From the Department of Clinical Neuroscience
Karolinska Institutet, Stockholm, Sweden

MULTISENSORY CONTROL OF GAZE STABILIZATION

FROM BRAINSTEM TO BEDSIDE

Tobias Wibble

Karolinska Institutet

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On the cover: The schematic of an eye with arrows indicating each distinct movement direction as caused by a dedicated extraocular muscle. Below is a vestibular apparatus, with a man and lamprey in motion. Created with BioRender.com.

Multisensory Control of Gaze Stabilization From Brainstem to Bedside
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Tobias Wibble

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Principal Supervisor:
Associate Professor Tony Pansell
Karolinska Institutet
Department of Clinical Neuroscience
Division of Eye and Vision

Co-supervisor(s):
Assistant Professor Juan Pérez-Fernández
Universidade de Vigo
Department of Functional Biology and Health Sciences
CINBIO

Dr. Frank Träisk
Karolinska Institutet
Department of Clinical Neuroscience
Division of Eye and Vision

Opponent:
Professor David S. Zee
Johns Hopkins University
Department of Neurology

Examination Board:
Professor Måns Magnusson
Lund University
Department of Otorhinolaryngology

Professor Tatiana Deliagina
Karolinska Institutet
Department of Neuroscience

Professor Bertil Lindblom
University of Gothenburg
Sahlgrenska akademin

To my grandparents, who made sure I did my homework.

POPULAR SCIENCE SUMMARY OF THE THESIS

There is rarely a moment when you're completely unaffected by motion. Even when sitting still in a still environment, the rhythm of your heartbeat will make your body shift ever-so-slightly. This usually doesn't bother us, and to our eyes the world appears still. This is because our inner ears, part of the vestibular system, continuously feed the brain with information on how the head is positioned and where it's going. The resulting signal activates the muscles controlling our eyes through the vestibulo-ocular reflex (VOR), which makes the eyes move in the opposite direction of the head. Visual motion can also recruit these muscles through the optokinetic reflex (OKR), which ensures that our eyes follow large scale movements in the visual field. Eye movements like thee are called gaze-stabilizing, as they aim to keep a visual scene stable on the retina. In everyday conditions, these two reflexes interact, and gaze-stabilization relies on both visual and vestibular information to keep us from perceiving the world as shaky or unstable. It is when these two systems supply the brain with mismatched information that a sense of vertigo or motion-sickness arises.

The brain's command centre for issuing gaze-stabilizing eye movements is located within the brainstem. This most primordial of vertebrate neural structures deals with many aspects of everyday life that we never think about, including balance. While several studies have explored the way our cortex influences our eye movement control, less is known about the brainstem's contribution to gaze-stability. This thesis aims to provide new insights into these mechanisms. In order to achieve this on a broad front, the studies attached to this work deals with 1) the neural network allowing gaze-stability in the brainstem, 2) quantifying the way vision and vestibular signals interact to produce gaze-stabilization in healthy humans, and 3) investigating how these eye movements relate to balance and how they serve a clinical purpose.

The lamprey represents our earliest vertebrate ancestor still alive today. Its brain is nevertheless remarkably similar to our own in many ways, and thanks to its accessible layout proved a valuable model for studying how multiple senses contribute towards gaze-stabilization. Our first trials showed that the multisensory interaction was very similar to that seen in humans, meaning that its supporting neural structure likely operates according to the same basic principles as our own. Through performing precise lesions, and recording activity in the eye muscles and several neural cores in the brain, we were able to produce a map of the fundamental pathway of gaze-stabilization. In this process, we recorded the earliest form of

the OKR, and showed that the vertebrate VOR has remained conserved throughout evolution, and that the merging of these two eye movements relies on very basic pathways in the brain.

The next set of studies aimed at finding a way to measure sensitive a person may be to visual and vestibular information. When a person tilts the head, the eyes will rotate in the opposite direction through the VOR. When that same person views a rotating visual scene, the eyes will rotate to follow it. These torsional eye movements aren't under any real voluntary control, meaning that they will reflect the brain activity at its most basal neural level. In order to test how the OKR and VOR interact, we rotated people in a mechanical chair while they viewed a visual scene. In addition, they were tasked with sitting still and watching that same scene rotate, as well as being rotated themselves in complete darkness. In this way we were able to get the eye movement responses to visual, vestibular, and combined visuovestibular stimulations. In a set of trials we could show that the relative importance of a visual scene goes up the more cluttered it gets, while it goes down as the movement goes faster. These results were expected based on what we know about the visual and vestibular systems, but the protocols we established here offer a new method through which their relative importance can be reliably quantified. In addition, we knew that a head tilt results in a vertical realignment of the eyes, but our results showed that this eye movement was also seen when watching the visual scene. This response is called vertical vergence, and is considered to reflect activity of the vestibular segment of the brainstem, or as a side-effect of a torsional eye movement brought on by the mechanical constraints of the eye. It was therefore curious that the size of the vertical vergence response appeared to be independent of the torsion.

In order to dig deeper into the nature of this vertical vergence of the eyes, we performed a study testing how it responses to changes in the visual scene. More specifically, we had subjects view a rotating image both with the eyes open as normal, and with one eye occluded with an infrared-translucent sheath that allowed the eye-tracker to still record the vertical vergence. It transpired that the vergence was increased with one eye closed, indicating that vision actually acts to supress the response even though it was caused by a rotation of the visual field. While further studies are needed, we believe this may stem from it being caused by a visual activation of the vestibular region of the brainstem; as more information is retrieved when both eyes are open, the visual input serves to tell the brain that no tilt is actually taking place. Going deeper into the relationship between visual motion and vestibular responses, we set out to see how cluttered visual surroundings may affect a person's balance. Once again we had people view a rotating visual scene, but this time we recorded their stress responses, reflected in an increased pupil size, as well as body-sway as indicated by the

change in pressure on a balance board that subjects were standing on. As the visual clutter was increased, subjects responded with greater sway, increased stress, and faster torsional eye movements. This may go a long way in explaining why some people experience discomfort in visually cluttered environments, as they provoke the balance system even if we aren't consciously aware of it.

The final step of this string of studies was to test these new protocols in a clinical setting. As stated before, vision and vestibular information must coalesce in order to give us a good perception of balance. The mismatch-model states that if these systems relay different types of information we may experience motion-sickness. Most people are familiar with the sensation, for example when traveling as a passenger in a car; our inner ears tell us that we are stationary, but visually we are moving at incredible speeds beyond what our body should be capable of performing. Antihistamines make up the most common form of treatment for motion-sickness. It has been theorized that they work through inhibiting the vestibular system though studies diverge in their conclusions, some stating that they instead inhibit our sensitivity to visual motion. As the protocols developed for this thesis were able to test both visual and vestibular influences on our brain, as recorded by the eye movement response, we set out to test how Meclizine, a common antihistamine, influences our senses. The results showed that under normal conditions, when exposed to a combination of visual and vestibular input under low accelerations, the drug has an inhibitory effect on gaze-stabilization. In contrast, this response is enhanced if the acceleration becomes too big, or when only the VOR is triggered by a head rotation in darkness. It would consequently seem as if Meclizine reaches its effect by inhibiting the merging of visual and vestibular information in normal conditions, which agrees with the notion that this type of 1st generation antihistamines have a stronger effect on the central nervous system than later generations, having stronger side-effects in the form of sedation. Still, the results reflect a complex effect of Meclizine, and further studies are needed to shed further light on this phenomenon.

Gaze-stabilization offers us valuable insights into the ways the brain handles visual and vestibular information. The works in this thesis touch upon a number of key elements that allow us to retain a stable perception of the world in spite of all the motion that surrounds us. There is plenty of room to delve deeper into the neural circuitry that allows this to happen, and the method established in this thesis could offer valuable new approaches to studying how damages to the brain may affect our control of eye movements, as well as our control of balance. The eye movements outlined in the published articles could also prove beneficial when diagnosing and rehabilitating people with sensory deficits or unclear problems of

dizziness or vertigo, as we can investigate which system may be affected by a drug or medical complaint. Visually induced vertigo is an example of such a condition where visual motion can trigger a person's balance response, and our findings provide further context for how such a condition may be reflected by reflexive eye movements. Altogether, this thesis outlines new ways in which we can use gaze-stabilization to aid in research and healthcare.

ABSTRACT

Without the continuous updates provided by the vestibular and visual systems, our world would appear blurry and unstable. In order to allow the retina to reliably record incoming light in a meaningful way, the vestibulo-ocular reflex (VOR) and optokinetic reflex (OKR) aim to stabilize our gaze on visual features. The VOR performs this function by causing the eyes to move in the opposite direction of the head, whereas the OKR allows the eyes to instinctively pursue a moving visual scene. Together, they make up the gaze-stabilizing eye movements available to us humans, and represent the origins of all vertebrate oculomotor control. The neurophysiological principles governing these responses appear to have been very well-conserved throughout evolution, although the basic mechanisms through which the VOR and OKR are integrated in the brainstem are still not fully mapped; in nominal conditions, gaze-stabilization will rely on seamless merging of visual and vestibular information at this level. While it is difficult to analyse the activity of such an integration in humans, tracking the VOR and OKR can offer valuable insights into how the brain responds to motion in different conditions. This thesis aims to further our understanding of gaze-stabilization by implementing a series of translational protocols which may be divided into three segments. 1) We will elucidate the basic neural principles and evolutionary origins of gaze-stabilization in the lamprey animal model, 2) investigate how eye movement responses reflects this visuovestibular integration in healthy subjects, and 3) test these findings in a clinical setting.

The basic science experiments featured a combination of behavioural and electrophysiological methodologies. In the first stage, video eye-tracking was used to monitor gaze-stabilizing eye movements in three dimensions. In this way we recorded both slow- and fast eye movements in all planes in what may be the phylogenetically oldest example of nystagmus. The next phase involved performing electrophysiological recordings to investigate the neural network that would allow for this primordial gaze-stabilization. As both visual and vestibular information is used to construct these eye movements, we constructed a rotating platform synchronized with two monitors for optokinetic stimuli. This platform was capable of holding an ex-vivo lamprey preparation while performing electrophysiological recordings of the extraocular muscles and vestibular nuclei. The next step was to establish whether the lamprey possess an OKR, which we were able to observe as eye muscle activity scaled with optokinetic velocities in the pitch and roll planes. Combining visual and vestibular stimulations in the tilting platform, we saw that the conjoined OKR-VOR exhibited clear additive properties, which favoured the vestibular modality as the velocity was

increased. Lesioning experiments showed that pretectum is vital for relaying the optokinetic signal towards the OKR, whereas tectum downregulates its intensity. Curiously, there was no clear OKR in the yaw plane. We did however record clear compensatory eye movements during locomotion using a semi-intact preparation in the corresponding directions. As a way to map the trajectory of the signals underlying these responses we performed tracer injections of key structures in the brain. As a result, we were able to visualize the fundamental neural network of gaze-stabilization.

Implementing similar principles as those outlined above, we tested how healthy human gaze-stabilization behaves in the roll plane under various circumstances. Using a mechanized sled capable of full-body rotations coupled with visual stimulations on a projected screen, healthy subjects wore an eye-tracking device while exposed to different levels of visual clutter and movement accelerations. Roll plane VOR and OKR responses can be monitored in terms of the ocular torsion, which sees the eyes torque around their axes. Unlike translational gaze-stabilizers this eye movement lacks any meaningful somatic innervation, meaning that it offers a clearer reflection of the fundamental reflex. Throughout these studies we recorded increased gain in ocular torsion to intensified visual and vestibular variables. In addition, when comparing the torsional response for the VOR and OKR to the joint visuovestibular response it became clear that they reliably produced summative responses. This phenomenon was then used to quantify the relative influence of each sense during movements.

Vertical vergence is a known physiological response to a head tilt and associated with ocular torsion. When performing the previously outlined studies we found that such a divergence of the eyes may also be triggered by optokinetic rotations. The torsion-vergence ratio increased to visual clutter and decreased with an upregulated acceleration of the stimulus motion. In order to ensure that the vergence was not secondary to torsion, we tested how it responded to visual information by having subjects view a rotating scene binocularly as well as monocularly. Results showed the vergence amplitude was increased during monocular viewing, suggesting that the response is suppressed during nominal binocular viewing. Furthermore, as postural control also involves similar multisensory modalities as gaze-stabilization, the relationship between postural sway and the OKR was tested. This involved a procedure where subjects watched a rotational optokinetic scene while standing on a balance board. Torsional gain was positively correlated with postural sway, as well as with the sympathetic response indicated by an increased pupillary dilation.

Having established that the OKR-VOR interaction could be used to infer their relative importance during a head roll, we implemented our visuovestibular protocol in a

pharmaceutical study testing the effects of Meclizine. Antihistamines like these are widely used as anti-emetic drugs, and are primarily attributed with reaching their desired effect by inhibiting the vestibular system. Contrary to this hypothesis, the torsional response showed increased vestibular influences during high velocities, although a converse inhibiting effect under nominal visuovestibular conditions at low accelerations. These findings may reflect the anti-emetic's inhibiting effect on the central integration of the sensory information.

Through its translational approach to gaze-stabilization, the studies in this thesis offer new insights into the neural activity that govern eye movement control. The results presented above can be further contextualized in relation to the overarching aims of this work.

1) As the lamprey exhibiting OKR, VOR and locomotion-supported eye movements, it is clear that gaze-stabilization was well-developed already at the dawn of vertebrate evolution. The presence of nystagmus indicates that the neural mechanism for goal-oriented saccades was present at this time, and that it likely operates through tectal downregulation of the OKR. Pretectum has been shown to act as the first integrator of visual motion in several vertebrate species, which holds true also for the lamprey. In addition, this thesis shows that a VOR-OKR interaction can be maintained without cerebellar or cortical influences. The fundamental network instead relies completely on a few subcortical structures.

2) Our subsequent studies in human subjects revealed even more robust visuovestibular integration, showing that torsional gain may be used to test the influence of the sensory modalities in any given scenario. Our studies on the rotational OKR reveal that vergence is an intrinsic part of the response, and as it suppressed by binocular vision it is tempting to suggest it may reflect a visual activation of the vestibular nuclei. This visuo-vestibular pathway could also explain the correlation between visual clutter, postural sway and increased sympathetic stress, as it would allow reflexive postural strategies without conscious interference.

3) We implemented the aforementioned visuovestibular protocols to evaluate the effects of Meclizine, which suggested that it may be through central inhibitory effects that its anti-emetic effects are achieved. This finding is in line with well-established notion that 1st generation antihistamines are more efficient due to their properties on the central nervous system, being associated with stronger side-effects in the form of general sedation.

In conclusion, this thesis approaches gaze-stabilization from a broad perspective, shining new light on the neural network as well as identifying new features of the human eye movement response. Having established the fundamental principles that govern the integration of these reflexes, our findings open up for investigating the next order of integration, as several neural

structures influence the gaze-stabilizing responses. The somatosensory influences towards this process remain poorly known, and future studies may benefit from mapping how the signal for locomotion-supported eye-movements is incorporated into the system. While we have indicated how torsion and vergence may hold utility, additional studies are needed to formalize the measuring of such processes for clinical use. Altogether, these findings could offer a valuable approach to evaluating sensory deficits, and aid in developing protocols for personalized medicine and rehabilitation.

LIST OF SCIENTIFIC PAPERS

Asterisk (*) denotes corresponding author:

I. **Wibble, T**., Pansell, T., Grillner, S., Pérez-Fernández, J*. Visuo-vestibular gaze control – Conserved subcortical processing. *Submitted*.

II. **Wibble, T***. & Pansell, T. Vestibular Eye Movements Are Heavily Impacted by Visual Motion and Are Sensitive to Changes in Visual Intensities. *Investigative Ophthalmology & Visual Science* **60**, 1021-1027 (2019).

III. **Wibble, T***., Engström, J., Pansell, T. Visual and Vestibular Integration Express Summative Eye Movement Responses and Reveal Higher Visual Acceleration Sensitivity than Previously Described. *Investigative Ophthalmology & Visual Science* **61**, 4-4 (2020).

IV. **Wibble, T***. & Pansell, T. Optokinetic stimulation induces vertical vergence, possibly through a non-visual pathway. *Scientific Reports* **10**, 15544, doi:10.1038/s41598-020-72646-8 (2020).

V. **Wibble, T***., Sodergard, U., Traisk, F., Pansell, T. Intensified visual clutter induces increased sympathetic signalling, poorer postural control, and faster torsional eye movements during visual rotation. *PLoS One* **15**, e0227370, doi: 10.1371/journal.pone.0227370 (2020).

VI. **Wibble, T***., Engström, J., Verrecchia, L., Pansell, T. The effects of meclizine on motion sickness revisited. *British Journal of Clinical Pharmacology* **86**, 1510-1518, doi:10.1111/bcp.14257 (2020).

LIST OF SCIENTIFIC PAPERS NOT INCLUDED IN THESIS

I. Frattini, D. & **Wibble, T***. Alertness and visual attention impact different aspects of the optokinetic reflex. *Investigative Ophthalmology & Visual Science.* **62**, 16, doi:10.1167/iovs.62.13.16 (2021)

II. Suzuki, DG., Pérez-Fernández, J., **Wibble, T.**, Kardamakis, AA., Grillner S*. The role of the optic tectum for visually evoked orienting and evasive movements. *Proceedings of the National Academy of Sciences* **116** (30), 15272–15281, doi: 10.1073/pnas.1907962116 (2019).

