Neuronal Basis of Horizontal Eye Velocity-to-Position Integration

By

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A dissertation submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

Department of Basic Medical Sciences

Program in Neuroscience & Physiology

New York University

January, 2007

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The reasonable man adapts himself to the world; the unreasonable one persists in trying to adapt the world to himself. Therefore, all progress depends on the unreasonable man.

-George Bernard Shaw (1856–1950)

ACKNOWLEDGEMENTS

Any thesis would not be possible without the help and support of many people. I would like to acknowledge those individuals whose help has made this work possible. First, my advisor Dr. Robert Baker, whose patience and countless support, as I struggled through this endeavor over the last six years, has shaped me into the scientist I am today. I would like to thank the members of my committee, Drs. Eric Lang, Rodolfo Llinas, Jerry Simpson and David Tank for their useful suggestions and encouragement. I would also like to thank Drs. Emre Aksay, James Beck, Josh Bassett, Georgi Gamkrelidze, Guy Major, and Hans Straka for their comments and critical feedback about the goldfish eye position integrator. Many thanks to Dr. Michael Pillinger and Dr. Elof Carlson for reading the various drafts of this thesis. Additional thanks to Akos and Alfred Benedict for their technical assistance and expertise. This work could not have been accomplished without the help of Dores Chu whose excellent care of the goldfish allowed me to concentrate on the more intellectual aspects of this project. Finally, I would like to thank my parents and my brother Daniel who have supported me through this process.

ABSTRACT

Motion of an image across the retina degrades visual accuracy, thus eye position must be held stationary. The horizontal eye velocity-to-position neural integrator (PNI), located in the caudal hindbrain of vertebrates, is believed to be responsible since the neuronal firing rate is sustained and proportional to eye position. The physiological mechanism for PNI function has been envisioned to be either 1) network dynamics within or between the bilateral PNI including brainstem/cerebellar pathways or 2) cellular properties of PNI neurons. These hypotheses were investigated by recording PNI neuronal activity in goldfish during experimental paradigms consisting of disconjugacy, commissurectomy and cerebellectomy.

In goldfish, the eye position time constant (τ) is modifiable by short-term (~1 hr) visual feedback training to either drift away from, or towards, the center of the oculomotor range. Although eye movements are yoked in direction and timing, disconjugate motion during τ modification suggested separate PNIs exist for each eye. Correlation of PNI neural activity with eye position during disconjugacy demonstrated the presence of two discrete neuronal populations exhibiting ipsilateral and conjugate eye sensitivity. During monocular PNI plasticity, τ was differentially modified for each eye corroborating coexistence of distinct neuronal populations within PNI.

The role of reciprocal inhibitory feedback between PNI was tested by commissurectomy. Both sustained PNI activity and τ remained with a concurrent nasal shift in eye position and decrease in oculomotor range. τ modification also was unaffected, suggesting that PNI function is independent of midline

connections.

The mammalian cerebellum has been suggested to play a dominant role for both τ and τ modification. In goldfish, cerebellar inactivation by either aspiration or pharmacology both prevented and abolished τ modifications, but did not affect eye position holding. PNI neurons still exhibited eye position related firing and modulation during training. By excluding all network circuitry either intrinsic or extrinsic to PNI, these results favor a cellular mechanism as the major determinant of sustained neural activity and consequently eye position holding. By contrast, cerebellar pathways are important for sustaining large τ (>20s), and unequivocally essential for τ modification.

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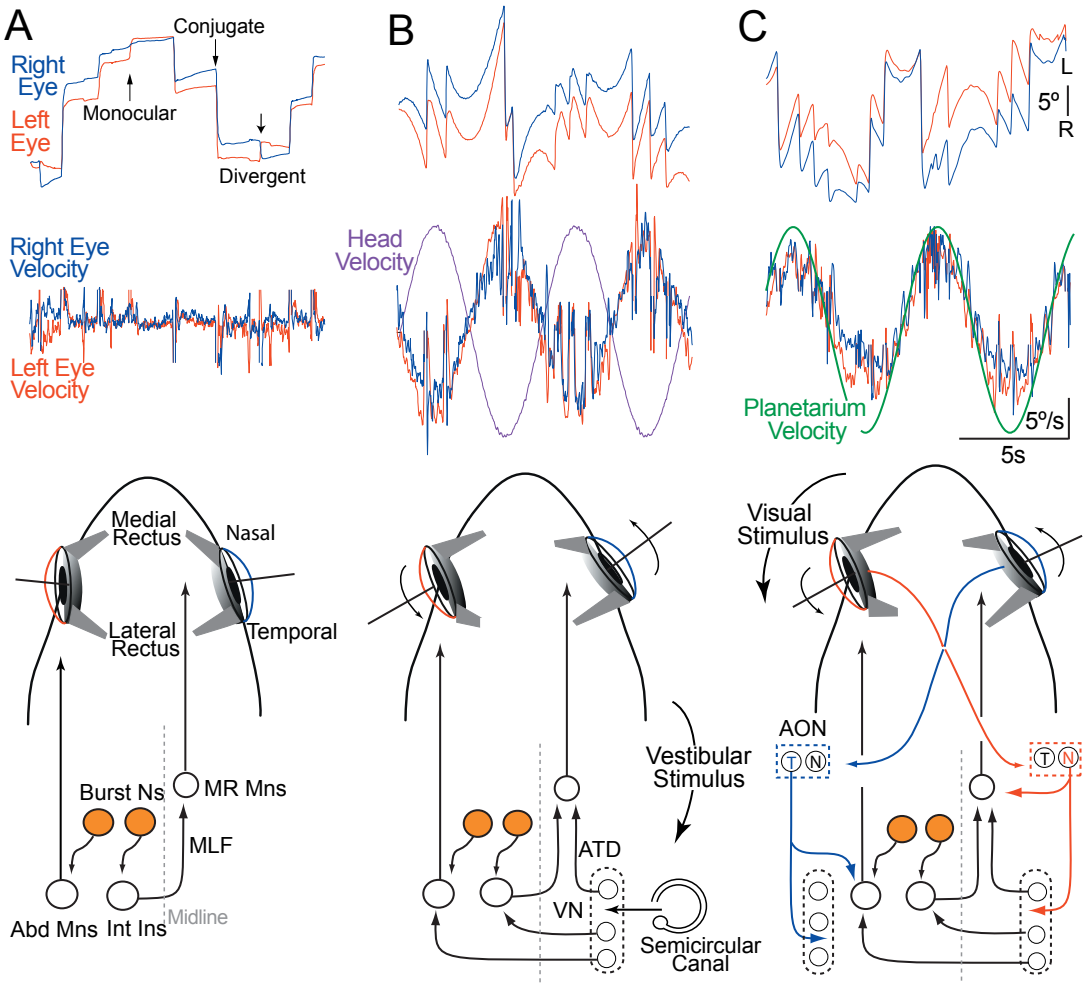
Chapter 1: Introduction

A recent review article posed the question “Why move the eyes if we can move the head?” (Delgado-Garcia 2000). Walls concluded that rather than eye movements having been developed to scan the visual world (Fig. 1-1A), eye movements developed in order to maintain visual acuity during movements of the head (Walls 1962). The effect of image motion without compensatory eye movements can be simulated by waving one’s hand as fast as possible in front of one’s face. Instead of seeing the distinct features of the hand, such as the fingers, the object becomes blurry. The magnitude of image motion necessary to cause blurring of the visual surround differs between species; however, in humans the threshold has been estimated to be ~1°/s (Land 1999). Thus it was postulated that to maintain visual acuity during movement, the speed at which an image crosses the retina, termed retinal slip, must be minimized (Walls 1962). This is the proposed role of the compensatory vestibuloocular and optokinetic reflexes (VOR and OKR)(Robinson 1968). Interestingly, recent experimental evidence has suggested that although retinal slip must be minimized, the complete absence of slip also degrades visual acuity (Land 1999).

