & Steinbach, M. J. (2008). Vision with one eye: a review of visual function following unilateral enucleation. *Spatial vision*, *21*(6), 509.
Straznicky, C., Tay, D., & Glastonbury, J. (1980). Regeneration of an abnormal ipsilateral visuotectal projection in Xenopus is delayed by the presence of optic fibres from the other eye.
Taylor, J., & Gaze, R. (1990). The course of regenerating retinal axons in the frog chiasma: the influence of axons from the other eye. *Anatomy and embryology*, *181*(4), 405-412.
Taylor, J., & Guillery, R. (1995). Effect of a very early monocular enucleation upon the development of the uncrossed retinofugal pathway in ferrets. *Journal of Comparative Neurology*, *357*(2), 331-340.
Thanos, S., Fujisawa, H., & Bonhoeffer, F. (1984). Elimination of ipsilateral retinotectal projections in mono-ophthalmic chick embryos. *Neuroscience letters*, *44*(2), 143-148.
Van Alphen, A., & De Zeeuw, C. (2002). Cerebellar LTD facilitates but is not essential for long-term adaptation of the vestibulo-ocular reflex. *European Journal of Neuroscience*, *16*(3), 486-490.
Van Hof-van Duin, J., & Mohn, G. (1986). Monocular and binocular optokinetic nystagmus in humans with defective stereopsis. *Investigative ophthalmology & visual science*, *27*(4), 574-583.
Wu, Y., Dal Maschio, M., Kubo, F., & Baier, H. (2020). An optical illusion pinpoints an essential circuit node for global motion processing. *Neuron*, *108*(4), 722-734. e725.
Yücel, Y., Jardon, B., Kim, M.-S., & Bonaventure, N. (1990). Directional asymmetry of the horizontal monocular head and eye optokinetic nystagmus: Effects of picrotoxin. *Vision research*, *30*(4), 549-555.



Suppl. fig. 1: Reproducibility of generation of one-eyed tadpoles. a) counts of animals generated from embryonic eye removals one week after extirpation. Monocular: successful generation of one-eyed animals. Regrowth: partial regrowth of a partial or full eye, retina, or a lens. Deformations: Malformed tissue structures in the region of the extirpation or at the brain. b), c), Immunohistochemistry for markers of muscle tissue (MyoVI), nervous tissue (Tubulin) and a nuclear counterstain (DAPI) of monocular (b) and control (c) animals at stage 46. d), e) Zoom-in into the eye region of a monocular (d) or control (e) tadpole at stage 46, 7 days after extirpations. e), f) *In-vitro* preparations of monocular (g) and control (f) tadpoles at stage 53, 4-5 Weeks after extirpations.



Supp. Fig. 2: Left-right asymmetries in binocular and monocular tadpoles. a) Conjugations of the left and right eye for leftward (blue) and rightward (red) unidirectional motion, and for sinusoidal motion (black). A slope of 1 represents conjugate eye motion, >1 stronger motion of the left, and <1 of the right eye. Slope of leftward and rightward motion are 1.12 and 0.69, respectively, while sinusoidal conjugation is intermediate at 0.92. b) Conjugations of the left and right eye for leftward (blue) and rightward (red) components of sinusoidal motion. Slope of leftward and rightward motion are 0.95 and 0.93, respectively c) Plot from Fig. 3b on nasal and temporal motion components of unidirectional stimuli of binocular and lesioned tadpoles. Added are grey dots which represent a summation of corresponding nasal and temporal values of lesioned animals, with corresponding statistics. d) Plot taken from Fig. 3c, with added grey dots which represent symmetry values of the lesioned nasal component, with the temporal component substituted with summed nasal and temporal gains calculated in panel c). e) Correlation of nasal (red) and temporal (blue) gains of embryonic monocular tadpoles (same individuals from Fig. 4f) with the amount of ipsilateral fraction of RGC projections. Nasal: $R^2 = 0.002$, fit: $y = -0.04y + 0.2$. Temporal: $R^2 = 0.67$, fit: $y = -0.87y + 0.23$. f) Substack of the thalamic areas of control (left) and monocular (right) tadpoles to highlight thalamic projections.

CHAPTER III:

Homeostatic plasticity of eye movement performance in *Xenopus* tadpoles following prolonged visual image motion stimulation

Publication 2

Michael Forsthofer[1,2] ,Hans Straka[1*]

[1]Faculty of Biology, Ludwig-Maximilians-University Munich, Planegg, Germany

[2]Graduate School of Systemic Neurosciences, Ludwig-Maximilians-University Munich, Großhaderner Str. 2, 82152 Planegg, Germany

[*]**Correspondence:** straka@lmu.de (HS)

Contribution of Authors:

MF and HS conceptualized and designed experiments for the study. MF conducted the behavioral and histological experiments, designed, and wrote analysis scripts, and analyzed and plotted the data. MF made initial figures, and MF and HS revised figures. MF wrote the initial version of the manuscript, and HS and MF edited the manuscript.

My contributions to this manuscript:

I, together with HS, designed the experiments. I conducted behavioral experiments, designed, and wrote analysis scripts, analyzed, and plotted the data. I performed immunohistochemistry, acquired, and analyzed microscopic images of the samples. I created initial versions of all figures and revised them together with HS. I wrote the initial version of the manuscript and revised it with HS.

For online access, please refer to: https://doi.org/10.1007/s00415-022-11311-8

Journal of Neurology (2023) 270:57–70
https://doi.org/10.1007/s00415-022-11311-8

ORIGINAL COMMUNICATION

Homeostatic plasticity of eye movement performance in *Xenopus* tadpoles following prolonged visual image motion stimulation

Michael Forsthofer[1,2] · **Hans Straka**[1]

Received: 17 March 2022 / Revised: 8 July 2022 / Accepted: 19 July 2022 / Published online: 10 August 2022

Abstract

Visual image motion-driven ocular motor behaviors such as the optokinetic reflex (OKR) provide sensory feedback for optimizing gaze stability during head/body motion. The performance of this visuo-motor reflex is subject to plastic alterations depending on requirements imposed by specific eco-physiological or developmental circumstances. While visuo-motor plasticity can be experimentally induced by various combinations of motion-related stimuli, the extent to which such evoked behavioral alterations contribute to the behavioral demands of an environment remains often obscure. Here, we used isolated preparations of *Xenopus laevis* tadpoles to assess the extent and ontogenetic dependency of visuo-motor plasticity during prolonged visual image motion. While a reliable attenuation of large OKR amplitudes can be induced already in young larvae, a robust response magnitude-dependent bidirectional plasticity is present only at older developmental stages. The possibility of older larvae to faithfully enhance small OKR amplitudes coincides with the developmental maturation of inferior olivary–Purkinje cell signal integration. This conclusion was supported by the loss of behavioral plasticity following transection of the climbing fiber pathway and by the immunohistochemical demonstration of a considerable volumetric extension of the Purkinje cell dendritic area between the two tested stages. The bidirectional behavioral alterations with different developmental onsets might functionally serve to standardize the motor output, comparable to the known differential adaptability of vestibulo-ocular reflexes in these animals. This homeostatic plasticity potentially equilibrates the working range of ocular motor behaviors during altered visuo-vestibular conditions or prolonged head/body motion to fine-tune resultant eye movements.

Keywords Optokinetic reflex · Cerebellum · Inferior olive · Ontogeny · Plasticity

Introduction

Gaze-stabilizing eye movements ensure unblurred visual images during head and/or body movements. Such image-stabilizing eye movements appeared early during evolution in freely swimming aquatic vertebrate ancestors with subsequent phylogenetic improvement of the performance, likely related to the increasing complexity of locomotion, visual acuity, and motion repertoires [1–3]. Optimal execution of these reflexes requires real-time evaluation of the motor performance through sensory reafferences and computational predictions of visual motion to allow animals to adapt the output [4, 5]. While being the dominant contributor to gaze stabilization during head perturbations, the open-loop vestibulo-ocular reflex (VOR) is supplemented by closed-loop visuo-motor reflexes such as the optokinetic reflex (OKR) [6]. This latter ocular motor behavior is driven by residual large-field visual image motion that remains uncompensated for by the VOR, and provides the sensory feedback to optimize gaze stability [7]. The visuo-motor system thus enhances the adequacy of eye movements following integration of visual motion and vestibular signals, which occurs predominantly within cerebellar circuits [8]. In fact, the various afferent and efferent brainstem–cerebellar connections integrate multisensory motion signals [9, 10], but also form the neural substrates for implementing changes in sensitivity during active *versus* passive head movements [11] or during development and aging [8].

✉ Hans Straka
straka@lmu.de

[1] Faculty of Biology, Ludwig-Maximilians-University Munich, Großhaderner Str. 2, 82152 Planegg, Germany

[2] Graduate School of Systemic Neurosciences, Ludwig-Maximilians-University Munich, Großhaderner Str. 2, 82152 Planegg, Germany

The role of the cerebellum in eye motion plasticity in mature vertebrates has been extensively studied using a variety of stimulus paradigms, species, and behavioral repertoires (e.g., [8]). The flexible interaction between vestibular and visual motion signals in cerebellar circuits has been the focus of numerous experimental studies (e.g., [12]; see [8]) largely based on earlier theoretical considerations [13, 14]. Key to the outcome of such studies was the importance of the connectivity between the inferior olive and the cerebellum in mediating adaptive plasticity [10, 15]. The majority of these studies explored the role of the cerebellum in adapting vestibular motion-evoked eye movements under specific sensory conditions (e.g., visuo-vestibular mismatch; [16]) or after a loss of head/body motion-related sensory signals (e.g., labyrinthectomy; [17]). In contrast, only few studies have so far evaluated the consequence of prolonged visuo-vestibular or pure visual motion stimulation and the potential role of the cerebellum in modifying ocular motor outputs (e.g., [15, 16, 18, 19]). Habituation of the VOR performance has been demonstrated during prolonged vestibular stimulation [20–22]. Conversely, improvement of VOR performance is possible but dependent on specific stimulus paradigms [23]. Increases of the OKR amplitude have been shown during prolonged vestibular or optokinetic stimulation [18, 24, 25]. Bidirectional, cerebellum-dependent changes of the angular VOR have been reported in *Xenopus laevis* tadpoles during prolonged rotation and were interpreted as homeostatic plasticity, adjusting the ocular motor output to a preset magnitude [26]. However, the ontogenetic onset and applicability of such a homeostatic plasticity to other ocular motor behaviors is so far unknown.

Here, we tested the impact of prolonged visual motion stimulation on the performance of the OKR in *Xenopus* tadpoles at different developmental stages, before and after the presumed onset of cerebellar function. The employment of semi-intact preparations for eye motion recordings, surgical manipulations, tract tracing, and immunohistochemical analyses, allowed demonstrating the involvement of the cerebellum in specific aspects of homeostatic OKR plasticity, once cerebellar neuronal elements have become functional.

Materials and methods

Animals and experimental preparation

Xenopus laevis embryos were obtained from the in-house breeding facility at the Biomedical Center or the Biocenter Martinsried of the Ludwig-Maximilians-University Munich. After hatching, tadpoles were reared at a temperature of 17–18 °C with a 12/12-h light/dark cycle in dechlorinated water.

All experiments were performed on semi-intact in vitro preparations of 97 tadpoles in compliance with the "Principles of animal care", publication No. 86-23, revised 1985 of the National Institute of Health and were carried out in accordance with the ARRIVE guidelines and regulations. Permission for the experiments was granted by the legally responsible governmental body (Regierung von Oberbayern) under the license code ROB-55.2–2532.Vet_03-17–24. In addition, all experiments were performed in accordance with the relevant guidelines and regulations of the Ludwig-Maximilians-University Munich.

Semi-intact preparations were obtained following a procedure described previously [27–29]. Animals of either sex at larval stages 50–51 (young tadpoles) and 55–56 (old tadpoles, [30]), respectively, were deeply anesthetized in 0.05% 3-aminobenzoic acid ethyl ester methanesulfonate (MS-222; Pharmaq Ltd.) dissolved in ice-cold frog Ringer solution (75 mM NaCl, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 2 mM KCl, 0.1 mM $MgCl_2$, and 11 mM glucose, pH 7.4). In a Ringer-filled, Sylgard-lined dish (Sylgard 184, Dow), larvae were decapitated under a binocular microscope (SZX16, Olympus) at the cervical spinal cord. Following removal of the lower jaws and visceral organs, the head was mechanically secured dorsal side-up onto the Sylgard-lined floor with *minutiae* pins (0.2 mm, Fine Science Tools). Subsequently, the skin of the head and the cartilaginous tissue of the skull were opened, the *choroid* plexus covering the fourth ventricle was removed and the forebrain disconnected. Both eyes, including all extraocular muscles, were left intact and remained connected to the brain via the optic nerve and the extraocular motor nerves, allowing presentation and sensory–motor processing of visual motion stimuli and the recording of eye movements (see below). Following the dissection, semi-intact preparations were transferred into fresh frog Ringer solution and allowed to recover for 3 h at 17 °C.

Disruption of the olivary–cerebellar pathway in semi-intact preparations was performed by a longitudinal midline transection in rhombomeres (*r*) 7 and 8, the hindbrain segmental level where the axons of cerebellum-projecting inferior olivary neurons cross the midline [31]. Following the initial 3-h recovery period and two cycles of stimulation to capture the reference OKR performance, the surgical intervention was performed under visual guidance using *minutiae* pins (0.1 mm). The target site for the transection was identified by external landmarks such as the lateral exit/entrance of cranial nerves IX–XI in r6 and r7 [32]. Thereafter, preparations were allowed to recover from the procedure for 30 min at 17 °C in darkness and were subsequently subjected to visual image motion stimulation as described below. The successful disruption of inferior olivary axons was histologically confirmed after each experiment (see below).

Visual motion stimuli, optokinetic training, and eye motion recording

For eye motion recordings, semi-intact preparations were mechanically secured dorsal side-up in the center of a Sylgard-lined circular dish (Ø 5 cm), surrounded by a visual virtual reality environment as described previously (Fig. 1a; [29]). The visual environment consisted of equally spaced, vertical black and white stripes subtending a visual angle of 16° per stripe. This pattern was projected onto a cylindrical screen (275° coverage, Ø 8 cm, 5 cm height; Fig. 1a) at 60 Hz by three digital light processing video projectors (Aiptek V60), affixed in 90° angles to each other on the experimental table. For the optokinetic training, the visual image motion pattern was continuously oscillated horizontally to the left and right for 30 min (Fig. 1b). Stimulus motion consisted of alternating bidirectional movements at two velocities: 4°/s or 8°/s, presented at different stimulus cycle durations—for 10 s or for 20 s—to generate visual image motion with different peak-to-peak position amplitudes. Accordingly, these stimulus parameter combinations produced four different profiles (Fig. 1c), and correspondingly elicited OKR responses with different eye motion amplitudes. Each animal was tested for only one stimulus profile to avoid transferring a possible training effect from an initial training stimulus onto a subsequent one. Accordingly, each stimulus profile was separately tested on a group of animals naïve to any training stimulus.

The movement of both eyes was captured non-invasively from above with a camera (Grasshopper 0.3 MP Mono FireWire 1394b, PointGrey, Vancouver, BC, Canada) equipped with an adequate objective lens and infrared-Filter (800 nm long pass) at a frame rate of 30 frames per second, while the preparation was illuminated from above by an infrared light source (840 nm). Eye positions were extracted in real time from the video by fitting an ellipse around each eye [27]. The angle between the long axis of each ellipse and the horizontal image axis was calculated in a frame-by-frame manner by a custom-written software [29, 33] and was recorded and stored for off-line analysis along with the visual motion stimulus (Spike2 version 7.04, Cambridge Electronic Design Ltd.).

Data analysis

Data acquired in Spike2 were exported in the MATLAB (The MathWorks Inc.) file format and analyzed off-line with custom-written Python scripts. Due to irregular sampling of stimulus and eye positions, stimulus and eye motion recordings were resampled at 200 Hz and filtered with a 4 Hz low-pass Butterworth filter. Traces were segmented into individual stimulus cycles from peak-to-peak, and

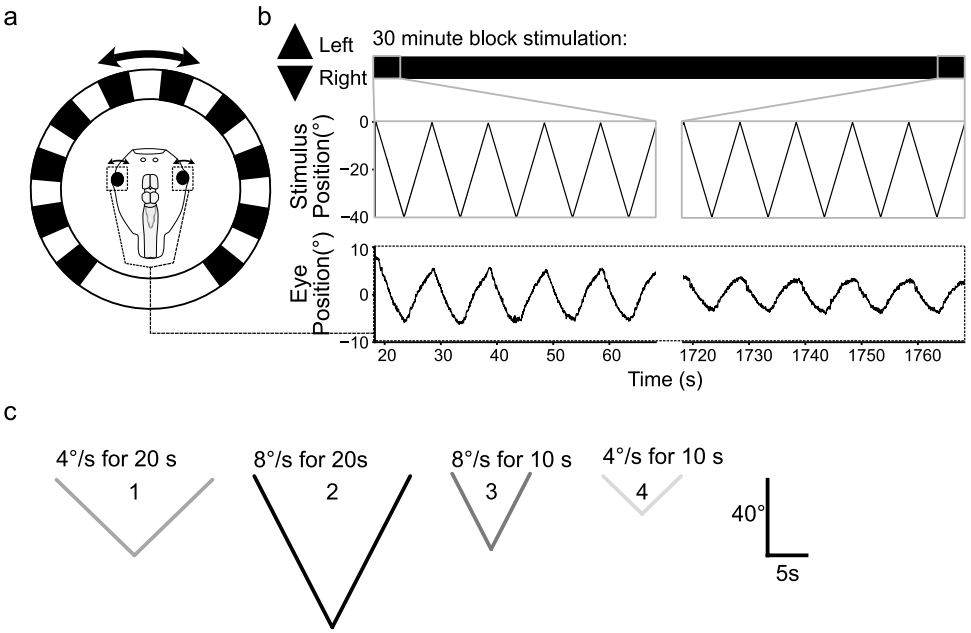


Fig. 1 Stimulation and recording of the optokinetic reflex in semi-intact preparations of *Xenopus laevis* tadpoles. **a** Schematic illustrating the experimental setting with a Ringer-filled circular recording chamber hosting the preparation; horizontal motion of vertical black and white stripes across the surrounding cylindrical screen serves as large-field visual motion stimulus and elicits eye movements (double arrows). **b** Representative example of horizontal positional oscillations of the eyes (lower trace) during prolonged (30 min) visual motion stimulation (upper traces), at the onset (left) and the end of the training period (right). **c** Single cycles of visual image motion profiles depicting the different combinations of stimulus velocities of either 4°/s (1, 4) or 8°/s (2, 3), presented in bidirectional alternation with a cycle duration of either 20 s (1, 2) or 10 s (3, 4)

conjugate motion traces of the left and right eye were averaged to generate a joint response of both eyes (see [28]). Infrequent cycles with either stimulus-evoked fast phases or spontaneous jerking movements were manually identified and excluded from further analysis. Following this preprocessing, peak-to-peak amplitudes of eye movements were determined for each motion cycle, to allow detection of amplitude changes across the training session. In addition, responses to the first and last five stimulus motion cycles of a given training period of 30 min were averaged, respectively, to generate a mean initial and a mean entrained response to directly compare the peak-to-peak motion amplitude of the original and entrained OKR response. Statistical analysis and plotting were performed in Prism 9 (GraphPad Software Inc.). Representative eye motion traces were normalized and averaged prior to plotting. Data, comparing responses of individual animals, were plotted as column scatter plots with or without mean ± SD. Statistical differences between experimental groups were calculated with the non-parametric Mann–Whitney *U*-test (unpaired parameters), the Wilcoxon signed-rank test (paired parameters), or the Kruskal–Wallis test and a Dunn's test (unpaired parameters) for multiple comparisons and indicated as *p*-values ($*p < 0.05$; $**p < 0.001$; $***p < 0.0001$).