CONTENTS

LIST OF ABBREVIATIONS

aCSF	Artificial cerebrospinal fluid
AON	Anterior octavolateral nucleus
AOS	Accessory optic system
ANS	Autonomic nervous system
C-ETD	Chronos eye-tracking device
COP	Centre of pressure
COR	Cervico-ocular reflex
Deg	Degree
DLR	Dorsal light reflex
DVD	Dissociated vertical divergence
EOM	Extraocular muscle
HIT	Head impulse test
IVN	Inferior vestibular nucleus
LC	Locus coeruleus
LVN	Lateral vestibular nucleus
MVN	Medial vestibular nucleus
nMLF	Nucleus of the medial longitudinal fasciculus
ms	Millisecond
N.III	Oculomotor nucleus
N.IV	Trochlear nucleus
N.VI	Abducens Nucleus
OCR	Ocular counter-roll
OKR	Optokinetic reflex
OT	Ocular torsion
PNS	Parasympathetic nervous system
r-VOR	Rotational vestibulo-ocular reflex
Sec/s	Second
SC	Superior colliculus
SCC	Semicircular canal
SNS	Sympathetic nervous system

SVN	Superior vestibular nucleus
t-VOR	Translational vestibulo-ocular reflex
VES	Vestibular
vHIT	Video head impulse test
VIS	Visual
VISVES	Visuovestibular
VMH	Visual motion hypersensitivity
VOR	Vestibulo-ocular reflex
VOT	Visually induced torsion
VN	Vestibular nucleus
VV	Vertical vergence
WBB	Wii balance board

1 INTRODUCTION

1.1 EVOLUTION OF VISION

1.1.1 General introduction of purpose and function

It is the overarching ambition of this thesis to approach gaze-stabilization from a broad perspective, from mapping its most fundamental neural network to characterizing how visual and vestibular information contribute to these compensatory reflexes in humans. Eye movements are ancient and were present already at the onset of vertebrate evolution [1]. Remarkably little has changed in terms of how our eyes remain fixed in space as we move through an ever-changing environment, and the extraocular muscles and their innervation have remained largely conserved from jawless fish to primates. Still, as the vertebrate brain increased in size to generate an increasingly complex set of behaviours, the neural network impacting gaze-stabilization will have expanded by employing cortical, subcortical and cerebellar regions in this automated response.

Vision has emerged as the primary sensory informant for humans, involving several interconnected pathways in a complex neural system [2]. The brain, in turn, relies on the eyes to provide it with the sensory data needed to produce said vision. In order to contextualize the principles of gaze-stabilization, it may be advantageous to present the process through which eye movements were formed. If we by the term *vision* refer to the detection of light, the first forms of light-detecting cells would have constituted simple binary on-off cells. These primordial sensory cues may have allowed their owners some simple phototactic responses. As photosensitive cells gathered into a concave surface, the inclination of incoming light would provide a sense of direction in an early form of image-forming eye. The earliest example of vision comes from fossils of trilobites, an extinct species of arthropods that evolved 530 million years ago [3,4]. The birth of image forming vision allow for more complex interpretations of the visual surroundings. In order to separate self-motion from that of external stimuli, and to maintain focus on an object moving across the visual scene, there arose a need to stabilize the image. Without compensatory eye movements the photoreceptors may fail to accurately register the viewed object properly, as the visual scene would risk crossing the photoreceptors too fast for them to activate properly [5].

Eyes exemplify the concept of convergent evolution, and with several sets of eyes abiding by the same general principles the gaze-stabilizing mechanisms have evolved independently across multiple taxa, eventually giving rise to several eye movement forms [6]. Vertebrate eyes generally tend to function according to the same principles, and although operating according

to the same basic mechanism as many invertebrates they make up a distinct building plan easily distinguishable from that of arthropods and cephalopods [7]. It is likely that goal-oriented gaze-shifts and fixating eye movements arose from the neural template of gaze-stabilization [6,8], and so understanding these fundamental sensorimotor reflexes are central for mapping the neurophysiology of eye-movements, reflecting the peripheral detection of a movement and its subsequent integration in the brain.

The two primary sensory contributors to gaze-stability are the vestibular system through the vestibulo-ocular reflex (VOR), and vision in the form of the optokinetic reflex (OKR). While some vertebrate classes exhibit additional contributors towards these compensatory responses, most notably locomotion, they constitute the basis for clamping a visual feature on the retina. Before concentrating on the specifics of the VOR and OKR, it may be beneficial to first illustrate how they fit within the framework of eye-movement control.

1.1.2 Vertebrate eye movements across the seven classes

As this thesis make use of the oldest extant vertebrate as an animal model, the lamprey, to infer visuovestibular control of primate eye movement control, this chapter will feature a brief comparative summary of vertebrate eye movements. These can be arranged into three separate classes: gaze-stabilizing, gaze-shifting, and fixational. Each can in turn be separated into further subclasses, as outlined in table 1.

Table 1. A summary of the major classes of vertebrate eye movements and their subordinate categories.

Gaze-stabilizations[8-11]	**Gaze-shifts**[12]	**Fixational eye movements**[13]
Optokinetic reflex	Saccades	Microsaccades
Vestibulo-ocular reflex	Vergence	Ocular drifts
Corollary discharges	Smooth-tracking	Ocular microtremors

Prior to the studies undertaken in this thesis, the phylogenetically oldest vertebrate eye movement was believed to be the lamprey VOR, allowing the vestibular system to produce a compensatory eye movement in the opposite direction of a head movement [1]. Studies performed in cartilaginous fish had yielded only inclusive optokinetic responses, and the earliest OKR had only been conclusively shown in osteichtyes as reflected in eye movement

reflexively following a moving visual scene [8]. Chondricthyes have however been shown to express compensatory eye movements through corollary discharge during locomotion [8]. This type of eye movements are not always counted towards those of gaze-stabilizers, though I believe a point can be made for its inclusion.

Eye movements compensating for locomotion through spinal efference copies have been shown in dogfish and tadpoles [14,15]. One may consider it appear likely that oculomotor compensation through locomotion efference copies is represented in the neural networks beyond those of cartilaginous fish and amphibians, though the extent of their utility may differ greatly between species [16]. It should also be noted that the presence of an eye movement within a vertebrate class does not necessarily mean that all members exhibit them; studies in humans have shown that proprioceptive feedback through the neck muscles can induce eye movements, though these responses appear to be subthreshold for providing meaningful image stabilization [17].

Fixational eye movements are perhaps the least explored of the eye movement types. These miniscule corrections ensure that the eyes can remain in position. They have been shown in all vertebrate classes from bony fish to mammals, in both foveated and afoveated species, and without fixation, the world would appear blurry [18]. One may postulate that the presence of such fixations imply a need to counteract the mechanical forces of the orbita caused by the flexibility of the extraocular muscles (EOM). As such, it appears likely that this set of eye movements would be intrinsically linked with the primordial gaze-stabilizers of the OKR and VOR.

Gaze-shifts are goal-oriented eye movements purposed to redirect visual attention [19]. Saccades are quick ballistic eye movements, redirecting visual focus from one part of the visual field to another in a movement which cannot be stopped once initiated [20]. These are the types of eye movements one would implement when reading this text. Vergence allows horizontal and vertical alignment of the eyes to allow for binocularity and visual acuity when focusing between objects at varying distances, or adjust for binocular disparity [21]. An example may include first looking at a bird through the window and then having the eyes converge inwards to shift focus to a smidge on the window glass. Smooth-tracking is seemingly limited to primates, and has the eyes pursue a moving visual feature as it moves, for example when following a car or a train passing by with your eyes [12].

Unlike stabilizing and fixating eye movements, gaze-shifts may be subject to voluntary somatic control [6]. Much like fixational eye movements, these are comprised of features that

were already present in the OKR and VOR; a vestibulo-ocular reflex involves an initial slow-phase component in the opposite direction of the head rotation followed by a quick-phase, and the same mechanism is found in the OKR's response to visual movements [12,22]. Vergence is also part of the VOR, as the eyes diverge vertically during head rolls (tilting) and horizontally during head surge (moving forward and backward) while maintaining visual fixation, though this horizontal vergence is also under voluntary control [23]. The voluntary initiation of these movements are issued from cortical input from several regions, most notably the frontal eye field [24]. Their implementation, however, rely on the subcortical activation of a number of key nuclei in the brainstem, which initially emerged to support gaze-stabilization [6,8,12,25]. Despite the importance of these structures, and the utility of the resulting eye-movements in clinical and scientific protocols, there are still many aspects that may benefit from further investigation. This thesis will consequently focus on the most basic level of control of gaze-stabilization in the brainstem and its integration of visual and vestibular information, and study the same sensory integration in humans during stimulation in the least studied direction of movement, the roll plane.

1.2 PRINCIPLES OF GAZE STABILIZATION

Having established the purpose and main functions of eye movement control, this chapter will provide further commentary on the specific components of gaze-stability. This will be done initially from the human perspective before introducing the conserved nature of lamprey oculomotion and how it compares to that of primates, with special reference to roll plane responses.

1.2.1 General principles

Vertebrate gaze stabilization is, as previously noted, primarily maintained through two reflexive eye movements. Movements in the visual field produce an optokinetic reflex (OKR), in which retinal information results in an activation of select extraocular muscles so that the eyes may follow the visual scene [26]. A head movement will cause an activation of the vestibulo-ocular reflex (VOR) to induce smooth eye movements in the opposite direction of the head, which relies on activity in the vestibular apparatus in the form of semicircular canals detecting fluid inertia, and the otolith organs relaying gravitational pull on the macula [27]. There is also evidence for somatosensory information contributing to stabilizing eye movements [10], with corollary discharges differentiating between passive and active self-motion [11]. The relative importance of these influences may differ between species, and in humans the somatosensory influences are somewhat debated, though evidence suggests activation of the neck muscles contribute to visual processing as well as the eye movement

response [28,29]. It has consequently been put forward that proprioceptive signals may aid human gaze-stability [10], and that corollary discharges allow for the separation and recognition of passive and active visual field movements [11]. In addition, several studies have investigated how stimulation of the neck muscles may produces compensatory eye movements through the colliculo-ocular pathway [28,30,31]. Its actual influence on gaze-stability has however been questioned, as it has only been shown to cause very small and slow compensatory responses, too weak to hold functional utility on its own [30].

In order to assess the functionality of these gaze-stabilizing eye movements in the roll plane, we may consider some of the mechanisms they aim to fulfil. Proper binocular vision relies on proper gaze-stabilization through distinct eye movements adhering to Donder's law, which states that a specific gaze position will yield an equally specific orientation of the eyes, including a torsional component [32]. Ocular torsion (OT) therefore plays an important role in motor fusion of the eyes. Additionally, OT aids in sensory fusion by increasing the range associated with Panum's area [33]. Panum's area, in turn, is the visual range in which single binocular vision can be maintained, and the torsional disparities between two images will be greater in the peripheral retina compared to the fovea[34]. In the context of gaze-stabilization, OT can be achieved both through the VOR and OKR. From a vestibular perspective, the VOR will produce a torsional eye movement response in the shape of ocular counter-roll (OCR) in response to a head tilt, which is primarily brought on by the vertical semi-circular canals and then maintained by the utricules [35,36]. The positional gain of this movement is estimated to be between 10-20%, decreasing as the amplitude of the head roll increases [37]. Visually induced ocular torsion (VOT) has a much smaller gain of about 1-4% [38]. It is also known that visual content can increase the gain of an OCR, indicating visual contribution to the VOR [38]. Torsional gain during combined visuovestibular stimulations may consequently yield an indication of how the two senses are weighed in terms of their stabilizing influence, and their relative importance could be quantified based on their proportional contribution towards the integrated response. While this thesis aims to explore this relationship, subsequent studies establishing control values across a large population could be useful for establishing clinical benchmarks, as the visual-vestibular ratio may give an indication of what system is afflicted in patients with sensory deficits.

It has long been known that a torsional eye movement is accompanied by a vertical vergence (VV) of the eyes, i.e. skewing [21]. These vertical and torqueing eye movements are intrinsically interconnected according to Donder's law [32]. This relationship could possibly be explained by mechanical constraints of the orbita [39], but it has been put forward that the

specific ratio between torsion and vergence may be neurologically driven, as the orbital dynamics cannot fully account for the specificity with which the eyes are positioned [32]. Vertical vergence is generally considered a vestibular brainstem response and a physiological component of the VOR; it is part of the ocular tilt reaction, where the utricles signal for the eyes to divergence along the vertical visual axis [40]. More specifically, a head roll will cause the contralateral eye to move downward in relation to its ipsilateral counterpart, which instead moves upward [41,42]. Much like its torsional counterpart, vertical vergence aims to increase the fusional range [23].

It has been known for some time that vertical vergence can be seen during rotational optokinetic stimulation, but it has been considered as purely secondary to the torsional response according to the principles described above [32]. Nevertheless, the full extent of this relationship remains poorly investigated. Studies have indicated that vertical divergence, in the form of skew deviation, can be caused by visual aberrations. These include visual disparities [21], and the notable observation that amblyopic children may grow up to express a constant vertical misalignment of the eyes [43]. A hypothesis aiming to explain this pathology suggests that amblyopia may cause a vestigial dorsal light reflex (DLR) to manifest. This is supported by the finding that children with this condition also experience a slight tilt to the visual scene, as indicated by testing their subjective visual vertical [44]. The DLR will be covered in more detail in the next segment, as it is most prominent in phylogenetically older vertebrates, but it serves as a light-guided reorientation mechanism and as such involves visual activation of the vestibular system [45]. This invites the question if vertical vergence, much like torsion, could be considered an eye movement response common for both the VOR and OKR; if so, it may prove valuable in evaluating multisensory control of gaze-stabilization in patients.

1.2.2 Vestibular activation and the vestibule-ocular reflex

This segment will briefly outline the main principles giving rise to a torsional gaze-stabilization from vestibular and visual stimulations. As the vestibular nuclei are comprised of four sub-nuclei, activation of the VOR will also be accompanied with a set of reflexive responses related to postural control. In order to understand the clinical utility in using gaze-stabilization, this segment will therefore offer an overview of the vestibular nucleus and its functions.

1.2.2.1 Vestibular registration

The vestibular apparatus consists of two distinct organs, the semicircular canals (SCC) and the otoliths [46]. The former detect angular acceleration through movement of endolymph, a potassium rich fluid, in the superior, horizontal and posterior canal, which are named for their position in the vestibular complex. As illustrated in figure 1, each canal opens into an ampulla, which houses the gelatinous cupula. When the canal moves with the head, the cupula is pushed into the endolymph with a force proportional to the fluid's inertia. As the cupula bends, stereocilia bend with it, giving rise to an electric signal that can be conveyed through the vestibular nerve; during constant velocity, the inertia is maintained for about 10 seconds before the endolymph reaches equilibrium, after which the convection of movement stops [47]. Depending on the flexion of the cupula, the hair cells will be hyperpolarized or depolarized, allowing directional information.

The otoliths, in addition to supporting the registration of angular acceleration, specialise in detecting static changes of head position [48]. These organs are composed of the saccule and utricle, responding to vertical and horizontal stimulations respectively due to the orientation of their hair cells. A gelatinous layer embedded with tiny crystals, otoconia, blanket these hair cells. As gravity causes the crystals to shift during head movements they pull on stereocilia in either direction, causing hyper- or depolarization according to the same principles as seen in the semicircular canals[49]. While the initial response will aid in detecting dynamic angular displacement (r-VOR), the hair cells will continue to signal the static position of the vestibular apparatus (t-VOR), as the otoconia continue to tug on the otolithic membrane covering the gelatinous layer [50].

Altogether, the semicircular canals and the otolith organs provide constant feedback on head position and acceleration through the vestibular nerve. Due to the polarizing-depolarizing nature of the stereocilia, this system works according to a push-pull mechanism; as one side hyperpolarizes the other side will depolarize, shifting the equilibrium.

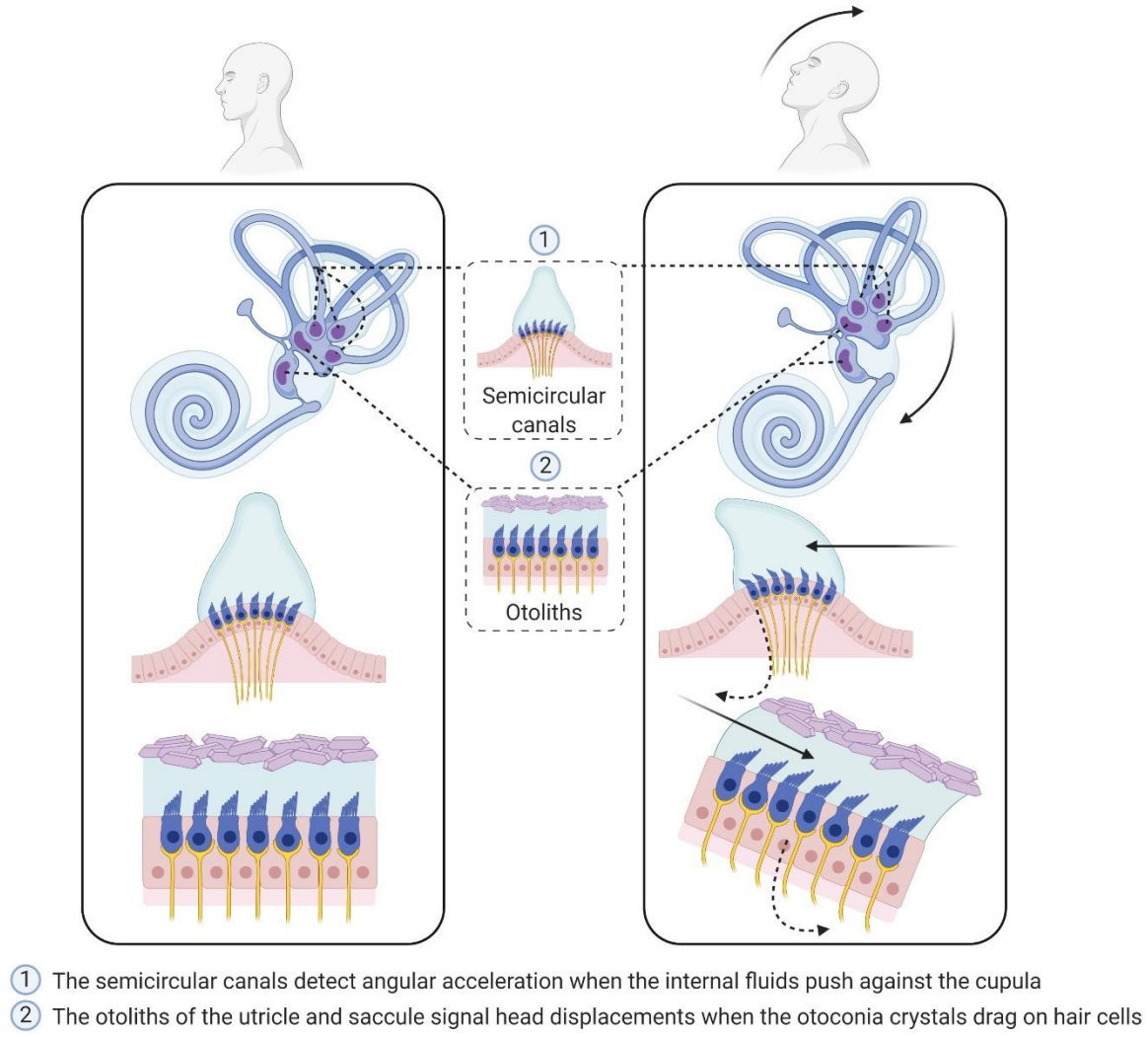

Figure 1. The peripheral vestibular system. The schematic illustrates how the vestibular apparatus and its components, the semicircular canals and the otolith organs, behave during a head movement as they translate inertia into neural signals. 1) The semicircular canals detect angular acceleration when the internal fluid pushes against the cupula which in turn produce movements of hair cells. 2) The otolith organs, the utricle and the saccule, signal head displacements when the otoconia crystals drag on the embedded hair cells. Resulting electrical impulses are relayed through the vestibulocochlear nerve to the vestibular nuclei. Created with BioRender.com.