Subsequent to the evolutionary appearance of eye movements to compensate for movements of the head, many animals acquired the ability to scan the visual environment and maintain the eyes in an eccentric rotational position in the orbit. In order to do so, a tonic force must be generated to offset the physical dynamics of the connective tissue. The central neuronal circuitry responsible for providing this specific oculomotor behavior became known as the “eye velocity-to-position neural integrator”. This name was chosen because

Figure 1-1: Stereotypical eye movement behaviors in goldfish *Carassius auratus*.

A: Position (upper) and velocity (lower) records during spontaneous eye movements of the Left and Right eye. Conjugate, monocular and divergent saccades are illustrated. A simplified schematic shows reticular (burst) neurons projecting to the abducens (Abd) motoneurons (Mns) and internuclear interneurons (Int Ins). The internuclear interneuron pathway decussates and ascends through the medial longitudinal fasiculus (MLF) to the contralateral medial rectus (MR) Mns (Suwa and Baker 1996). B: Eye position and velocity traces during vestibular stimulation at 0.125 Hz and 15.7°/s peak head velocity (purple). Excitatory second order vestibuloocular reflex pathways are shown for rightward head rotation. Inhibitory connections are omitted for clarity. Neurons from different vestibular subgroups project to 1) ipsilateral MR Mns via the Ascending Tract of Dieters (ATD), 2) contralateral Abd Mns and 3) contralateral Abd Int. C: Eye position and velocity traces during the optokinetic reflex elicited at 0.125 Hz and 15.7°/s peak planetarium velocity (green). The schematic depicts temporal and nasal excitatory pathways in response to leftward planetarium movement. Both nasal-to-temporal (N) and temporal-to-nasal (T) visual motion direction cells are present. Retinal slip is conveyed through pretectal accessory optic nuclei (AON) directly to the Abd and MR Mns complemented by signaling through the vestibular nucleus that is shown here to occur as a direct connection.


A
Conjugate
Right Eye
Left Eye
Monocular
Divergent
Right Eye Velocity
Left Eye Velocity
Medial Rectus
Nasal
Lateral Rectus
Temporal
Burst Ns
MR Mns
MLF
Abd Mns
Int Ins
Midline
B
Head Velocity
Vestibular Stimulus
ATD
VN
Semicircular Canal
C
L
5º
R
Planetarium Velocity
5º/s
5s
Visual Stimulus
AON
T
N

the neuronal circuitry integrated, in the mathematical sense, eye velocity dominated afferent signals into eye position related firing rates (Skavenski and Robinson 1973). The biological mechanism by which these neurons and circuitry accomplish integration is unknown. The purpose of this thesis was to provide experimental evidence to distinguish from among the theoretical models which one best accounts for eye velocity-to-position neural integration.

Basic eye movement control

In vertebrates, each eye rotates within the orbit through the action of six extraocular muscles arranged in three antagonistic pairs (Buttner-Ennever 2005). The anatomical origins and insertions of the extraocular muscles in vertebrates are described elsewhere (Porter et al. 1995). Kinematics of the extraocular muscles in goldfish are beyond the scope of this thesis, but were studied by Graf and McGurk (Graf and McGurk 1985). The eyes can rotate around three distinct axis of motion: horizontal, vertical, and torsional (Wong 2004). Torsional, or cyclotorsional, movements are described as intorsional or extorsional. Intorsion is defined as the rotation of the upper half of the eye towards the nose, whereas extorsion is defined as the rotation of the upper-half of the eye away from the nose (Chernyak 2004).

Lateral rotations

Horizontal rotations are accomplished by the simultaneous contraction/ relaxation of the lateral and medial rectus muscles (Fig. 1-1A) such that each eye can move either rightward or leftward (Fig. 1-1). Temporal rotation, referred to as **abduction**, is caused by contraction of the lateral rectus muscle. The lateral rectus muscle is innervated by cranial nerve VI, whose motoneurons are located in a caudal hindbrain nucleus termed the abducens (Buttner-Ennever 2005).

The abducens motoneurons are embryologically derived from rhombomeres 5 and/or 6 in vertebrates (Buttner-Ennever 2005; Gilland and Baker 2005). In most vertebrates, the abducens motoneurons are contained within one nucleus on each side (Baker and Highstein 1975; Evinger et al. 1987; Glicksman 1980; Steiger and Buttner-Ennever 1978). In goldfish, the abducens motoneurons are contained in two separate nuclei on each side, located about 200 microns apart and termed the rostral and caudal abducens nucleus, respectively (Sterling and Gestrin 1975). Both abducens subgroups are located about 300 microns from the midline and close to the ventral pial surface of the brain (Cabrera et al. 1992). No differences in somatic size were observed between neurons located in rostral versus the caudal nucleus (Cabrera et al. 1992). Injection of horseradish peroxidase into the lateral rectus muscle in goldfish, retrogradely labeled between 42-94 motoneurons on each side, distributed equally between the rostral and caudal nuclei (Cabrera et al. 1992; Graf and McGurk 1985; Pastor et al. 1991).

Abducens motoneurons fire in a phasic-tonic manner, with increasing firing rate at more lateral eye positions (Pastor et al. 1991). The average eye position sensitivity of abducens motoneurons was 7.13 (sp/s)/° and 6.32 (sp/s)/° for the rostral and caudal abducens nuclei (Pastor et al. 1991). The velocity sensitivity during head rotation at 0.125 Hz was 0.80 (sp/s)/°/s and 0.96 (sp/s)/°/s respectively (Pastor et al. 1991). The abducens motoneurons have an eye position threshold that varied across the neuronal population with the mean recruitment centered about 0° (Pastor et al. 1991). Significant eye position and velocity sensitivity differences were not found between the activity of the rostral and caudal subgroups contrary to an earlier hypothesis suggesting that the caudal subgroup controlled phasic contraction of the lateral rectus muscle

and rostral subgroup tonic contraction (Gestrin and Sterling 1977; Sterling and Gestrin 1975). Thus the abducens motoneurons can be modeled as one nucleus in the goldfish.