Neuronal tracing

The connectivity between inferior olivary neurons and the cerebellum was anatomically profiled after completion of the behavioral experiments by fluorescent labeling of the neuronal projections in control tadpoles and those which received an experimental midline transection (see above). Tetramethylrhodamine, conjugated to 3000 MW Dextran (Invitrogen, D3308), was dissolved in ddH_2O, and crystallized onto 0.1 mm *minutiae* pins. Preparations were mechanically secured in Sylgard-lined dishes as those used for eye motion tracking. During the staining process, the Ringer solution was temporarily removed from the dish to prevent tracer crystals from undesired spreading and contaminating adjacent tissue [31]. Crystals were deposited in the left half of the single-foliated cerebellum. Thereafter, preparations were incubated in 500 ml freshly oxygenated frog Ringer solution at 16 °C for 24 h, fixed in freshly prepared 4% paraformaldehyde (PFA) for 24 h, washed 3 × in phosphate-buffered saline (PBS; 0.3 mM Na_3PO_4, 15 mM NaCl, 0.105 mM K_3PO_4) for 10 min, counterstained with DAPI (Thermo Scientific, 62,248) at 1:500 for 2 h at room temperature and subsequently cleared using the uDISCO protocol [34]. In short, whole mount preparations were dehydrated for 2 h each in stepwise increasing concentrations of butanol (30, 50, 70, 80, 90, 96, 100%), and cleared for at least 4 h in a 1:2 solution of benzylbenzoate and benzylalcohol, mixed 15:1 with diphenylether. The cleared tissue was mounted in the clearing solution using custom built metal spacers and was scanned on a Leica SP5-2 confocal microscope (LAS-X software) with ~ 400 optical sections of 1.2 µm thickness.

Immunohistochemistry

Calbindin immunostaining was performed on 10 µm or 30 µm thick parasagittal or coronal brain sections. Following isolation (see above), brains were fixed in freshly prepared 4% PFA for 3 h at 4 °C and were subsequently stored in PBS. Prior to cutting, brains were cryoprotected for at least 48 h in 30% sucrose at 4 °C, and subsequently embedded in tissue freezing medium (Leica, 14,020,108,626) on dry ice, frozen onto a sample holder with the same medium and cut directly onto Superfrost Plus slides (Epredia, J1810AMNZ). Sections were left to dry for 24 h, rehydrated in PBS for 15 min, incubated with a rabbit-anti-Calbindin (Abcam, ab108404) and a mouse-anti-GAD67 antibody (Abcam, ab26116), diluted 1:400 in PBS with 0.05% Triton-X 100 (Roth, 3051.3) and 10% normal goat serum (Millipore, S26) for 18 h at 4 °C. Slides were then washed 3 × for 10 min in PBS and subsequently incubated with an Alexa 488-conjugated goat-anti-rabbit antibody (Invitrogen, A11008), Alexa 546-conjugated goat-anti-mouse antibody (Invitrogen, A21045) and DAPI at a dilution of 1:400, 1:400 and 1:500, respectively, in PBS with 0.05% Triton-X. Slides were then washed 3 × for 10 min in PBS and finally cover-slipped with Poly-Mount (Polysciences, 18,606–20). Sections were scanned on a Leica SP5-2 confocal microscope at an optical thickness of 1.2 µm (10 × objective) or 0.63 µm (20 × objective), with ~ 10 (10x) or ~ 30 (20x) optical sections. For quantification, image stacks were loaded into ImageJ [35] and on each image, the area in μm^2 of Purkinje cell somata and Calbindin-positive dendritic trees, respectively, was measured. Areas were then multiplied by the thickness of each slice for an approximation of the volume, and summed up across slices for each individual animal.

Results

Semi-intact preparations of larval *Xenopus* were placed in the center of a circular recording chamber, surrounded by a vertical striped pattern oscillating horizontally at a constant velocity (Fig. 1a). This large-field visual motion pattern elicited eye movements that faithfully followed the stimulus (Fig. 1b), indicative of a robust OKR as demonstrated previously for these animals [27, 29, 36]. Prolonged presentation of this pattern for 30 min, here referred to as optokinetic training, provoked changes in the performance of the OKR. Such OKR modulations were observable in both young (stage 50–51) and old (stage 55–56) *Xenopus* tadpoles, although to different extents in the two groups.

Irrespective of age-dependent entrained characteristics, the training evoked plasticity measures with corresponding behaviorally relevant changes.

Plasticity of optokinetic responses

Old tadpoles

Optokinetic training with the standard reference motion profile (±40° positional excursion, ±4°/s velocity, 20 s period, Stimulus 1; Fig. 1c) consistently elicited changes of the OKR amplitude that varied considerably in extent between individual preparations (Fig. 2a, left). Most tadpoles exhibited an early decrease of the response amplitude within the first 5 min of the training (Fig. 2b), however, the effect of the entrainment on OKR magnitude was rather variable at the end of the 30-min training. While some of the stage 55–56 tadpoles exhibited an amplitude increase or approximately maintained the pre-training amplitude, others showed a considerable attenuation of the OKR by the

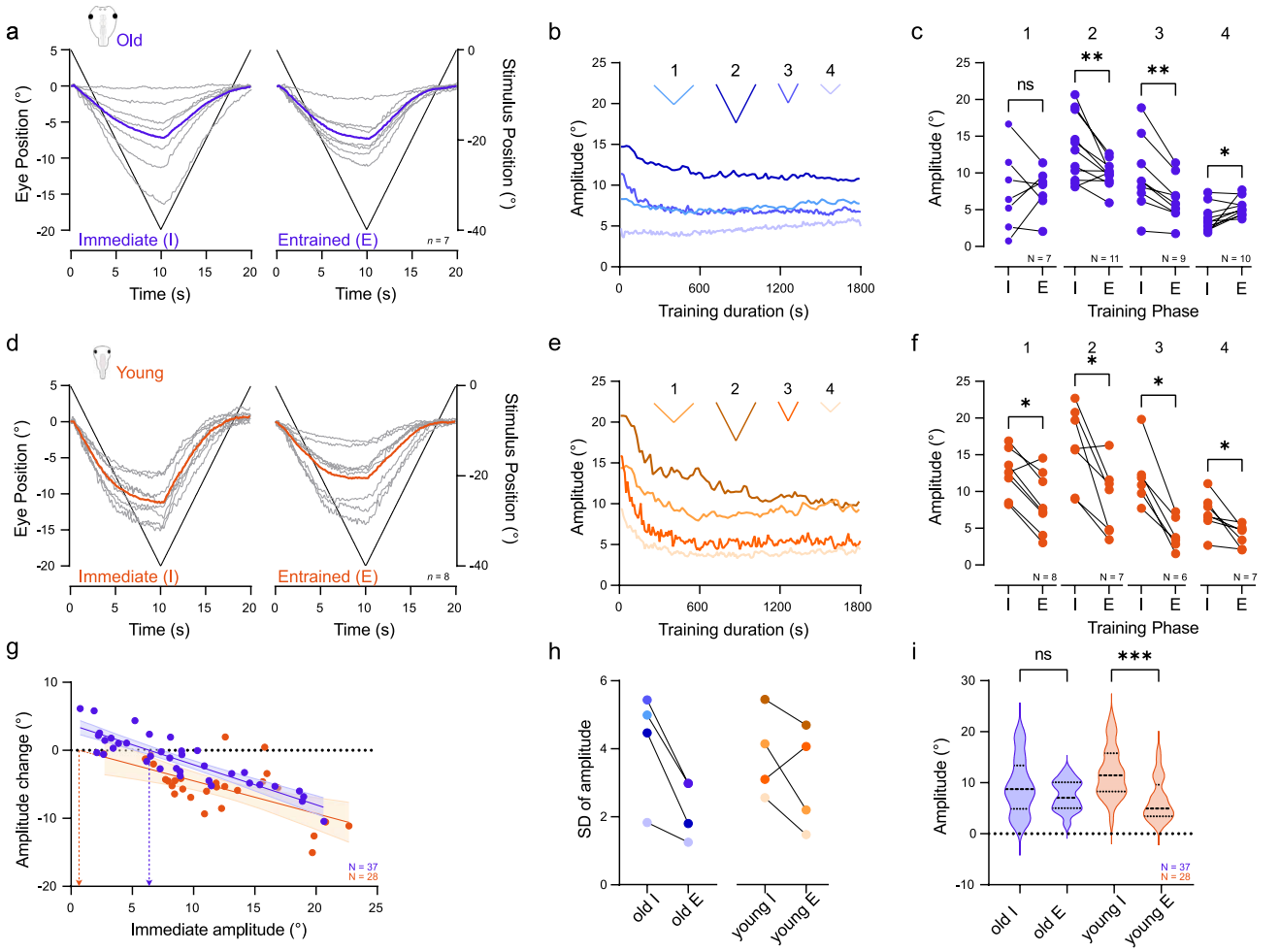


Fig. 2 Ontogeny of OKR plasticity. **a–f** Differential effects of prolonged visual image motion in old (**a–c**) and young (**d–f**) larvae; individual eye motion cycles (gray traces) and population average (colored traces, $n = 7$ (old, **a**), $n = 8$ (young, **d**) at the onset (left) and the end (right) of prolonged visual motion stimulation with profile 1 (velocity: 4°/s, cycle duration: 20 s; see 1 in Fig. 1c); population-averaged response amplitudes across 30 min of training (**b, e**), measured on a *per*-cycle basis for stimulus paradigms 1–4, respectively; mean response of five cycles (**c, f**) obtained immediately (I) and at the end of the 30-min OKR entrainment (E) on a *per*-animal basis for stimulus paradigms 1–4 (performed on different, independent sets of naïve tadpoles, respectively; see Fig. 1c). **g** Amplitude changes plotted against immediate OKR amplitudes for old (blue) and young (orange) tadpoles; solid lines indicate linear regression fit to data; shaded areas indicate 95% confidence interval of the fit; horizontal dotted line indicates no amplitude change; dots above indicate OKR amplitude increase, dots below OKR amplitude decrease; blue (old tadpoles) and orange (young tadpoles) vertical dashed arrows indicate the theoretical initial amplitudes, above or below which a decrease or increase is expected. **h** Standard deviation (SD) of the averaged response amplitudes immediately (I) and at the end of the entrainment period (E) for old and young tadpoles and stimulus paradigms 1–4 (for color-code see **b**, **e**). **i** Violin plots of immediate (I) and entrained response amplitudes (E), in young and old tadpoles respectively, pooled across stimulation paradigms 1–4

entrainment (Fig. 2a, right, 1 in Fig. 2c). A detailed evaluation of the emerging pattern suggested that this variability was systematically correlated with the initial amplitude of the OKR prior to the training session (Fig. 2a, 1 in Fig. 2c). Despite the use of the same stimulus (1 in Fig. 1c), the initial, pre-training amplitude differed markedly between individual preparations, with apparently differential consequences for the direction of the training-induced plasticity. While the average response amplitude of the population before (mean ± SD: 7.4° ± 5.4) and after 30 min training (mean ± SD: 7.6° ± 3.0) was very similar ($p = 0.99$; Wilcoxon signed-rank test; 1 in Fig. 2c), the overall variability of the post-training OKR amplitudes became markedly reduced. This effect derived from an overall homogenization of the OKR amplitude of the population after the training. OKR responses with large initial peak-to-peak amplitudes became attenuated during the prolonged visual motion stimulation, while small initial OKR magnitudes became enhanced by the training (1 in Fig. 2c). To test whether the degree and direction of the plasticity indeed depended on the initial response magnitude, motion stimulus parameters that more invariantly elicited either large (2, 3 in Fig. 1c) or small pre-training OKR amplitudes (4 in Fig. 1c) were used.

Presentation of visual motion stimuli with higher velocity and/or excursion magnitudes of the stimulus pattern (2, 3 in Fig. 1c) consistently evoked an OKR with large amplitudes prior to the onset of the training in practically all tadpoles (pattern 2: mean ± SD: 13.4° ± 4.5; pattern 3: mean ± SD: 9.7° ± 5.0; Fig. 2b, c). Prolonged exposure to either of these two visual motion paradigms reliably caused a significant attenuation of the entrained responses (pattern 2: mean ± SD: 9.9° ± 1.8; $p = 0.0049$, Wilcoxon signed-rank test; pattern 3: mean ± SD: 6.4° ± 3.0; $p = 0.0039$, Wilcoxon signed-rank test; Fig. 2c). However, despite reacting to the same stimulus profile, tadpoles with an initial OKR of intermediate amplitude exhibited a weaker attenuation of the entrained OKR amplitudes. We, therefore, suspected that the effective parameter for initiating the robust decrease in OKR amplitude is likely the initial eye motion performance itself, rather than large motion stimulus amplitudes per se. This was tested by presenting a visual motion pattern with smaller stimulus amplitudes (4 in Fig. 1c), which, prior to the training, reliably elicited an OKR with only small response amplitudes (4 in Fig. 2c, mean ± SD: 3.8° ± 1.8). Prolonged exposure to this visual motion stimulus for 30 min robustly provoked a significant increase of the entrained OKR amplitudes (4 in Fig. 2c, mean ± SD: 5.3° ± 1.3; $p = 0.0137$, Wilcoxon signed-rank test).

These experiments demonstrated a response amplitude-dependent plasticity of OKR magnitudes following prolonged exposure to visual motion stimuli in the cohort of old tadpoles. The observed changes comply with the reported cerebellum-dependent homeostatic eye movement plasticity previously demonstrated for the VOR in these animals [26], albeit with a stronger emphasis on motor output magnitude rather than stimulus strength. Based on the arguments presented in the latter study, the currently observed harmonization of the OKR amplitudes towards a preset response magnitude following prolonged visual motion stimulation might also be triggered by a cerebellum-dependent homeostatic plasticity. Within the group of animals with a variety of initial OKR amplitudes, this suggests that with ongoing training, responses become more similar between animals. This interpretation is supported by the clear diminishment of the variability as indicated by the dependency of training-induced changes on initial eye motion amplitudes across different stimulus paradigms, which shows a linear relationship between the initial response amplitude and resultant change by the entrainment (Fig. 2g, blue, $R^2 = 0.765$). The linear regression analysis further allows estimates about the plasticity pattern. The slope of the regression line indicates the dependency of the plasticity on the initial amplitude, with 0 signifying no, and higher/lower values indicating a pronounced dependency. For old, stage 55–56 tadpoles, the slope was -0.59, indicating a clear dependency of the plasticity on the initial OKR amplitude. Furthermore, the x-axis zero-intercept (horizontal dotted line) of the linear regression (blue dashed vertical arrow in Fig. 2g) yielded the theoretical initial amplitude, above or below which a respective decrease or increase is expected. In old tadpoles, this value was 6.4°. Also, the training was accompanied by a reduction of the standard deviation (see above, Fig. 2h), which together with the smaller variation of post-training response amplitudes across the different stimuli (Fig. 2i), indicates an overall reduced inter-animal variability of OKR amplitudes after the training in these animals.

Young tadpoles

Given the likely influence of the cerebellum in modulating the observed plasticity in visuo-motor behavior, younger tadpoles, with a potentially immature cerebellum, were assessed for similar visuo-motor training-induced changes. Anatomical studies identified the presence of the olivo-cerebellar circuitry in *Xenopus laevis* as early as stage 45 [37, 38]. However the functional onset of all relevant morphological structures occurred only much later at stage 53 [37, 38]. Even though these findings suggest that young tadpoles have defects in the cerebellar control of the OKR, a confirmation by respective behavioral studies is absent. Accordingly, young tadpoles (stages 50–51), which previously have been shown to exhibit a robust OKR [39, 40] were subjected to the same OKR training paradigm as the group of old tadpoles (see above). In general, OKR responses in young tadpoles prior to the training were similarly variable in amplitude for each stimulus paradigm (Fig. 2d, f). The

subsequent optokinetic training of young larvae also induced an amplitude decrease early during the prolonged stimulation (Fig. 2e) as in older tadpoles. However, in contrast to the latter group, this decrease became manifested over the 30-min training period such that all young tadpoles exhibited a consistent and significant OKR amplitude reduction after the entrainment ($p < 0.05$ for 1–4 in Fig. 2f; Wilcoxon signed-rank test) independent of the stimulus profile and thus of the initial response magnitude (compare "I" and "E" in Fig. 2f).

Furthermore, and also in contrast to the outcome reported for older tadpoles, the training-induced diminishment of the OKR response within this younger age-group was not accompanied by a strong, concurrent reduction in the variability of the respective peak-to-peak amplitudes as observed in older animals (Fig. 2g). This is illustrated by the retention of the relatively large variability of entrained response amplitudes in young tadpoles (orange dots in Fig. 2g; $R^2 = 0.378$) as compared to the group of older tadpoles (blue dots in Fig. 2g; $R^2 = 0.765$). This notion was substantiated by the rather limited reduction of the standard deviation of the entrained as compared to the immediate response amplitudes (Fig. 2h) and by the reduced distribution of post-training amplitudes across all stimuli (Fig. 2i). While initial peak-to-peak responses in young animals appeared to be larger in magnitude (compare Fig. 2a and d), the effect was not significant across paradigms (compare young "I" and old "I" in Fig. 2i). Nonetheless this finding might have been influenced by the overall larger peak-to-peak amplitudes of the OKR for the smallest stimulus, specifically at young stages, as compared to older tadpoles (Supplemental Fig. 1a, stimulus profile 4, $p = 0.0185$, Mann–Whitney U-test). While this fact does not explain the absent homogenization of inter-animal variations after the entrainment in young tadpoles, this aspect might well contribute to the lack of amplitude increases. This effect might emerge because even the smallest stimulus (4 in Fig. 1c) triggered OKR responses in younger larvae with amplitudes that generally exceeded those encountered in older tadpoles and which were increased after training in the latter (see vertical dashed line indicating the transition from amplitude increases (above) to decreases (below) in Fig. 2g). However, an estimate can be made based on the linear regression analysis shown in Fig. 2g. The slope of the regression line in young larvae (– 0.48) was lower than that of old tadpoles, indicating a rather limited dependency of the direction and strength of the plasticity on the initial amplitude of stage 50–51 larvae. In addition, the x-axis zero-intercept (horizontal dotted line) at 0.6° (orange dashed vertical arrow in Fig. 2g) indicates that much smaller amplitudes are theoretically required for an amplitude increase. The lack of amplitude increases by the entrainment in young tadpoles and the initially larger OKR amplitudes in these animals might derive from immature cerebellar circuits at this developmental stage [38], with a consequent lack of modifiable inhibitory influences of Purkinje cells on, e.g., vestibulo-ocular neurons and thus on the activation of extraocular motoneurons [6]. To test and eventually confirm the role of the cerebellum in OKR plasticity in *Xenopus* tadpoles, the neuronal connection between inferior olivary neurons and the cerebellum, which would serve as a critical pathway for such a computation [10], was next surgically interrupted in old tadpoles.