1.2.2.2 Activating the brainstem

The resulting signal conveyed through the vestibular nerve terminates in the vestibular nucleus (VN) of the brainstem [51]. The distribution of these projections is presented in figure 2, and it is reported that the superior vestibular nucleus (SVN) is targeted by semicircular afferents, whereas the lateral vestibular nucleus (LVN) is innervated by otolith input [52]. The

remaining inferior and medial nuclei (IVN; MVN) receive afferents by both peripheral structures.

VN efferents will subsequently project to a number of structures involved in the reflexive response to movement. The LVN will activate the vestibulospinal tract, causing reflexive recruitment of muscles that may prevent a fall if the vestibular activity was not triggered by a self-generated movement [52]. In contrast, if the vestibular activity was part of a planned head movement, the cerebellum will attenuate this response through interactions with the vestibular nucleus, ensuring that we are in control of our own locomotion [53]. The MVN will also issue signals leading to stabilizing head movements by recruiting neck muscles [51].

The MVN and SVN act as the main initiators of the VOR [54]. This is achieved primarily through excitatory projections to the contralateral abducens nucleus (N.VI), which in turn activates its contralateral oculomotor nucleus (N.III) and ensures that both eyes move in the same direction during a translational head movement [55]. The MVN will also excite or inhibit activity in the contralateral N.III and trochlear nucleus (N.IV) [51], whereas the SVN projects to the ipsilateral N.III and N.IV through the medial longitudinal fasciculus [56].

A vestibular signal may consequently produce a cascade of reflexive responses related to self-movement. Considering the range of functions of the VN, one may claim that the VOR reflects neuronal activity representing how the brain integrates motion, and postulate that the ocular movement may be correlated to reflexive postural commands. As will be outlined later (See Optokinetic reflex), the visual system also recruits the VN in its producing the OKR. A gaze-stabilizing response may therefore serves as a valuable proxy for assessing both peripheral and central systems of movement-integration. For example, this is routinely done through the head impulse test, where an examiner evaluates the inner ear through quickly moving the patient's head, or identifying vertical or rotational nystagmus beats as having central aetiologies [57].

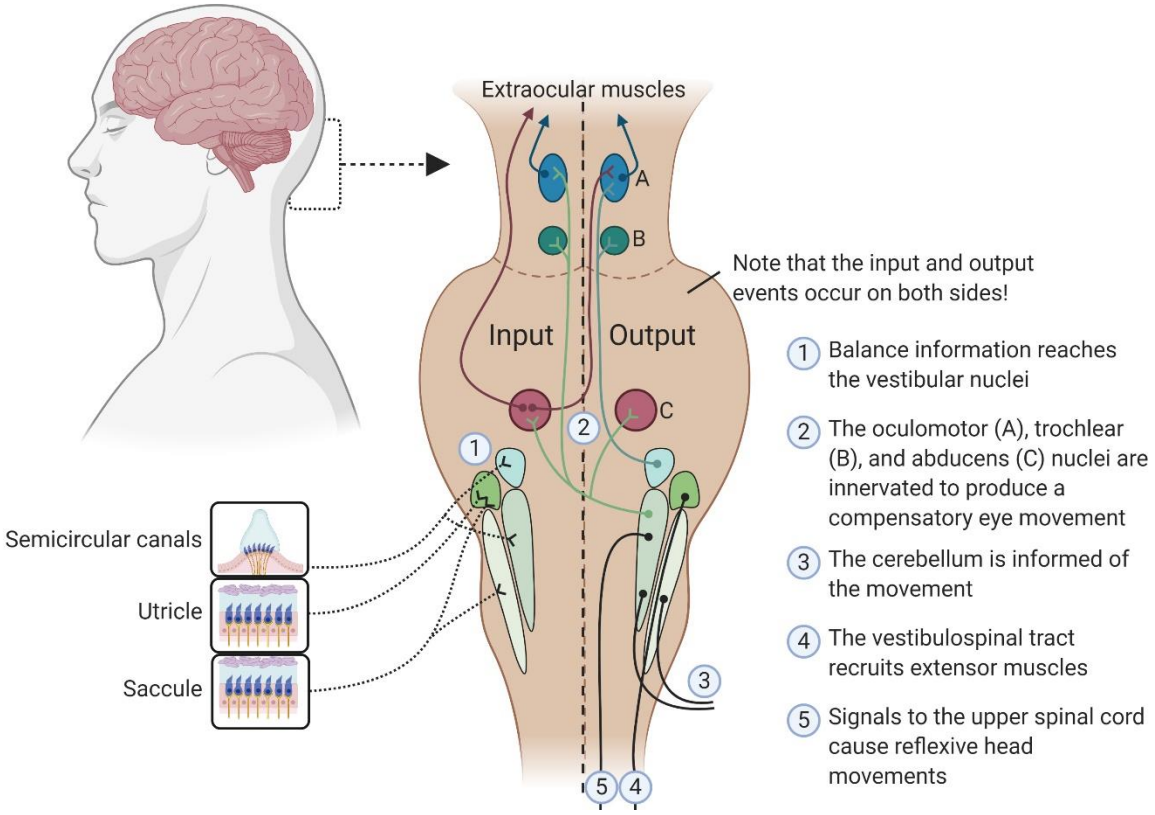

Figure 2. The central vestibular system. Peripheral vestibular information reaches the four sub-nuclei of the central vestibular complex of the brainstem. The basic and reflexive oculomotor and postural responses are outlined in the schematic, with no chronological order. Created with BioRender.com.

1.2.2.3 Recruiting the eyes

Motor neurons originating in N.III, N.IV and N.VI innervate the extra-ocular muscles (EOM) responsible for rotating the eyes. This complete network is illustrated in figure 3. The following segment will briefly outline the route through which torsion is achieved.

Torsional VOR, the OCR, involves rotating the eye around its visual axis without causing any horizontal or vertical movements. The following example accompanies a head tilt to the right [58]. From the perspective of the semicircular canals, the anterior SCC produces an excitatory signal that activates the ipsilateral superior rectus muscle and the contralateral inferior oblique. The posterior SCC will instead recruit the ipsilateral superior oblique and contralateral inferior rectus. Hering's law of equal innervation allows for this conjugation, controlling for the fact that an eye movement in one direction will be innervated by the antagonistic EOM of the contralateral eye [59]. For the same reason, the left anterior SCC

inhibits the contralateral inferior rectus and the ipsilateral superior oblique, while the posterior SCC inhibits the contralateral inferior oblique and the ipsilateral superior rectus.

As the EOM are always innervated by their respective cranial nerves, this step holds true for any torsional eye movement, whether it is vestibular or optokinetic in origin. As ocular torsion lacks any meaningful somatic voluntary innervation it may serve as an objective indicator of the visual, vestibular, and visuovestibular contributions to gaze-stabilization.

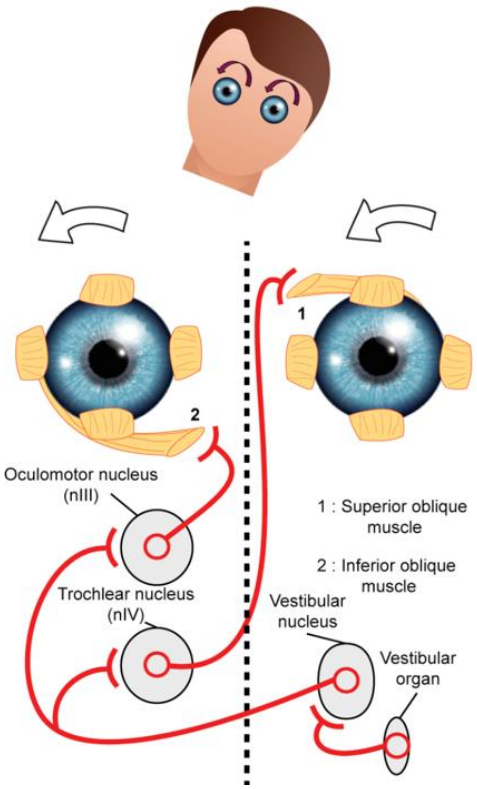

Figure 3. Neural activation of the extraocular muscles in humans. The schematic illustrates how the eyes move during a head roll. The red lines illustrate neural innervation between the structures, illustrated as grey ellipses. Input originates in the respective vestibular organs and move upwards in the figure. The lamprey schematic features an adult lamprey with the frontal section of the eye-brain-labyrinth preparation to its right.

1.2.3 The optokinetic reflex

Whereas the VOR is purposed to compensate for a head movement, the OKR evolved to implement direct visual feedback in its task to stabilize gaze. Being phylogenetically younger

than the VOR but having evolved in parallel, this fundamental sensorimotor integration involves neural structures unique to visual feedback incorporated in the vestibular pathway [8].

1.2.3.1 The neural pathway of the OKR

As an object moves across the retina, the photoreceptors translate incoming light into electrical impulses through the optic nerve in order to giving rise to a signal that carries both spatial and temporal information [60]. The neural network allowing optokinetic data to be translated into an OKR is less direct than its vestibular counterpart. Almost all nodes in this system receive afferents from a number of cortical and subcortical structures whose importance is difficult to deduce in the highly evolved vertebrate brain [61]; as outlined in the next chapter, we aim to map the fundamentals of the OKR using a more primitive neural model. Nevertheless, figure 4 illustrates an overview of the OKR pathway.

After light has been registered by the eye, a retinal signal from the nasal quadrants will be relayed through the optic nerve and cross to the other cerebral hemisphere at the level of the optic chiasm, while the temporal input remains on the ipsilateral side [62]. After the chiasma the information is carried through the optic tract [62]. This tract projects to pretectum which influences a number of visually guided reflexes, including the OKR; other examples include smooth-tracking, conveying the accommodation and pupillary reflexes, as well as supporting a circadian rhythm [63,64]. There are a number of pretectal outputs that could influence an eye movement response [63]. In the context of the OKR, direct projections to N.III and MVN are of particular note; the former controls eye movement responses directly and the latter is involved in the VOR and other vestibular responses as outlined above. Pretectum also projects to the superior colliculus (SC), which holds a key function in visually guided behaviours and eye movement control [65]. Projections via the accessory optic system (AOS) of the pretectal area also influence the OKR and it synergy with the vestibular system [66].

In addition to activating pretectum, the optic tract also provides input to the superior colliculus, whose function is mentioned above. The tract terminates in the lateral geniculate nucleus (LGN) of the thalamus. From here, fibres reach several cortical areas where visual motion and content can be interpreted and perceived before modulating the subcortical network [67,68] . One such example of feedback is the cortical projections to pretectum that modify its afferent signalling [69]. Significant cortical input to the superior colliculus also allows for voluntary motor commands, such as gaze-shifts.

Dissecting the network described above gives rise to a few clear, direct contributors towards the OKR, notably pretectal projections to N.III and MVN. Naturally, this invites the question

on why only the N.III receives direct innervation, as compared to the N.IV and N.VI. Lacking any such direct projections, a fulminant OKR would have to occur through crosstalk between the EOM nuclei, or by making use of the VOR network through the MVN. Studies have for example shown that pretectum, through the AOS, is capable of producing an optokinetic response through the velocity storage mechanism (VSM), which integrates motion information through vestibular-cerebellar interactions in the brainstem [70]; this mechanism will be further addressed in the next chapter.

One may infer some information regarding the OKR pathway by juxtapositioning its temporal properties with that of the VOR. For comparison, the VOR has a latency of 8.6 milliseconds (ms) [71], while an OKN is initiated after 70-75 ms [72]. Naturally, the vestibular system can be expected to exhibit a greater sensitivity to motion due to its mechanical and direct translation of inertia. This cannot be explained solely by different response rates of the peripheral organs however. A study comparing the latency of pretectal activity and the OKR in monkeys showed that the earliest pretectal activity occurred 20 ms before the eye movement, with the average pretectal neuron exhibiting a lead time of 7.5 ms [73]. Considering the substantially longer onset of the OKN, one may suggest that its fundamental operation is maintained through a more complex network of nodes than the VOR.

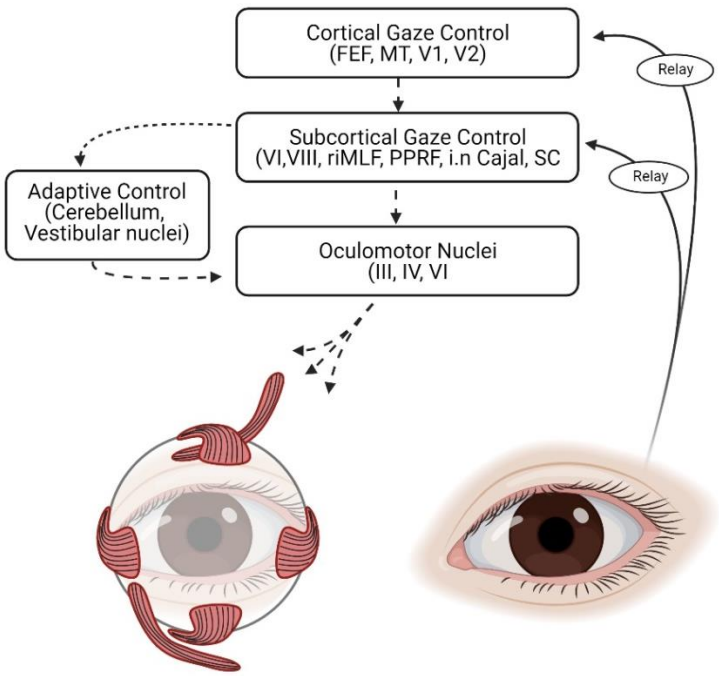

Figure 4. The basic neural pathway for the optokinetic reflex. The schematic presents the hierarchy of the neural pathways involved in producing an optokinetic oculomotor response. Visual motion is registered by the eye (right), and then relayed to cortical and subcortical structures that produce and adapt the reflexive oculomotor output. Created with BioRender.com.

1.2.4 From lamprey to man

Despite the clear differences between primates and cyclostomes the basic principles governing eye movement control appear remarkably similar (see figure 5) [74]. Nevertheless, there are undoubtedly vast differences in habitat and behavioural complexity between our species that put different demands on the eyes and their motility. Therefore, it may be advantageous to touch on the comparative nature of gaze-stability between lamprey and man before going into the details of how vestibular and visual information is translated into reflexive gaze realignments.

Inferring the neural pathways responsible for maintaining the VOR and OKR in humans, and how they relate to pharmacodynamics and various pathologies, is far from straight-forward. The primate visual neural network is complex and its hierarchical processing involves several feedback-loops dispersed throughout the system [61]. For example, the previous segment outlined how the pretectal signal may be integrated through a process involving the cerebellum, though other candidates exist, such as the medial vestibular nucleus or gaze centres [75]. One may keep this in mind when trying to bridge the gaps between brainstem functions and bedside evaluations. In order to address the core principles governing gaze-stabilization, this thesis implements the lamprey animal model, which thanks to their conserved neural mechanisms have been referred to as a "blueprint of the mammalian nervous system" [76].

The VOR likely emerged as the first form of reflexive gaze-stabilization, having evolved in early vertebrates and remaining highly conserved [74]. The lamprey, a jawless vertebrate which diverged from the vertebrate evolutionary line 560 million years ago, features a nervous system which in many ways is representative of that seen in mammals [76]. As the lamprey is considered one of the first animals to possess the VOR, the animal model may provide us with valuable insights into the origins and organization of the multisensory control of gaze-stabilization [74]. The comparison can be further justified by their possessing well-developed image-forming eyes and a layered retina similar to mammals [77,78]. Importantly, the extraocular muscles are organized through the same brainstem nuclei as in mammals, with only some minor deviations due to the varying homology of the eye muscles [79]. Regarding the VOR, the reflex is caused by an activation of the animal's vestibular area as detected by the labyrinth, and relayed to the extraocular muscles in a similar fashion to that of other vertebrates [1,80].

In contrast, it is unclear whether lamprey possess an OKR. Its visual system is, as previously noted, well-developed, and its optic tectum corresponds to the mammalian superior colliculus in its function of dictating gaze shifts based on multisensory input [81]. Much like its human homologue, the optic tectum is layered, and its superficial segment receives visual information from the retina according to a retinotopic map [78]. A corresponding motor map is maintained in deep layer neurons, whose dendrites extend into the superficial layer in order to receive retinal input, and which projects to brainstem regions according to a motor map, correlating eye movements with those of the head and body [82,83]. This network, crucial for body posture, has been well-researched and its neural mechanisms mapped [84].