Medial rotations

Nasal horizontal rotation of the eye, referred to as **adduction**, is correlated with contraction of the medial rectus muscle (Miller and Robins 1992) innervated by a division of cranial nerve III (Porter et al. 1995). In all vertebrates, medial rectus motoneurons develop embryologically in the caudal midbrain (Buttner-Ennever 2005; Gilland and Baker 2005). In goldfish, medial rectus motoneurons are located in a midline neuronal column of the ventral mesencephalon with an anterior-posterior extent of about 350-400 microns (Pastor et al. 1991). In each oculomotor nucleus there are an average of between 26-41 medial rectus motoneurons (Graf and McGurk 1985; Pastor et al. 1991). Similar to abducens motoneurons, medial rectus motoneurons fire in phasic-tonic pattern with an average firing rate sensitivity of 8.4 (sp/s)/° (Pastor et al. 1991). The velocity sensitivity during head rotation at 0.125 Hz was 1.22 (sp/s)/°/s (Pastor et al. 1991). Medial rectus motoneurons also demonstrated clear eye position thresholds and hysteresis in firing rate, similar to abducens motoneurons (Pastor et al. 1991).

Abducens internuclear interneurons

Coordination of the movement of one eye temporally with the contralateral eye movement nasally is believed to be accomplished through the activity of the abducens internuclear interneurons (Buttner-Ennever 2005; Carpenter and Batton 1980; Highstein and Baker 1978). These neurons also have been termed abducens internuclear neurons or abducens interneurons. (De la Cruz et al. 1991; Maloney et al. 1992). The abducens internuclear interneurons project to

the contralateral medial rectus motoneurons and were initially hypothesized to receive similar afferent connections as the abducens motoneurons (Carpenter and Batton 1980; Highstein and Baker 1978). The extent of separation of the motoneuron population from the internuclear interneuron population within the abducens nucleus varies in vertebrates. In frontal eyed mammals, such as primates and cats, the abducens motoneurons and internuclear interneurons are co-localized and intermingled within the abducens nucleus (Cabrera et al. 1992; Kairada 1985; McCrea et al. 1986; Steiger and Buttner-Ennever 1978). However, even in cats, some separation between the two populations of neurons has been observed with rostral sections of the abducens nucleus containing more interneurons and the caudal sections containing more motoneurons (Steiger and Buttner-Ennever 1978) raising the possibility that these neuronal populations may not receive identical innervations. Supporting this assertion, abducens internuclear interneuron activity was found to be similar, but not identical, to the activity of the abducens motoneurons in cats during spontaneous scanning and OKR (Delgado-Garcia et al. 1977).

In lateral eyed mammals, the abducens internuclear interneurons and motoneurons are spatially separated to varying degrees (Cabrera et al. 1988; Evinger et al. 1987; Glicksman 1980). In goldfish, separation of the abducens motoneurons and internuclear interneurons is the most complete of any animal investigated, since internuclear interneurons are located in different nuclei than the motoneurons (Cabrera et al. 1992). Similar to the abducens motoneurons, rostral and caudal abducens internuclear nuclei have been described (Cabrera et al. 1992). An average of 54 abducens internuclear interneurons are present on each side in the goldfish with 48% in the rostral nucleus and 52% in the

caudal nucleus (Cabrera et al. 1992).

Function of abducens internuclear interneurons

It was originally proposed and the predominant opinion was that a primary role of the abducens internuclear interneurons was to mediate conjugate motion of the eyes (Baker and Highstein 1975; Buttner-Ennever 2005). However, evidence suggested that the major function of abducens internuclear interneurons was to control nasal eye motion independent of abducens motoneuron activity (Delgado-Garcia et al. 1977). Regression analysis supported this viewpoint as abducens internuclear interneuron firing rate versus eye position showed a better correlation for the contralateral eye position than the correlation of the firing rate versus the ipsilateral eye position (Delgado-Garcia et al. 1977). This observation in the derived mammalian frontal eyed cat clearly implied that the abducens internuclear interneurons control the contralateral eye, and indirectly conjugate eye motion.

Control of vertical and torsional eye movements

In contrast to horizontal eye movements, which are primarily controlled by the action of one muscle pair, vertical and torsion eye movements are due to the action of two muscle pairs (Porter et al. 1995). The four extraocular muscles controlling vertical and torsional eye movements are the superior rectus, inferior rectus, superior oblique and inferior oblique.

The superior rectus is innervated by cranial nerve III by motoneuron axons that originate in the contralateral oculomotor nucleus (Glicksman 1980), and the primary action of the muscle is elevation of the eye (Porter et al. 1995). However, contraction of the superior rectus also produces some intorsion and adduction (Porter et al. 1995). The inferior rectus is innervated by cranial

nerve III, whose motoneurons are located in the ipsilateral oculomotor nucleus (Glicksman 1980). The primary action of the inferior rectus is depression (Porter et al. 1995). Contraction of the inferior rectus also produces extorsion and adduction (Porter et al. 1995). The inferior oblique is innervated by cranial nerve III whose motoneurons are located in the ipsilateral oculomotor nucleus. The primary action of the muscle is extorsion and abduction (Porter et al. 1995). If the eyes are medial in position, contraction of the inferior oblique will elevate the eye (Porter et al. 1995).

The superior oblique muscle is innervated by cranial nerve IV, referred to as the trochlear nerve, whose motoneurons originate in the contralateral trochlear nucleus (Glicksman 1980). The superior oblique produces intorsion when the eyes are lateral and depression when the eyes are medial (Porter et al. 1995). The trochlear motoneurons are derived from the first rhombomeric segment (Buttner-Ennever 2005; Gilland and Baker 2005).

Oculomotor compensatory reflexes: vestibuloocular reflex (VOR)

Since the original proposed purpose for eye movements was to keep the visual world fixed upon the retina, it appeared evolutionarily advantageous for a system to develop in which head movements would directly evoke compensatory eye movements. This compensatory reflex has been termed the vestibuloocular reflex (VOR)(Delgado-Garcia 2000). A brief review of the vestibuloocular system will be provided in the introduction although the major focus of the thesis is the horizontal eye velocity-to-position neural integrator and not the VOR. A detailed review article on the mechanisms and function of the VOR has been published (Straka and Dieringer 2004). Although vestibular responses occur after both rotation and translation of the head, the neuronal circuitry responsible for the

angular VOR is much better understood (Rohregger and Dieringer 2002).