Impact of inferior olivary projections on ocular motor plasticity

The climbing fiber input to the cerebellum (Fig. 3a) arising from the inferior olive (IO) (Fig. 3a–c) was surgically interupted by a midline transection at the level of the caudal hindbrain in the group of old tadpoles (Fig. 3a, green line; d, e, inset). This manipulation was preferable over a pharmacological or surgical inactivation of the cerebellum itself, because transection of the climbing fiber pathway specifically eliminates visual reafferent signals required for the execution of plastic alterations, while leaving other neuronal elements of the cerebellum, such as parallel fiber inputs, and their effect on the OKR anatomically and functionally intact. Dye labeling of these projections was used to confirm the integrity or interruption of the midline-crossing axonal pathway ventrally in the caudal hindbrain (Fig. 3c green arrowheads). Labeled fibers in controls represent axons of inferior olivary neurons (Fig. 3c, green rectangle). Following midline transection, these axons were disconnected from their parent cell bodies (Fig. 3d, *), as indicated by the failure to retrogradely label the latter somata from the cerebellum (Fig. 3e, green square). These results anatomically confirmed the efficacy and success of the approach in functionally disconnecting the cerebellum from the inferior olivary nucleus. Following transection of the climbing fiber pathway, respective preparations were subjected to prolonged OKR training as described above with a stimulus pattern that either elicited large (3 in Fig. 1c; 3f; mean ± SD: 7.5° ± 3.7) or small (4 in Fig. 1c; 3f; mean ± SD: 5.3° ± 1.8) peak-to-peak response amplitudes. The magnitude of the initial OKR after the surgery was comparable to those of controls (Supplemental Fig. 1b, $p = 0.2359$ for 3; $p = 0.1088$ for 4, Mann–Whitney U-test), suggesting that the lesion had no significant impact on the general performance of the OKR. The retention of pre-lesion OKR amplitudes likely derived from the employed lesion protocol that left cerebellar circuits anatomically unimpaired. In addition, a reversal of potential long-lasting, downstream manifestation of previously acquired cerebellar influences on the OKR circuitry [41, 42] was avoided by the brevity of the experiments. In addition, prolonged visual motion stimulation consistently provoked an OKR amplitude decrease in all preparations

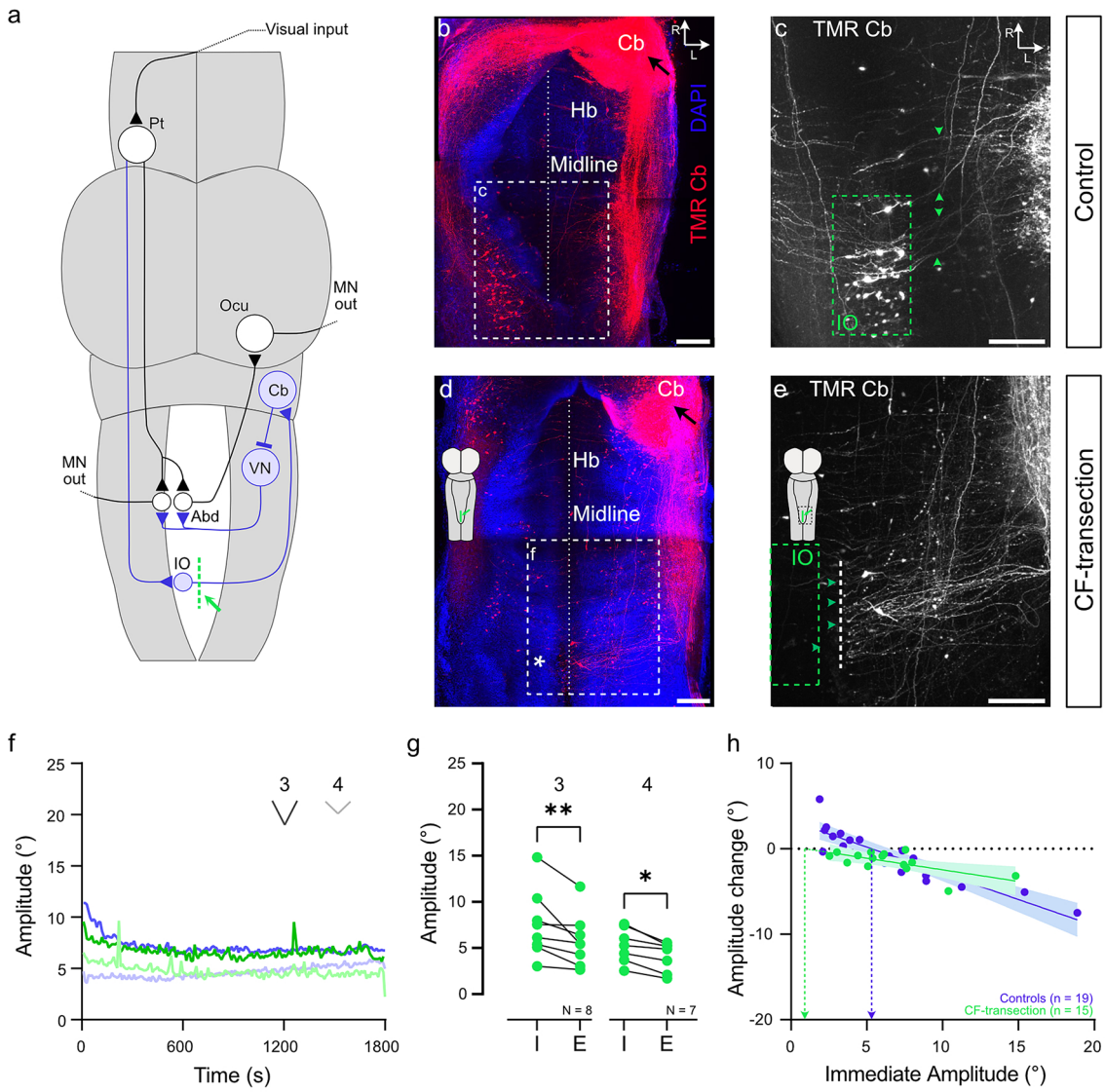


Fig. 3 Anatomical and functional consequences of climbing fiber transection. **a** Schematic of a *Xenopus* tadpole brain depicting the direct (black) and indirect (blue) OKR pathways for eliciting a horizontal OKR during rightward motion stimulation of the right eye; the site of climbing fiber transection in the caudal hindbrain is indicated by the green dashed line. **b–e** Whole-mount confocal reconstructions of the hindbrain (Hb) of a stage 55 control tadpole (**b–c**) and after midline transection (**d–e**) at the level of the caudal hindbrain (green line in **a**, scheme in **d**) counterstained with DAPI (blue nuclei); unilateral cerebellar injections of Tetramethylrhodamine (TMR; black arrows), outlined the climbing fiber (CF) axonal pathway (green arrow heads in **c, e**) and parent inferior olivary cell bodies in the contralateral ventral hindbrain in controls (**b**) and lack thereof after the lesion (* in **d**), illustrated at higher magnification in **c, e** (green dashed rectangles); **f** Population-averaged response amplitudes across 30 min of training, measured on a *per*-cycle basis for stimulus paradigms 3 and 4, in controls (blue) and after CF transection (green). **g** Mean response of five cycles obtained immediately (I) and at the end of the 30-min OKR entrainment (E) on a *per*-animal basis for stimulus paradigms 3 and 4 (see Fig. 1c) in CF-transected animals. **h** Amplitude changes plotted against immediate OKR amplitudes for controls (blue) and CF-transected animals (green); solid lines indicate linear regression fit to data, and shaded area the 95% confidence interval of the fit; horizontal dotted line indicates no amplitude change; dots above the line indicate amplitude increase, dots below amplitude decrease. Blue (control tadpoles) and green (CF-transected tadpoles) vertical dashed arrows indicate the theoretical initial amplitudes, above or below which a decrease or increase is expected. *Abd* abducens nucleus, *Cb* cerebellum, *D* dorsal, *IO* inferior olive, *L* lateral, *MN* motoneurons, *Ocu* oculomotor nucleus, *Pt* pretectum, R rostral, *VN* vestibular nuclei. Scale bars in all panels represent 100 µm

during the early phase of the training (Fig. 3f, green traces). However, in contrast to old control tadpoles with intact climbing fibers, animals with a climbing fiber transection only expressed a consistent and significant down-regulation

of the OKR amplitude after the 30-min training, irrespective of the initial amplitude (3 in Fig. 3g: mean ± SD: 7.5° ± 2.6, $p = 0.0078$; 4 in Fig. 3g: mean ± SD: 4.1 ± 1.6, $p = 0.0156$; Wilcoxon signed-rank test). This result was not surprising for tadpoles with large initial amplitudes prior to the training as shown in the age-matched controls (Fig. 3f, top green and blue traces, 3 g, left). However, that this decrease was observed in animals subjected to stimuli which normally provoke a robust OKR increase over time was in stark contrast to controls (Fig. 3f, bottom green and blue traces). The rather invariant and unidirectional training effect, irrespective of the initial amplitude (Fig. 3g, h), resembled the exclusive attenuation of the OKR amplitude by prolonged visual motion stimulation in young larvae (Fig. 2f, g), with an even lower slope of the regression line of -0.29 and a comparable x-axis zero-intercept (horizontal dotted line) at 0.94 (green vertical dashed arrow in Fig. 3h). This suggests that the climbing fiber input to the cerebellum is involved in an up-, but not in a down-regulation of the OKR amplitude. Accordingly, the lack of an amplitude increase of the OKR at young larval stages might derive from the fact that the cerebellar circuitry and/or respective cellular elements are incomplete or insufficiently mature to exert a homeostatic increase of the ocular motor output.

Ontogeny of cerebellar elements

To explore whether developmental differences at the level of the cerebellum might explain the observed differential OKR plasticity in younger as compared to older tadpoles, immunohistochemical analyses of key cellular elements at stage 50 and stage 56 were performed (Fig. 4). These developmental stages coincided with the condition prior to and after the known onset of Purkinje cell and climbing fiber development in frogs [43]. The calcium-binding protein Calbindin reliably outlined Purkinje cell somata and dendrites at both developmental stages and allowed a comparison of the general cerebellar anatomy and cell morphology (Fig. 4). Apart from the expected size difference of the cerebellum due to body and brain growth between younger and older tadpoles (Fig. 4a, d), the cerebellum at stage 56 revealed a more elaborate structural diversification (Fig. 4b, e). Combined with immunohistochemical labeling for GAD67, a marker for GABAergic neurons, younger tadpoles revealed the characteristic Purkinje cell layer and a molecular layer. The corresponding structural elements at stage 56, in contrast, had a very different and considerably layered organization of these zones (Fig. 4e), in addition to another group of Calbindin-positive cells that were smaller in size when compared to Purkinje cells (* in Fig. 4e). In comparison to young tadpoles, the molecular layer at stage 56 was expanded and encompassed a large region with GABAergic cell bodies and fibers and a predominantly Calbindin-immunopositive dorsal ridge. In addition, while cerebellar structures at stage 50 were rather homogenous in the medio-lateral direction (Fig. 4b, c, g), the molecular layer, and in particular the GABAergic components, increased in extent towards the midline of the cerebellum (Fig. 4e, f, h). Quantification of the volume of both the Purkinje cell somata and dendrites revealed a significant increase for both parameters during development. The approximate Purkinje cell layer volume increased significantly from 1.3×10^6 µm^3 ± 0.2×10^6 (mean ± SD) in young to 3.8×10^6 µm^3 ± 1.4×10^6 (mean ± SD) in old tadpoles (Fig. 4i, Somata, $p = 0.012$, Mann–Whitney *U*-test). The approximate dendritic volume also markedly increased from 1.8×10^6 µm^3 ± 0.6×10^6 (mean ± *SD*) in young to 7.4×10^6 µm^3 ± 1.6×10^6 (mean ± SD*)* in old tadpoles (Fig. 4i, Dendrites, $p = 0.012$, Mann–Whitney *U*-test). While general increases in volume were largely due to the brain growth during development, the overall Purkinje cell dendritic volume appeared to increase disproportionately compared to the layer of Purkinje cell somata. While not quite significant ($p = 0.07$), the dendritic volume was 2.1 ± 0.5 (mean ± SD) times larger than the area occupied by the cell bodies in old tadpoles, while this ratio was only 1.4 ± 0.5 (mean ± SD) in young tadpoles (Fig. 4i). Therefore, in addition to the lack of a layered population of small Calbindin-positive cells, those layers, which were already present in the cerebellum of young tadpoles are considerably smaller compared to those in older animals. As climbing fiber–Purkinje cell interactions conveying visual sensory feedback to the cerebellum occur in the molecular layer at Purkinje cell dendrites, the anatomical findings provide suggestive evidence for a potential substrate of the observed developmental differences in OKR plasticity.

Discussion

The OKR in *Xenopus* tadpoles at mid-larval stages exhibits a bidirectional plasticity during prolonged visual motion stimulation. The outcome of the entrainment was rather variable, and in different animals consisted of an increase, a decrease, or no alteration of OKR magnitudes. The overall variable gain changes, however, were inversely correlated with the pre-training response amplitude, suggesting the activation of a homeostatic plasticity that likely adjusts the motor performance to a preset value during prolonged stimulation. In contrast to this bidirectional plasticity of older tadpoles, younger larvae exclusively exhibited a decrease of the OKR amplitude, suggesting the presence of two separate plasticity mechanisms with different ontogenetic onsets. The neuronal site for the delayed up-regulatory plasticity likely includes inferior olivary–cerebellar projections and requires functionally mature cerebellar elements, suggested by respective morphological differences between young and old tadpoles.

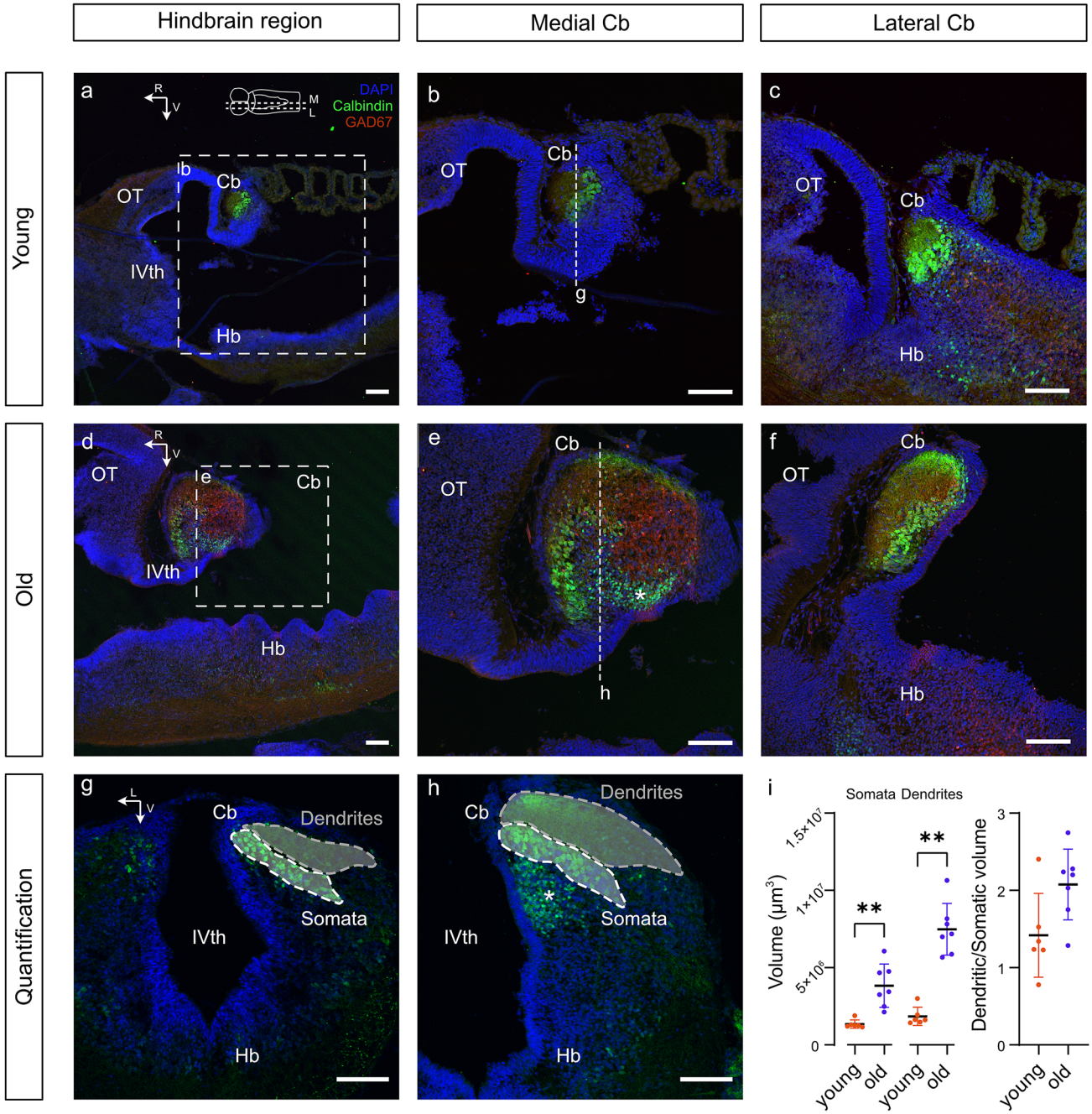


Fig. 4 Ontogenetic plasticity of calbindin- and glutamate-decarboxylase (GAD67)-immunopositive cerebellar structures. **a–f** Parasagittal sections at two medio-lateral planes (see scheme on top in **a**) through the hindbrain (Hb) depicting calbindin- (green) and GAD67-labeled (red) and DAPI-counterstained (blue) morphological structures in young (**a–c**) and old tadpoles (**d–f**); medially (M in scheme on top in **a**) located sagittal sections depicting overviews (**a, d**) and higher magnifications (**b, e**) of calbindin- and GAD67-immunopositive structures in the hindbrain and cerebellum (Cb); laterally (L in scheme on top in **a**) located sagittal sections (**c, f**) depicting respective immunopositive elements in the lateral cerebellum of young (**c**) and old (**f**) tadpoles. **g, h** Coronal sections at the level of the cerebellum of young (**g**) and old (**h**) tadpoles, outlining the region used to measure the location of Purkinje cell (PC) somata (white) and the extension of the dendritic tree (gray). **i** Quantification of the volumes of Purkinje cell somata (left), dendritic tree (middle) and ratio of dendritic tree/Purkinje cell somatic volumes (right) in young and old tadpoles. *OT* optic tectum, *R* rostral, *V* ventral, *IVth* fourth ventricle. Scale bars in all panels represent 100 µm

Substrate and development of OKR performance

The sensory–motor transformation subserving the OKR occurs along two different neuronal pathways (Fig. 3a) [3]. In both circuits, information about visual image motion is mediated by sets of retinal ganglion cells in the accessory optic tract that terminate in several, motion direction-specific, nuclei in the pretectum [44]. These pretectal nuclei represent the hub for a direct projection to extraocular motoneurons as demonstrated, e.g., in frogs, forming a short-latency OKR circuitry (Fig. 3a, black) [45, 46]. A second, more indirect pathway encompasses projections from the pretectal area through the inferior olive, cerebellum, and vestibular nuclei to finally access motoneurons in the extraocular motor nuclei (Fig. 3a, blue) [3]. This longer-latency pathway, involving the cerebellum, allows integration of multimodal signals related to motion in space and provides the likely substrate for spatio-temporal plasticity of ocular motor reactions in all vertebrates [47] including amphibians [26]. This pathway is shared with the VOR, through the projection of Purkinje cells onto floccular target neurons in the mammalian vestibular nuclei [12] or the correspondent cell type in amphibians to modulate the VOR gain through direct projections onto ocular motor nuclei (Fig. 3a). In fact, visual feedback in the form of climbing fiber activity onto Purkinje cells is essential for VOR adaptation [42, 48], linking OKR and VOR plasticity. Thus, visuo- and vestibulo-motor plasticity are at least in part governed by shared substrates and neuronal principles, indicating that the mechanistic features, such as spatio-temporal as well as eco-physiological circumstances under which behavioral adaptations are induced might be similar and likely cross-linked between the visuo- and vestibulo-motor system, once the cerebellum has become functional.

As precocial animals, locomotor activity in *Xenopus laevis* emerges very soon after hatching [49], which necessitates concurrent gaze-stabilizing eye movements [50]. While this is achieved at very early developmental stages by feedforward locomotor efference copies and utricular signals [40, 51, 52], the OKR is required to provide visual feedback and to initiate modifications of eye movements [52–54]. In fact, a functional OKR has been observed in *Xenopus* tadpoles as early as stage 45, with a moderate performance that remains largely invariant across the subsequent developmental period [51, 52]. These visuo-motor responses are thus well suited to provide appropriate visual feedback [55] to fine-tune gaze-stabilizing eye movements evoked by locomotor efference copies and/or vestibulo-ocular reflexes [40, 51, 52] at both developmental stages of *Xenopus* larvae employed in the current study.

Despite the presence of a robust OKR, the age-dependent differences in the capacity to exert plastic adaptations of the performance indicate that part of the sensory–motor circuitries or individual cellular elements might still be incomplete or afunctional at early larval stages. The robust performance, though without the ability for bidirectional changes at early developmental stages, suggests that visuo-motor responses in young larvae are exclusively mediated by the direct pathway (Fig. 3a, black). Given that the cerebellum is the predominant site for initiating modifications of eye movements in all vertebrates [10] including amphibians [26], it is possible that the indirect visuo-motor pathway through the cerebellum is still insufficiently established in young larvae, in compliance with the morphological findings of the current study (Fig. 4). This interpretation depends on the assumption that the rather stereotypic down-regulation of OKR performance across animals and stimuli can occur independent of intact climbing fiber connections as demonstrated by the respective lesion (Fig. 3), and may be caused by habituation or fatigue. While the overall consequence on OKR amplitude is similar between lesioned old and un-lesioned young animals, the underlying mechanism of the down-regulation most likely differs from the amplitude decreases previously observed during long-term visuo-vestibular mismatch training, based on the considerably slower time-course of the latter experimental outcome and the multiple timescales and mechanisms identified for motor learning [16, 56, 57].