Altogether, the lamprey animal model lends itself well for studying the neural mechanisms underlying gaze stabilization, as well as its evolutionary origins. The lamprey possesses a small region in its lateral pallium, corresponding to the mammalian cortex, which deals with visual input relayed through the thalamus [85]. In addition, there is a palliopretectal fibre bundle connecting pretectum and the lamprey visual cortex [85]. In this regard, the lamprey is in possession of pathways supporting both cortical and subcortical control of the VOR and OKR. Recording lamprey eye movement responses to optokinetic stimuli would shed further light on the nature of its OKR, and should it exist the well-conserved neural pathways would allow for in-depth studies of the underlying mechanisms. Considering the reflexive nature of the eye movement responses one could expect these pathways to be highly similar to the human network. One matter of particular interest when addressing gaze-stabilization is that of visuovestibular integration, i.e OKR-VOR interaction. According to the network driving the VOR and OKR in mammals, the two mechanisms come together at the level of the vestibular nuclei [86]. Consequently, several interesting candidates for studying the neural mechanisms underlying mammalian gaze-stabilization can be found in the lamprey brain. In humans, particular importance has been placed on the cerebellum for combining visual and vestibular data, with reference to the projections travelling through the inferior olive and cerebellum before reaching the vestibular nuclei [69]. However, experiments in rats have indicated pretectum to be the initial relay point for the OKR, and that the gaze-stabilizing response may be retained even after a cerebellectomy [87]. The layout of the lamprey brain lends itself well to investigating the OKR's point of convergence with the VOR through electrophysiological recordings. One should note that while the lamprey possesses what is referred to as a cerebellar plate, its functionality is somewhat unclear, and has been likened to that of a commissural area [80]. In this way, should the lamprey exhibit an OKR, the absence of a functional cerebellum would provide valuable context for its importance in producing gaze-stability.

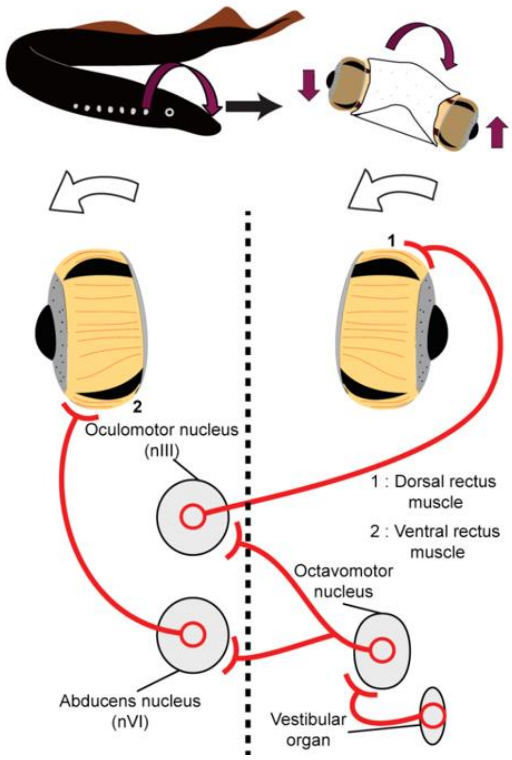

Figure 5. Neural activation of the extraocular muscles in lamprey. The schematic illustrates how the eyes move during a head roll in an adult lamprey with the frontal section of the eye-brain-labyrinth preparation to its right. The red lines illustrate neural innervation between the structures, illustrated as grey ellipses. Input originates in the respective vestibular organs and move upwards in the figure.

1.3 READING THE BRAIN THROUGH EYE MOVEMENTS

Eye movement analysis offers insights into a range of neural processes. As outlined above, the recruitment of the EOM involves several regions of the brain; the activity produced in these regions is often not limited in their function of gaze-stabilization, as illustrated by the VN [46]. In order to maintain proper gaze-stability, visual and vestibular information need to coalesce, and possibly receive modulation by somatosensory contributors as well. Another, mostly reflexive, biological response that rely on these three senses is balance control [88]. In order to function properly, both gaze-stability and balance share a need to quickly decipher movements and produce appropriate and reliable responses. With this in mind, it is unsurprising that several structures in the vertebrate brain play a part in both vision and balance [89,90]. Below will follow a brief contextualization of the pathways relevant for postural control, and how they may be related to gaze-stability.

1.3.1 Reflexive postural control

Vision is key for maintaining balance. This can easily be illustrated by attempting to stand on one leg with eyes opened, and then repeating the same endeavour with eyes closed. Through providing proximal cues, we continuously rely on our eyes to guide our perception of upright. Many people complain of vertigo in scenarios where distinguishable visual features are absent, such as looking down from a tall building; in reality, such symptoms rarely constitute true vertigo, though this will be discussed in a later chapter [91].

In order to maintain balance, humans rely on sensory feedback from three sensory systems: Vision, vestibular input, and somatosensation [88]. Input from the respective sensory organs are integrated across multiple neural structures as outlined below. Many studies have focused on cortex while, much like the integrative aspects of gaze-stabilization, the subcortical mechanisms remain comparatively poorly understood. Through the use of fMRI there has emerged a set of areas thought to be key for balance control. These involve the human middle temporal complex (hMT+), which is sensitivity to motion [92-95], and the parieto-insular vestibular cortex (PIVC), which is believed to integrate the three sensory modalities [96-98]. After the cortical processing, the resulting activity can then be further processed in the cerebellum and vestibular nuclei, where the appropriate postural adjustments may be carried out [11,99-102].

In order to sustain proper postural control, visual, vestibular and somatosensory data are required to convey the same level of information [88]; if we walk forward, optic flow will move visual elements further into the periphery, the semicircular canals will detect the angular acceleration of the head moving with each step, and receptors in the legs, knees and feet will confirm that each step is carried out and that the feet have landed safely on the ground. It is when these systems differ in their input that motion-sickness or vertigo may arise [88]. This mismatch-model system will be further contextualized later in this thesis (The clinical use of gaze-stabilization, motion-sickness intervention). Out of these sensory modalities, one could argue that vision may be the most prone to manipulation. Notably, this occurs when afferent visual data processed through the cortical network cause vection, a faulty sensation of self-motion [103]. While this sensation certainly influences our postural strategies, studies have shown that vection alone cannot account for the postural shifts seen during visual motion, and that a shorter-latency system leading to reflexive balance responses must exist; it has consequently been put forward that this visual modulation of postural equilibrium works in tandem with an extraocular mechanism [104]. An activation of the EOM, through efference copies or re-afferents, would in such a scenario also activate subcortical commands relating

to posture [104]. This hypothesis would offer an explanation for the shorter latency responses, though by suggesting that eye movements themselves give rise to postural shifts it may invite the question of how such strategies were carried out prior to the evolution of mobile eyes.

Considering the network employed in gaze-stabilization, one may expect that the same signal giving rise to an eye movement could contribute toward a reflexive adjustment of posture. Direct projections from pretectum to the MVN would arguably allow for such a pathway, and offer shorter latencies than an extraocular mechanism. Additionally, tectum, corresponding to the superior-colliculus in mammals, is known to play a key role in visually guided behaviours influencing locomotion and postural shifts [83]. As both the optic tract and pretectum project to the superior colliculus, one could expect that such a relationship is likely present in mammals as well, though more heavily modulated by cortical processes.

Gaze-stabilization reflects fundamental principles relating to motion and multisensory integration, and may consequently serve as a prime candidate for offering further insights into how postural control is maintained. As torsional eye movements lack any meaningful voluntary control, compared to horizontal and vertical directions, this thesis aim to study how the roll-plane OKR may be correlated with reflexive responses related to postural control.

1.3.2 Stress responses and the autonomic nervous system

Regulatory mechanisms in our body are maintained through the autonomic nervous system (ANS) [105]. This in turn consists of the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS) [105]. When we find ourselves at rest during unthreatened conditions, the PNS acts as the primary influencer on bodily functions [105]. In stressful situations, such as those seen when our balance is threatened, will instead cause a surge in activity of the SNS, readying the body for immediate action [105].

In psychometric testing, researchers often implement a range of measurements to quantify a stress response, such as skin conductance due to increased sweating, stress hormones in the saliva, pulse rate and pupil-size [106]. In the context of gaze-stability, the latter offers a convenient approach to inferring the effects of visual motion on eye-movements, as many video eye-trackers allow for pupil detection and measuring. When the SNS signals the onset of a fight-or-flight response, the pupil size increases [105]. As the response has a comparatively short latency of approximately 0.25 seconds (s) [107], it is also suitable for detecting both acute and dynamic stress responses [108], such as those that may be expected during postural instability. Pupillometry consequently offers insight into a person's level of alertness and stress.

In nominal conditions, the pupil diameter shifts in response to light and is under the jurisdiction of the PNS; poor lighting conditions cause a relaxation of the iris sphincter, whereas a well-lit scene causes the inverse, aiming to adjust the amount of light entering the eye [105]. The mechanism responsible for this response is mediated through the pretectum, which projects to the Edinger-Westphal nucleus, leading to activation of the ciliary ganglion that ultimately recruits the intraocular muscles [109]. The dilatation seen to matters involving stressful or cognitively challenging scenarios can instead be linked to activity in the locus coeruleus (LC), with later responses seen in the superior and inferior colliculus, as well as in the cingulate cortex dealing with emotional responses [110]. The LC conveys physiological reactions to stress at the level of the brainstem, and has been shown to express causal inference of stress-induced pupil diameter [111].

This invites the question on how the LC is associated with gaze-control. Noradrenaline acts as the primary neurotransmitter involved in the LC, and a recent study in humans has noted that an increase in noradrenaline will result in increased consistency of visually evoked potentials in the visual cortex [112]. Activity in the visual cortex naturally imposes modulatory influences on gaze-stability; visual attention has for example been shown to upregulate both OKR and VOR [113,114] and acute stress has been shown to enhance motion-perception [115]. Considering the location of the LC in the brainstem and its relationship with the SC, subcortical processes may be expected to modify these eye movement response as well.

Pupillometry consequently reflects activity across the neural visual network, and pupil reactions may serve as an indication for how the brain reacts to visual motion. Correlating such responses to that of body-sway may offer further insights into the interconnectivity between visual motion, alertness, and postural control. In order to further the use of gaze-stabilization in clinical testing, this thesis aims to present new insights into this relationship.

1.4 THE CLINICAL USE OF GAZE-STABILIZATION

The ultimate aim of this thesis is to investigate ways through which roll-plane eye-movement responses may contribute to the understanding of gaze-control, which may by extension aid in patient care. As it stands, gaze-stabilization is a key component in a neurological assessment, and a skilled practitioner can infer precise topographical details through a few procedures. This chapter will outline a few general principles pertaining to this type of testing.

1.4.1 Peripheral findings

The peripheral sensory systems that may be clinically tested using the OKR and VOR are the eyes and the inner ear. However, OKR testing generally involves a suspicion of a defunct central pathway; it is certainly possible that damaged EOM may inhibit the OKR, but simpler oculomotor protocols testing smooth-tracking may be better suited for such suspicions [116]. The VOR by contrast offers significant clinical use when assessing its peripheral system.

Most notably, VOR tests are performed on patients with vertigo or balance disorders [57]. Emergency room clinicians are generally reliant on their own subjective interpretation of the patient's response, possibly with the aid of Frenzel-goggles that distort the patient's vision while allowing the tester clear line-of-sight of the eyes [57]. A typical procedure involves rapidly moving the subjects head and checking if the eyes remain fixed in space, as ordained through the VOR [57]. Absence of a reflexive response, altered latencies, or hyperactive nystagmus activity can reveal a great deal of information regarding the structural integrity of the vestibular apparatus [57].

Most advanced vertigo and balance centres are equipped with eye-tracking systems in the form of video oculography [117]. The most common implementation of this technology is through the video head impulse test (vHIT), which allows for bedside vestibular testing of the semi-circular canals [118]. Head impulse tests (HIT) or vHIT have been shown comparable to MRI or PET in discerning between dangerous and harmless causes for vertigo, i.e peripheral damages to the vestibular system or central causes such as stroke or cerebral haemorrhaging [119]. Many tertiary vertigo centres are also equipped with software that allows for immediate and objective evaluations of the recorded eye movements. In this regard there is a wide platform available for implementing novel forms of eye movement analyses, and as eye tracking software is becoming more available the user base is likely to expand.

While this thesis does not aim to separate the central and peripheral systems' sensory contributions towards a gaze-stabilizing response, it is important to recognize the conditions in which similar methodologies to those implemented in this thesis are used. As the ultimate goal of this work is to contribute towards patient care, clinical availability of the methodologies involved should be taken into consideration. All findings presented in this thesis reflect the central integration of visual and vestibular data towards gaze-stability. Thanks to the widespread use of eye-tracking in healthcare, they may emerge in evaluations of the peripheral systems, according to the principles outlined above. The clinical framework for eye-movement analysis is consequently quite well-developed. New findings may benefit from this, as many protocols could be adopted using the equipment already in use.

1.4.2 Central findings

Altered states of gaze-stability can reveal a range of central disorders [57]. Nystagmus is a physiological feature of both OKR and VOR, but many injuries to the brainstem can cause pathological nystagmus beats, either during gaze-shifts or at rest; the direction of these ballistic eye movements may indicate the localization of an injury [57]. Similarly, skew deviation is an eye movement response associated with lesions at the vestibular level in the brainstem, causing a vertical divergence of the eyes similar to that seen during a head rotation [120]. The dynamic integration of multisensory signals are equally prone to modification through structural damages or drugs [121,122].

1.4.2.1 Non-vestibular vertigo

Structural damages may manifest in impaired VOR or OKR, caused by lesions of the brainstem, cerebellum and a number of cortical areas [123-126]. As introduced in the previous chapter, this thesis will focus on postural control, and how gaze-stabilization may reflect central causes of non-vestibular vertigo. Vertigo encompasses all conditions in which a person experiences a false sensation of movement, either self-generated or external. There are many causes for such sensations, and as vertigo constitutes a symptom rather than a clear pathology it may be difficult to diagnose and treat [127,128]. Considering that approximately half of all vertiginous patients exhibit no clear peripheral pathologies [128], identifying additional biomarkers and treatment interventions for central causes are much needed. At this stage, it may be important to address that many patients refer to their symptoms as vertigo, when they in fact experiences dizziness, unsteadiness, or a general discomfort to motion. While vertigo is an active sensation of motion, dizziness encompasses all feelings of general imbalance [127]. For example, the vertigo experienced when looking down a tall building caused by a lack of proximal cues rarely constitutes real vertigo, but rather an uneasy feeling of balance discomfort that could better be described as dizziness. This distinction is important for diagnostic purposes, but may be confusing to a patient for whom the terms may appear interchangeable. Non-vestibular vertigo, consequently, often covers a mix of various symptoms related to either set of symptoms. Many patients experience this motion discomfort from visual movements, giving rise to the term visual vertigo, or visual motion hypersensitivity (VMH); as symptoms are often aggravated in visually cluttered surroundings, it is sometimes called super-market syndrome [129]. VMH can originate from a range of disorders, including migraines, Parkinson's disease, traumatic brain injuries and vestibular disorders [130-133]. Symptoms are often aggravated by non-organic aetiologies or stem from psychiatric conditions [134,135]. It may therefore appear likely that VMH symptoms

could arise from a variety of damages to the central integration involving visual motion. Considering the integrative network merging visual and vestibular data, gaze-stabilization offer promising biomarkers for these conditions, allowing objective and quantifiable values that may be used for identifying the extent of an injury and allow for personalized follow-up protocols. For example, studies have shown VMH patients to express aberrant OKR [136,137]. As vertigo and dizziness constitute disproportionate costs for society, both economic and in terms of quality of life [138], developing gaze-stability analyses for the clinical care of such patients would be of significant importance to the health-care system. By furthering our knowledge of visuovestibular integration in gaze-stability, this thesis aims to identify new approaches through which non-vestibular vertigo may be studied.

1.4.2.2 Motion-sickness intervention

As previously outlined, gaze-stabilization and postural control share several common denominators, such as responding to motion and relying on seamless multisensory integration. Most people have experienced motion-sickness, and while often nothing more than a discomfort, it can lead to debilitating conditions requiring medical intervention or even hospitalisation [139]. Car sickness is an example that may be useful in illustrating this common phenomenon [88]: while the person is seated in a moving car, the vestibular system is at an equilibrium as the head is moving at a constant velocity, somatosensory input corroborates this feeling of being stationary, but visual input will continuously relay a message of intense movement beyond the physiological confines of our evolutionary origins. The resulting sensory discrepancy is what gives rise to nausea and motion-sickness, signalling that something is wrong with our perception of motion as the brain is not in tune with the body [88].

Both the OKR and VOR have been used for assessing the effects of anti-emetic drugs targeting motion-sickness [140,141]. The most commonly used forms of motion-sickness medication are antihistamines, the anticholinergic properties of which are believed to work primarily through inhibiting the peripheral vestibular system [142], possibly exhibiting different levels of efficiency on the SCCs and otoliths [141]. Nevertheless, antihistamines have also been shown to reduce optokinetic nystagmus (OKN) after oral administration, while having no effect on the VOR [140]. It is noteworthy that 1st generation antihistamines are the most efficient in targeting motion-sickness. It has been argued that this may be due to them expressing stronger central properties compared to later generations, also causing stronger side effects, most notably sedation [143]. Meclizine, a common anti-emetic, has been ascribed such a central inhibitory effect on the MVN, a hypothesis deduced by observing decreased VOR gain after administration [141]. As the MVN is involved in both postural and gaze-stabilizing responses,

and receives both visual and vestibular afferents, pharmaceutical interventions such as Meclizine could be tested using the visuovestibular testing protocols, which may in turn be evaluated through monitoring gaze-stabilization.

Considering the fact that gaze-stability has yielded promising but somewhat inconclusive results relating to the effects of antihistamines, further studies are warranted to add context on its central effects. As the torsional response has not yet been used in this line of investigation, its response will be presented in this thesis as an example on how torsional gaze-stabilization may be used in clinical research, and by extension improving patient care.

2 RESEARCH AIMS

The aims of this thesis can be divided into three separate themes. Their purpose is to further our understanding of gaze-stability both for future clinical utility as well as relating to its basic governing principles. Each article compiled for this thesis will deal with one of these objectives according to their respective subdivisions. Ultimately, their findings will be contextualized and the main conclusions summarised.

2.1 THE NEURAL NETWORK

The first part of this thesis is aimed at elucidating the fundamental neural mechanisms allowing gaze-stabilization through visuovestibular integration. This endeavour will employ the lamprey animal model, and as such will also aim to investigate the evolutionary origins of vertebrate eye-movement control.

2.2 NEW INSIGHTS INTO THE PHYSIOLOGICAL RESPONSE

The second set of studies aims to present new insights into VOR and OKR integration. This will involve developing a method for quantifying the sensory contribution to gaze-stability in different scenarios, and investigate how the OKR can be correlated to reflexive responses normally associated with a vestibular response.