Signal transduction in the angular VOR

Located within the vertebrate head is a series of fluid filled ducts called the semi-circular canals, which are responsible for detecting rotational vestibular motion (Highstein et al. 2005). A review of the spatial and temporal coding of the semicircular canals has been published (Highstein et al. 2005). In most vertebrates, including goldfish, three semicircular canals exist on each side of the head: horizontal, anterior, and posterior (Straka and Dieringer 2004). The anterior, and horizontal canals are also known as the superior (Cullen and Minor 2002; Schratzenstaller et al. 2005), and lateral canals respectively (Highstein et al. 2005). The three canal system allows rotation to be detected in three orthogonal planes: horizontal (0°), 45° and 135°. The anterior canal on one side of the head and the posterior canal on the opposite side of the head form the 45° and 135° planes while the horizontal canals form the horizontal plane (Straka and Dieringer 2004). Each canal has a widening called the ampulla (Highstein et al. 2005). Within the ampulla is a membrane called the crista ampullaris, in which the mechanotransductive hair cells are located (Highstein et al. 2005). When the head accelerates, inertia causes the endolymph to exert a force against a gelatinous membrane called the cupula, deflecting it and causing the hair cells to bend (Highstein et al. 2005). Bending of the hair cells in the "on" direction increases the release of neurotransmitter which depolarizes the primary vestibular afferents (Highstein et al. 2005).

The relationship between head acceleration and activity of primary vestibular afferents has been largely studied with sinusoidal waveforms since acceleration, velocity and position can be related mathematically by a phase

value. Due to fluid mechanics within the semicircular canals, the primary vestibular afferent neuronal signal is phase lagged compared to the rotational acceleration (Ezure et al. 1978). The response of most primary afferents is more closely related to head velocity, although in fish some afferents encode acceleration (Rabbitt et al. 1996). The three neuron horizontal angular VOR pathway has been the most thoroughly studied and it will be detailed below.

A simplified schematic of the horizontal rotational VOR pathway and eye movements for goldfish is shown in Fig. 1-1B. The horizontal primary afferents project to 'second-order' vestibular neurons in the vestibular nuclei (Buttner and Buttner-Ennever 2005; Buttner-Ennever 2005). Secondary excitatory neurons project to the contralateral abducens motoneuron and the abducens internuclear interneurons (Fig. 1-1) (Buttner-Ennever 2005). The abducens internuclear interneurons cross the midline and terminate on the contralateral medial rectus motoneurons. Thus, both eyes move counter to the direction of head rotation (Baker and Highstein 1975). In addition, a distinct population of vestibular neurons first described in mammals and named the Ascending Tract of Dieters (ATD), provides an ipsilateral excitatory connection to the medial rectus motoneurons (Baker and Highstein 1978). Inhibitory (not shown) second order vestibular neurons project to the ipsilateral abducens motoneurons and internuclear neurons, but not to either the ipsilateral or contralateral medial rectus motoneurons (Baker and Highstein 1978). These excitatory and inhibitory pathways form the "direct" pathway, or the so-called "3 neuron arc" (Szentagothai 1950). At low frequencies of vestibular rotation, i.e. <0.1 Hz, an additional pathway is required for proper eye movement compensation. The anatomical connections of the anterior and posterior canal's three neuron pathways are

described elsewhere (Graf 1988).

Role of proprioception in VOR

Although proprioceptive signals have been shown to originate from the extra-ocular muscles, the presumed afferent feedback has not been shown to affect VOR response dynamics (Donaldson 2000). Proprioception has been hypothesized to serve as a “long-term recalibration signal”, since the VOR is an “open loop feedback system” (Porter et al. 1995). Such signals might allow corrections in eye movements initiated by head motion in the absence of vision.

Oculomotor compensatory reflexes: optokinetic reflex (OKR)

Eye tracking in response to external world motion, called the optokinetic reflex, is another major compensatory mechanism (Fig. 1-1C). The OKR is induced by retinal slip conveyed in the vast majority, if not all, vertebrates through the accessory optic system (Giolli et al. 2005). In contrast to the “open-loop” feedback VOR, the OKR is described as a “closed loop” feedback system since errors in eye velocity compensation will cause retinal slip and initiate a compensatory OKR (Schweigart et al. 1999) (Fig. 1-2). A review of the known anatomy and connectivity of the accessory optic system in vertebrates has been recently published (Giolli et al. 2005). While not a focus of this thesis, the relevant connections are described below.

The accessory optic system (AOS)

It has been stated that the optic chiasm of goldfish completely decussates such that the right side of the brain only receives retinal afferent signals from the left eye (Sharma 1972). However, some anatomical studies questioning this assertion suggest the presence of ipsilateral retinal pathways (Springer

and Gaffney 1981; Springer and Landreth 1977). The extent of retinal signal decussation is not critically important since detailed knowledge of AOS efferent connections in teleosts is limited (Finger and Karten 1978; Uchiyama et al. 1988). Direct projections are proposed to exist between the accessory optic system and the ipsilateral abducens and medial rectus motoneurons in teleosts as illustrated in Fig. 1-1C (Uchiyama et al. 1988). Electrophysiological responses of pretectal neurons in rainbow trout has shown at least two separate populations of neurons. One type is responsive to nasal-to temporal visual motion while the other responds to temporal-to-nasal visual motion (Klar and Hoffmann 2002). Thus both directions of horizontal eye motion, temporal and nasal, can in theory be elicited monocularly by visual stimuli (Fig. 1-1C).

Both anatomical and electrophysiological evidence suggests that accessory optic signals are also relayed through hindbrain vestibular pathways. In cats, the nucleus of the optic tract was shown to project to vestibular neurons mediating the horizontal VOR (Watanabe et al. 2003). In addition, electrophysiological recordings from second order vestibular neurons in mammals (Boyle et al. 1985; Cazin et al. 1980) and goldfish (Allum et al. 1976; Dichgans et al. 1973) have demonstrated firing rate modulation in response to optokinetic stimuli. Thus optokinetic behaviors in goldfish are partially mediated by direct connections to the extraocular motoneurons as well as through the vestibular nuclei.

During constant velocity rotation of the visual surround, second order vestibular neurons in goldfish exhibit sustained elevated firing rates indicating that the adequate stimulus for OKR is retinal slip velocity (Dichgans et al. 1973). Sinusoidal analysis, however, showed that vestibular neurons modulation was

closely correlated with eye velocity rather than slip velocity, indicating a signal transformation between AOS and vestibular nuclei (Green et al. 1997).

Response dynamics of VOR and OKR

Normally, during combined vestibular-visual viewing conditions the VOR and OKR work synergistically as depicted in Fig. 1-2, to ensure compensatory eye movements across a wide range of frequencies and velocities (Baarsma and Collewijn 1974). The optokinetic and vestibular reflexes can be experimentally independently studied. The OKR response is better during low frequency motion (Fig. 1-2 black curve) (Keng and Anastasio 1997; Marsh and Baker 1997), while the VOR response is better during high frequency motion (Fig. 1-2 yellow curve) (Baarsma and Collewijn 1974; Godaux et al. 1983a; Schweigart et al. 1995). The more accurate eye movements during high frequency head rotation are illustrated by the absence of a blurring hand and even clear recognition of the fingers when asked to rotate your head (VOR) as fast as possible, while holding your hand stationary. If the behavioral conditions are reversed and the head is stationary while the hand moves (OKR) then the image is blurred.