Ocular motor plasticity

While young *Xenopus* tadpoles prior to stage 51 exhibit already some degree of visuo-motor plasticity, the full range of OKR adaptability appears only later during ontogeny. The OKR magnitude-dependent bidirectional adaptation caused an overall harmonization of the response amplitudes across different animals as well as across different stimuli. As such, these data suggest a rather more homogenous entrained performance, indicative of a homeostatic mechanism. Prerequisite to this homeostatic plasticity is the variability of the naïve OKR before training. A comparably variable inter-individual performance has been observed for the goldfish [58] and *Xenopus* tadpole OKR [55], as well as for the *Xenopus* VOR [59], and is likely related to intrinsic sensory–motor noise levels in the system as indicated in the latter study. This also includes variations in retinal sensitivity to the stimulus, as well as different muscle strengths and differences in the activity levels in the underlying neuronal circuits. Furthermore, the conduction of the experiments in developing brains likely enhances these effects, potentially inducing a larger degree of output variability. The displayed adaptive plasticity likely aims at maintaining a defined and preset level of synaptic drive within the visuo-motor circuitry during continuous activation. However, such an adaptation,

specifically the attenuating component, is in contrast to those previously observed in fish [19] or mammals [53, 60], where prolonged OKR training consistently induced an increase of the OKR response, ultimately leading to a higher and thus more efficient visual motion tracking. This difference might derive from differences in stimulus conditions or might represent a species-specific feature. The bidirectional changes in older *Xenopus* tadpoles suggest that the homogenization of OKR amplitudes through training aims at an optimization of motor performance rather than an enhancement of tracking a large-field visual scene. This concept of an adaptive plasticity, i.e., dynamic context-dependent increase or decrease of eye movements to achieve a leveled response, has previously been demonstrated for the VOR in age-matched *Xenopus* tadpoles [26]. For the respective training paradigm in the latter study, the direction of VOR amplitude changes were governed by stimulus, rather than response strength as demonstrated here. This difference complies with the fact that the OKR is a closed-loop system, operating on sensory feedback, allowing a graded increase or decrease of the amplitude rather than a stereotypic enhancement or attenuation. The cellular substrate for such differentially tuned plasticity is located in the molecular layer of the cerebellum (see [8, 15, 61]), and at least partially conveyed by climbing fiber input from the inferior olive, which has been shown to correlate with an increase, but not a decrease of VOR gain [62, 63]. As two of several cerebellar learning mechanisms, long-term potentiation (LTP) of parallel fiber–Purkinje cell synapses, diminishes the performance of gaze-stabilizing reflexes, while long-term depression (LTD) of such synapses, induced by climbing fiber and Purkinje cell co-activation, increases reflex performance [64, 65]. In both cases, this is achieved by modulating Purkinje cell firing rates, which by itself is sufficient to decrease or increase VOR performance [66]. This increase *versus* decrease is supplemented by the modulatory activity of GABAergic molecular layer interneurons, known to fine-tune the impact of climbing fiber activity for more nuanced gain changes, potentially facilitating adaptive plasticity as observed in the current study [67]. A comparable role of GABAergic neurons in the molecular layer of the cerebellum in *Xenopus* occurs likely in old, but not young tadpoles (Fig. 4). The fact that young *Xenopus* larvae lack a cerebellar granule cell layer [38], required for LTP-induced response decreases, supports the notion that the observed amplitude decrease at early developmental stages might be conveyed by a non-cerebellar pathway, although this remains to be experimentally validated.

Development of cerebellar circuits as anatomical substrate for ocular motor plasticity in *Xenopus*

The differences between the two larval age-groups correlate with known checkpoints in cerebellar development, and the ontogenetic appearance of cerebellar structures in this species, further tying behavioral differences to this brain structure. While inferior olivary–cerebellar connections are present as early as stage 45, climbing fiber axons are sparse, unlikely to be functional, and Purkinje cell dendritic trees are only rudimentary in extent [37]. In addition, the external granule cell layer in the *Xenopus* cerebellum remains inconspicuous for a substantial part of the larval life and becomes identifiable only at stage 53 [37]. The involvement of cerebellar granule cells in OKR plasticity [68] renders these neurons in particular a possible substrate for the observed training effect. In fact, the immunohistochemical identification of selected cerebellar markers demonstrated rather negligible numbers of major cellular elements at stage 51 (Fig. 4), in compliance with the relatively delayed onset of cerebellar function in comparison to the principal visuo-motor performance (Fig. 2) [52]. Most importantly, however, the volume of Purkinje cell dendrites, the primary site of climbing fiber–Purkinje cell interactions associated with eye motion plasticity [42, 69, 70], is much less pronounced in such young tadpoles as compared to those at stage 56. The rather immature cerebellum at stage 51 likely renders this structure incapable of appropriate, sensory feedback-based neuronal computations to alter visuo-motor responses on a meaningful level. Confirmation for the necessary integrity of the inferior olive and climbing fiber connectivity with Purkinje cells as potential substrates for OKR modifications derives from studies in mice [18]. Based on the outcome of the latter study, pharmacological inactivation of the inferior olive completely eliminates a successful OKR gain-up training, in agreement with the outcome of the transection of climbing fibers in the present study on *Xenopus* tadpoles. According to the collectivity of findings and assumptions therefore, plastic increases of eye movement amplitudes also in *Xenopus,* appear to require a functionally intact inferior olive and ascending connections with cerebellar Purkinje cells, which in *Xenopus* tadpoles reach functional maturity only between stages 51 and 55. Thus, during larval development, a fast onset of the OKR to facilitate gaze stabilization during locomotion from early on in development is only later complemented by a second, plastic pathway recruitment that allows more mature animals to integrate and modify visual feedback. This renders the visual system able to rapidly respond to immediate visual scene motion, within a motor range that is tuned to the animal's visual surroundings through means of homeostatic plasticity.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00415-022-11311-8.

Acknowledgements The authors acknowledge financial support from the German Science Foundation (CRC 870 and RTG 2175). Microscopy was performed at the center for advanced light microscopy (CALM) at the LMU Munich. The authors thank the Rupp lab at the Biomedical Center of the LMU Munich for providing *Xenopus laevis* embryos. The authors thank Clayton Gordy for valuable comments on this manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL.

Declarations

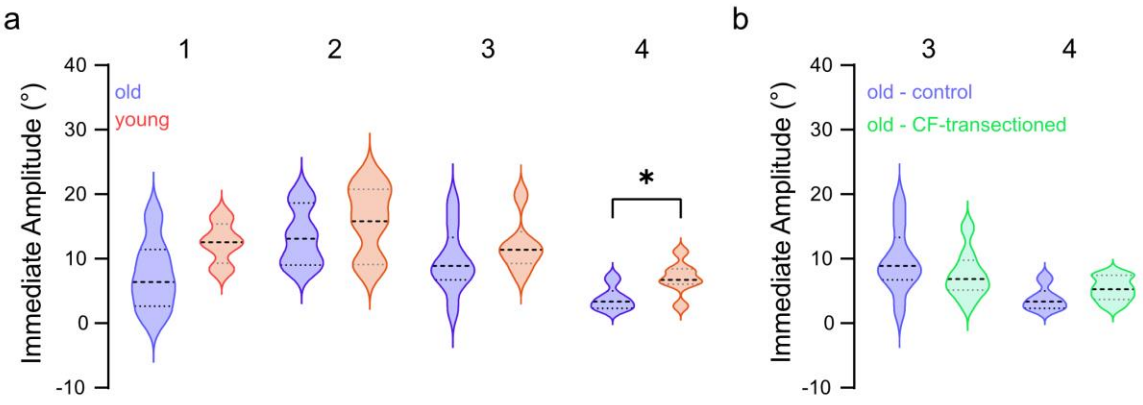
Conflicts of interest The authors declare no competing financial interests.

References

1. Land M, Mennie N, Rusted J (1999) The roles of vision and eye movements in the control of activities of daily living. Perception 28:1311–1328
2. Straka H, Gordy C (2020) The Vestibular system: The "LeathermanTM" among sensory systems. In: Fritzsch B, Straka H (eds) The Senses: A Comprehensive Reference, vol 6. Elsevier, Academic Press, pp 708–720
3. Horn AKE, Straka H (2021) Functional organization of extraocular motoneurons and eye muscles. Annu Rev Vis Sci 7:793–825
4. Straka H, Simmers J, Chagnaud BP (2018) A new perspective on predictive motor signaling. Curr Biol 28:R232–R243
5. Markov DA, Petrucco L, Kist AM, Portugues R (2021) A cerebellar internal model calibrates a feedback controller involved in sensorimotor control. Nat Comm 12:1–21
6. Straka H, Dieringer N (2004) Basic organization principles of the VOR: lessons from frogs. Progr Neurobiol 73:259–309
7. Collewijn H (1989) The vestibulo-ocular reflex: is it an independent subsystem? Rev Neurol 145:502–512
8. Boyden ES, Katoh A, Raymond JL (2004) Cerebellum-dependent learning: the role of multiple plasticity mechanisms. Annu Rev Neurosci 27:581–609
9. Cullen KE (2019) Vestibular processing during natural self-motion: implications for perception and action. Nat Rev Neurosci 20:346–363
10. Blazquez PM, Hirata Y, Pastor AM (2020) Functional organization of cerebellar feed-back loops and plasticity of influences on vestibular function. In: Fritzsch B, Straka H (eds) The senses: a comprehensive reference, vol 6. Elsevier, Academic Press, pp 389–413
11. Brooks JX, Carriot J, Cullen KE (2015) Learning to expect the unexpected: rapid updating in primate cerebellum during voluntary self-motion. Nat Neurosci 18:1310–1317
12. Lisberger S, Miles F, Zee D (1984) Signals used to compute errors in monkey vestibuloocular reflex: possible role of flocculus. J Neurophysiol 52:1140–1153
13. Marr D (1969) A theory of cerebellar cortex. J Physiol 202:437–470
14. Albus JS (1971) A theory of cerebellar function. Math Biosci 10:25–61
15. Miki S, Urase K, Baker R, Hirata Y (2020) Velocity storage mechanism drives a cerebellar clock for predictive eye velocity control. Sci Rep 10:6944
16. França de Barros F, Schenberg L, Tagliabue M, Beraneck M (2020) Long term visuo-vestibular mismatch in freely behaving mice differentially affects gaze stabilizing reflexes. Sci Rep 10:20018
17. Beraneck M, McKee JL, Aleisa M, Cullen KE (2008) Asymmetric recovery in cerebellar-deficient mice following unilateral labyrinthectomy. J Neurophysiol 100:945–958
18. Pham NC, Kim YG, Kim SJ, Kim C-H (2020) Differential effects of inferior olive lesion on vestibulo-ocular and optokinetic motor learning. NeuroReport 31:9–16
19. Miki S, Baker R, Hirata Y (2018) Cerebellar role in predictive control of eye velocity initiation and termination. J Neurosci 38:10371–10383
20. Jäger J, Henn V (1981) Vestibular habituation in man and monkey during sinusoidal rotation. Ann N Y Acad Sci 374:330–339
21. Dow ER, Anastasio TJ (1998) Analysis and neural network modeling of the nonlinear correlates of habituation in the vestibulo-ocular reflex. J Comput Neurosci 5:171–190
22. Gutierrez-Castellanos N, Winkelmann BHJ, Tolosa-Rodriguez L, De Gruijl JR, de Zeeuw CI (2013) Impact of aging on long-term ocular reflex adaptation. Neurobiol Aging 34:2784–2792
23. Miles F, Eighmy B (1980) Long-term adaptive changes in primate vestibuloocular reflex. I. Behavioral observations. J Neurophysiol 43:1406–1425
24. Miyoshi T, Pfaltz C (1973) Studies on optokinetic habituation. Int J Equilib Res 3:94
25. Inoshita T, Hirano T (2018) Occurrence of long-term depression in the cerebellar flocculus during adaptation of optokinetic response. Elife 7:e36209
26. Dietrich H, Straka H (2016) Prolonged vestibular stimulation induces homeostatic plasticity of the vestibulo-ocular reflex in larval *Xenopus laevis*. Eur J Neurosci 44:1787–1796
27. Gravot CM, Knorr AG, Glasauer S, Straka H (2017) It's not all black and white: visual scene parameters influence optokinetic reflex performance in *Xenopus laevis* tadpoles. J Exp Biol 220:4213–4224
28. Soupiadou P, Gordy C, Forsthofer M, Sanchez-Gonzalez R, Straka H (2020) Acute consequences of a unilateral VIIIth nerve transection on vestibulo-ocular and optokinetic reflexes in *Xenopus laevis* tadpoles. J Neurol 267(Suppl 1):62–75
29. Knorr AG, Gravot CM, Glasauer S, Straka H (2021) Image motion with color contrast suffices to elicit an optokinetic reflex in *Xenopus laevis* tadpoles. Sci Rep 11:1–12
30. Nieuwkoop PD, Faber J (1994) Normal table of *Xenopus laevis* (Daudin): a systematical and chronological survey of the development from the fertilized egg till the end of metamorphosis. Garland Publisher, New York
31. Straka H, Baker R, Gilland E (2001) Rhombomeric organization of vestibular pathways in larval frogs. J Comp Neurol 437:42–55

32. Straka H, Baker R, Gilland E (2006) Preservation of segmental hindbrain organization in adult frogs. J Comp Neurol 494:228–245
33. Beck JC, Gilland E, Tank DW, Baker R (2004) Quantifying the ontogeny of optokinetic and vestibuloocular behaviors in zebrafish, medaka, and goldfish. J Neurophysiol 92:3546–3561
34. Pan C, Cai R, Quacquarelli FP, Ghasemigharagoz A, Lourbopoulos A, Matryba P, Plesnila N, Dichgans M, Hellal F, Ertürk A (2016) Shrinkage-mediated imaging of entire organs and organisms using uDISCO. Nat Meth 13:859–867
35. Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Meth 9:671–675
36. Knorr, A.G., Gravot, C.M., Gordy, C., Glasauer, S., Straka, H. (2018) I spy with my little eye: a simple behavioral assay to test color sensitivity on digital displays. Biol Open 7:bio035725
37. van der Linden JAM, ten Donkelaar JH, De Boer-van Huizen R (1990) Development of olivocerebellar fibers in the clawed toad, *Xenopus laevis*: A light and electron microscopical HRP study. J Comp Neurol 293:236–252
38. van der Linden JAM (1990) The development of cerebellar connections in the clawed toad, *Xenopus laevis*. PhD Thesis. Nijmegen. ISBN 90–9003324–6
39. Lambert FM, Beck JC, Baker R, Straka H (2008) Semicircular canal size determines the developmental onset of angular vestibuloocular reflexes in larval *Xenopus*. J Neurosci 28:8086–8095
40. Lambert FM, Bacqué-Cazenave J, Le Seach A, Arama J, Courtant G, Tagliabue M, Eskiizmirliler S, Straka H, Beraneck M (2020) Stabilization of gaze during early *Xenopus* development by swimming-related utricular signals. Curr Biol 30:746–753
41. Pastor AM, de la Cruz RR, Baker R (1994) Cerebellar role in adaptation of the goldfish vestibuloocular reflex. J Neurophysiol 72:1383–1394
42. Gittis AH, du Lac S (2006) Intrinsic and synaptic plasticity in the vestibular system. Curr Opin Neurobiol 16:385–390
43. Nieuwenhuys R, ten Donkelaar HJ, Nicholson C (1998) The central nervous system of vertebrates. Springer
44. McKenna OC, Wallman J (1985) Accessory optic system and pretectum of birds: comparisons with those of other vertebrates. Brain Behav Evol 26:91–103
45. Cochran S, Dieringer N, Precht W (1984) Basic optokinetic-ocular reflex pathways in the frog. J Neurosci 4:43–57
46. Gruberg ER, Grasse KL (1984) Basal optic complex in the frog (*Rana pipiens*): a physiological and HRP study. J Neurophysiol 51:998–1010
47. Masseck OA, Hoffmann KP (2009) Comparative neurobiology of the optokinetic reflex. Ann N Y Acad Sci 1164:430–439
48. Faulstich BM, Onori KA, du Lac S (2004) Comparison of plasticity and development of mouse optokinetic and vestibulo-ocular reflexes suggests differential gain control mechanisms. Vision Res 44:283419–283427
49. Sillar KT, Simmers AJ, Wedderburn JF (1992) The post-embryonic development of cell properties and synaptic drive underlying locomotor rhythm generation in *Xenopus* larvae. Proc R Soc Lond B Biol Sci 249:65–70
50. Straka H (2010) Ontogenetic rules and constraints of vestibulo-ocular reflex development. Curr Opin Neurobiol 20:689–695
51. Bacqué-Cazenave J, Courtand G, Beraneck M, Lambert FM, Combes D (2018) Temporal relationship of ocular and tail segmental movements underlying locomotor-induced gaze stabilization during undulatory swimming in larval *Xenopus*. Front Neural Circuits 12:95
52. Bacqué-Cazenave J, Courtand G, Beraneck M, Straka H, Combes D, Lambert FM (2022) Locomotion-induced ocular motor behavior in larval *Xenopus* is developmentally tuned by visuo-vestibular reflexes. Nat Comm 13:2957. https://doi.org/10.1038/s41467-022-30636-6
53. Collewijn H, Grootendorst A (1979) Adaptation of optokinetic and vestibulo-ocular reflexes to modified visual input in the rabbit. Progr Brain Res 50:771–781
54. Shin S-L, Zhao GQ, Raymond JL (2014) Signals and learning rules guiding oculomotor plasticity. J Neurosci 34:10635–10644
55. Schuller J (2017) Functional organization and ontogeny of the optokinetic reflex in *Xenopus laevis*. Dissertation, LMU München: Graduate School of Systemic Neurosciences (GSN). Doi: https://doi.org/10.5282/edoc.25344
56. Yang Y, Lisberger SG (2010) Learning on multiple timescales in smooth pursuit eye movements. J Neurophysiol 104:2850–2862
57. Van Alphen AM, de Zeeuw CI (2002) Cerebellar LTD facilitates but is not essential for long-term adaptation of the vestibulo-ocular reflex. Eur J Neurosci 16:486–490
58. Marsh E, Baker R (1997) Normal and adapted visuooculomotor reflexes in goldfish. J Neurophysiol 77:1099–1118
59. Glasauer S, Straka H (2022) Low gain values of the vestibulo-ocular reflex can optimize retinal image slip. Front Neurol 13:897293. https://doi.org/10.3389/fneur.2022.897293
60. Kodama T, du Lac S (2016) Adaptive acceleration of visually evoked smooth eye movements in mice. J Neurosci 36:6836–6849
61. Giolli RA, Blanks RH, Lui F (2006) The accessory optic system: basic organization with an update on connectivity, neurochemistry, and function. Progr Brain Res 151:407–440
62. Kimpo RR, Rinaldi JM, Kim CK, Payne H, Raymond JL (2014) Gating of neural error signals during motor learning. Elife 3:e02076
63. Lisberger SG (2021) The Rules of cerebellar learning: around the Ito hypothesis. Neuroscience 462:175–190
64. Linden DJ, Connor JA (1995) Long-term synaptic depression. Annu Rev Neurosci 18:319–357
65. Lev-Ram V, Wong ST, Storm DR, Tsien RY (2002) A new form of cerebellar long-term potentiation is postsynaptic and depends on nitric oxide but not cAMP. Proc Nat Acad Sci U S A 99:8389–8393
66. Nguyen-Vu TB, Kimpo RR, Rinaldi JM, Kohli A, Zeng H, Deisseroth K, Raymond JL (2013) Cerebellar Purkinje cell activity drives motor learning. Nat Neurosci 16:1734–1736
67. Rowan MJ, Bonnan A, Zhang K, Amat SB, Kikuchi C, Taniguchi H, Augustine GJ, Christie JM (2018) Graded control of climbing-fiber-mediated plasticity and learning by inhibition in the cerebellum. Neuron 99:999–1015
68. Galliano E, Gao Z, Schonewille M, Todorov B, Simons E, Pop AS, D'Angelo E, van den Maagdenberg AMJM, Hoebeek FE, de Zeeuw CI (2013) Silencing the majority of cerebellar granule cells uncovers their essential role in motor learning and consolidation. Cell Rep 3:1239–1251
69. du Lac S, Raymond JL, Sejnowski TJ, Lisberger SG (1995) Learning and memory in the vestibulo-ocular reflex. Annu Rev Neurosci 18:409–441
70. Wakita R, Tanabe S, Tabei K, Funaki A, Inoshita T, Hirano T (2017) Differential regulations of vestibulo-ocular reflex and optokinetic response by β-and α2-adrenergic receptors in the cerebellar flocculus. Sci Rep 7:3944

Supplemental Figure 1
Forsthofer and Straka



Supplemental fig. 1 Comparison of OKR amplitudes at training onset (immediate). a Violin plots of immediate OKR amplitudes (prior to the training) in old (blue; n = 37) and young (orange; n = 28) control tadpoles for the different stimulation paradigms (1–4) depicted in Fig. 1c. b Violin plots of immediate OKR amplitudes (prior to the training) in old unmanipulated (blue; n = 19) and climbing fiber (CF)-transected tadpoles (green; n = 15) for stimulus paradigms 3 and 4. * $p < 0.05$, Mann–Whitney U-test

DISCUSSION AND FUTURE DIRECTIONS

In this thesis, I investigate functional plasticity and its dependence on anatomical structures. In both cases, the focus was on how function depends on the underlying circuitry and its maturation, and whether plasticity can improve motor performance. Unsurprisingly, categorizing ‘compensation’ or ‘improvement’ in context of behavior is not a straightforward task, and plasticity affects various parameters of the same behavior in different ways. Following identification of general behavioral plasticity, I subsequently characterized changes in timing, amplitude, and directionality to gain a more nuanced understanding on how behavior changes, and potentially, to what end. I quantified changes in different aspects of motor performance, such as strength or timing of a response, and correlated the extent of these changes with variations in the anatomy of the underlying circuits, caused either by developmental differences or surgical manipulations. In my first study, I found that during development, axons from both eyes are necessary to facilitate normal axonal pathfinding in *Xenopus laevis* from the eye to the brain. I further observed that RGC pathfinding errors in embryonically induced one-eyed impaired plasticity of the OKR. This suggests that these rerouted RGC axons are functional, but do not contribute to compensation and may in fact be detrimental for optokinetic behavior. One-eyed animals with little axonal pathfinding errors on the other hand showed compensation. These results therefore suggest that molecular cues and interaction with the contralateral eye are important factors for RGCs to target the correct brain hemisphere. RGC activity seems to not contribute in a compensatory fashion to retino-tectal pathfinding, and the ability to compensate only with near unaltered pathfinding suggests plasticity to be conveyed by canonical mechanisms in, for example, the cerebellum.