2.3 CLINICAL IMPLEMENTATIONS

The third aim focuses on testing the clinical utility of the eye-movement variables outlined in the pursuit of the aforementioned goals. In this context, the sensory specific effects of Meclizine, a commonly used antihistaminergic anti-emetic drug, will be investigated through analysing changes in gaze-stabilizing responses.

3 SUMMARY OF STUDIES

3.1 VISUO-VESTIBULAR GAZE CONTROL – CONSERVED SUBCORTICAL PROCESSING

3.1.1 Aim

In order to better understand gaze-stabilization and its implication in humans, we need to unravel the underlying neural mechanisms responsible for producing these reflexive eye movements. This study aimed to comprehensively analyse the neural structures involved in the visuovestibular integration of the OKR and VOR, while also investigated the evolutionary origins of vertebrate eye movements.

3.1.2 Outline

Gaze-stability relies on reflexive and reliable compensatory eye movements in order to prevent image blurring [8]. As denoted in the introduction of this thesis, this is achieved through the VOR and OKR, two systems having evolved in parallel [10]. In our natural world, these two mechanisms continuously need to integrate, combining self-motion with an ever-changing visual scene. Despite the primordial origins of these reflexive eye movements, most studies have focused on their cortical and cerebellar mechanisms [144]. While the VOR is phylogenetically well-conserved throughout vertebrate evolution, the earliest example of OKR has currently been shown in bony fish, Osteichtyes [26]. Investigating the presence of an OKR in the oldest extant vertebrate, the lamprey, would consequently add important context for how gaze-stabilization operates on the most fundamental neuronal level. As it is unclear whether the lamprey is in possession of saccadic, quick-phase, eye movements, this study also opened up for establishing whether the template for all eye movement control existed already at the dawn of vertebrate evolution.

Aiming to reliably introduce vestibular, visual, and combined visuovestibular stimulations to an animal model, we designed and constructed a moving platform synchronized with visual stimulations on two screens. Having developed an eye-brain-labyrinth preparation of the lamprey animal model, this system allowed for electrophysiological recordings and pharmacological and mechanical intervention across the entire brain. We subsequently implemented four different stimulation protocols, featuring combinations of low and high velocities and amplitudes while measuring activity in the extra-ocular muscles as well as in the vestibular nuclei.

By performing lesions of key structures we could monitor any changes in the way visual and vestibular data were integrated along this pathway, and through injecting tracers into several

structures sustaining eye movement control we could illustrate the network allowing for these shifts. Responses were correlated to behavioural trials where intact lamprey were rotated in three planes, roll, yaw and pitch, while having their eyes filmed so as to allow for eye tracking and characterisation of the VOR. To provide comparative analysis on the gain between different stimulation velocities, we constructed a platform holding a tube of cold water in which an intact and immobile lamprey could fit comfortably. The VOR was analysed using eye-tracking to a series of 180 degree (deg) rotations in this platform, with special reference to identifying slow- and quick-phases.

As several other vertebrate classes have been shown to exhibit gaze-stabilization through spinal corollary discharges during locomotion, we also constructed a semi-intact lamprey preparation where eye movements could be monitored using video eye tracking during swimming with the head fixed in place. Altogether, this set of trials illustrated the conserved nature of the neural mechanisms allowing gaze-stabilization. We were then able to contextualize these findings in an updated phylogenetic tree of eye movement control.

3.1.3 Results

The behavioural trials employed in this study produced reliable VOR responses in all three planes. The subsequent eye tracking focusing on the yaw planed showed that lamprey exhibit clear nystagmic eye movements throughout a vestibular stimulation, producing intermittent slow- and quick phases in a saw-tooth pattern that is representative of a VOR. This shows that the lamprey, as the phylogenetically oldest vertebrate, is capable of the most basic subset of eye movements from which all others are believed to be descended. In assessing the OKR, eye muscle activity scaled with optokinetic velocities in the roll and pitch planes, but not in yaw.

Having established that lamprey possess both OKR and VOR, their integrative properties were investigated. Muscle activity reflected a visuovestibular integration similar to that seen in humans, with visual and vestibular contributions being equivalent in the low intensity protocol and subsequently shifting in favour of the vestibular input as velocities or amplitudes were increased. From a temporal perspective, the peak amplitude for the visuovestibular response was reached at a point in time between those measured for isolated visual and vestibular trials.

Tracer injections showed pretectal neurons extending dendrites into the optic tract and projecting axons to several regions key for oculomotor control, including the vestibular nucleus and oculomotor nuclei. There were also projections to a cluster within the nucleus of

the medial longitudinal fasciculus (nMLF), a region shown to function as a vertical gaze centre in several vertebrate species [145]. Lesioning of pretectum terminated the OKR, showing the structure to be key for integrating optokinetic information. Lesioning of tectum, the agnathan counterpart to the superior colliculus, had no impact on the OKR, though it did cause an upregulation of the integrated visuovestibular response, causing greater contractions and faster onsets of the extraocular muscles. These pretectal and tectal influences on the OKR were also reflected in the neural activity of the VN. Lesioning of the lamprey pallium, hosting the primordial visual cortex, had no discernible effect on the optokinetic responses.

In order to assess the effects of corollary discharges on gaze-stabilization, a semi-intact preparation was made to swim either from mechanical stimulation of the tail or through pharmacological activation of the spinal cord CPGs. The latter did not produce any compensatory eye movements, while mechanically triggering swimming resulted in conjugated eye shifts in the yaw plane synchronous with the tail movements.

Altogether, these experiments allowed us to delineate the integrative neural pathway of visual and vestibular information on its most fundamental level (see figure 6a). These new insights also allowed for creating a phylogenetic tree of eye movements across the vertebrate spectrum (see figure 6b).

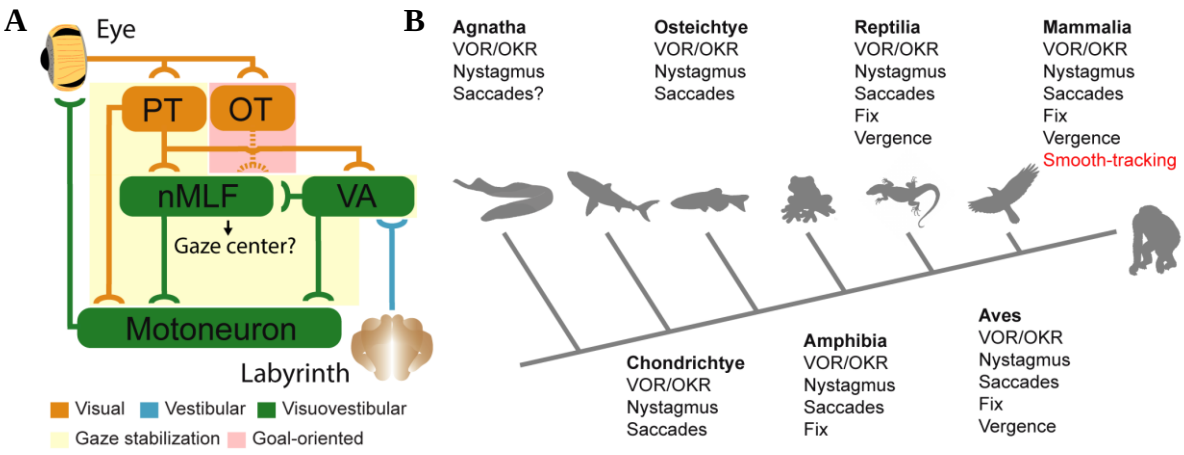

Figure 6. The gaze-stabilizing neural network of lamprey and the phylogenetic tree of eye movements. A) The fundamental neural pathway relaying visual, vestibular, and visuovestibular information in order to produce a gaze-stabilizing response is outlined in yellow, with the visual contribution towards goal-oriented eye movements featured in pink. Abbreviations: PT, Pretectum; OT, Optic tectum; nMLF, nucleus of the medial longitudinal fasciculus; VA, Vestibular area. B) The phylogenetic tree outlines the presence of well-defined eye movements across the vertebrate groups. While studies have indicated that lamprey, an agnathan, possess goal-orienting ballistic eye movements, the presence of

saccades is yet to be established. Smooth-tracking is highlighted in red due to being largely limited to primates.

3.1.4 Discussion

This study established that lamprey possess OKR as well as a VOR featuring nystagmus. Considering the well-preserved nature of vertebrate eye movements [26,74], this sets the stage for using the lamprey animal model for investigating the neural mechanisms allowing gaze-stabilization. It has recently been shown that the lamprey possesses a basic visual cortex [146], which allowed us to investigate the cortical influences on this most fundamental eye movement control. As resection of pallium left the OKR and VOR unaltered, it can be concluded that gaze-stabilization does not rely on cortical input. It is quite possible that their integration is altered by this input however, which future studies may benefit from investigating. Furthermore, the lamprey lacks a functional cerebellum, which allows the conclusion that multisensory control of gaze-stabilization relies solely on subcortical neural processes.

Our study also shows that the first stage integration of optokinetic data into the OKR occurs at the level of the pretectum. This is in line with previous studies on the vertebrate accessory optic system [66]. It is consequently clear that gaze-stabilization has been conserved from the onset of vertebrate evolution, allowing us to produce the neural map and phylogenetic tree of eye movements seen in figure 6b. The fact that tectal inactivation upregulated the integrated visuovestibular response may have implications for how tectum, and subsequently the mammalian superior colliculus, codes for goal-oriented eye movements. This study shows that tectum can downregulate the optokinetic reflex. In the context of the lamprey tectum having been shown to feature a motor map allowing for targeted head-eye movements [82], this finding likely reflects the tectal-pretectal hierarchy of eye movement control, where tectum imposes inhibitory input to pretectum in favour of goal-oriented gaze-shifts. Elucidating the nature of this interaction hence appears quite interesting for understanding voluntary eye movement control, warranting further studies on the matter.

As gaze-stabilization through corollary discharge during locomotion has been shown in several vertebrate classes [8,15], we were interesting in investigating whether this feature was descendent from the agnathan lamprey. Our results indicate that swimming, brought on by mechanical stimulation of the tail, was capable of producing conjugated and synchronous eye movements coupled with forward locomotion. The fact that pharmacological activation of the spinal cord did not activate this clear pattern of eye movements would suggest that while this

response may contribute to a gaze-stabilizing response, it may not be sufficient to produce one without vestibular or visual input. The results nevertheless illustrate that locomotion modulates lamprey gaze-stabilization. As for fictive locomotion, work in the chondricthyan dogfish has shown the spinal cord to be essential in locomotive gaze-stabilization [8]. It is noteworthy that we did observe the odd eye movement during fictive swimming, synchronous with particularly strong body movements. It is therefore possible that a strong locomotive motor command may result in a gaze-shifting eye movement. While this question remains open, our results clearly indicate that the lamprey is in possession of the neural template allowing for gaze-stabilization through corollary discharge, though it is insufficient to produce compensatory eye movements on its own accord.

In conclusion, the presence of clearly defined OKR and VOR in the lamprey shows that gaze-stabilization has been conserved throughout vertebrate evolution, relying on subcortical mechanisms for their integration. As the VOR features both slow- and quick-phases it is clear that the neural template for both smooth and ballistic eye movements existed already at the origin of vertebrate life, offering valuable context for how advanced oculomotor control subsequently emerged. Our results allowed the construction of a neural network showing how visual and vestibular integration leads to gaze-stabilization, and offered new insights into pretectal-tectal interactions for the control of goal-oriented gaze-shifts. Altogether, these findings expand on the fundamentals of vertebrate eye movement control.

3.2 VESTIBULAR EYE MOVEMENTS ARE HEAVILY IMPACED BY VISUAL MOTION AND ARE SENSITIVE TO CHANGES IN VISUAL INTENSITIES

VISUAL AND VESTIBULAR INTEGRATION EXPRESS SUMMATIVE EYE MOVEMENT RESPONSES AND REVEAL HIGHER VISUAL ACCELERATION SENSITIVITY THAN PREVIOUSLY DESCRIBED

3.2.1 Aim

By combining visual, vestibular, and visuovestibular stimulations for two levels of visual clutter and movement acceleration, these studies aimed to outline the effects of visual information density (study 2) and acceleration (study 3) on visuovestibular integration.

3.2.2 Outline

Visually cluttered surroundings are known to cause distractions and increased levels of stress in the work-place [147,148]. Such responses may be perfectly physiological, but many patients with non-vestibular vertigo express severe symptoms in the presence of visually complex surroundings, showing intense aversion to visual motion [129,149]. Visual clutter has also been

attributed in increased fall-risks in the elderly [150], further inviting the question of how visual information density is integrated in the brain. Considering that content added to a visual scene is known to enhance the OKR in the form of increased OT to visual rotations [38], a combination of visual (VIS), vestibular (VES) and visuovestibular (VISVES) stimulations could offer a means to quantify the relative importance of the visual and vestibular systems when integrating cluttered motion. This relationship was evaluated in study 2, which saw subjects view two intensity levels of rotating visual clutter. Based on the results, as presented below, study 3 involved assessing the effects of optokinetic and vestibular accelerations on the eye movement responses. It is well-known that optokinetic input shares several subcortical integration points with vestibular afferents, and that the optokinetic velocity is reflected in the neural activation in the vestibular nuclei [151,152]. For this reason, study 3 employed two levels of acceleration rather than clutter, keeping the visual information density level constant between the intensities. While visual clutter levels are more associated with visual processing, acceleration is thought to be of little influence on vision, as compared to the vestibular system which serves to detect motion [153].

To study the impact of visual clutter and stimulation acceleration on gaze-stabilization, both studies employed a mechanized chair capable of full-body roll-rotations, which were synchronized with a visual scene presented on a projector screen. In this way, it was possible to evaluate the eye movement response to isolated visual and vestibular stimulations, as well as the combined visuovestibular protocol. The visual element in this setup was divided into two intensity levels, featuring low- and high levels of information density (see figure 7). The OKR and VOR responses were tracked in terms of OT and VV using the Chronos Eye-Tracker (C-ETD), and the slow-phase velocities were measured and compared between intensity levels.

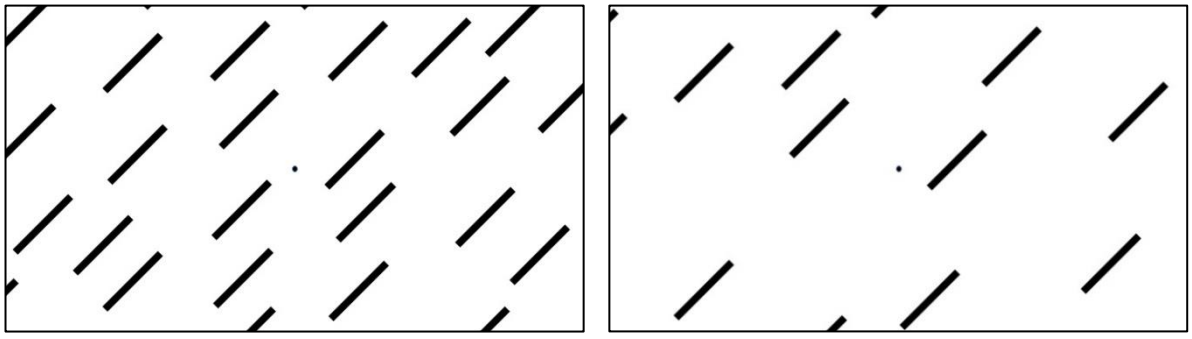

Figure 7. Low and high visual stimulation intensities. The high intensity (left) contains 38 lines centred on a fixation point angled at 45 degrees, while the low intensity (right) features 19 lines.

3.2.3 Results

In study 2, study participants were balanced in terms of sex and age, with age having a significant effect on OT with younger subjects expressing faster slow-phases and a greater increase between VES and VISVES modalities. While visual clutter levels had no effect on neither torsional nor vergence velocities, the ratio between them was significantly altered, favouring torsion over vergence to intensified visual clutter. The eye movement responses are illustrated in figure 8.

In addition, the torsional responses showed robust summative effects when comparing the results between modalities, i.e. the torsional velocity during VIS added to that seen during VES was equal to the VISVES results. This allowed for the quantification of each sense's relative influence on the eye movement response. By dividing the VIS and VES results by that of VISVES, this influence could be retrieved as a percentage. This relationship was not affected by clutter levels.

Study 3 saw no effect of age or sex on the eye movement response. Torsion was significantly altered by acceleration for VES and VISVES modalities, while vergence was affected in all three. The torsion-vergence ratio exhibited a strong trend towards decreasing with increased acceleration intensities, meaning that higher acceleration increased vergence velocities more than that of torsion.

Much like in study 2, torsion exhibited robust summative effects between the three modalities, with the sum of VIS and VES equating the VISVES results. As noted above, acceleration had no impact on torsion for VIS, allowing this comparison despite differences in accelerations between the modalities. Study 3 revealed that the relative importance of vision and vestibular input favoured the vestibular system as the acceleration is increased, through the summative nature of the torsional response was retained.

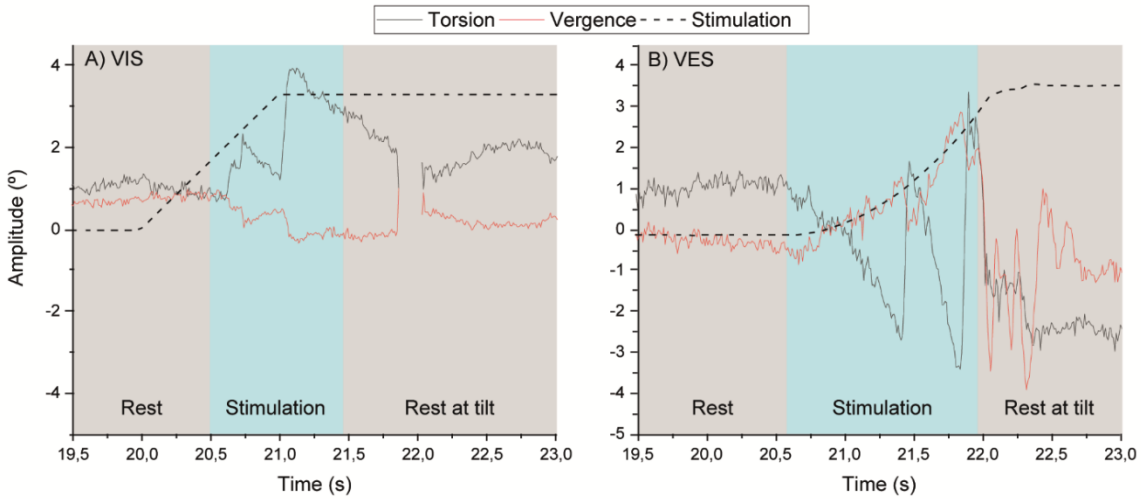

Figure 8. Torsional and vergence eye movement responses to visual (A) and vestibular (B) rotational motion. The visual (VIS) signal has been inverted to facilitate comparisons with the vestibular (VES) responses. Values for the stimulation positions have been divided in half for fitting purposes.