The better gain and phase of the OKR during low frequency as opposed to high frequency movements can be partially explained by the relative latencies of the vestibular and optokinetic responses. In goldfish, the latency of the VOR to velocity steps is ~19 ms for adduction and ~25 ms for abduction, reaching peak velocity within ~ 100 ms (Pastor et al. 1992). This is in stark contrast to the latency of the optokinetic system which is ~76 ms in goldfish, and reaches a peak velocity at ~300 ms (Marsh and Baker 1997). However, in the natural life of the animal the differences in response dynamics is unimportant as both the OKR and VOR function in tandem to ensure a compensatory gain of 1

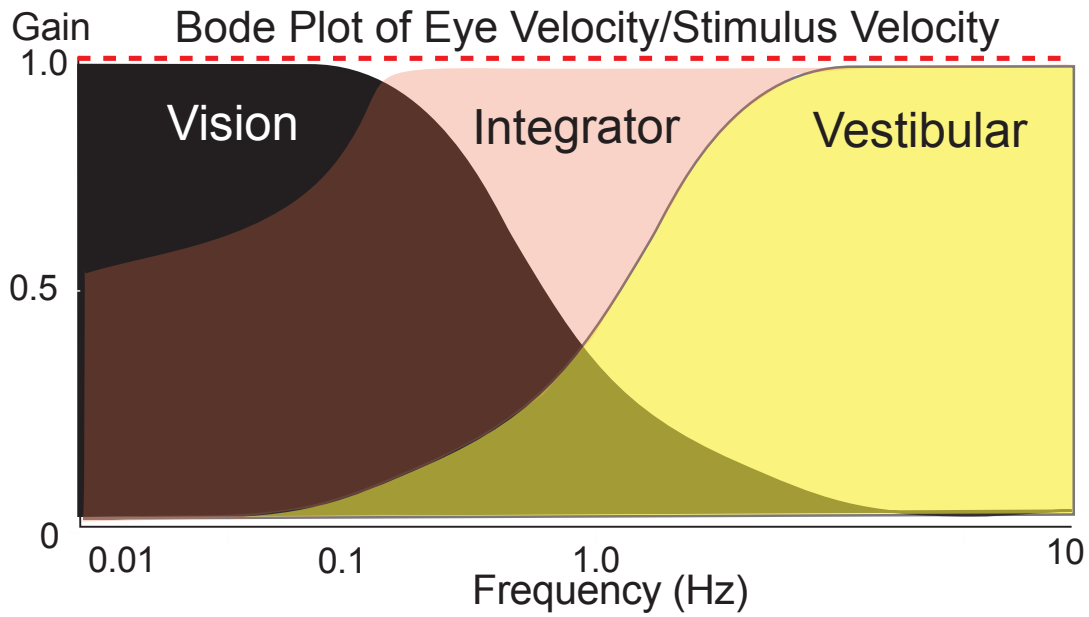


Figure 1-2: Visual and vestibular contribution to horizontal eye movement in goldfish.

Bode plot vignette of optokinetic (black) and vestibular (dark yellow) induced eye velocity vs. stimulus velocity (Gain) during rotation from 0.01 Hz to 10 Hz. The additional compensation provided by the eye velocity-to-position integrator (pink) allows perfect compensation to be achieved throughout the frequency range. The integrator serves to extend the VOR gain range. Adapted from Marsh 1997, Keng 1997, and Pastor 1992.

regardless of either frequency or amplitude of motion.

Oculomotor terminology

The oculomotor field has developed a long list of terms used to describe eye motions. Many of the words are used interchangeably; however, the definitions adopted by authors differ, which has confounded many issues. The first concept which is fundamental to understanding oculomotor physiology is that although most experiments record and comment on eye position, eye velocity and eye acceleration, these measures are an *indirect* assessment of the neuronal output. The oculomotor system encodes extraocular muscle forces; however, since these are difficult to measure without disturbing the dynamics of the system, behavioral characteristics are used as a surrogate assessment.

Oculomotor performance (Fig. 1-1 traces) is defined as how closely an animal's eye motion tracks an optokinetic stimulus (OKR) or compensates for angular head rotation (VOR). Depending upon the task, performance can be measured as a gain, or phase relationship. The ability to maintain an eccentric gaze, known as **fixation stability**, is also a measure of oculomotor performance.

Plasticity is any modification of oculomotor performance and is composed of two separate but related processes, **learning** and **memory**. The definitions of learning and memory used in this thesis are the simplest variations of the definitions provided by Morris (Morris et al. 1988). **Learning** is the acquisition of a new behavioral level. Improvement in the performance of an oculomotor task over time is envisioned as a surrogate of learning. The second component of plasticity is **memory,** defined as the ability to recall a previously learned behavioral level. Memory is also viewed as the retention of a new behavior.

Memory is tested when the learning stimulus is withdrawn.

Saccades and nystagmus

When compensatory eye movements require displacements larger than the oculomotor range of the eyes, quick ballistic movements are generated counter to the direction of slow phase motion thereby allowing continuous tracking. These resetting movements are termed **saccades** (Fig. 1-1A arrows) and are generated by burst neurons in the pontine reticular formation (Fuchs et al. 1985; Moschovakis et al. 1996). **Nystagmus** is an oculomotor behavior in which the eyes are driven to drift in one direction and saccadic “fast phases” occur in the opposite direction to re-center eye position (Zee 1985). Nystagmus can be either a compensatory response or it can indicate a **velocity bias** present throughout the oculomotor range. A velocity bias is a non-eye position dependent constant eye drift and is believed to be generated by an imbalance of activity between the vestibular nuclei (Zee 1985). Since it is assumed that a velocity bias is not generated by the eye velocity-to-position neural integrator, its presence is not considered a measure of integrator function, but rather a global measure of the state of the oculomotor system.

The phenomenon of compensatory nystagmus to extend the oculomotor range is classically illustrated by the vignette of the ocular responses when riding a train. When visualizing by a subject looking out the window at the passing visual environment, their eyes are noted to exhibit a saw-tooth pattern of tracking motion with saccades in the opposite direction. However, the individual’s view of the scene from the window is perceived as a smooth constant motion of the world.

The saccadic system can be utilized in many species including goldfish to

scan the visual environment in the absence of motion (Fig. 1-1A) (Dieringer et al. 1992; Easter 1971). The long periods of stable eye position between saccades are named the intersaccadic fixations, or more simply **fixations** (Mensh et al. 2004) (Fig. 1-1A).