Correspondingly, my second project focused on the role of the cerebellum in plasticity of optokinetic eye motions. Specifically, I investigated changes in the OKR in response to training, and found that changes are not homogenous across tadpoles, with individuals showing either an increase or decrease of the OKR depending on initial OKR amplitude. This is congruent with studies on VOR plasticity, and suggests that tadpoles gravitate towards a standardized motor response when trained to a repetitive motion with the aim of achieving homeostasis rather than improved compensatory eye motion. In the second part of the study, I identified that the cerebellum is specifically tied to the up-regulation of gain, which only appears later in development long after OKR onset.

In the following, I will discuss the novelties of both studies in context of literature. In the first part of the discussion, I will give an overview of the goals and mechanisms observed in homeostatic plasticity in tadpoles. In the current and previous studies (Dietrich & Straka,

2016; Forsthofer & Straka, 2022; Gordy & Straka, 2022), responses did lead to an improved motor output as is the case in many other species, and I will discuss what 'optimization' of a response may mean for *Xenopus* in comparison to other vertebrates. In the second part, I will elaborate on the role of primary visual targets in the diencephalon and midbrain in homeostatic plasticity. This is based on findings on how the midbrain itself contributes to plasticity, but also how anatomical impairments of the pretectal OKR circuit may limit the extent of compensation. Finally, I will talk about the role of the cerebellum in plasticity and homeostasis of eye movements and propose future experiments to further dissect the role of the cerebellum versus primary visual areas in adaptively changing visual behavior.

Plasticity in tadpole gaze stabilization

To understand the purpose of behavioral changes, one needs to understand what the 'ideal' motor output would be for a specific stimulus in context. For gaze stabilization, the goal seems obvious at first: if a scene moves on the retina, that movement should be fully compensated for by an equal degree of eye motion, which would be called a unity gain. While normally this is not the case for one single component of gaze stabilization, as normally the OKR and VOR work conjointly, prolonged repetitive stimulation can increase the gain of the OKR in isolation to almost one, achieving near perfect compensation (Pham et al., 2020). However, there is no free lunch: Such an investment into improving one singular behavior is associated with a cost. This can be energy that is needed to move muscles or drive motor neuron activity, or generating a large enough population of neurons during development that may be necessary for such an increased motor output (Glasauer & Straka, 2022; Rangel & Hare, 2010). In addition, such a maximization of eye motion can impair the dynamic range of responses to further changes in the visual environment. If a given visual scene motion in the environment is half compensated for by the eye moving only 50% of it's total motion, it seems logical to increase eye motion to 100% of its range to fully compensate for the stimulus motion. However, if stimulus motion is now further increased, the eye is now physically incapable of responding to that increased stimulus motion. The question then becomes whether it is more important for an animal to perfectly respond to its current environmental parameters, or whether it is more beneficial to be ready to respond to changes in the environment, while keeping responses to the current environment just 'good enough.'

Homeostasis in tadpole gaze stabilization

It appears that in *Xenopus* tadpoles, optokinetic circuits are geared towards efficiency as opposed to maximized responses. Optokinetic and vestibulo-ocular training experiments show that tadpoles gravitate towards a stereotyped motor pattern rather than improving OKR performance. While initially weak responses increase in amplitude as would be expected for

improvement of compensation, this improvement stops far below unity gain. Large, but still below unity eye motions on the other hand are attuned with training (Forsthofer & Straka, 2022). Similar results are obtained from experiments on VOR training, where firing rates in response to vestibular stimulation initially depend on the stimulus, but in time become more homogenous across stimuli (Dietrich & Straka, 2016). As in the current study, this effect is dependent on the cerebellum, and ablations thereof eliminate both up- and downregulation of motor neuron output. While these results are largely congruent, two main differences exist. First, in the OKR the decision for up- or downregulation of gain depends on the initial motor response rather than the stimulus. This likely stems from the fact that the VOR receives no feedback about the efficacy of its motor output in the dark without the OKR closing the feedback loop, which only happens during OKR training (Collewijn, 1989; Straka & Dieringer, 2004). Therefore, the VOR can only operate based on the strength of its sensory input, whereas for the OKR, the adequacy of eye motion can be evaluated by visual feedback. The second difference is the observation that in case of the OKR, amplitude decreases did not require functional visual feedback to the cerebellum, which suggests that downregulation of OKR gain may be facilitated elsewhere in the brain, which will be discussed later in this thesis.

Behavioral data from my studies on embryonic development with only one eye provides further indication of a preference of consistency over full compensation. Under normal conditions, tadpoles show a per-eye asymmetry of the OKR response and a preference for temporally directed optokinetic motion. Monocular stimulus conditions heavily impair the temporal motion direction but not the nasal one, leading now to an inverse asymmetric OKR that preferentially follows nasal motion. While embryonically one-eyed tadpoles without anatomical aberrations compensate for the selective impairment of temporal motion, they did not restore to control conditions with a preferred temporal motion. Instead, the temporal OKR only improved to be similar to the nasal one, achieving a symmetric OKR in response to temporal and nasal motion. Thus, instead of restoring the maximal temporal response, only a partial compensation occurs which induces a now consistent response to both leftward and rightward stimuli (Forsthofer & Straka, 2022). These results are based on embryonic lesions with an inherent variability, and therefore allow only a weak link between cause and effect in comparison to optokinetic training on an unmanipulated circuit. One explanation is that the sensory input that is lost with one eye, and thus half the visual input, simply cannot be fully replaced by within-circuit plasticity such as increased synaptic efficacy or higher baseline firing rates. However, restoration of consistency instead of maximum responses provide an alternative explanation in line with the hypothesis of this thesis. Similar results, achieving a symmetric VOR instead of full compensation and indeed at the cost of the unimpaired motion direction, were previously gathered from embryonic unilateral ear removals and lend credibility

to the current study (Gordy & Straka, 2022). Symmetry of the remaining eye may be preferrable, as it ensures symmetric OKR response across the entire visual system, now comprised of only the remaining eye. Viewed from this angle, symmetry across the visual system is also the case in binocular animals: the directional preference of one eye is compensated for by the opposite direction preference of the other eye, and the compensation I observe does induce a restoration to control conditions in this regard. Symmetry is also the case in adult frogs, where the predominant motor output of gaze stabilization is provided by head and body motion, and thus like in one-eyed animals, by a single effector. Here, too, the head (unsurprisingly) performs symmetrical OKR under binocular conditions, and produces asymmetric OKR under monocular conditions due to an impairment of the nasal direction of the obstructed eye (Birukow, 1937; Jardon & Bonaventure, 1992a). As also observed in the current study, this impairment of the temporal direction is compensated for and OKR reverts to bilaterally symmetric head optokinetic nystagmus, showing both the ability of adult frogs to compensate for loss of one eye and symmetry as the preferred and expected output of visual gaze stabilization (Jardon & Bonaventure, 1992a, 1992b).

Both, optokinetic training and compensation for unilateral eye loss fall in line with the hypothesis that optokinetic plasticity in tadpoles may differ from what has been observed in many other vertebrates. In addition to homeostatic motor output after optokinetic training and restoration of symmetry in monocular tadpoles, the VOR is also subject to homeostatic control through the cerebellum, providing data from two different sensory systems and two different modes of causing plasticity (Dietrich & Straka, 2016). More recently, a modelling approach has been taken to explain the low gain of tadpole VOR. This model predicts that if sensory and motor noise, i.e. spontaneous activity in elements of the VOR circuit, are taken into account, a gain below unity is to be expected to efficiently minimize retinal image slip (Glasauer & Straka, 2022). Therefore, theoretical approaches give merit to experimental data in tadpoles suggesting the strive for an efficient, rather than perfect, gaze stabilization strategy, potentially with the benefit of maintaining a dynamic response range to further environmental changes.

From an eco-physiological point of view, such an approach is fitting for a transitory stage like the *Xenopus* tadpole. In most animals with continuous development, visual and motor improvements are accumulated and improved during development until adulthood, where a full behavioral repertoire is reached. This implies that immature animals display limited behavior (Distler & Hoffmann, 2011). However, tadpoles possess another difference to e.g. mice, in that they undergo a condensed period of rapid behavioral changes in metamorphosis. First, tadpoles are precocial animals, and from a very young stage require functional locomotor and sensory systems in order to survive, necessitating a very early onset

of the OKR (Lambert et al., 2008). This goes as far as that size-imposed limitations, such as non-functional semicircular canals in young larvae, being compensated for by changes in the locomotor pattern to substitute semicircular canal signals with utricular ones (Lambert et al., 2020). While one might assume that this immaturity decreases with age, this is not entirely true: Adult frogs are known to perform gaze stabilization predominantly through head and body motion, and show only limited eye motion (Birukow, 1937; Straka & Dieringer, 2004), and tadpoles approaching metamorphosis show a decrease in VOR and OKR performance past stage 55 (Schuller, 2017). It may well be that due to this low incentive to 'perfect' OKR performance throughout development, the more advantageous approach is to rapidly develop a 'good enough' OKR circuit with limited plasticity that is sufficient for reduction of retinal image slip. Instead, the resources required for formation of this circuit can be applied to maturation into a post-metamorphic frog, and to develop brain structures required for the novel behavioral repertoire required at this stage (Glasauer & Straka, 2022; Schuller, 2017). While this is a creative explanation, other possible reasons have been mentioned above in simply developmental immaturity of the OKR. Specific to embryonic lesion studies, plasticity may also simply not be potent enough to overcome erroneous sensory input caused by errors in connection formation, and leading to competing motor output. The following two chapters will therefore discuss the involvement of both the diencephalon/midbrain and the cerebellum in plastic changes of optokinetic behavior, as well as manipulation-induced limitations thereof.

Plasticity in the pretectum

Despite the cerebellum's major role as a plasticity-conveying area in the OKR, processing of sensory stimuli and the resultant motor output can be modulated at any neuron or synapse along the core OKR pathway. Besides distribution of plasticity mechanisms to where they may be most efficiently applied to e.g. selectively modulate sensory processing or motor output, this creates redundancy, where loss of a plasticity-associated brain area does not abolish any and all ability of adapting behavior (Drachman, 2005). Multiple studies in fish and frog confirmed that the pretectum and tectum are capable of plastic changes in optokinetic behaviors.

Plasticity in the diencephalon and midbrain

In the diencephalon of frogs, multiple studies suggest an involvement of the nLM in compensatory plasticity. Following the demonstration that frogs are unable to respond to temporally directed optokinetic stimuli upon suture of one eye, as well as the subsequent recovery of this impairment back to a bidirectional OKR after 8 days, multiple studies show the contribution of different neurotransmitters in the pretectum to a symmetric and asymmetric optokinetic response. Agonists of muscarinic acetylcholine receptors are sufficient to entirely

abolish asymmetry similarly to the one eye sutured condition and therefore show the same effect as one week of adaptation to unilateral sensory deprivation. Vice versa, agonists of GABA-receptors abolish the effect of adaptation and revert a compensated symmetric OKR back to one with no response to temporal stimuli (Jardon & Bonaventure, 1992a, 1992b; Yücel et al., 1990). While these experiments do not show that tuning of neurotransmitter release in the pretectum is indeed the mechanism involved in compensation, they would be sufficient to do so. More compelling is the observation that application of NMDA to one pretectal hemisphere following monocular deprivation accelerates the process of regaining a symmetrical optokinetic nystagmus (Jardon & Bonaventure, 1997). Relating this to the studies at hand, restoration of symmetry that is observed in embryonically generated one-eyed animals may involve pretectal mechanisms similar to the ones in adult frogs. Further, this implicates innate plasticity mechanisms to facilitate compensation in embryonically, as well as acute one-eyed animals, rather than embryonic plasticity. The fact that such compensation happened only in embryonically one-eyed tadpoles with no pathfinding changes corroborates this notion. Indeed, OKR performance of animals with aberrant ipsilateral retinotectal connectivity rather demonstrate that embryonic pathfinding errors impair functional compensation in the OKR. While these studies show that the pretectum is sufficient to mediate plasticity, and appropriate activation of either of the bilateral nLM is essential to facilitate it, initiation of plasticity may still require an evaluation of sensori-motor performance in other brain areas, such as the cerebellum (Straka et al., 2018).

A form of plasticity that does not seem to depend on the cerebellum is a gain decrease in the OKR, which still occurs in absence of functional climbing fiber connections to the cerebellum (Forsthofer & Straka, 2022). Zebrafish data suggests that the tectum is involved in OKR gain decreases through habituation based on electrophysiology studies (Perez-Schuster et al., 2016), and such habituation-associated neurons are also present in the nLM (Niu et al., 2006). These neurons are normally selective for one motion direction, and therefore likely involved in the OKR (Perez-Schuster et al., 2016; Thompson & Burr, 2009). Both these studies corroborate the finding that downregulation of gain can be conveyed without the cerebellum similarly to our observations in lesioned or immature tadpoles. The current hypothesis for gain regulation is therefore that gain-down regulation happens through habituation within the core OKR circuit, and ontogenetically likely appears together with the OKR (Lambert et al., 2008). Gain-up regulation is implemented later through maturation of cerebellar structures and allows for the full extent of OKR plasticity. While such gain-up regulation could also be involved in the selective recovery of temporal motion direction in one-eyed frogs, the hypothesis that the cerebellum indeed initiates plasticity following monocular

deprivation, or whether this is exclusively mediated by pretectal and tectal nuclei and their commisures, could not be tested in this thesis.

Detrimental effects of anatomical aberrations

Since adult frogs can compensate for the loss of one eye and previous studies show a large degree of activity-driven compensatory plasticity in embryonic visual development (Blackiston & Levin, 2013; Ruthazer & Cline, 2004), it is intriguing that non-canonical ipsilateral fibers are detrimental for behavioral compensation. The more RGCs project to ipsilateral, instead of contralateral midbrain targets, the less compensation occurs. At least in retinotectal development, genetics are likely the key players for gross connectivity to the correct nuclei, while function serves to fine-tune connectivity within nuclei. Those conclusions are suggested by a large body of literature on early visual deprivation or RGC pathfinding mutants in binocular animals (Baier, 2000; Barabási et al., 2022; Huang et al., 2006; Mumm et al., 2006). In this study, the high degree of observed phenotypic variability in chiasmatic crossing revealed a negative correlation between anatomical to behavioral variability and provides basis for a hypothesis on the potential mechanisms impairing OKR plasticity. Under binocular conditions, direction selectivity of each eye means that leftward motion is mainly detected by the right eye, which connects to nasal direction specific cells in the left nLM and drives leftward eye motions through ocular motor nuclei. Rightward motion is only weakly detected by the right eye, and from the contralateral nLM rightward eye motion is initiated through commissural fibers. The inverse of this is true for the left eye and the right nLM (Kubo et al., 2014; Naumann et al., 2016; Portugues et al., 2014). In animals with aberrant ipsilateral projections, the same eye now transmits temporal or nasal direction motion to both nLMs. Unilateral tectal ablations in embryonic frog and fish demonstrated that RGCs from the ipsilateral eye can connect to the ipsilateral tectum, and retain a retinotopic innervation pattern (Ramdya & Engert, 2008; Ruthazer & Cline, 2004). If, in monocular, rather than single-tectum miswiring, RGCs likewise innervate their appropriate targets in both brain hemispheres, this would mean that nasal-sensitive RGCs wire to nasal selective neurons in the ipsi-, as well as the contralateral nLM. These will then initiate competing eye motions to both the left and the right, cancelling nasally directed eye motions with an increasing fraction of ipsilateral fibers. This is not the case in our data for nasal motion, the response to which is unimpaired in one-eyed animals, and suggests that this effect is either counteracted by plasticity, or that nasal-sensitive RGCs are not miswired. Indeed, it is specifically temporal motion that is impaired and fails to be improved in animals with aberrant circuitry. Detection of motion in this direction is thought to depend on pretectal commissures from the contralateral nLM back to the ipsilateral one (Burgess et al., 2009; Portugues et al., 2014). Specifically, rightward motion activates temporal motion

sensitive inhibitory RGCs in the right eye, alleviating inhibition of the left nLM onto the right pretectum. This facilitates activity in the right pretectum, inducing rightward eye motion and a functional, albeit weak, OKR. In animals with bilateral RGC projections, reduced inhibition of the right nLM may be countered by direct inhibitory input of miswired RGCs, and impair eye motion, or reciprocal inhibition between the hemispheres may occur (Kubo et al., 2014; Wu et al., 2020). Future studies could test this hypothesis by recording the activity of the miswired fraction during OKR, to identify if the miswired RGCs and their axon show direction preference. Continued sensory input from miswired RGCs would persist to exhibit an influence of the bilateral complements of the nLM, and this may impair compensatory plasticity which does occur in animals with acute eye loss, and therefore absent activity in one of the bilateral nLM. The inability for compensation may suggest that plasticity happens only after the RGC-pretectum synapse by altering intra-pretectal signaling, which may be insufficient to overcome erroneous RGC input. Studies in zebrafish with entirely uncrossed retinotectal projections corroborate this, as in those cases, the OKR is entirely inverted (Huang et al., 2006). Overall, my data show that for compensation, anatomical miswiring may be more detrimental than the complete absence of functional connections to targets that lost their canonical sensory input. Normally complementary binocular integration now receives competing input, limiting plasticity of sensorimotor performance within the pretectum. Nonetheless, further plasticity may happen at other centers along the OKR pathway, such as the cerebellum.

Plasticity in the cerebellum

The cerebellum is uniquely poised in gaze-stabilization circuits due to sensory and motor convergence, allowing for assessment of appropriateness of gaze-stabilization (Ito, 2013; Knogler et al., 2019). This requires three key components: A motor or context signal from granule cells, a sensory instructive signal from the inferior olive, and following integration of the two, context-dependent activity of PCs. Previous studies on plasticity in frog gaze stabilization functionally removed cerebellar output, and while effectively abolishing cerebellar induced plasticity, also increased general eye motion responses due to the removed inhibitory PC influence (Dietrich & Straka, 2016; Pastor et al., 1994). My study employed a more selective approach in disconnecting specifically the visual instructive signal through climbing fiber lesion, leaving cerebellar output through PF-mediated PC activation intact (Forsthofer & Straka, 2022; Galliano et al., 2013; Knogler et al., 2017). This selective manipulation allowed identifying that in larval frogs, CF feedback is specifically associated with gain-up regulation.

While it is likely that this is achieved by reduction of PC firing and therefore decreased inhibition onto the OKR circuitry, plasticity initiated in the cerebellum is not permanently

maintained there. As discussed in the previous section, the cerebellum can, through deep cerebellar nuclei, induce plasticity in downstream nuclei themselves (Carcaud et al., 2017; de Barros et al., 2020). Projections from the cerebellar nucleus in frogs have been identified to target the midbrain, and could therefore induce plasticity there, the likes of which could be involved in restoring a symmetric OKR after monocular deprivation (Gonzalez et al., 1984; Jardon & Bonaventure, 1997). Such an involvement could be tested in future experiments, by inactivating the cerebellar circuit before functional maturation, and thus before cerebellar plasticity chronically manifests in its downstream targets (Van Alphen & De Zeeuw, 2002). On a shorter timescale, such as optokinetic training, the cerebellum itself is critical to induce timing or amplitude changes in optokinetic responses, and cerebellar lesions entirely abolish these effects. However, interspecies comparisons revealed that even in this direct cerebellar involvement, velocity storage centers in either the hindbrain or the midbrain were essential to initiate cerebellar plasticity (Miki et al., 2018; Miki et al., 2020; Sumbre et al., 2008). These examples demonstrate that the cerebellum may play a key role in initiating plasticity and is sufficient to induce it, but requires connectivity to other integrative nuclei, and eventually induces long-term plasticity in its target areas. This may, once again, serve to 1) distribute plasticity across the brain, creating redundancy, and 2) allow the cerebellum to maintain homeostasis of PC modulation rather than eventually exhausting its dynamic range of fine-tuning behavior.