3.2.4 Discussion

This study explored the influences of visual clutter on the integration of the VOR and OKR, finding a significant effect on the relationship between torsional and vergence eye movements. Older individuals are known to be more readily influenced by visual motion in their postural control [154]. As indicated by the eye movement responses, this is also reflected in the VOT, with the younger subjects expressing more adaptive responses between VES and VISVES trials.

It is well established that torsion and vergence are intrinsically linked [155]. This relationship has generally been attributed to their orbital mechanics and joint neurophysiological innervations [23,156]. We show here that a rotating optokinetic stimulation will produce a vertical vergence of the eyes, and that torsion and vergence respond differently to low and high levels of visual clutter. Considering that this cannot be explained by the physical constraints of the orbita, it appears likely that the two eye movements are modified through different neural pathways. While neither eye movement was significantly affected by clutter levels, the ratio favoured that of torsion when the information density was intensified, increasing in relation to vergence. The lack of significance within each eye movement may be due to the short duration of the protocol. In contrast, acceleration exhibited a greater impact on vertical vergence, even affecting the VIS response. This finding was somewhat unexpected considering the previously mentioned notion that the visual system is relatively

unaffected by accelerations. Such a result further highlights the likelihood of torsion and vergence relying on separate neural pathways. Furthermore, as vergence is generally considered a brainstem reflex of a vestibular nature, and that the present study shows it to be sensitive to acceleration over visual information density, one may hypothesise that vergence reflects a visual activation of the vestibular nuclei. Naturally, such a relationship demands further investigation.

The torsional responses showed robust summative effects between modalities for both visual clutter and acceleration levels, allowing for analysing the relative influence of visual and vestibular input to the integrative VOR-OKR response. Unsurprisingly, the vestibular response proved more prominent, guiding ca. 75% of the total reflex as compared to vision's 20% in study 2. Acceleration had a greater impact on the nature of this contribution, lowering vision's portion from ca. 30% during the low intensity to 20% during the high. As these studies did not implement any proprioceptive component, it may be worth reflecting on the possible influences of the cervico-ocular reflex (COR); evidence points towards passive movements of the neck muscles being able to influence gaze-stabilization [28]. Subjects in this study wore an extraction collar to alleviate any effects of the COR, but considering the robust nature of the VOR and OKR that allows for this quantification of multisensory influences, it may be of interest to address proprioception in future studies. Nevertheless, these values provide new insights into the integrative properties of gaze stabilization to varying visual and vestibular conditions. It appears likely that patients suffering from varying degrees of sensory deficits may benefit from being evaluated according to the same principles, allowing for a concrete value which can be used to assess their multisensory integration and consequently follow over time to provide personalized rehabilitation and care.

To conclude, this study is the first to show that visually induced torsion and vergence may express different patterns to rotating visual motion. Such a relationship may indicate separate neural pathways modulating the two eye movement responses; as the visual clutter level increased, the torsion-vergence ratio also increased, while a reverse trend was seen during increased accelerations. Additionally, the torsion response reflected a more dynamic response pattern in young adults compared to older subjects, showing the former to possess greater integrative properties in merging VOR and OKR. Finally, the robust summative nature of the VOT between modalities allowed a quantifiable description of how vision and vestibular information are prioritized in gaze-stabilization under different conditions.

3.3 INTENSIFIED VISUAL CLUTTER INDUCES INCREASED SYMPATHETIC SIGNALLING, POORER POSTURAL CONTROL, AND FASTER TORSIONAL EYE MOVEMENTS DURING VISUAL ROTATION

3.3.1 Aim

This study aimed to investigate how visual clutter influences traditional postural responses through analysing and correlating the OKR with changes in stress- and body-sway measurements.

3.3.2 Outline

In study 2 we showed how visual clutter influences the torsion-vergence ratio during optokinetic rotations, favouring a relative increase in torsion as the visual information was enhanced. The stimulation implemented in the aforementioned study was relatively short, which may have contributed to neither torsion nor vergence being significantly altered independently. For that reason, a study focusing on the isolated OKR was carried out. Additionally, as visual clutter is known to negatively influence postural control [157], the present study employed simultaneous posturographic measurements to monitor body-sway.

The visual stimulation was adapted from study 2, and featured the same general outline of inclined bars (see figure 7). However, as this study was not to be compared to results retrieved in darkness, the colour scheme was inverted, yielding black bars on white backgrounds. In a similar fashion to studies 2 and 3, the visual stimulation started with subjects viewing the static visual scene for 20s, acquiring a baseline. In order to stress the oculomotor responses to the visual rotation, the scene was then rotated for 20s at 72 deg/s^2, after which the static scene was presented for another 20s. Subjects were asked to fixate their gaze on a central fixation point during the entire protocol. Ocular torsion and vertical vergence were recorded using the C-ETD. Additionally, the C-ETD also captures pupil size, which may be correlated to the instinctive stress response to a stimulus[158]. For this reason we also collected pupil size data throughout the protocol.

In order to ascertain that the luminosity of the visual scene did not change during the procedure we used a photometer to measure candela levels during several trials, which revealed no significant divergence across the visual scene. Aiming to correlate OKRs to subjects' postural shifts, we recorded the centre of pressure (CoP), before, during, and after each stimulation as an indication of body-sway; this was done using the Wii Balance Board (WBB). Participants were asked to remain standing upright as straight as possible during the entire protocol. OT and VV velocities were collected across three periods during the active stimulation phase: early (0-5s), middle (7.5-12.5s), and late (15-20s). Values were averaged

for all slow-phases during these periods, allowing a detailed description of the OKR across the stimulation. The average pupil size and body-sway were calculated and compiled into three time periods, before, during and after the visual rotation. Participants were balanced in terms of sex, male or female, and age, young or old. The order of presentation was also balanced between the low and high intensities.

3.3.3 Results

It is well-established that older individuals have a smaller pupil size compared to a younger population [159], which was also reflected in this study. This did however not affect the change in pupil size as a response to the stimulation. There was no other effect of sex or age on the dependent variables.

The visual stimulation produced clear OT and VV responses (see figure 9). OT expressed clear reduction in average velocity as the stimulation progressed, while VV decreased only from the early to middle period. Importantly, visual clutter levels only had a significant effect on OT, with velocities increasing to the higher level of visual information density. Consequently, the increase in OT-VV ratio observed was caused by the increase in the OT response. This eye movement was affected by the order of presentation, as subjects who viewed the lower intensity stimulation first expressed higher velocities during both intensities.

Both body-sway and pupil size were affected by the level of visual clutter (see figure 9). Contrary to our hypothesis, both parameters decreased from the initial baseline to the optokinetic stimulation, reflecting improved postural control and a decreased stress response. While the new level of body-sway was retained after the stimulation had ended, the pupil size increased in size. An increase in visual clutter caused increased values to the higher visual intensity level for both body-sway and pupil size. Notably, this was true for all time periods, even while subjects were viewing static scenes. These results consequently indicate that intensified visual clutter causes poorer balance and increased stress, even when no active movement is involved. One may also note that while the CoP did not follow the direction of visual motion, as the posturographic findings in this study showed that subjects generally exhibited little movement in the lateral axis as compared to substantial anteroposterior sway, most notably towards the visual scene.

Correlation analyses showed that OT and VV were moderately correlated only for the low intensity stimulation, meaning that the two reflexes diverged in their response patterns as the visual clutter was increased. Torsion could also be correlated to pupil size for both visual

intensities, and to body-sway for the high intensity, meaning that OT may prove a valuable proxy for determining postural effects from visual clutter, particularly when exposed to high levels of visual information densities.

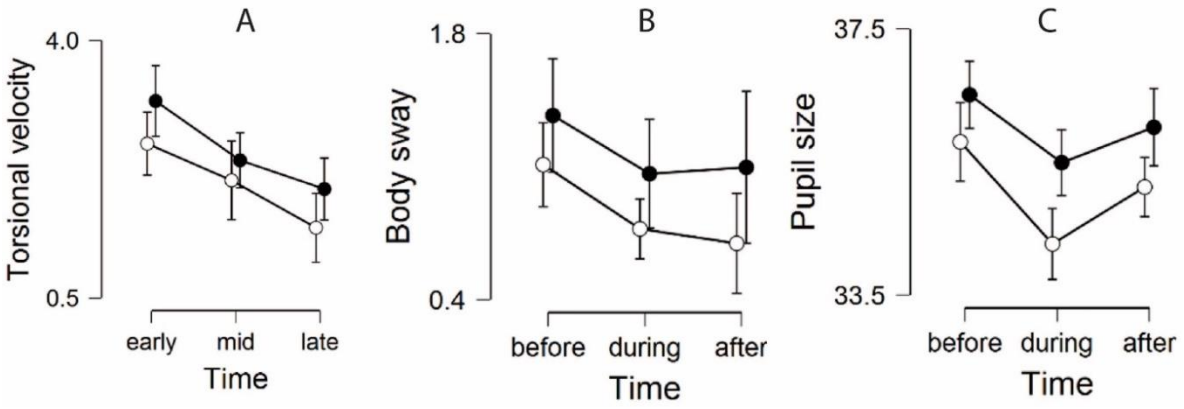

Figure 9. Torsional (A), body sway (B), and pupillary (C) responses to optokinetic stimulation. Torsional velocities refer to degrees per second, while body-sway in the shape of centre-of-pressure changes and mean pupil diameters were retrieved through a method of indexation. The figures are reprinted from Wibble T, Södergård U, Träisk F, Pansell T. Intensified visual clutter induces increased sympathetic signalling, poorer postural control, and faster torsional eye movements during visual rotation. PLoS One. 2020 Jan 3;15(1):e0227370. Published by PLoS One.

3.3.4 Discussion

This study investigated the effects on visual clutter on the OKR and the general balance response, as indicated by body-sway and pupil-size. The reduction in OT over time reflects a well-known fading effect [160]. While OT velocities are known to reflect the speed of an optokinetic stimulation [160], also inducing increased vection [161], this study shows that OT velocities are affected by visual clutter in a similar fashion. It consequently appears likely that the energy levels contained in a rotating visual scene, velocities or clutter, have similar neurophysiological influences on the oculomotor network. We also found a significant effect on both body-sway and the autonomic stress response as reflected by the pupil-size. Altogether, these findings reflect an innate sensitivity to visual clutter, reflected in how visual information is integrated in the balance response. It should be noted that subjects expressed no subjective worsening of discomfort between intensity levels, suggesting primarily subcortical mechanisms involved in the process. Additionally, building on our findings in study 2, the fact that VV was less influenced by visual intensity levels compared to OT suggests that the shift in OT-VV ratio in the previous study may have been due to a shift in

OT velocities, which only became significant during the prolonged stimulation implemented here.

One ambition with this study was to investigate whether OT or VV could be implemented as a proxy for how visual motion affects postural control. As reflected by the correlation analyses, OT proved to be such a candidate, particularly during the high intensity level which produced moderate correlations to both body-sway and the autonomic stress response. The improved postural response and lowered stress response seen during the active optokinetic stimulation were unexpected, considering that visual motion has been shown to negatively affect these parameters [162]. One possible explanation for such a phenomenon could be the loss of directional cues during the static viewing caused by Troxler's effect, caused by the suppression of static visual input during visual fixation [163]. This was frequently reported by subjects, who reported that the visual scene was perceived as blank with the black bars re-appearing during the active rotation. It may consequently seem likely that the loss of visual cues has a stronger effect on the balance reflexes than visual motion. It is also clear that visual clutter has a negative effect on these responses, as higher levels of visual clutter caused increased stress and body-sway during both static and active visual stimulations.

The findings in this study add important context for future studies investigating vertigo of non-vestibular origins. Patients with visual vertigo frequently describe an increased sensitivity to visual clutter and moving visual scenes [129]. This study shows that subjects, even though not being subjectively aware of it, will present with symptoms related to poorer postural control when exposed to visual clutter. Considering that this reflects a nominal physiological reaction, one may expect that patients expressing visual motion hypersensitivities may exhibited stronger responses due to an altered capacity for integrating visual information. Future studies investigating these fine oculomotor responses may serve as potential biomarkers for visually induced postural discomfort, enabling healthcare providers to follow patients over time with reference to objective and quantifiable parameters.

In conclusion, we have in this study shown how intensified visual clutter affects healthy individuals by causing an increased stress response and poorer postural control, as well as an altered OKR with faster torsional velocities during optokinetic rotations. These physiological responses may explain why many people experience discomfort to visual clutter, while also serving to contextualise the symptoms experienced by patients suffering from non-vestibular vertigo. As OT could be correlated to both postural and stress responses, it may serve as a possible proxy for studying visual motion integration in such patients.

3.4 OPTOKINETIC STIMULATION INDUCES VERTICAL VERGENCE, POSSIBLY THROUGH A NON-VISUAL PATHWAY

3.4.1 Aim

Our previous studies have shown that visually induced torsion and vertical vergence can respond in different ways to optokinetic motion, indicating modification through separate neural mechanisms. This study aims to investigate how vision influences the vergence response to optokinetic rotation.

3.4.2 Outline

Vertical vergence, or skew deviation, is a well-known brainstem reflex associated with a head tilt and which may serve as a diagnostic reference for vestibular conditions [40]. Vergence can also be induced by vertical disparities in a visual scene [21]. Vertical vergence is, due to the mechanical constraints of the orbita, expected to be associated with ocular torsion [23]. Our previous studies have shown that OT and VV behave differently depending on the level of visual clutter or motion acceleration of a rotating visual scene, indicating that the two eye movements are not necessarily constrained by their mechanical interactions. Additionally, the rotating optokinetic stimulations present no vertical disparities, inviting the question of why, and how, visually induced VV is induced and maintained.

In order to investigate the sensory drive of visually induced VV, we designed a testing protocol involving binocular and monocular viewing conditions. Subjects were standing in front of the projected visual scene, identical to the one implemented in study 3; they were instructed to stand shoulder-length and remain straight during the entire protocol according to similar principles as outlined in study 4, with the notable exception that the CoP was not quantified. The optokinetic stimulation was subsequently presented in two viewing conditions, the order of which was balanced through stratified randomization. The binocular viewing conditions involved subjects viewing the scene while their eye movements were recorded by the C-ETD in the same fashion as outlined in studies 2-4. The monocular trials involved placing an IR-translucent sheath in front of the left eye, allowing the camera to record binocular eye movements but blocking subject's line of sight of one eye.

Ocular torsion and vertical vergence were consequently monitored throughout these testing conditions. The outcome of the study could indicate one of three possible relationships between the two eye movements: 1) VV remains the same regardless of viewing condition or is continuously correlated to OT, indicating that it is secondary to OT, 2) VV is decreased

during monocular viewing, suggesting a visual drive, or 3) VV is increased during monocularity, signifying visual suppression.

3.4.3 Results

Visually induced vertical vergence was markedly increased during monocular viewing, while ocular torsion remained constant (see figure 10). Additionally, there were no distinct correlations between the two eye movements. Within-subject analyses also revealed significant differences between OT and VV, further signifying that the two are influenced by visual motion in different ways.

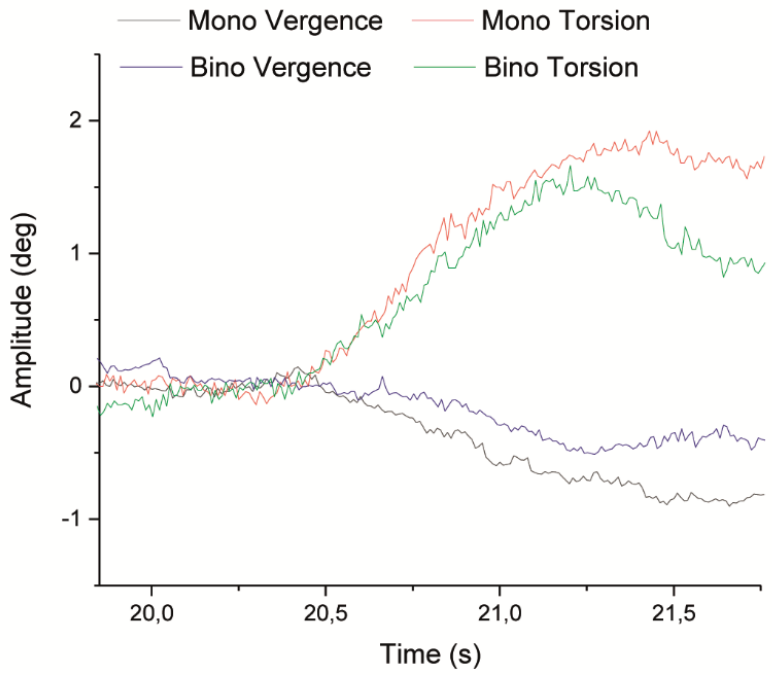

Figure 10. Ocular torsional and vergence responses to optokinetic stimulation during binocular (Bino) and monocular (Mono) viewing. Baseline levels have been adjusted to facilitate comparisons between viewing conditions. All oculomotor responses were collected from one individual.

3.4.4 Discussion

This study adds further evidence that visually induced vergence and ocular torsion rely on separate neural mechanisms. As outlined in our predictions, the fact that VV was increased during monocular viewing suggests that the eye movement response is in fact suppressed by vision. This naturally invites the follow-up question of how, and why, VV is induced in the first place.