Fixation stability, leak, instability

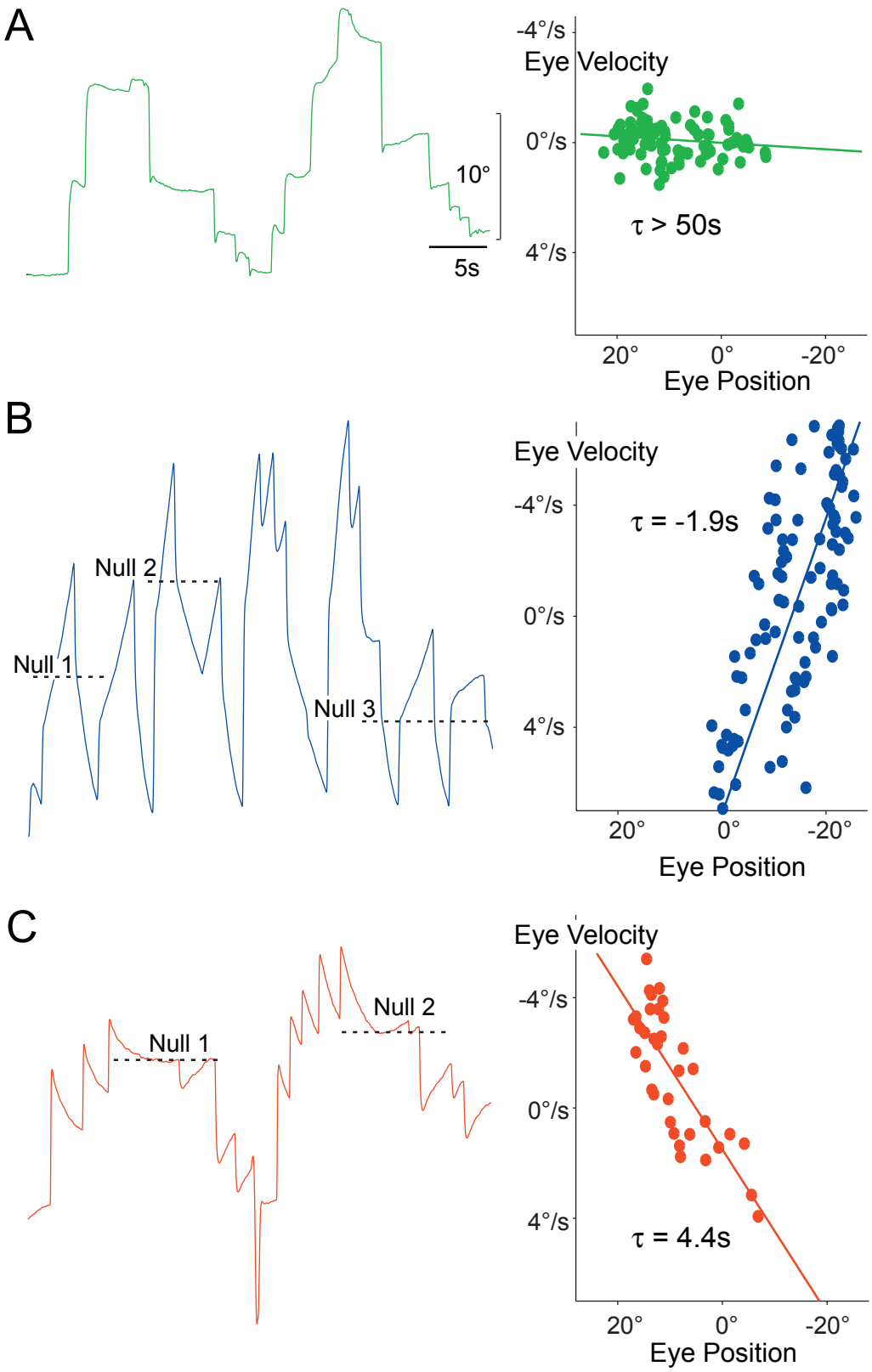
In this thesis, the term **stability** will be used in reference to the post-saccadic drift in the absence of visual feedback. Stability is measured by the time constant of eye position decay (τ) during fixations. The time constant is mathematically defined as the time at which the eye position would reach 1/e of the initial eye position. In the literature, the time constant has been computed by either fitting an exponential equation to each fixation or by using position-velocity (P-V) plots (Anastasio and Robinson 1991; Cheron and Godaux 1987; Major et al. 2004a; Mensh et al. 2004). In this thesis, P-V plots were used and determination of the time constant of decay will be explained in Chapter 2.

Various terms have been previously adopted to describe the stability of fixations based upon the presumed adequacy of the eye velocity-to-position neural integrator response (Goldman et al. 2003; Major et al. 2004b; Major and Tank 2004; Seung et al. 2000). If fixations are **stable,** as depicted in Fig. 1-3A, post saccadic drift is small at each eye position and the time constant of decay is large, i.e. > 20s. If fixations are not stable, they can be described as either leaky or unstable, depending on the direction of the drift. **Leak,** shown in Fig. 1-3C, occurs when the post-saccadic drift is *towards* a position within the oculomotor range, referred to as the null position, and thus the drift is centripetally directed due to undercompensation, presumably from the integrator. An **unstable** fixation, as illustrated in Fig. 1-3B, occurs when the eye drifts *away* from a null

Figure 1-3: Quantification of eye position time constant stability, instability and leak.

A: Eye position and position-velocity (P-V) plots of spontaneous stable eye movement, in which the slope of the P-V plot is close to zero (i.e., < 0.05°/s).

B: Eye position and P-V plot showing instability after 4 hrs of training at 20°/s. Fixations have large eccentric drifts, the P-V slope is positive, and the time constant is negative. Changes in null position can be observed after instability training. C: Eye position and P-V plot showing leak after 4 hrs training. Large centripetal drifts can be observed in which the slope of the P-V plot is negative and the time constant is positive. Changes in null position can also be observed after leak training


A
10°
5s
-4°/s
Eye Velocity
0°/s
4°/s
τ > 50s
20°
0°
-20°
Eye Position
B
Null 2
Null 1
Null 3
Eye Velocity
-4°/s
τ = -1.9s
0°/s
4°/s
20°
0°
-20°
Eye Position
C
Null 1
Null 2
Eye Velocity
-4°/s
0°/s
4°/s
τ = 4.4s
20°
0°
-20°
Eye Position