All in all, previous literature as well as my own work highlight two key findings. First, while the cerebellum clearly takes a key role in plasticity of the OKR, it is embedded in a network of different sensory and motor nuclei that are likewise critical to establish and maintain plasticity, and that are usually more specific to a certain aspect of adaptation such as timing, symmetry or amplitude. Second, in *Xenopus* tadpoles, homeostasis rather than perfect compensation is a recurring pattern in gaze stabilization. Whether this is owed to their environment, which may display high variability and therefore encourage fast adaptation with no point of long-term adaptation, or to limitations in their optokinetic circuitry as they rapidly approach metamorphosis into a lifeform that displays little to no eye motion is not yet known, and warrants further eco-physiological investigation extending into metamorphic changes in this species.

Summary and outlook: The necessity of change.

In this thesis, I aimed to help unravel how plasticity is conveyed by the brain, and to what end. I did this in a species that is renowned for both its embryonic and developmental plasticity, dating back to experiments conducted by Roger Sperry (Sperry, 1956). It turns out that despite an incredible capability for circuit restructuring and behavioral adaptation, even in

this species, induced circuit reorganization can indeed lead to strong impairments of plasticity. This raises the question of how embryonic and adult plasticity mechanisms are interdependent, how induction of plasticity is weighted on sensory versus motor parts in sensorimotor circuits and how behavioral plasticity is limited by altered sensory input. The strong behavioral plasticity in this species also appears to be geared towards keeping behavior constant, rather than perfectly adapting the tadpole to its surroundings. Especially this second aspect begs the question of how plasticity mechanisms act to maintain the behavior of tadpoles constant, despite their ever evolving neuronal circuits as they approach metamorphosis into an adult frog with an entirely different lifestyle and behavioral repertoire. For future studies, it is therefore highly intriguing to extend the snapshots taken by investigating tadpoles at the beginning and end of their rather 'stable' larval phase and investigate plasticity at their metamorphic peak, ideally involving functional recordings and potential connectivity changes from the sensory nLM to new downstream motor targets.

Cerebellar maturation and its role in homeostatic plasticity also warrant investigation. As up-regulation of OKR gain has been specifically tied to cerebellar maturation in tadpoles, the developmental trajectory of up- versus downregulation is still unclear. Continuation of OKR training through later developmental stages could reveal whether, with ongoing maturation of the cerebellum, adult stages of *Xenopus* eventually conform with most vertebrates and begin to respond to optokinetic training with gain increases rather than homeostasis. Indeed, metamorphosis presents itself as a pinnacle of plasticity, shifting a freely swimming prey species to a bottom-dwelling predator with no intermediate resting or pupal stage. Thus, here, an interesting interplay of large scale changes in neural circuits, facilititating novel behaviors such as limb-based locomotion, prey-capture or head-based gaze stabilization coincides with continued maintenance of essential sensory functions such as predator avoidance, motion detection and so on.

Any of these potential follow-up studies serve to showcase the potential of *Xenopus* as a model of behavioral plasticity. Despite its employment as an embryonic model, mostly with anatomical or genetic questions in mind, there is clear potential to investigate the behavioral consequences of embryonic changes in mature, behaving tadpoles. *Xenopus* in this study provides useful insights into how plasticity is gradually manifested throughout development, how it depends on correct wiring of sensory input, and perhaps most intriguingly, what the aim of plasticity might be in a transitory life stage, approaching major restructuring of the visual system.

REFERENCES

Ahrens, M. B., Li, J. M., Orger, M. B., Robson, D. N., Schier, A. F., Engert, F., & Portugues, R. (2012). Brain-wide neuronal dynamics during motor adaptation in zebrafish. *Nature*, *485*(7399), 471-477.

Albus, J. S. (1971). A theory of cerebellar function. *Mathematical Biosciences 10*(1-2), 25-61.

Anderson, D. J. (2016). Circuit modules linking internal states and social behaviour in flies and mice. *Nature Reviews Neuroscience*, *17*(11), 692-704.

Angelaki, D. E., & Cullen, K. E. (2008). Vestibular system: the many facets of a multimodal sense. *Annual Review of Neuroscience*, *31*, 125-150.

Baden, T., Berens, P., Franke, K., Román Rosón, M., Bethge, M., & Euler, T. (2016). The functional diversity of retinal ganglion cells in the mouse. *Nature*, *529*(7586), 345-350.

Baier, H. (2000). Combining physiology and genetics in the zebrafish retina. *The Journal of Physiology*, *524*(Pt 1), 1.

Baird, R. (1992). Morphological and electrophysiological properties of hair cells in the bullfrog utriculus. *Annals of the New York Academy of Sciences*, *656*(1), 12-26.

Balleine, B. W. (2019). The meaning of behavior: discriminating reflex and volition in the brain. *Neuron*, *104*(1), 47-62.

Barabási, D. L., Schuhknecht, G. F., & Engert, F. (2022). Nature over nurture: Functional neuronal circuits emerge in the absence of developmental activity. *BioRxiv*, 2022.2010. 2024.513526.

Barlow, H., & Levick, W. R. (1965). The mechanism of directionally selective units in rabbit's retina. *The Journal of Physiology*, *178*(3), 477.

Bath, A., Harris, N., & Yardley, M. (1998). The vestibulo-collic reflex. *Clinical Otolaryngology & Allied Sciences*, *23*(5), 462-466.

Bauer, J., Weiler, S., Fernholz, M. H., Laubender, D., Scheuss, V., Hübener, M., Bonhoeffer, T., & Rose, T. (2021). Limited functional convergence of eye-specific inputs in the retinogeniculate pathway of the mouse. *Neuron*, *109*(15), 2457-2468. e2412.

Beazley, L. (1975). Factors determining decussation at the optic chiasma by developing retinotectal fibres in Xenopus. *Experimental Brain Research*, *23*, 491-504.

Bechterew, W. v. (1883). Ergebnisse der Durchschneidung des N. acusticus, nebst Erörterung der Bedeutung der semicirculären Canäle für das Körpergleichgewicht. *Archiv für die gesamte Physiologie des Menschen und der Tiere*, *30*(1), 312-347.

Beck, J. C., Gilland, E., Tank, D. W., & Baker, R. (2004). Quantifying the ontogeny of optokinetic and vestibuloocular behaviors in zebrafish, medaka, and goldfish. *Journal of Neurophysiology*, *92*(6), 3546-3561.

Benson, A. (1990). Sensory functions and limitations of the vestibular system. In R. Warren & A. H. Wertheim (Eds.), *Perception and Control of Self-motion* (pp. 145-170).

Birukow, G. (1937). Untersuchungen über den optischen Drehnystagmus und über die Sehschärfe des Grasfrosches (Rana temporaria). *Zeitschrift für vergleichende Physiologie*, *25*(1), 92-142.

Blackiston, D. J., & Levin, M. (2013). Ectopic eyes outside the head in Xenopus tadpoles provide sensory data for light-mediated learning. *Journal of Experimental Biology*, *216*(6), 1031-1040.

Blanks, R. H., Clarke, R. J., Lui, F., Giolli, R. A., Van Pham, S., & Torigoe, Y. (1995). Projections of the lateral terminal accessory optic nucleus of the common marmoset (Callithrix jacchus). *Journal of Comparative Neurology*, *354*(4), 511-532.

Boyden, E. S., Katoh, A., & Raymond, J. L. (2004). Cerebellum-dependent learning: the role of multiple plasticity mechanisms. *Annual Review of Neuroscience 27*, 581-609.

Branoner, F., Chagnaud, B. P., & Straka, H. (2016). Ontogenetic development of vestibulo-ocular reflexes in amphibians. *Frontiers in Neural Circuits 10*, 91.

Branoner, F., & Straka, H. (2018). Semicircular canal influences on the developmental tuning of the translational vestibulo-ocular reflex. *Frontiers in Neurology*, *9*, 404.
Brecha, N., Karten, H., & Hunt, S. (1980). Projections of the nucleus of the basal optic root in the pigeon: an autoradiographic and horseradish peroxidase study. *Journal of Comparative Neurology*, *189*(4), 615-670.
Britto, L. (1983). Retinal ganglion cells of the pigeon accessory optic system. *Brazilian Journal of Medical and Biological Research= Revista Brasileira de Pesquisas Medicas e Biologicas*, *16*(4), 357-363.
Buhl, E. H., & Peichl, L. (1986). Morphology of rabbit retinal ganglion cells projecting to the medial terminal nucleus of the accessory optic system. *Journal of Comparative Neurology*, *253*(2), 163-174.
Burgess, H. A., Johnson, S. L., & Granato, M. (2009). Unidirectional startle responses and disrupted left–right co-ordination of motor behaviors in robo3 mutant zebrafish. *Genes, Brain and Behavior*, *8*(5), 500-511.
Burrill, J. D., & Easter Jr, S. S. (1994). Development of the retinofugal projections in the embryonic and larval zebrafish (Brachydanio rerio). *Journal of Comparative Neurology*, *346*(4), 583-600.
Burt, A., & Flohr, H. (1991). Role of the visual input in recovery of function following unilateral vestibular lesion in the goldfish. I. Short-term behavioural changes. *Behavioural brain research*, *42*(2), 201-211.
Büttner-Ennever, J., & Büttner, U. (1992). Neuroanatomy of the ocular motor pathways. *Bailliere's clinical neurology*, *1*(2), 263-287.
Büttner-Ennever, J., & Akert, K. (1981). Medial rectus subgroups of the oculomotor nucleus and their abducens internuclear input in the monkey. *Journal of Comparative Neurology*, *197*(1), 17-27.
Calhoun, A., & Hady, A. E. (2021). What is behavior? No seriously, what is it? *BioRxiv*, 2021.2007. 2004.451053.
Carcaud, J., de Barros, F. F., Idoux, E., Eugène, D., Reveret, L., Moore, L. E., Vidal, P.-P., & Beraneck, M. (2017). Long-lasting visuo-vestibular mismatch in freely-behaving mice reduces the vestibulo-ocular reflex and leads to neural changes in the direct vestibular pathway. *Eneuro*, *4*(1), ENEURO.0290–0216.2017.
Carew, T. J., Walters, E. T., & Kandel, E. R. (1981). Classical conditioning in a simple withdrawal reflex in Aplysia californica. *Journal of Neuroscience*, *1*(12), 1426-1437.
Chagnaud, B. P., Simmers, J., & Straka, H. (2012). Predictability of visual perturbation during locomotion: implications for corrective efference copy signaling. *Biological Cybernetics 106*(11), 669-679.
Chan, S., & Guillery, R. (1993). Developmental changes produced in the retinofugal pathways of rats and ferrets by early monocular enucleations: the effects of age and the differences between normal and albino animals. *Journal of Neuroscience*, *13*(12), 5277-5293.
Chan, S. O., Chung, K., & Taylor, J. (1999). The effects of early prenatal monocular enucleation on the routing of uncrossed retinofugal axons and the cellular environment at the chiasm of mouse embryos. *European Journal of Neuroscience*, *11*(9), 3225-3235.
Chang, W., Brigande, J. V., Fekete, D. M., & Wu, D. K. (2004). The development of semicircular canals in the inner ear: role of FGFs in sensory cristae. *Development*, *131*(17), 4201–4211.
Cima, C., & Grant, P. (1980). Ontogeny of the retina and optic nerve of Xenopus laevis: IV. Ultrastructural evidence of early ganglion cell differentiation. *Developmental Biology*, *76*(1), 229-237.
Clément, G., & Reschke, M. F. (2008). Sensory Functions in Space. In G. Clément & M. F. Reschke (Eds.), *Neuroscience in Space* (pp. 101-132).
Cline, H. T. (1991). Activity-dependent plasticity in the visual systems of frogs and fish. *Trends in Neurosciences*, *14*(3), 104-111.

Cochran, S., Dieringer, N., & Precht, W. (1984). Basic optokinetic-ocular reflex pathways in the frog. *Journal of Neuroscience*, *4*(1), 43-57.
Collewijn, H. (1989). The vestibulo-ocular reflex: is it an independent subsystem? *Revue Neurologique 145*(8-9), 502-512.
Collewijn, H., & Grootendorst, A. (1979). Adaptation of optokinetic and vestibulo-ocular reflexes to modified visual input in the rabbit. In R. Granit & O. Pompeiano (Eds.), *Progress in Brain Research* (Vol. 50, pp. 771-781). Elsevier.
Constantine-Paton, M., & Law, M. I. (1978). Eye-specific termination bands in tecta of three-eyed frogs. *Science*, *202*(4368), 639-641.
Cruz-Martín, A., El-Danaf, R. N., Osakada, F., Sriram, B., Dhande, O. S., Nguyen, P. L., Callaway, E. M., Ghosh, A., & Huberman, A. D. (2014). A dedicated circuit links direction-selective retinal ganglion cells to the primary visual cortex. *Nature*, *507*(7492), 358-361.
Cullen, K. E., & Zobeiri, O. A. (2021). Proprioception and the predictive sensing of active self-motion. *Current Opinion in Physiology 20*, 29-38.
Curthoys, I. S. (2000). Vestibular compensation and substitution. *Current Opinion in Neurobiology 13*(1), 27-30.
Darwin, C. (1859). *On the origin of species*. Routledge.
Day, B. L., & Fitzpatrick, R. C. (2005). The vestibular system. *Current Biology*, *15*(15), R583-R586.
de Barros, F. F., Schenberg, L., Tagliabue, M., & Beraneck, M. (2020). Long term visuo-vestibular mismatch in freely behaving mice differentially affects gaze stabilizing reflexes. *Scientific Reports 10*(1), 1-12.
de Burlet, H. (1934). Handbuch der vergleichenden Anatomie der Wirbeltiere. *Bd. II/2*.
Dehmelt, F. A., Meier, R., Hinz, J., Yoshimatsu, T., Simacek, C. A., Huang, R., Wang, K., Baden, T., & Arrenberg, A. B. (2021). Spherical arena reveals optokinetic response tuning to stimulus location, size, and frequency across entire visual field of larval zebrafish. *Elife*, *10*, e63355.
Dewey, J. (1896). The reflex arc concept in psychology. *Psychological Review 3*(4), 357.
Dews, P., & Wiesel, T. (1970). Consequences of monocular deprivation on visual behaviour in kittens. *The Journal of Physiology*, *206*(2), 437-455.
Dhande, O. S., Estevez, M. E., Quattrochi, L. E., El-Danaf, R. N., Nguyen, P. L., Berson, D. M., & Huberman, A. D. (2013). Genetic dissection of retinal inputs to brainstem nuclei controlling image stabilization. *Journal of Neuroscience*, *33*(45), 17797-17813.
Dichgans, J., & Brandt, T. (1978). Visual-vestibular interaction: Effects on self-motion perception and postural control. In R. Held, H. W. Leibowitz, & H.-L. Teuber (Eds.), *Perception* (pp. 755-804). Springer.
Dieringer, N. (1995). 'Vestibular compensation': neural plasticity and its relations to functional recovery after labyrinthine lesions in frogs and other vertebrates. *Progress in Neurobiology 46*(2-3), 97-129.
Dietrich, H., & Straka, H. (2016). Prolonged vestibular stimulation induces homeostatic plasticity of the vestibulo-ocular reflex in larval Xenopus laevis. *European Journal of Neuroscience*, *44*(1), 1787-1796.
Ding, H., Smith, R. G., Poleg-Polsky, A., Diamond, J. S., & Briggman, K. L. (2016). Species-specific wiring for direction selectivity in the mammalian retina. *Nature*, *535*(7610), 105-110.
Dingwell, K. S., Holt, C. E., & Harris, W. A. (2000). The multiple decisions made by growth cones of RGCs as they navigate from the retina to the tectum in Xenopus embryos. *Journal of Neurobiology*, *44*(2), 246-259.
Distler, C., & Hoffmann, K.-P. (1992). Early development of the subcortical and cortical pathway involved in optokinetic nystagmus: the cat as a model for man? *Behavioural Brain Research 49*(1), 69-75.
Distler, C., & Hoffmann, K.-P. (2011). The optokinetic reflex. In S. P. Liversedge, I. D. Gilchrist, & S. Everling (Eds.), *The Oxford handbook of eye movements* (pp. 65-83).
Drachman, D. A. (2005). Do we have brain to spare? *Neurology*, *64*(12), 2004-2005.

Eberhorn, A. C., Büttner-Ennever, J. A., & Horn, A. K. (2006). Identification of motoneurons supplying multiply-or singly-innervated extraocular muscle fibers in the rat. *Neuroscience*, *137*(3), 891-903.
Eccles, J., Llinás, R., & Sasaki, K. (1966). The excitatory synaptic action of climbing fibres on the Purkinje cells of the cerebellum. *The Journal of Physiology*, *182*(2), 268-296.
Egelhaaf, M., Borst, A., & Reichardt, W. (1989). Computational structure of a biological motion-detection system as revealed by local detector analysis in the fly's nervous system. *Journal of the Optical Society of America A*, *6*(7), 1070-1087.
Ekerot, C.-F., & Jörntell, H. (2008). Synaptic integration in cerebellar granule cells. *The Cerebellum*, *7*, 539-541.
Elliott, K. L., & Fritzsch, B. (2018). Ear transplantations reveal conservation of inner ear afferent pathfinding cues. *Scientific Reports 8*(1), 13819.
Engelmann, J., Hanke, W., Mogdans, J., & Bleckmann, H. (2000). Hydrodynamic stimuli and the fish lateral line. *Nature*, *408*(6808), 51-52.
Engström, H., Ades, H. W., & Hawkins Jr, J. E. (1962). Structure and functions of the sensory hairs of the inner ear. *The Journal of the Acoustical Society of America*, *34*(9B), 1356-1363.
Faulstich, B. M., Onori, K. A., & Du Lac, S. (2004). Comparison of plasticity and development of mouse optokinetic and vestibulo-ocular reflexes suggests differential gain control mechanisms. *Vision Research 44*(28), 3419-3427.
Fite, K. V. (1969). Single-unit analysis of binocular neurons in the frog optic tectum. *Experimental Neurology*, *24*(4), 475-486.
Fite, K. V. (1985). Pretectal and accessory-optic visual nuclei of fish, amphibia and reptiles: Theme and variations (part 1 of 2) *Brain, Behavior and Evolution*, *26*(2), 71-80.
Fite, K. V., Reiner, A., & Hunt, S. P. (1979). Optokinetic nystagmus and the accessory optic system of pigeon and turtle. *Brain, Behavior and Evolution*, *16*(3), 192-202.
Forsthofer, M., & Straka, H. (2022). Homeostatic plasticity of eye movement performance in Xenopus tadpoles following prolonged visual image motion stimulation. *Journal of Neurology*, *270*(1), 57-70.
Franke, K., & Baden, T. (2017). General features of inhibition in the inner retina. *The Journal of Physiology*, *595*(16), 5507-5515.
Fraser, S. E., & Hunt, R. K. (1980). Retinotectal specificity: models and experiments in search of a mapping function. *Annual Review of Neuroscience 3*(1), 319-352.
Frost, B. (1978). The optokinetic basis of head-bobbing in the pigeon. *Journal of Experimental Biology*, *74*(1), 187-195.
Gaede, A. H., Goller, B., Lam, J. P., Wylie, D. R., & Altshuler, D. L. (2017). Neurons responsive to global visual motion have unique tuning properties in hummingbirds. *Current Biology*, *27*(2), 279-285.
Galliano, E., Gao, Z., Schonewille, M., Todorov, B., Simons, E., Pop, A. S., D'Angelo, E., Van Den Maagdenberg, A. M., Hoebeek, F. E., & De Zeeuw, C. I. (2013). Silencing the majority of cerebellar granule cells uncovers their essential role in motor learning and consolidation. *Cell Reports 3*(4), 1239-1251.
Gambrill, A. C., Faulkner, R. L., & Cline, H. T. (2018). Direct intertectal inputs are an integral component of the bilateral sensorimotor circuit for behavior in Xenopus tadpoles. *Journal of Neurophysiology*, *119*(5), 1947-1961.
García-Frigola, C., Carreres, M. I., Vegar, C., Mason, C., & Herrera, E. (2008). Zic2 promotes axonal divergence at the optic chiasm midline by EphB1-dependent and-independent mechanisms. *Development*, *135*(10), 1833-1841.
Gaze, R., & Straznicky, C. (1980). Regeneration of optic nerve fibres from a compound eye to both tecta in Xenopus: evidence relating to the state of specification of the eye and the tectum. *Development*, *60*(1), 125-140.
Gebhardt, C., Auer, T. O., Henriques, P. M., Rajan, G., Duroure, K., Bianco, I. H., & Del Bene, F. (2019). An interhemispheric neural circuit allowing binocular integration in the optic tectum. *Nature Communications 10*(1), 1-12.

Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology 21*(3), 394-407.
Giolli, R. A., Blanks, R. H., & Lui, F. (2006). The accessory optic system: basic organization with an update on connectivity, neurochemistry, and function. *Progress in Brain Research 151*, 407-440.
Gittis, A. H., & du Lac, S. (2006). Intrinsic and synaptic plasticity in the vestibular system. *Current Opinion in Neurobiology 16*(4), 385-390.
Glasauer, S., & Straka, H. (2022). Low gain values of the vestibulo-ocular reflex can optimize retinal image slip. *Frontiers in Neurology*, *13*.
Glastonbury, J., & Straznicky, K. (1978). Aberrant ipsilateral retinotectal projection following optic nerve section in Xenopus. *Neuroscience Letters 7*(1), 67-72.
Godement, P., Salaün, J., & Métin, C. (1987). Fate of uncrossed retinal projections following early or late prenatal monocular enucleation in the mouse. *Journal of Comparative Neurology*, *255*(1), 97-109.
Gonzalez, A., Ten Donkelaar, H., & De Boer-van Huizen, R. (1984). Cerebellar connections in Xenopus laevis: an HRP study. *Anatomy and Embryology 169*, 167-176.
Gordy, C., & Straka, H. (2021). Vestibular influence on vertebrate skeletal symmetry and body shape *Frontiers in Systems Neuroscience 15*, 753207.
Gordy, C., & Straka, H. (2022). Developmental eye motion plasticity after unilateral embryonic ear removal in Xenopus laevis. *Iscience*, *25*(10), 105165.
Gordy, C., Straka, H., Houston, D. W., Fritzsch, B., & Elliott, K. L. (2018). Transplantation of ears provides insights into inner ear afferent pathfinding properties. *Developmental Neurobiology 78*(11), 1064-1080.
Graf, W., Spencer, R., Baker, H., & Baker, R. (1997). Excitatory and inhibitory vestibular pathways to the extraocular motor nuclei in goldfish. *Journal of Neurophysiology*, *77*(5), 2765-2779.
Grant, S., & Keating, M. (1989). Changing patterns of binocular visual connections in the intertectal system during development of the frog, Xenopus laevis. *Experimental Brain Research*, *75*(1), 99-116.
Grüsser, O.-J., Grüsser-Cornehls, U., & Licker, M. (1968). Further studies on the velocity function of movement detecting class-2 neurons in the frog retina. *Vision Research 8*(9), 1173-1185.
Gudden, B. v. (1870). Experimentaluntersuchungen über das peripherische und centrale Nervensystem. *Archiv für Psychiatrie und Nervenkrankheiten*, *2*, 693-723.
Guillery, R. (1995). The optic chiasm of the vertebrate brain. In *Contributions to Sensory Physiology* (Vol. 7, pp. 39-73).
Hardcastle, B. J., & Krapp, H. G. (2016). Evolution of biological image stabilization. *Current Biology*, *26*(20), R1010-R1021.
Hassenstein, B., & Reichardt, W. (1956). Systemtheoretische analyse der zeit-, reihenfolgen-und vorzeichenauswertung bei der bewegungsperzeption des rüsselkäfers chlorophanus. *Zeitschrift für Naturforschung B*, *11*(9-10), 513-524.
Haverkamp, S., & Wässle, H. (2000). Immunocytochemical analysis of the mouse retina. *Journal of Comparative Neurology*, *424*(1), 1-23.
Hayhow, W. (1959). An experimental study of the accessory optic fiber system in the cat. *Journal of Comparative Neurology*, *113*(2), 281-313.
Herrera, E., & Garcia-Frigola, C. (2008). Genetics and development of the optic chiasm. *Frontiers in Bioscience*, *13*(5), 1646-1653.
Herrera, E. s., Brown, L., Aruga, J., Rachel, R. A., Dolen, G., Mikoshiba, K., Brown, S., & Mason, C. A. (2003). Zic2 patterns binocular vision by specifying the uncrossed retinal projection. *Cell*, *114*(5), 545-557.
Hettinger, L. J., Berbaum, K. S., Kennedy, R. S., Dunlap, W. P., & Nolan, M. D. (1990). Vection and simulator sickness. *Military Psychology 2*(3), 171-181.

Hoffmann, K.-P., Bremmer, F., Thiele, A., & Distler, C. (2002). Directional asymmetry of neurons in cortical areas MT and MST projecting to the NOT-DTN in macaques. *Journal of Neurophysiology*, *87*(4), 2113-2123.
Hoffmann, K.-P., & Schoppmann, A. (1975). Retinal input to direction selective cells in the nucleus tractus opticus of the cat. *Brain Research*, *99*(2), 359-366.
Holt, C. E. (1989). A single-cell analysis of early retinal ganglion cell differentiation in Xenopus: from soma to axon tip. *Journal of Neuroscience*, *9*(9), 3123-3145.
Höpker, V. H., Shewan, D., Tessier-Lavigne, M., Poo, M.-m., & Holt, C. (1999). Growth-cone attraction to netrin-1 is converted to repulsion by laminin-1. *Nature*, *401*(6748), 69-73.
Horn, A. K. (2020). Neuroanatomy of central vestibular connections. In B. Fritzsch & H. Straka (Eds.), *The Senses: a Comprehensive Reference* (Vol. 6, pp. 21-37).
Horn, A. K., & Hoffmann, K.-P. (1987). Combined GABA-immunocytochemistry and TMB-HRP histochemistry of pretectal nuclei projecting to the inferior olive in rats, cats and monkeys. *Brain Research 409*(1), 133-138.
Horn, A. K., & Straka, H. (2021). Functional organization of extraocular motoneurons and eye muscles. *Annual Review of Vision Science*, *7*, 793-825.
Hoskins, S., & Grobstein, P. (1985). Development of the ipsilateral retinothalamic projection in the frog Xenopus laevis. II. Ingrowth of optic nerve fibers and production of ipsilaterally projecting retinal ganglion cells. *Journal of Neuroscience*, *5*(4), 920-929.
Hua, J. Y., Smear, M. C., Baier, H., & Smith, S. J. (2005). Regulation of axon growth in vivo by activity-based competition. *Nature*, *434*(7036), 1022-1026.
Huang, C.-C., Sugino, K., Shima, Y., Guo, C., Bai, S., Mensh, B. D., Nelson, S. B., & Hantman, A. W. (2013). Convergence of pontine and proprioceptive streams onto multimodal cerebellar granule cells. *Elife*, *2*, e00400.
Huang, Y.-Y., Rinner, O., Hedinger, P., Liu, S.-C., & Neuhauss, S. C. (2006). Oculomotor instabilities in zebrafish mutant belladonna: a behavioral model for congenital nystagmus caused by axonal misrouting. *Journal of Neuroscience*, *26*(39), 9873-9880.
Hudspeth, A., & Corey, D. (1977). Sensitivity, polarity, and conductance change in the response of vertebrate hair cells to controlled mechanical stimuli. *Proceedings of the National Academy of Sciences*, *74*(6), 2407-2411.
Hudspeth, A., & Jacobs, R. (1979). Stereocilia mediate transduction in vertebrate hair cells (auditory system/cilium/vestibular system). *Proceedings of the National Academy of Sciences*, *76*(3), 1506-1509.
Ilg, U. J. (1997). Slow eye movements. *Progress in Neurobiology 53*(3), 293-329.
Ito, M. (1982). Cerebellar control of the vestibulo-ocular reflex--around the flocculus hypothesis. *Annual Review of Neuroscience 5*(1), 275-297.
Ito, M. (2006). Cerebellar circuitry as a neuronal machine. *Progress in Neurobiology 78*(3-5), 272-303.
Ito, M. (2013). Error detection and representation in the olivo-cerebellar system. *Frontiers in Neural Circuits 7*, 1.
Jardon, B., & Bonaventure, N. (1992a). Plasticity of the frog monocular OKN: involvement of pretectal GABAergic and cholinergic systems. *Neuroscience Letters 137*(2), 189-192.
Jardon, B., & Bonaventure, N. (1992b). The pretectal cholinergic system is involved through two opposite ways in frog monocular OKN asymmetry. *Experimental Brain Research 90*, 72-78.
Jardon, B. L., & Bonaventure, N. (1997). Involvement of NMDA in a plasticity phenomenon observed in the adult frog monocular optokinetic nystagmus. *Vision Research 37*(11), 1511-1524.
Jeffery, G. (2001). Architecture of the optic chiasm and the mechanisms that sculpt its development. *Physiological Reviews*, *81*(4), 1393-1414.
Jeffery, G., & Erskine, L. (2005). Variations in the architecture and development of the vertebrate optic chiasm. *Progress in Retinal and Eye Research 24*(6), 721-753.
Jørgensen, J., Shichiri, M., & Geneser, F. (1998). Morphology of the hagfish inner ear. *Acta Zoologica*, *79*(3), 251-256.

Karlstrom, R. O., Trowe, T., Klostermann, S., Baier, H., Brand, M., Crawford, A. D., Grunewald, B., Haffter, P., Hoffmann, H., & Meyer, S. U. (1996). Zebrafish mutations affecting retinotectal axon pathfinding. *Development*, *123*(1), 427-438.
Katz, L. C., & Shatz, C. J. (1996). Synaptic activity and the construction of cortical circuits. *Science*, *274*(5290), 1133-1138.
Keating, M., & Feldman, J. D. (1975). Visual deprivation and intertectal neuronal connexions in Xenopus laevis. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, *191*(1105), 467-474.
Kennard, C. (1981). Factors involved in the development of ipsilateral retinothalamic projections in Xenopus laevis. *Journal of Embryology and Experimental Morphology*, *65*(1), 199-217.
Kim, J. S., Greene, M. J., Zlateski, A., Lee, K., Richardson, M., Turaga, S. C., Purcaro, M., Balkam, M., Robinson, A., & Behabadi, B. F. (2014). Space–time wiring specificity supports direction selectivity in the retina. *Nature*, *509*(7500), 331-336.
Kist, A. M., & Portugues, R. (2019). Optomotor swimming in larval zebrafish is driven by global whole-field visual motion and local light-dark transitions. *Cell Reports 29*(3), 659-670. e653.
Klar, M., & Hoffmann, K.-P. (2002). Visual direction-selective neurons in the pretectum of the rainbow trout. *Brain Research Bulletin 57*(3-4), 431-433.
Knogler, L. D., Kist, A. M., & Portugues, R. (2019). Motor context dominates output from purkinje cell functional regions during reflexive visuomotor behaviours. *Elife*, *8*, e42138.
Knogler, L. D., Markov, D. A., Dragomir, E. I., Štih, V., & Portugues, R. (2017). Sensorimotor representations in cerebellar granule cells in larval zebrafish are dense, spatially organized, and non-temporally patterned. *Current Biology*, *27*(9), 1288-1302.
Knorr, A., Gravot, C., Straka, H., & Glasauer, S. (2017). I spy with my little eye: A simple behavioral assay to test color perception in animal virtual reality setups. *Journal of Vision*, *17*(10), 661-661.
Koser, D. E., Thompson, A. J., Foster, S. K., Dwivedy, A., Pillai, E. K., Sheridan, G. K., Svoboda, H., Viana, M., Costa, L. d. F., & Guck, J. (2016). Mechanosensing is critical for axon growth in the developing brain. *Nature Neuroscience 19*(12), 1592-1598.
Kubo, F., Hablitzel, B., Dal Maschio, M., Driever, W., Baier, H., & Arrenberg, A. B. (2014). Functional architecture of an optic flow-responsive area that drives horizontal eye movements in zebrafish. *Neuron*, *81*(6), 1344-1359.
Lac, S., Raymond, J. L., Sejnowski, T. J., & Lisberger, S. G. (1995). Learning and memory in the vestibulo-ocular reflex. *Annual Review of Neuroscience 18*(1), 409-441.
Lambert, F. M., Bacqué-Cazenave, J., Le Seach, A., Arama, J., Courtand, G., Tagliabue, M., Eskiizmirliler, S., Straka, H., & Beraneck, M. (2020). Stabilization of gaze during early Xenopus development by swimming-related utricular signals. *Current Biology*, *30*(4), 746-753. e744.
Lambert, F. M., Beck, J. C., Baker, R., & Straka, H. (2008). Semicircular canal size determines the developmental onset of angular vestibuloocular reflexes in larval Xenopus. *Journal of Neuroscience*, *28*(32), 8086-8095.
Land, M. (2019). Eye movements in man and other animals. *Vision Research 162*, 1-7.
Land, M. F. (2015). Eye movements of vertebrates and their relation to eye form and function. *Journal of Comparative Physiology A*, *201*(2), 195-214.
Lázár, G. (1972). Role of the accessory optic system in the optokinetic nystagmus of the frog. *Brain, Behavior and Evolution*, *5*(6), 443-460.
Lázár, G. (1983). Transection of the basal optic root in the frog abolishes vertical optokinetic head-nystagmus. *Neuroscience Letters 43*(1), 7-11.
Lettvin, J. Y., Maturana, H. R., McCulloch, W. S., & Pitts, W. H. (1959). What the frog's eye tells the frog's brain. *Proceedings of the IRE*, *47*(11), 1940-1951.
Lev-Ram, V., Wong, S. T., Storm, D. R., & Tsien, R. Y. (2002). A new form of cerebellar long-term potentiation is postsynaptic and depends on nitric oxide but not cAMP. *Proceedings of the National Academy of Sciences*, *99*(12), 8389-8393.

Lewis, E., & Li, C. (1975). Hair cell types and distributions in the otolithic and auditory organs of the bullfrog. *Brain Research*, *83*(1), 35-50.
Li, Z., & Fite, K. V. (2001). GABAergic visual pathways in the frog Rana pipiens. *Visual Neuroscience 18*(3), 457-464.
Lisberger, S., & Fuchs, A. (1978). Role of primate flocculus during rapid behavioral modification of vestibuloocular reflex. I. Purkinje cell activity during visually guided horizontal smooth-pursuit eye movements and passive head rotation. *Journal of Neurophysiology*, *41*(3), 733-763.
Lisberger, S., Miles, F., & Optican, L. (1983). Frequency-selective adaptation: evidence for channels in the vestibulo-ocular reflex? *Journal of Neuroscience*, *3*(6), 1234-1244.
Lisberger, S., Miles, F., & Zee, D. (1984). Signals used to compute errors in monkey vestibuloocular reflex: possible role of flocculus. *Journal of Neurophysiology*, *52*(6), 1140-1153.
Llinás, R., & Sugimori, M. (1980). Electrophysiological properties of in vitro Purkinje cell somata in mammalian cerebellar slices. *The Journal of Physiology*, *305*(1), 171-195.
Lupandin, A. (2005). Effect of flow turbulence on swimming speed of fish. *Biology Bulletin*, *32*, 461-466.
Macé, É., Montaldo, G., Trenholm, S., Cowan, C., Brignall, A., Urban, A., & Roska, B. (2018). Whole-brain functional ultrasound imaging reveals brain modules for visuomotor integration. *Neuron*, *100*(5), 1241-1251. e1247.
Maekawa, K., & Takeda, T. (1979). Origin of descending afferents to the rostral part of dorsal cap of inferior olive which transfers contralateral optic activities to the flocculus. A horseradish peroxidase study. *Brain Research*, *172*(3), 393-405.
Magherini, P., Giretti, M., & Precht, W. (1975). Cerebellar control of vestibular neurons of the frog. *Pflugers Archiv: European Journal of Physiology*, *356*(2), 99-109.
Marr, D. (1969). A theory of cerebellar cortex. *The Journal of Physiology*, *202*, 437-470.
Marsh, E., & Baker, R. (1997). Normal and adapted visuooculomotor reflexes in goldfish. *Journal of Neurophysiology*, *77*(3), 1099-1118.
Masseck, O. A., & Hoffmann, K. P. (2009). Comparative neurobiology of the optokinetic reflex. *Annals of the New York Academy of Sciences*, *1164*(1), 430-439.
Matesz, C., & Székely, G. (1977). The dorsomedial nuclear group of cranial nerves in the frog. *Acta Biologica Academiae Scientiarum Hungaricae*, *28*(4), 461-474.
Matsuda, K., & Kubo, F. (2021). Circuit organization underlying optic flow processing in zebrafish. *Frontiers in Neural Circuits 15*, 709048.
Matsui, H., Namikawa, K., Babaryka, A., & Köster, R. W. (2014). Functional regionalization of the teleost cerebellum analyzed in vivo. *Proceedings of the National Academy of Sciences*, *111*(32), 11846-11851.
Matsumoto, A., Briggman, K. L., & Yonehara, K. (2019). Spatiotemporally asymmetric excitation supports mammalian retinal motion sensitivity. *Current Biology*, *29*(19), 3277-3288. e3275.
Mauss, A. S., Vlasits, A., Borst, A., & Feller, M. (2017). Visual circuits for direction selectivity. *Annual Review of Neuroscience 40*, 211-230.
McLaughlin, T., & O'Leary, D. D. (2005). Molecular gradients and development of retinotopic maps. *Annual Review of Neuroscience 28*, 327-355.
Michnovicz, J., & Bennett, M. (1987). Effects of rapid cerebellectomy on adaptive gain control of the vestibulo-ocular reflex in alert goldfish. *Experimental Brain Research*, *66*, 287-294.
Miki, S., Baker, R., & Hirata, Y. (2018). Cerebellar role in predictive control of eye velocity initiation and termination. *Journal of Neuroscience*, *38*(48), 10371-10383.
Miki, S., Urase, K., Baker, R., & Hirata, Y. (2020). Velocity storage mechanism drives a cerebellar clock for predictive eye velocity control. *Scientific Reports 10*(1), 1-13.
Montgomery, N. M., Fite, K. V., & Grigonis, A. M. (1985). The pretectal nucleus lentiformis mesencephali of Rana pipiens. *Journal of Comparative Neurology*, *234*(2), 264-275.

Mumm, J. S., Williams, P. R., Godinho, L., Koerber, A., Pittman, A. J., Roeser, T., Chien, C.-B., Baier, H., & Wong, R. O. (2006). In vivo imaging reveals dendritic targeting of laminated afferents by zebrafish retinal ganglion cells. *Neuron*, *52*(4), 609-621.

Murcia-Belmonte, V., & Erskine, L. (2019). Wiring the binocular visual pathways. *International Journal of Molecular Sciences*, *20*(13), 3282.

Nakagawa, S., Brennan, C., Johnson, K. G., Shewan, D., Harris, W. A., & Holt, C. E. (2000). Ephrin-B regulates the Ipsilateral routing of retinal axons at the optic chiasm. *Neuron*, *25*(3), 599-610.

Naumann, E. A., Fitzgerald, J. E., Dunn, T. W., Rihel, J., Sompolinsky, H., & Engert, F. (2016). From whole-brain data to functional circuit models: the zebrafish optomotor response. *Cell*, *167*(4), 947-960. e920.

Nevin, L. M., Robles, E., Baier, H., & Scott, E. K. (2010). Focusing on optic tectum circuitry through the lens of genetics. *BMC Biology 8*(1), 1-10.

Niell, C. M., & Smith, S. J. (2005). Functional imaging reveals rapid development of visual response properties in the zebrafish tectum. *Neuron*, *45*(6), 941-951.

Nieuwkoop, P. D., & Faber, J. (1994). *Normal table of Xenopus laevis (Daudin): A systematical and chronological survey of the development from the fertilized egg till the end of metamorphosis* Garland Publishing Inc.

Niu, Y. Q., Xiao, Q., Liu, R. F., Wu, L. Q., & Wang, S. R. (2006). Response characteristics of the pigeon's pretectal neurons to illusory contours and motion. *The Journal of Physiology*, *577*(3), 805-813.

Oster, S., Deiner, M., Birgbauer, E., & Sretavan, D. (2004). Ganglion cell axon pathfinding in the retina and optic nerve. Seminars in cell & developmental biology,

Oyster, C. W., Simpson, J. I., Takahashi, E. S., & Soodak, R. E. (1980). Retinal ganglion cells projecting to the rabbit accessory optic system. *Journal of Comparative Neurology*, *190*(1), 49-61.