As touched upon in the outline of this study, VV is generally considered to be a brainstem reflex stemming from the vestibular nuclei. There is no evidence for any other sensory drive of this phenomenon, with the possible exception of vision through vertical disparities, though even then it is closely coupled with OT [21]. It has been previously suggested that patients with dissociated vertical divergence (DVD) may do so due to an otherwise vestigial human dorsal-light reflex [43]. DVD is characterized by one eye drifting upward when not in use, and studies have shown that children who grew up amblyopic, and later developed DVD, also show deviating subjective visual verticals, perceiving an increased rotation of a visual scene [44]. The dorsal light reflex serves as a postural re-alignment mechanisms based on incoming sunlight rather than gravity, and studies in early vertebrates have shown significant visuovestibular interaction as reflected in vestibular nuclei activity [45]. It is well known that the vestibular nuclei responds to optokinetic stimulations also in mammals [164,165]. In study 3 we showed how visually induced vergence can be affected by acceleration, which the visual system is relatively insensitive to while it is a key vestibular parameter [166]. For this reason, it is tempting to suggest that vertical vergence may reflect an oculomotor response to a visual activation of the vestibular nuclei.

Research in primordial vertebrates has suggested that ocular motility was driven by the development of a vestibular apparatus [167]. From this perspective, the VOR appears to be the driving force of gaze-stabilization, with the subsequent addition of the OKR. This perspective is given further credence from the fact that visual motion employs the vestibular nuclei, indicating that the neural integration of visual motion occurred secondary to that of vestibular input [165]. Should vertical vergence have remained as a vestigial dorsal light reflex in humans, only activated during monocular suppression in childhood, it is tempting to suggest that visually induced vertical vergence reflects a similar mechanism. In Brodsky's studies, patients with vertical divergence perceive and increased tilt due to monocular suppression, while this study showed that healthy subjects viewing a real visual tilt will exhibit increased vertical divergence during monocular viewing. With these two findings reflecting opposite sides of the same coin, it would appear likely that visually induced VV reflects a typically vestibular response in the perception of tilt. As our human visual system has developed to forego the dorsal light reflex, one may postulate that this vestibular response has likely been depressed in favour of the complex processing of visual information allowed by the human cortical-subcortical network. Vertical vergence may then be viewed as a primordial gaze-stabilizing reflex, aiming to maintain a visual horizon on the retina through an activation of the first gaze-stabilizing response in the vestibular nuclei.

In conclusion, optokinetic stimulation will induce a vertical vergence of the eyes, uncoupled to ocular torsion. While visually induced, this response is suppressed by vision as the vergence amplitude was increased during monocular viewing. Based on evolutionary evidence for vertebrate gaze-stabilization, we put forward that visually induced vertical vergence reflects the optokinetic activation of the vestibular nuclei. As such, this eye movement response may be of clinical relevance when assessing patients suffering from visually induced dizziness.

3.5 THE EFFECTS OF MECLIZINE ON MOTION SICKNESS REVISITED

3.5.1 Aim

Our previous studies have shown that the VOR and OKR exhibit robust summative responses in the torsional eye movement reflex. Meclizine is a commonly used antiemetic, but its effects on the sensory systems are still debated. This study investigates the sensory-specific effects of Meclizine on the visual and vestibular system.

3.5.2 Outline

Motion sickness and vertigo are generally believed to stem from a mismatch of multisensory integration, particularly visual and vestibular input [88]. Meclizine is an antihistaminergic H1-antagonist, and while its antiemetic mechanism has generally ascribed to a depression of the vestibular system this efficiency has come into question [168,169]. Studies have shown antihistamines to exhibit different effects on different parts of the vestibular system, while others have yielded no effect at all, instead revealing reduced sensitivities to visual motion [140,141]. For this reason, the methodological principle outlined in studies 2 and 3, deducing visual and vestibular influences through ocular torsion, would lend itself well for evaluating these sensory-specific responses.

The gaze-stabilizing reflexes of the OKR and VOR constitute well-established methods for evaluation antiemetic medications [142,170]. Visuovestibular integration is reflected in a number of neural structures, cortical and subcortical in the brainstem and cerebellum [89,165,171]. Any depression of sensory neural pathway should consequently be reflected in a deviation of their influences on gaze-stabilization.

Studies 2 and 3 allowed us to quantify the influences of the visual and vestibular systems on gaze-stabilization, as they retained a robust summative effect in the torsional OKR and VOR. As motion sickness is frequently associated with velocities and accelerations, the present study opted to implement the same methodological setup as outlined in study 3, introducing

visual, vestibular, and visuovestibular stimulations according to the previously described principles. In order to test the efficiency of Meclizine, subjects participated in all trials to produce baseline values. All participants were then administered either two tablets of 25mg Meclizine, or two sugar pills constituting the placebo intervention; all trials were then repeated after a two hour waiting period. The intervention was triple blinded, with the administration key not being revealed to all researchers until the analysis was complete. The gaze-stabilizing eye movements were analysed in terms of changes in torsional velocities, shifts in amplitudes, and number of nystagmus beats during the stimulation, compared between baseline and post-intervention trials.

3.5.3 Results

The use of a repeated ANOVA model allowed intricate analyses of within-subject effects across the three modalities, comparing the outcomes in relation to pre- and post-medical intervention. Meclizine's effects on gaze-stabilization proved complex. Analyses needed to account for possible learning effects between trials as well as inter-individual differences. Using a Bayesian ANOVA approach, results showed a significant interaction effect where the intervention, Meclizine or placebo, showed significant influences dependant on the stimulation intensity.

Contrary to the current hypothesis, the torsional velocity was increased to vestibular stimulations in the Meclizine group relative to the placebo group. Optokinetic responses remained unaffected, while the strongest effect was seen for the visuo-vestibular trials, where a strong Bayesian correlation showed that torsional velocities decreased during low intensity trials, only to increase in the high intensity protocol.

3.5.4 Discussion

Aiming to evaluate the effects on Meclizine on motion sickness through gaze-stabilization, our results revealed a complex interaction which ultimately suggests that the antihistaminergic efficacy is maintained not through any sensory-specific depression, but rather a general sedation as indicated by the lowered integrative visuovestibular response. Considering the well-described effects of Meclizine on the subjective feeling of motion-sickness, this offers valuable insight into how it may reach its antiemetic effects.

In order to contextualize the findings further, it may be helpful to illustrate how the stimulation parameters could be reflected in real-world scenarios. A train, for example, regularly tilts at angles of 5-7 degrees, while a car sees essentially no movement in the roll plane, compared to fighter pilots who may experience 90 degree rolls at a maximum rate of

125 deg/s [172,173]. In this regard, the accelerations implemented in this study are in the higher end of intensities of what an everyday traveller may expect. The decreased responses during this study's low intensity visuovestibular trials may, therefore, be considered the closest to nominal travelling conditions, i.e. a passenger with intact visual and vestibular input during low accelerations. The facts that the isolated vestibular trial produced increased torsional gain goes against the hypothesis that Meclizine downregulates the vestibular response, conversely indicating an enhancement. Such an enhancement may well be the reason behind the subsequent increase in visuovestibular activity during the high intensity stimulations, as the relative importance of the vestibular system is increased with heightened accelerations as shown in study 3.

As previously outlined, studies diverge on the effects of Meclizine, and the relatively small sample size in this study does pose a limitation for extrapolating the results to a general population. Furthermore, at least one previous study has shown that vestibular function in healthy subjects may only be affected during linear acceleration [174]. Other studies implementing rotatory stimuli often make use of quite high accelerations, up to 400 deg/s^2, which serves an important purpose in stress testing the vestibular system though it may not reflect daily activities [141]. One may also theorize that the outcome could be dosage-dependant; the present protocol provided subjects with twice the recommended intake for motion sickness, though still within the recommended use for the drug. As such, a subclinical effect may be misinterpreted as drug-induced although it may have stemmed from a decreased vestibular gain due to habituation.

Considering the complex response patterns found, this opens up the discussion for what alternate pharmacodynamics may be in place to sustain the anti-emetic effects. Animal studies have recently indicated that the effects of antihistamines are relayed through central receptors, influencing several factors such as respiration, thermoregulation and gastric activity [175]. As antihistamines, primarily early generation iterations, famously cause drowsiness, it is tempting to suggest that Meclizine's desired effects are achieved through general sedation, downregulating the multisensory integration of visual and vestibular inputs. Naturally, such a claim invites neurophysiological trials which cannot be performed on human subjects. It is noteworthy that the majority of antihistamines in clinical use for treating motion sickness are first generation, impacting both central and peripheral systems, whereas second generation iterations are less effective which may be due to their milder central effects [176,177].

In conclusion, Meclizine did not exhibit any inhibitory effect on neither VOR nor OKR. In contrast, VOR gain was increased in the Meclizine group compared to controls, suggesting enhancement of vestibular input. A decreased gaze-stabilization gain was however observed during the combined visuovestibular trial during low intensities, reflecting the protocol closest to nominal travelling conditions. Considering the efficiency of first generation antihistamines over later models, Meclizine's anti-emetic effects are likely due to the central effects of Meclizine and other first antihistamines, possibly in part through sedative influences on sensory integration as indicated in this study. The optokinetic and vestibulo-ocular reflexes summarily offer valuable insight into how visuovestibular integration is affected by antihistaminergic medication. Future studies targeting these systems may benefit from considering gaze-stabilization when assessing the sensory specific effects of clinical interventions.

4 SUMMARY

Eye movements reflect the activity of a complex sensorimotor network. Ultimately, the neural decision to recruit the extra-ocular muscles is carried out by the brainstem. Gaze-stabilization represents the most fundamental level of eye movement control, illustrated by the vertebrate VOR and OKR having evolved already at the dawn of vertebrate life. The neural circuitry of these two mechanisms serves as a progenitor for all vertebrate eye movements [6]. This thesis aimed to approach gaze-stabilization from a wide perspective, unravelling the neural mechanisms conserved from the onset of vertebrate evolution, investigating the integration of VOR and OKR in healthy adults and finally implementing the new insights in a clinical setting.

The first study of this thesis made use of the lamprey animal model to study the neural network allowing for visuovestibular integration in gaze-stabilization. Of particular notice was the preserved role of the pretectal nucleus which served as the first step of visual motion integration; through projections into the optic tract for retrieving optokinetic information this nucleus could then relay data to the vestibular and oculomotor nuclei. Combined with the clear OKR and VOR, featuring both slow- and fast-phase eye movements, these findings show that the lamprey serves as a valid model for studying gaze-stability, serving as the neural template on which vertebrate evolution rests. While voluntary gaze-shifts in humans, such as goal-oriented saccades, are initiated by cortical command, this thesis suggests that gaze-stabilization is achieved through subcortical mechanisms that do not rely on cerebellar or cortical input for merging visual and vestibular data. Furthermore, the interaction between tectal and pretectal influences on visuovestibular integration indicate that the superior colliculus likely served as the first neural structure involved in voluntary eye movements through a downregulation of the reflexive optokinetic response.

Having provided behavioural, neurophysiological and anatomical context for eye movement control in agnathan vertebrates, this thesis allowed us to create an updated phylogenetic tree of eye movements, illustrating the origins of all oculomotor control. Through evaluating the neural basis of the OKR, the VOR, and their subsequent interaction, we are now in a better position for contextualizing human eye movement responses. It was from this perspective we moved forward assessing ocular torsion and vertical vergence in healthy humans.

Before addressing the integrative nature of the OKR onto the VOR in gaze-stabilization, we may first contextualize the findings of the studies dealing with isolated optokinetic stimuli. The aim of studying gaze-stabilization in healthy subjects was to identify features that may be

of clinical utility. As shown in this thesis, ocular torsion could be correlated to body-sway and pupil-size, the former being indicative of postural imbalance and the latter an increased stress response. In addition, it is noteworthy that increased visual information density, or clutter, increased OKR gain. With the OKR reflecting a need to stabilize a visual scene on the retina, one may ask why the same movements would produce different responses; an image with additional visual features should logically demand the same amount of clamping as one with less clutter, as long as they move with the same amplitude and velocity. In contextualizing the eye movement response with its evolutionary origins, i.e the OKR building on the pre-existing VOR network, one possible hypothesis takes shape.

While intensified visual clutter does not necessitate greater OKR gain, it does produce increased retinal flow. As a result, more information is carried through the optic tract. The increased OKR velocity appears to cause additional pretectal activity, as this structure serves as the first integrator of optokinetic information towards a gaze-stabilizing response. It may be that pretectal neurons fail to differentiate between optokinetic velocities or visual content carried through the optic tract, instead relaying visual input based on signal strength as relayed through the optic nerves. Such a relationship may offer important context for visual deficits in the general population, as peripheral ocular damages may lead to altered visual motion processing through the pretectal efferents.

The second order of optokinetic integration occurs at the level of the vestibular nuclei. Approaching this from the perspective of sensorimotor control, we may hypothesise as to why visual clutter affects postural and autonomic responses. Increased pretectal activity may be reflected in a greater engagement of the vestibular nuclei [164], and with no clear way of separating optokinetic velocities from visual content an increase in either would result in upregulated gaze-stabilizing reflexes. Such an interaction would also explain the increased postural and stress responses to intensified visual clutter found in this thesis; an increased vestibular activation will naturally have consequences for postural control, which here was illustrated in increased body-sway and sympathetic activity.

Studies 2-4 also raised the question as to why vertical vergence behaved differently to ocular torsion. The two are known to be intrinsically linked, and there is no clear reason why they should exhibit different response patterns to the same optokinetic stimulus. As previously outlined, vertical vergence is generally considered a vestibular response, secondary to ocular torsion, or driven by visual disparities, aiming to maintain intact stereovision. Several studies in this thesis found deviations from this hypothesis; the torsion-vergence ratio skewed in different conditions, favouring torsion with increased visual clutter and favouring vergence

during increased accelerations. These findings indicate separate neural mechanisms sustaining the two eye movements. As vertical vergence is considered vestibular in nature, we designed a study to investigate the role of visual input to this response. Results reliable showed how the response was increased during monocularity, signifying that binocular visual information inhibits this reflexive eye movement.

The fact that optokinetic stimulation can induce vertical vergence, and that it is suppressed during binocular viewing, constitutes an anomaly in traditional gaze-stabilization. These findings show that vergence is not only part of the VOR, but also the OKR. In addition to adding novel insights into the constituent parts of the optokinetic reflex, it also highlights the fundamental neural connectivity of the VOR and OKR; it is unlikely that visually induced vertical vergence is relayed through direct projections from pretectum to oculomotor nuclei, but like other optokinetic responses are modified by activity in the vestibular nuclei. In study five, we discuss the possibility that visual input supressed this response, though it may also be that monocular viewing simply results in lower pretectal activity, which in turn translates into decreased vestibular signalling; pretectal neurons are known to be differentially tuned to monocular or binocular input in zebrafish, establishing that a neural correlate for such a relationship exists in vertebrates [178]. The fact that ocular torsion remained constant would in such a case indicate that OT may be more readily modified by purely visual pathways, which its increase to enhanced visual clutter would also suggest. The visual-vestibular dichotomy is further exemplified in the studies investigating OKR and VOR interactions. As previously noted, the torsion-vergence ratio was increased to intensified visual clutter and decreased to heightened accelerations. These results fit well within the proposed model of ocular torsion reflecting optokinetic content and vergence signifying vestibular activation, as acceleration is known to have little effect on visual responses but act as a primary variable for the vestibular system.

In investigating the integrative effects of the OKR and VOR in humans, it was clear that the torsional response expressed a clearer summative effect, which was not seen for the vergence reflex. This was manifest in torsional velocities being consistently robust in that when added together from the isolated trials, the OKR and VOR summated to the joint visuovestibular response. Torsion is a known component of both OKR and VOR, whereas we have just recently shown that the same is true for vergence. It is consequently not necessarily surprising that vergence did not reflect this robust summative nature, especially if this eye movement response intrinsically favours the vestibular system. Another possible explanation may be

that, as human vision expresses the least amount of leeway for torsional disparities compared to vertical and horizontal input [179], the torsional reflex is better equipped for realignment.

The summative nature of the torsional response between visual and vestibular modalities paves the way for implementing this eye movement reflex in investigating the sensory specific effects of medical interventions. Meclizine, due to its somewhat contrasting findings regarding its pharmacodynamics effects, offered an appropriate first approach. Contrary to the ruling hypothesis, we found no inhibitory effect on the VOR of this first generation anti-histamine. We rather saw the inverse effect, as VOR velocities were enhanced. A reduction seen in the nominal, visuovestibular, condition could however offer an explanation for the drug's effect through an inhibition of the sensory integration. The conclusion, therefore, was that the anti-emetic effects may not be due to the antihistaminergic influence on the peripheral vestibular system, but rather through a central effect of general sedation. As outlined in previous sections, this is in line with first generation antihistamines proving the most efficient against motion-sickness, as they have stronger central and sedative effects.

5 CONCLUSIONS

The previous segment contextualized the findings of the individual studies, both in relation to each other as well as the general framework of gaze-stabilization. Here follows a brief overview of the main findings of this thesis:

- The VOR and OKR are well-developed in lamprey and show additive properties during visuovestibular stimulations.
- Prectectum appears as the primary contributor to visual motion information to gaze-stabilization.
- Nystagmic eye movements are featured in the lamprey VOR, presenting the evolutionary oculomotor basis for goal-oriented saccades.
- Ocular torsion reflects the visual information density, or clutter, in a visual scene and can be correlated to automated postural responses in the form of body-sway and increased autonomic stress.
- Vertical vergence can be optokinetically induced, though it is inhibited by binocular visual input, suggesting a vestibular drive.
- The OKR, like the VOR, recruits vergence eye movements in its reflexive response. Both means of gaze-stabilization consequently involves slow-phases, quick-phases, and vergence eye movements.
- Visual and vestibular input exhibit clear summative effects on gaze-stabilization in humans as indicated by ocular torsion.
- Roll plane gaze-stabilization offers a valid methodological platform for clinical trials investigating interventions targeting the visual or vestibular systems. This thesis used ocular torsion to show that the anti-emetic effects of Meclizine, a first generation antihistamine, may achieve efficacy through a general sedative effect on the central integration of visuovestibular information.