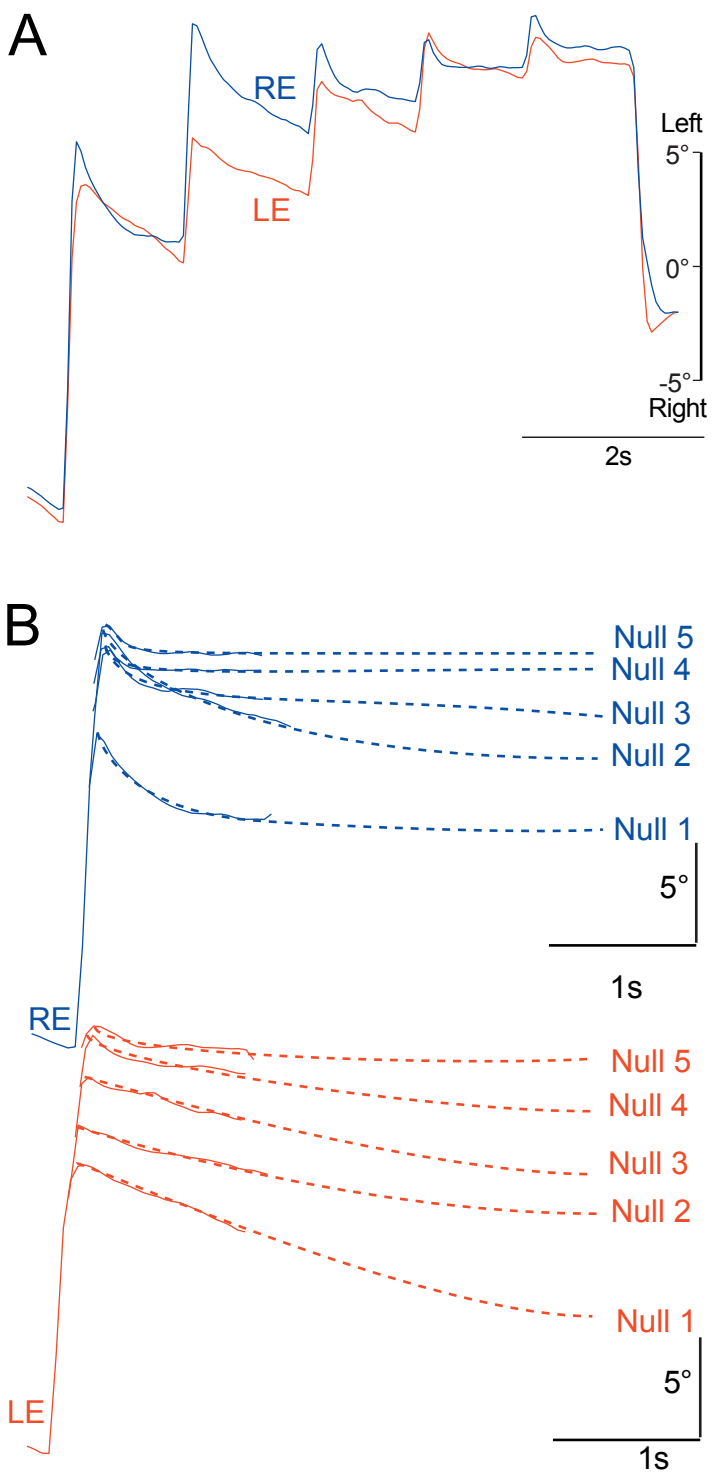


Figure 1-4: Variation in null position shifts during scanning. A: Spontaneous scanning behavior in darkness for the right and left eye. B: Fixations and saccades are superimposed to illustrate multiple null positions.

position within the oculomotor range and thus the drift is centrifugally directed due to an overcompensation, presumably arising from the integrator (Tiliket et al. 1994; Zee et al. 1980).

The conventional definition for **null position** is the eye position obtained when agonist-antagonist muscles, e.g., lateral and medial rectus, exhibit either no or equal force. Theoretically and practically this is usually close to the center of the oculomotor range. In this thesis, as in previous studies, **null position** is behaviorally defined as an eye position without drift (Tiliket et al. 1994). In addition, since the position of a zero drift may not be obvious, the null position can be defined as the eye position at which the drift changes directions from leftward to rightward or vice-versa. These definitions allow for multiple null positions within a scanning range and the null position may shift during the course of an experiment. The presence of multiple null positions is shown in Fig. 1-4. During the half minute record obtained from a naïve goldfish during spontaneous scanning in darkness, eye position decay shifted illustrating five unique null positions.

Version and vergence

In the majority of animals, motion of the two eyes appears to be linked or yoked during scanning of the visual environment. This parallel behavior is called conjugate eye motion. The term **version** is often used to describe conjugate motions of the eyes (King and Zhou 2000). During **version,** the eyes rotate by identical amplitudes in the same direction (Fig. 1-5) (King and Zhou 2002). The pathways controlling horizontal versional eye movements are located in the hindbrain (King and Zhou 2000). Previous studies have assumed that the neuronal signal controlling eye movements encode a versional eye position

which is mathematically defined as the average of the left and right eye positions. (Cova and Galiana 1995).

Vergence occurs when the eyes rotate in *opposite* directions (Fig. 1-5) (King and Zhou 2002). If the eyes rotate towards each other it is called **convergence.** Rotation away from each other is referred to as **divergence** (King and Zhou 2002). A commonly used stimulus to elicit vergence is to alter the apparent distance of the target from the eyes. Previous studies have defined the degree of vergence by computing a vergence angle which is the difference between the left and right eye position (Cova and Galiana 1995). Version and vergence are probably controlled by different neuronal circuitry, (Semmlow et al. 1998) with the latter responses largely initiated in the midbrain (Mays and Gamlin 1995; Saida et al. 2001). Frontal-eyed and lateral-eyed animals appear to have vergence-specific neurons, that also were found in goldfish within the vicinity of the medial rectus motor nucleus (Hermann 1971).

Conjugate, ipsilateral and contralateral neuronal sensitivity

In spite of the fact that versional and vergence eye movements are elicited by different spatial stimuli and sites of initiation in the brain, central neurons were previously analyzed and computed as a single unit, with separate vergence and versional sensitivities (Gamlin et al. 1989; Sylvestre and Cullen 2002). Recently neurons have been described in terms of right eye and left eye sensitivities rather than vergence and version sensitivities (Sylvestre et al. 2003; Sylvestre and Cullen 2002; Zhou and King 1996, 1998). When both approaches were used, it was determined that analysis of abducens motoneurons by left versus right eye sensitivity was a much better method (Sylvestre and Cullen 2002). This thesis adapted a similar terminology to that of Sylvestre and Cullen, in which neurons