Pak, W., Hindges, R., Lim, Y.-S., Pfaff, S. L., & O'Leary, D. D. (2004). Magnitude of binocular vision controlled by islet-2 repression of a genetic program that specifies laterality of retinal axon pathfinding. *Cell*, *119*(4), 567-578.

Pastor, A. M., de la Cruz, R. R., & Baker, R. (1994). Cerebellar role in adaptation of the goldfish vestibuloocular reflex. *Journal of Neurophysiology*, *72*(3), 1383-1394.

Pastor, A. M., Rosa, R., & Baker, R. (1997). Characterization of purkinje cells in the goldfish cerebellum during eye movement and adaptive modification of the vestibulo-ocular reflex *Progress in Brain Research 114*, 359-381.

Perez-Schuster, V., Kulkarni, A., Nouvian, M., Romano, S. A., Lygdas, K., Jouary, A., Dipoppa, M., Pietri, T., Haudrechy, M., & Candat, V. (2016). Sustained rhythmic brain activity underlies visual motion perception in zebrafish. *Cell Reports 17*(4), 1098-1112.

Petros, T. J., Rebsam, A., & Mason, C. A. (2008). Retinal axon growth at the optic chiasm: to cross or not to cross. *Annual Review of Neuroscience 31*(1), 295-315.

Pham, N. C., Kim, Y. G., Kim, S. J., & Kim, C.-H. (2020). Differential effects of inferior olive lesion on vestibulo-ocular and optokinetic motor learning. *NeuroReport*, *31*(1), 9-16.

Platt, C., & Straka, H. (2020). Vestibular endorgans in vertebrates and adequate sensory stimuli. In B. Fritzsch & H. Straka (Eds.), *The Senses: A Comprehensive Reference* (Vol. 6, pp. 108-128).

Poggio, G. F., & Poggio, T. (1984). The analysis of stereopsis. *Annual Review of Neuroscience 7*(1), 379-412.

Popper, A. N., Ramcharitar, J., & Campana, S. E. (2005). Why otoliths? Insights from inner ear physiology and fisheries biology. *Marine and Freshwater Research 56*(5), 497-504.

Portugues, R., Feierstein, C. E., Engert, F., & Orger, M. B. (2014). Whole-brain activity maps reveal stereotyped, distributed networks for visuomotor behavior. *Neuron*, *81*(6), 1328-1343.

Precht, W. (1978). *Neuronal operations in the vestibular system* (Vol. 2). Springer Science & Business Media.

Precht, W., & Llinás, R. (1969). Functional organization of the vestibular afferents to the cerebellar cortex of frog and cat. *Experimental Brain Research*, *9*, 30-52.
Prusky, G. T., Alam, N. M., & Douglas, R. M. (2006). Enhancement of vision by monocular deprivation in adult mice. *Journal of Neuroscience*, *26*(45), 11554-11561.
Qian, H., Zhu, Y., Ramsey, D. J., Chappell, R. L., Dowling, J. E., & Ripps, H. (2005). Directional asymmetries in the optokinetic response of larval zebrafish (Danio rerio). *Zebrafish*, *2*(3), 189-196.
Rabbitt, R. D., Damiano, E. R., & Grant, J. W. (2004). Biomechanics of the semicircular canals and otolith organs. In S. M. Highstein, R. R. Fay, & A. N. Popper (Eds.), *The Vestibular System* (Vol. 19, pp. 153-201).
Ramdya, P., & Engert, F. (2008). Emergence of binocular functional properties in a monocular neural circuit. *Nature Neuroscience 11*(9), 1083-1090.
Rangel, A., & Hare, T. (2010). Neural computations associated with goal-directed choice. *Current Opinion in Neurobiology 20*(2), 262-270.
Roeser, T., & Baier, H. (2003). Visuomotor behaviors in larval zebrafish after GFP-guided laser ablation of the optic tectum. *Journal of Neuroscience*, *23*(9), 3726-3734.
Rose, T., Jaepel, J., Hübener, M., & Bonhoeffer, T. (2016). Cell-specific restoration of stimulus preference after monocular deprivation in the visual cortex. *Science*, *352*(6291), 1319-1322.
Ruthazer, E. S., Akerman, C. J., & Cline, H. T. (2003). Control of axon branch dynamics by correlated activity in vivo. *Science*, *301*(5629), 66-70.
Ruthazer, E. S., & Cline, H. T. (2004). Insights into activity-dependent map formation from the retinotectal system: a middle-of-the-brain perspective. *Journal of Neurobiology*, *59*(1), 134-146.
Schönenberger, N., Escher, G., & Van der Loos, H. (1983). Axon number in oculomotor nerves in Xenopus: removal of one eye primordium affects both sides. *Neuroscience Letters 41*(3), 239-245.
Schuller, J. (2017). Functional organization and ontogeny of the optokinetic reflex in Xenopus laevis [Dissertation, Ludwig-Maximilians-University Munich].
Shewan, D., Dwivedy, A., Anderson, R., & Holt, C. (2002). Age-related changes underlie switch in netrin-1 responsiveness as growth cones advance along visual pathway. *Nature Neuroscience 5*(10), 955-962.
Siju, K., De Backer, J.-F., & Grunwald Kadow, I. C. (2021). Dopamine modulation of sensory processing and adaptive behavior in flies. *Cell and Tissue Research 383*, 207-225.
Simpson, J. I. (1984). The accessory optic system. *Annual Review of Neuroscience 7*(1), 13-41.
Simpson, J. I., & Graf, W. (1981). Eye-muscle geometry and compensatory eye movements in lateral-eyed and frontal-eyed animals. *Annals of the New York Academy of Sciences*, *374*(1), 20-30.
Simpson, J. I., Leonard, C. S., & Soodak, R. E. (1988). The accessory optic system of rabbit. II. Spatial organization of direction selectivity. *Journal of Neurophysiology*, *60*(6), 2055-2072.
Soodak, R. E., & Simpson, J. I. (1988). The accessory optic system of rabbit. I. Basic visual response properties. *Journal of Neurophysiology*, *60*(6), 2037-2054.
Soupiadou, P., Gordy, C., Forsthofer, M., Sanchez-Gonzalez, R., & Straka, H. (2020). Acute consequences of a unilateral VIIIth nerve transection on vestibulo-ocular and optokinetic reflexes in Xenopus laevis tadpoles. *Journal of Neurology*, *267*(1), 62-75.
Spencer, R. F., & Porter, J. D. (1988). Structural organization of the extraocular muscles. *Reviews of Oculomotor Research 2*, 33-79.
Sperry, R. W. (1945). Restoration of vision after crossing of optic nerves and after contralateral transplantation of eye. *Journal of Neurophysiology*, *8*(1), 15-28.
Sperry, R. W. (1956). The eye and the brain. *Scientific American*, *194*(5), 48-53.
Sretavan, D. W., & Reichardt, L. F. (1993). Time-lapse video analysis of retinal ganglion cell axon pathfinding at the mammalian optic chiasm: growth cone guidance using intrinsic chiasm cues. *Neuron*, *10*(4), 761-777.

Steeves, J. K., Gonzalez, E. G., & Steinbach, M. J. (2008). Vision with one eye: a review of visual function following unilateral enucleation. *Spatial Vision 21*(6), 509.
Steinhausen, M. (1986). Vestibuläres System. *Lehrbuch der Animalischen Physiologie: Nach dem Gegenstandskatalog*, 537-549.
Straka, H., & Chagnaud, B. P. (2017). Moving or being moved: that makes a difference. *Journal of Neurology*, *264*(Suppl 1), 28-33.
Straka, H., & Dieringer, N. (1991). Internuclear neurons in the ocular motor system of frogs. *Journal of Comparative Neurology*, *312*(4), 537-548.
Straka, H., & Dieringer, N. (2004). Basic organization principles of the VOR: lessons from frogs. *Progress in Neurobiology 73*(4), 259-309.
Straka, H., Fritzsch, B., & Glover, J. C. (2014). Connecting ears to eye muscles: evolution of a 'simple'reflex arc. *Brain, Behavior and Evolution*, *83*(2), 162-175.
Straka, H., Lambert, F. M., Pfanzelt, S., & Beraneck, M. (2009). Vestibulo-ocular signal transformation in frequency-tuned channels. *Annals of the New York Academy of Sciences*, *1164*(1), 37-44.
Straka, H., & Simmers, J. (2012). Xenopus laevis: An ideal experimental model for studying the developmental dynamics of neural network assembly and sensory-motor computations. *Developmental Neurobiology 72*(4), 649-663.
Straka, H., Simmers, J., & Chagnaud, B. P. (2018). A new perspective on predictive motor signaling. *Current Biology*, *28*(5), R232-R243.
Strauss, S., Korympidou, M. M., Ran, Y., Franke, K., Schubert, T., Baden, T., Berens, P., Euler, T., & Vlasits, A. L. (2022). Center-surround interactions underlie bipolar cell motion sensitivity in the mouse retina. *Nature Communications 13*(1), 5574.
Straznicky, C., Tay, D., & Glastonbury, J. (1980). Regeneration of an abnormal ipsilateral visuotectal projection in Xenopus is delayed by the presence of optic fibres from the other eye. *Development*, *57*(1), 129–141.
Sumbre, G., Muto, A., Baier, H., & Poo, M.-m. (2008). Entrained rhythmic activities of neuronal ensembles as perceptual memory of time interval. *Nature*, *456*(7218), 102-106.
Szekely, G., & Szentagothai, J. (1956). Elementary nervous mechanisms underlying optokinetic responses, analyzed by contralateral eye grafts in urodele larvae. *Acta physiologica Academiae Scientiarum Hungaricae*, *10*(1), 43-55.
Szentágothai, J. (1950). The elementary vestibulo-ocular reflex arc. *Journal of Neurophysiology*, *13*(6), 395-407.
Taylor, J. (1987). Fibre organization and reorganization in the retinotectal projection of Xenopus. *Development*, *99*(3), 393-410.
Taylor, J., & Gaze, R. (1990). The course of regenerating retinal axons in the frog chiasma: the influence of axons from the other eye. *Anatomy and Embryology 181*(4), 405-412.
Taylor, J., & Guillery, R. (1995). Effect of a very early monocular enucleation upon the development of the uncrossed retinofugal pathway in ferrets. *Journal of Comparative Neurology*, *357*(2), 331-340.
ten Donkelaar, H. J., de Boer-van Huizen, R., Schouten, F. T. M., & Eggen, S. J. H. (1981). Cells of origin of descending pathways to the spinal cord in the clawed toad (Xenopus laevis). *Neuroscience*, *6*(11), 2297-2312.
Thanos, S., Fujisawa, H., & Bonhoeffer, F. (1984). Elimination of ipsilateral retinotectal projections in mono-ophthalmic chick embryos. *Neuroscience Letters 44*(2), 143-148.
Thompson, P., & Burr, D. (2009). Visual aftereffects. *Current Biology*, *19*(1), R11-R14.
Tomita, T. (1970). Electrical activity of vertebrate photoreceptors. *Quarterly Reviews of Biophysics 3*(2), 179-222.
Udin, S. B., & Fawcett, J. W. (1988). Formation of topographic maps. *Annual Review of Neuroscience 11*(1), 289-327.
Van Alphen, A., & De Zeeuw, C. (2002). Cerebellar LTD facilitates but is not essential for long-term adaptation of the vestibulo-ocular reflex. *European Journal of Neuroscience*, *16*(3), 486-490.

Van Buskirk, J., Candolin, U., & Wong, B. (2012). Behavioural plasticity and environmental change. In U. Candolin & B. B. M. Wong (Eds.), *Behavioural Responses to a Changing World. Mechanisms and Consequences* (pp. 145-158).
Van Egmond, A., Groen, J., & Jongkees, L. (1949). The mechanics of the semicircular canal. *The Journal of Physiology*, *110*(1-2), 1.
Van Hof-van Duin, J., & Mohn, G. (1986). Monocular and binocular optokinetic nystagmus in humans with defective stereopsis. *Investigative Ophthalmology & Visual Science 27*(4), 574-583.
Vigouroux, R. J., Duroure, K., Vougny, J., Albadri, S., Kozulin, P., Herrera, E., Nguyen-Ba-Charvet, K., Braasch, I., Suárez, R., & Del Bene, F. (2021). Bilateral visual projections exist in non-teleost bony fish and predate the emergence of tetrapods. *Science*, *372*(6538), 150-156.
Wagner, H., Pappe, I., Brill, S., & Nalbach, H.-O. (2022). Development of the horizontal optocollic reflex in juvenile barn owls (Tyto furcata pratincola). *Journal of Comparative Physiology A*, *208*(4), 479-492.
Wagner, H., Pappe, I., & Nalbach, H.-O. (2021). Optocollic responses in adult barn owls (Tyto furcata). *Journal of Comparative Physiology A*, *208*, 239–251.
Walley, R. E. (1967). Receptive fields in the accessory optic system of the rabbit. *Experimental Neurology*, *17*(1), 27-43.
Wallman, J., & Letelier, J.-C. (1993). Eye movements, head movements, and gaze stabilization in birds. In H. P. Zeigler & H.-J. Bischofs (Eds.), *Vision, Brain, and Behavior in Birds* (pp. 245–263).
Walls, G. (1962). The evolutionary history of eye movements. *Vision Research 2*(1-4), 69-80.
Wang, K., Hinz, J., Haikala, V., Reiff, D. F., & Arrenberg, A. B. (2019). Selective processing of all rotational and translational optic flow directions in the zebrafish pretectum and tectum. *BMC Biology 17*(1), 1-18.
Wässle, H. (2004). Parallel processing in the mammalian retina. *Nature Reviews Neuroscience*, *5*(10), 747-757.
West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press.
Williams, S. E., Grumet, M., Colman, D. R., Henkemeyer, M., Mason, C. A., & Sakurai, T. (2006). A role for Nr-CAM in the patterning of binocular visual pathways. *Neuron*, *50*(4), 535-547.
Williams, S. E., Mann, F., Erskine, L., Sakurai, T., Wei, S., Rossi, D. J., Gale, N. W., Holt, C. E., Mason, C. A., & Henkemeyer, M. (2003). Ephrin-B2 and EphB1 mediate retinal axon divergence at the optic chiasm. *Neuron*, *39*(6), 919-935.
Wilson, M., Taylor, J., & Gaze, R. (1988). A developmental and ultrastructural study of the optic chiasma in Xenopus. *Development*, *102*(3), 537-553.
Wizenmann, A., Thanos, S., Von Boxberg, Y., & Bonhoeffer, F. (1993). Differential reaction of crossing and non-crossing rat retinal axons on cell membrane preparations from the chiasm midline: an in vitro study. *Development*, *117*(2), 725-735.
Wu, Y., Dal Maschio, M., Kubo, F., & Baier, H. (2020). An optical illusion pinpoints an essential circuit node for global motion processing. *Neuron*, *108*(4), 722-734. e725.
Wylie, D., & Frost, B. (1990). The visual response properties of neurons in the nucleus of the basal optic root of the pigeon: a quantitative analysis. *Experimental Brain Research*, *82*, 327-336.
Yau, K.-W. (1994). Phototransduction mechanism in retinal rods and cones. The Friedenwald Lecture. *Investigative Ophthalmology & Visual Science 35*(1), 9-32.
Yuasa, J., Hirano, S., Yamagata, M., & Noda, M. (1996). Visual projection map specified by topographic expression of transcription factors in the retina. *Nature*, *382*, 632-635.
Yücel, Y., Jardon, B., Kim, M.-S., & Bonaventure, N. (1990). Directional asymmetry of the horizontal monocular head and eye optokinetic nystagmus: Effects of picrotoxin. *Vision Research 30*(4), 549-555.

APPENDIX A:

PUBLICATION LIST

Forsthofer, M., & Straka, H. (2023). Homeostatic plasticity of eye movement performance in Xenopus tadpoles following prolonged visual image motion stimulation. *Journal of Neurology*, *270*(1), 57-70.

Gordy, C., **Forsthofer, M.**, Soupiadou, P., Özugur, S., & Straka, H. (2022). Functional Neurobiology in Xenopus Provides Insights into Health and Disease. In *Xenopus* (pp. 277-288). CRC Press.

Forsthofer, M., Schutte, M., Luksch, H., Kohl, T., Wiegrebe, L., & Chagnaud, B. P. (2021). Frequency modulation of rattlesnake acoustic display affects acoustic distance perception in humans. *Current Biology*, *31*(19), 4367-4372.

Soupiadou, P., Gordy, C., **Forsthofer, M.**, Sanchez-Gonzalez, R., & Straka, H. (2020). Acute consequences of a unilateral VIIIth nerve transection on vestibulo-ocular and optokinetic reflexes in Xenopus laevis tadpoles. *Journal of Neurology*, *267*, 62-75.

APPENDIX B: PERMISSIONS

Figure 1a:

Permission for reprint of Figure 1a, adapted from the article:

Gordy C and Straka H (2021) Vestibular Influence on Vertebrate Skeletal Symmetry and Body Shape. *Front. Syst. Neurosci.* 15:753207. doi: 10.3389/fnsys.2021.753207

published in *Frontiers of Systems Neuroscience* has been granted to the author under the Creative Commons Attribution 4.0 International License (CC BY 4.0).

Figure 1b:

Permission for reprint of Figure 1b, adapted from the article:

Branoner F, Chagnaud BP and Straka H (2016) Ontogenetic Development of Vestibulo-Ocular Reflexes in Amphibians. Front. Neural Circuits 10:91. doi: 10.3389/fncir.2016.00091

published in *Frontiers in Neural Circuits* has been granted to the author under the Creative Commons Attribution 4.0 International License (CC BY 4.0).

Figure 2a:

Permission for reprint of Figure 2a, adapted from the article:

Franke, K. and Baden, T. (2017), General features of inhibition in the inner retina. J Physiol, 595: 5507-5515. https://doi.org/10.1113/JP273648

published in the *Journal of Physiology* has been granted to the author by John Wiley and Sons under the license number 5507141147684.

AFFIDAVIT

Eidesstattliche Versicherung/Affidavit

Hiermit versichere ich an Eides statt, dass ich die vorliegende Dissertation **Same same but different: Plasticity of a 'conserved' reflex** selbstständig angefertigt habe, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

I hereby confirm that the dissertation **Same same but different: Plasticity of a 'conserved' reflex** is the result of my own work and that I have only used sources or materials listed and specified in the dissertation.

31.03.2023 Michael Forsthofer

München, den Unterschrift

Munich, date signature

DECLARATION OF AUTHOR CONTRIBUTIONS

The following information details the authorship contributions for the data presented in this dissertation:

CHAPTER II:

Bilateral retinotectal miswiring impairs behavioral compensation in embryonic one-eyed *Xenopus laevis*

Michael Forsthofer, Clayton Gordy, Meghna Kolluri, Hans Straka

(Unpublished Manuscript)

Contribution of Authors:

HS and CG conceptualized, and HS, CG and MF designed experiments for the study. CG, MF and MK performed embryonic extirpations and quantified successful removals. CG and MK performed immunohistochemistry. MF and CG acquired behavioral data, and MF performed and imaged dye tracings. MF and CG established analysis scripts, and MF analyzed data. MF and CG created figures, and CG, MF and HS edited the manuscript. HS acquired funding and supervised the project.

My contributions to this manuscript:

I contributed to design of experiments and analysis and contributed to coding of analysis scripts. I performed the majority of embryonic extirpations and subsequent assessment of successful eye removals. I performed anatomical dye tracings, microscopy and analysis thereof. I acquired the majority of behavioral data, and performed analysis thereof. I created initial versions of figure 1 panel b) and c), as well as all other main and supplemental figures, and contributed to revisions on all figures. I wrote the first version of the manuscript, and contributed to the revision process.

CHAPTER III:

Homeostatic plasticity of eye movement performance in *Xenopus* tadpoles following prolonged visual image motion stimulation

Michael Forsthofer, Hans Straka

(Published in *Journal of Neurology*, 2022)

Contribution of Authors:

MF and HS conceptualized and designed experiments for the study. MF conducted the behavioral and histological experiments, designed and wrote analysis scripts, and analyzed and plotted the data. MF made initial figures, and MF and HS revised figures.. MF wrote the initial version of the manuscript, and HS and MF edited the manuscript.

My contributions to this manuscript:

I, together with HS, designed the experiments. I conducted behavioral experiments, designed, and wrote analysis scripts, analyzed and plotted the data. I performed immunohistochemistry, acquired, and analyzed microscopic images of the samples. I created initial versions of all figures and revised them together with HS. I wrote the initial version of the manuscript and revised it with HS.

I hereby confirm the accuracy of the above author contributions.

München, 31.03.2023	Michael Forsthofer (doctoral student)
München, 31.03.2023	Prof. Dr. Ruben Portugues (supervisor)