6 POINTS OF PERSPECTIVE

In breaching the topic of gaze-stabilization from a broad perspective, this thesis presents a number of new findings that warrant further investigation. Starting with the basic principles of visuovestibular integration towards VOR/OKR interaction, the conserved nature of gaze-stabilization throughout the vertebrate lineage invites further studies using the lamprey animal model. While we have shown that pretectum serve as the first level of visual integration, we have only superficially addressed second order integration points. For example, the interaction between pretectum and tectum emerges as a key aspect of eye-movement control. It is still not known through what mechanisms tectum influences the pretectal relay of visual data, and the lamprey preparation offers an accessible approach to such an investigation. The same holds true for cortical input; pallial lesioning did not abolish the OKR, but its impact on visuovestibular integration remains unknown. These trials could be carried out using the methodologies outlined in this thesis, and serve an important role in mapping the foundation of eye movement control. While we focused primarily on the anterior octavolateral nucleus (AON), the lamprey brainstem features three distinct vestibular nuclei [74], and the interaction between these remains largely unknown. The human vestibular nuclei are some of the largest cranial nerve cores, featuring several sub-nuclei. We have investigated visual input into the lamprey AON using both extra- and intracellular recordings, but further studies could benefit from investigating how these signals are relayed throughout this network. Apart from mapping visuovestibular integration, it could also provide evolutionary context for the merging of the octavomotor areas into the clusters that constitute the mammalian nuclei. Additionally, considering the close relationship to locomotion, the vestibular nuclei would likely offer further insights into how somatosensory information influences gaze-stabilization, which occurs through corollary discharge in the lamprey during locomotion. Parallels may be drawn with the human attenuation of the vestibular responses during self-generated movements [53]. Furthermore, tracer injections in the lamprey oculomotor as well as vestibular nuclei showed axons connecting to a region near the nMLF, which may correspond to a primordial gaze-centre. The lamprey consequently offers several promising approaches to studying eye-movement control from both evolutionary and neurophysiological perspectives.

Regarding eye-movement analysis in humans, we introduce the notion that vertical vergence can be induced through optokinetic stimulations. Considering the limited population size, this hypothesis would benefit from additional studies expanding on the nature of such a response. Our hypothesis that it reflects a visual recruitment of the vestibular system requires further

studies correlating vestibular activity to vergence eye movements. This is naturally difficult to do accurately in humans, though MEG or fMRI may be of some assistance. Electrophysiological experiments in primate animal models would provide clearer results if combined with scleral coil eye tracking, as per the golden standard. Nevertheless, it would be interesting to study optokinetic vergence, or the torsion-vergence ratio, in patients who suffer from visually induced vertigo. One may speculate that such symptoms may be correlated with a relative increase in the vergence response that could reflect a hyper-excitability of the vestibular nuclei from optokinetic sources. Such a finding would give important context for non-vestibular vertigo, and offer an objective and quantifiable parameter to implement for clinical use and testing.

The robust summative effect of ocular torsion from the OKR and VOR onto a visuovestibular response opens up for studying sensory specific effects of medical conditions and interventions. We have already implemented such a protocol in our study on Meclizine, and similar designs may be successfully implanted in detailing the effects of any intervention involving visual or vestibular input. Furthermore, the relative influence of each sense onto the visuovestibular sum may be indicative of how patients with sensory deficits integrate motion. For example, patients with non-vestibular vertigo may exhibit greater visual influences. It could also indicate how an individual with visual field loss is affected by optokinetic stimuli; clinical tests can assess visual acuity and the extent of the visual loss, but there is currently no objective way of quantifying how visual motion is affected. As the vestibular response in such patients would be expected to remain unaffected, the torsional VOR may offer a point of reference to what an expected response rate may look like. Similar testing protocols may be implemented for patients with vestibular deficits, or non-specific symptoms of altered motion sensitivity.

7 METHODOLOGICAL CONSIDERATIONS

Through its incorporation of both basic and clinical studies this thesis employs a wide range of techniques. In order to offer an overview of the general principles involved these will be presented in two separate segments, dedicated to the lamprey and human protocols respectively. The article summaries have introduced the general outline these methodologies apply to each specific study. For more detailed descriptions we would refer to the individual papers attached to this compilation.

7.1 ETHICAL CONSIDERATIONS

Due to the translational nature of this thesis, several ethical permits were issued for the range of studies undertaken. All studies consequently complied with the Declaration of Helsinki. Ethical approvals were issued by the local ethics committees for clinical (EPN 2018-1768-31-1.) as well as basic science (N195/14; 5806-2019) protocols. This segment will present an overview of the ethical considerations taken into account for the lamprey and human trials respectively.

The animal trials of this thesis required the decerebration of all lamprey preparations. This was carried out under general anaesthesia induced by MS-222, with every effort made to use as few animals as possible and minimize their suffering. Animals were housed in aquaria that were continuously perfused with oxygen, equipped with filtration systems, and monitored through daily inspections to ensure that the water temperature was kept at the appropriate level and that the animals appeared content. Upon processing the animal for electrophysiological recordings or tracer injections, one animal would be retrieved, anaesthetized in water, before a quick incision at the level of the third gill was administered using a sharp scalpel to ensure a smooth lesioning of the rostral spinal cord. The use of our newly developed lamprey eye-brain-labyrinth preparation allowed us to then record the physiological gaze-stabilizing responses to visual, vestibular, and somatosensory stimulations. The fact that we were able to perform the research protocols in a close to real-life setting is of significant importance when discussing the ethical considerations of the animal trials; as a key component of handling animal trials involves ensuring that the outcome is met with as few animals as possible, the neural integrity of our design lowers the number of animals one may need, providing clear and quantifiable responses reflecting those found in the behavioural protocols. These latter trials involving intact lamprey involved exposing the animals to an alien environment for a short period of time, as they were enclosed in a small cylinder which allowed breathing but discouraged swimming, while a

bright light was shone onto the head to ensure good video-quality. We monitored the lamprey throughout this entire period for signs of duress. The ideal condition for the testing protocol involved the animal attaching to the wall of the cylinder, at which point it would be relaxed, immobile, and offer the clearest eye movement response. For that reason we would take a break if the testing period became unnecessarily prolonged, during which the animal was placed in a larger container of water and the content of the cylinder could be renewed. After the behavioural trials, the animal would be released back into an aquarium.

The clinical studies employed a very mild set of methodologies. The only consistent complaint was relating to the eye-tracker proving a poor fit for some participants. We attempted to assuage this by remoulding the face-mask holding the tracker in place, and offering cotton swabs to alleviate the discomfort. Study six was the only one where subjects reported any negative side-effects from participation, exclusively in the form of increased drowsiness from the antihistamines. Testing a pharmaceutical intervention naturally involves rigorous ethical considerations. It should be noted that the drug, Meclizine, is available over-the-counter, and the administered dosage was within the daily-recommended intake although it was set to twice that of the anti-emetic starting dose. As with all of our other clinical studies, written consent was obtained and subjects invited to ask questions after having the procedure including possible side effects explained to them. It should be noted that no subject expected any improvement from any present concern, and the purpose was solely to test the drug's effect on gaze-stability during the stimulations. Several subjects reported drowsiness as a side-effect, though these were reported from several members of the placebo-group as well, so may well have been due to the repetitive nature of the trials. All subjects had the option to terminate the procedure by pressing a stop-button at any given time, or simply close their eyes during optokinetic conditions. In addition, it was our policy to not award participation with any material and monetary compensations, as we wanted to limit the risk of subjects participating against their own interests. We routinely asked participants on how they perceived the trials and for their advice on possible improvements on subject comfort. Ultimately, we received no indication that any subject perceived any trial to be excessive or anything more abrasive than slightly uncomfortable.

7.2 LAMPREY STUDIES

The preclinical study involved three sets of protocols: behaviour, electrophysiology, and tract tracing. The initial set of behavioural studies employed feature-tracking algorithms on videos obtained with a dedicated camera (Grasshopper3, GS3-U3-23S6M-C, FLIR Systems, Wilsonville) which was used across all trials. This way we could quantify the VOR in all

three planes. In order to visualize its trajectory more specifically, we constructed the lamprey-rotating platform, where an intact specimen was housed in a fitted chamber filled with cold water. As we could control the velocity and amplitude of these rotations, we were able to compare slow eye-movements and fast eye-movements in the yaw plane across multiple intensities. Using the same video-tracking protocols, we were also able to capture the eye-movement response during locomotion induced either through mechanical stimulation of the tail in a semi-intact preparation, or by chemically triggering the central nervous system with glutamate in a brain-spinal cord dissection; the former would cause the lamprey to issue the command for motion, whereas the latter would stem from downstream excitation at the level of the spinal cord, allowing some context for how any locomotion-induced gaze-stabilization would be conveyed. Attempts were made to film the OKR, using mirrors placed at several locations. While we noted some indications of movements, and as shown in the electrophysiological recordings, the eye movements proved rapid and transient, and our video camera was not able to reliable quantify the response. All trials were carried out in darkness, save for a bright light source located just above the video camera in order to allow sufficient lighting conditions for images to be captured while also blinding the lamprey so as to eliminate any optokinetic contribution during the rotation.

Having built the spinning platform using the Arduino microcontroller and servo-motors (see figure 11), we produced a rotating platform capable of sustaining a lamprey brain for several hours while presenting both visual and vestibular stimulations. With our novel lamprey-brain-labyrinth preparation in mind we linked this system with electrophysiological recording equipment in order to construct an integrated platform through which visuovestibular integration could be quantified and subsequently tested through lesioning interventions. Preparations were enclosed in a see-through plastic cylinder, which was cooled through induction via a holding-tube made out of copper attached to a Peltier cooler, keeping the artificial cerebrospinal fluid (aCSF) at 6-8°C. Electrophysiological trials, using tungsten microelectrodes (~1-5 MΩ) connected to a 4-channel amplifier to capture local field potentials (LFPs), aimed to reproduce the physiological responses seen during the behavioural protocols while monitoring neural and muscular responses in some key areas, notably the vestibular nuclei and extraocular muscles. Both vestibular and visual motion triggered activity in these regions, validating the animal model for future use. As the lamprey brain is quite thin, most structures are reachable from a dorsal opening. We were subsequently able to perform a set of stimulations, carry out precise lesions either mechanically or pharmacologically, and repeat said protocols in order to test the effect of specific neural regions on gaze-stabilization. Vestibular stimulations were carried out using a

servo motor and coded in the Arduino IDE. This signal was triggered using Matlab and the Psychophysics Toolbox extension, which also was used to create the optokinetic stimuli synchronized with the servo engine. All experiments were carried out in darkness with the only light sources being the two monitors which presented a white light at baseline; this scene was intermixed with black lines during the optokinetic trials, minimizing the influence of a dorsal light response. Preparations were left to adapt to this light for ca. 1 hour before stimuli were applied. The integrity of the neural network was tested by manually tilting the platform and observing the EMG recordings of the VOR.

Tracer injections were performed as a compliment to the electrophysiological findings, allowing us to visualise the network involved. Tracers were pressure injected into the oculomotor nucleus or the vestibular nucleus, specifically the AON, in order to ascertain how their respective projections interact. This allowed further context for the results found during the lesioning protocols and enabled us to create a map of the fundamental network controlling gaze-stabilization in the brainstem. In addition, injections were performed to aid during the intracellular electrophysiological recordings; unilateral injections into the tract projection from the AON to N.III with dextran allowed us to identify cells carrying the relevant motor response. Patch-clamp whole-cell recordings were subsequently performed on a brain preparation with the AON and pretectum intact, with stained AON neurons being recorded during pretectal stimulations. Recordings were made using pipettes of 7-10 MΩ with an intracellular solution, and the stimulations were made using the same borosilicate glass microcapillaries at one-to-two times the threshold strength of a postsynaptic potential (10-100 μA). As it was not possible to perform physiological optokinetic stimulations, these electrical prompts served to reflect the activity of pretectum as the first level integrator of visual motion, as outlined in the previous trials.

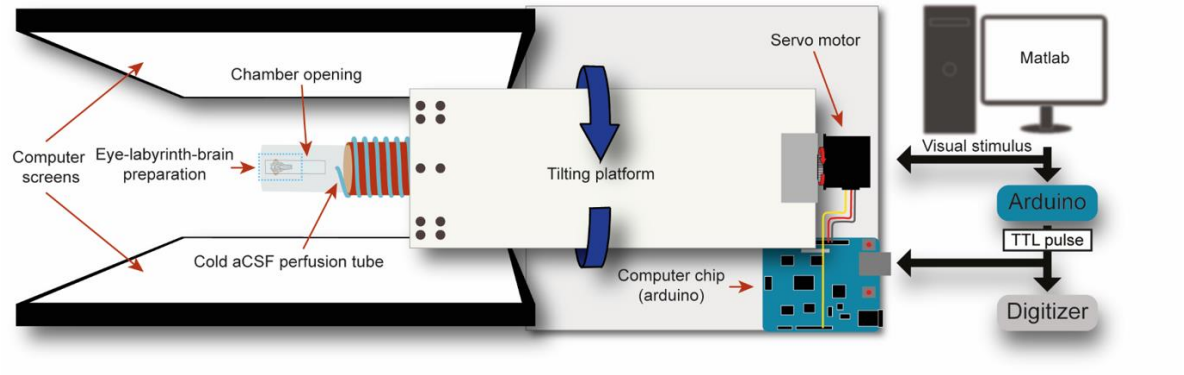

Figure 11. The lamprey tilting platform allowing synchronized vestibular and visual stimulations. Matlab commands allow the system to tilt with the aid of a servo motor while optokinetic stimuli are presented on two monitors. This is relayed through an Arduino microcontroller which also allows for simultaneous electrophysiological recordings. A lamprey preparation can be submerged in artificial cerebrospinal fluid in the transparent chamber, which is continuously cooled through induction using a Peltier system.

7.3 HUMAN STUDIES

In order to quantify the relative contribution of visual and vestibular input on the multisensory control of gaze-stabilization, we exposed healthy human subjects to optokinetic and whole-body roll-rotations of comparable magnitudes while recording the eye movement responses of ocular torsion and vertical vergence. To this effect, an in-house constructed motorized chair was used (see figure 12). Moving on two independent belts connected to two AC Brushless Servo Motors (Baldor BSM90C, 400 V), it is capable of precis translational and rotational full-body movements through its associated software. The chair was implemented in studies 2, 3 and 6, moving subjects with predetermined amplitudes and accelerations around a rotation-point that was readjusted for each individual to be centred on the glabella. This vestibular stimulation was matched with optokinetic rotations presented on a projector screen (res 1024 x 768; contrast 2000:1; update frequency 60Hz) by a front video projector (NEC NP-M350X, NEC Display Solutions Ltd., Tokyo). The visual scene was projected two meters in front of the subject, covering 50° of the field-of-view. The visual motion was produced using PowerPoint for Windows 10 (Powerpoint; Microsoft, Redmond, WA). This experimental setup also allowed for carrying out independent optokinetic and vestibular protocols. This was done by having either the subject sit at rest while the visual scene was rotated according to the study-specific protocol (visual only), or by performing the whole-body rotation in complete darkness (vestibular only). The outcome variables for these two sensory-specific trials could then be compared to the combined trial, featuring the subject

being tilted in the chair while viewing the static visual scene (visuovestibular). The specific layout of the visual scene is presented in the attached manuscripts of each study.

Studies 4-5 implement isolate optokinetic stimulations according to the principles described above, with the important distinction that subjects viewed the screen while standing. The standing position was designated to the same physical location as the mechanized chair would otherwise have occupied. In order to correlate postural sway with the reflexive eye movement responses in study 4, subjects were instructed to stand as still as possible on a Wii Balance Board (WBB; Nintendo, Kyoto, Japan) with feet placed at shoulder width. The CoP was measured using a dynamic sampling rate averaging 99.1Hz, and formatted into a scatter plot in the Origin software where a 95% confidence ellipse was used to quantify the area of movement. This value was taken to be representative of the postural sway and was correlated with the eye movement responses to the respective visual stimulation.

Study 5 tested the impact on binocularity on vertical vergence while viewing a rotating visual scene. Subjects were asked to stand within a marked area, located at the same spot the WBB was placed on in study 4 and according to the same principles, though their postural sway was not monitored. Monocular occlusion was carried out by placing an infrared-translucent sheath in front of the subjects' left eyes, blocking visible light while appearing as translucent to the eye-tracker cameras, thus allowing binocular recordings during monocular viewing.

Eye movements were measured using the Chronos Eye-Tracker (Chronos Inc, Berlin, Germany), employed to monitor the torsional and vergence eye movements known to be shared between the roll plane OKR and VOR. The C-ETD was set to binocular recording at 200Hz for study 2, which was altered to 100Hz for studies 2, 3 and 6 as it was deemed adequate for the slow-phase analyses undertaken. The head mount featured a source for infrared radiation for each eye, and with infrared compatible cameras the eyes could be recorded monitored in complete darkness. In addition to tracking pupil displacements with a high spatial resolution (<0.05°), the C-ETD implements iris feature tracking to calculate the OT (<0.1°). This is done by comparing each frame to an initial reference segment of the iris. The Chronos software provides each frame with a quality score between 0 and 1, with 1 equating the highest level of quality. In order to ascertain high quality data and preclude the eyelid interfering with the iris segment, all frames below a score of 0.5 were removed. The C-ETD also hosts two accelerometers that track the head in three rotational and three translational dimensions. Eye, head and chair positions were exported to Origin (OriginPro 2017; OriginLab, Northampton, MA) where the movement amplitudes were translated into degrees and plotted over time. Displacements in the form of amplitudes, slow-phases and

nystagmus beats were then identified and calculated manually according to the aim of each specific study, as reflected in the respective result sections of this thesis. Nystagmus beats were identified visually as being separated by, and in the opposite direction of, slow-phase eye movements. Head position was compared to the chair location so as to ascertain vestibular activity at any given time. This ensured that each eye movement response could be attributed to the correct sensory system.

Figure 12. The mechanized sled used for whole-body manipulation during vestibular stimulations. The head is stabilized using an extriction collar and hook-and-loop straps. The chair rotates on two belts powered by dedicated servo motors, while the eye movement responses are monitored using the head mounted Chronos Eye Tracker worn by the subject. Reprinted from Wibble T, Pansell T. Vestibular eye movements are heavily impacted by visual motion and are sensitive to changes in visual intensities. *Invest Ophthalmol Vis Sci.* 2019;60:1021–1027. © 2019 The Authors. Published by the Association for Research in Vision and Ophthalmology (ARVO).

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