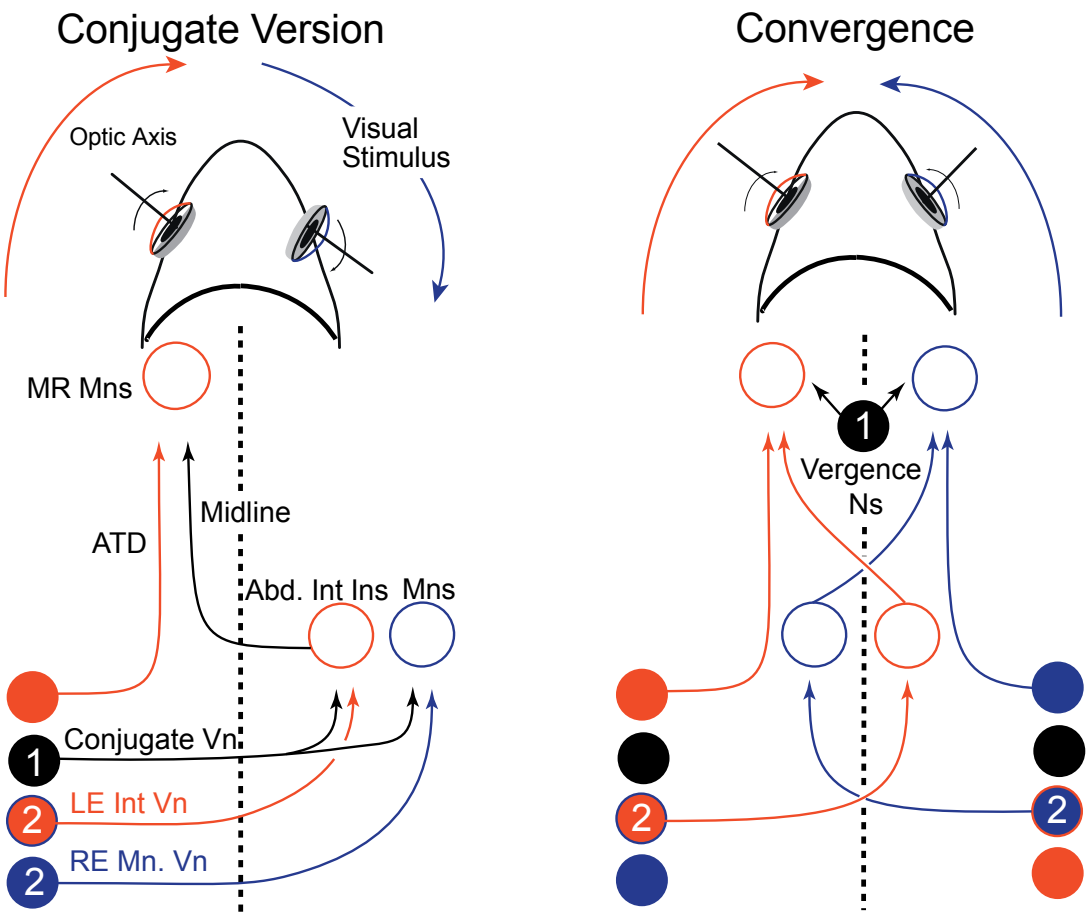


Figure 1-5: Version and vergence eye movements.

Movements of the left and right eyes are shown during version and convergence. Hindbrain pathways controlling horizontal conjugate versional eye movements include the Ascending Tract of Dieters (ATD) to medial rectus (MR) motoneurons (Mns) and either a conjugate vestibular pathway (1) to the Abducens (Abd) Mns and internuclear (Int) interneurons (Ins) or (2) innervation by separate monocular vestibular neurons (Vn). Convergence-like eye movements are either controlled by (1) midbrain vergence neurons (Ns) or (2) innervation by seperate monocular vestibular neurons

correlated better with one eye than the other eye were categorized as either **ipsilateral** or **contralateral** neurons (Sylvestre et al. 2003). If the neurons were statistically equally well correlated with either eye, they were categorized as **conjugate** (Sylvestre et al. 2003). Conjugate neurons also have been referred to as **binocular** neurons (Sylvestre et al. 2003; Zhou and King 1996)

Theories for behaviorally monocular eye movements

Although movement of the eyes appear to be yoked, or linked, during spontaneous behavior, both theory and careful measurement has shown that the magnitude of each eye motion must differ during most stimulus paradigms in order to ensure proper compensation. The typical example offered for disconjugate eye motion is during the VOR when the object of interest is nearer to, but not centered, between the eyes (Snyder and King 1992).

Two theories were proposed in the 19th century to explain monocularity. Individual eye motion may be the result of separate versional oculomotor control pathways for each eye (Von Helmholtz 1910). Alternatively, individual eye motion could actually be a arithmetic combination of conjugate versional and vergence eye movement pathways as championed by Hering, in his law of equal innervation (Hering et al. 1977).

> *'The two eyes are so related to one another that one cannot be moved independently of the other; rather the musculature of both reacts simultaneously to one and the same impulse of will . . . We are just unable to innervate the muscles of one eye alone for right or left movement.*' (Hering et al. 1977)

One of the goals of this thesis was to determine whether eye movements are monocularly or conjugately encoded within the goldfish hindbrain.

Anatomical support for Hering's Law

Hering's explanation was favored due to the discovery of the mammalian

abducens internuclear interneurons in the 1970's. The horizontal versional eye movements were believed to be conjugately controlled since the abducens motoneurons and internuclear neurons were found to receive nearly identical synaptic innervations, with the latter pathway controlling the contralateral eye (Baker and Highstein 1975; Carpenter and Batton 1980; Highstein and Baker 1978).

Electrophysiological evidence supporting Von Helmholtz's theory

The issue of whether versional eye movements are encoded as a conjugate (Busettini et al. 1996; Gamlin et al. 1989; Ramat et al. 1999; Sylvestre et al. 2002) or monocular (Dell'Osso 1994; Miyoshi et al. 1981; Zhou and King 1997, 1996, 1998) signal is important for understanding the functional organization of the eye movement control pathways. Recently the conjugacy of horizontal eye movement control has been challenged (King and Zhou 2002, 2000). Electrophysiological evidence has suggested that the hindbrain pre-motor and vestibular neurons firing rates are monocularly encoded (McConville et al. 1994; Sylvestre et al. 2003; Zhou and King 1996). Recordings from prepositus hypoglossi neurons in rats has shown that a subpopulation of neurons appear to be driven by optokinetic stimulation of the contralateral eye only, implying monocular motor pathways (Lannou et al. 1984). Finally, the cat abducens motoneurons were shown to be better correlated with eye movements of the ipsilateral eye while the abducens internuclear interneurons were better correlated with eye movements of the contralateral eye (Delgado-Garcia et al. 1977; Delgado-Garcia et al. 1986a, 1986b). This finding of monocularity suggested that the abducens motoneurons and abducens internuclear interneurons did not control the same eye movements even in frontal eyed animals.

In goldfish, the firing rate of medial rectus motoneurons appeared to be monocularly related (Hermann 1971). Based on morphological and electrophysiological criteria, the activity of medial rectus motoneurons are almost exclusively mediated by the activity of the abducens internuclear interneurons in goldfish (Suwa and Baker 1996). Thus since the medial rectus motoneurons are monocular, the abducens internuclear interneurons should also be monocularly related in goldfish.

Monocularity of abducens motoneurons

Interestingly, there is also evidence to suggest that the abducens motoneurons and abducens internuclear interneurons encode the same eye movements. In primates, some abducens motoneurons appear to encode conjugate eye movements, however the disjunctive eye positions were elicited using distance mediated vergence (King et al. 1994; Sylvestre and Cullen 2002; Zhou and King 1996). Gamlin has reported that during vergence primate abducens internuclear interneurons exhibited firing rates inappropriate for contralateral eye movements (Gamlin et al. 1989). This finding is in agreement with the vergence response of cat abducens internuclear interneurons whose firing rates were inappropriate for contralateral eye movement (Delgado-Garcia et al. 1986a, 1986b). The vergence-version ambiguity of feline abducens internuclear interneurons responses illustrates a weakness of most monocular studies in mammals since monocularity is elicited by distance-mediated vergence, hence the results provide limited insight into the control of versional horizontal eye movements by hindbrain neurons and circuitry.

Oculomotor training and plasticity paradigms provide evidence for separate encoding of signals for each eye. Visuomotor training can induce

monocular changes in the VOR, OKR and saccades (Averbuch-Heller et al. 1999; Lemij and Collewijn 1991a, 1992, 1991b; McElligott and Wilson 2001; Oohira and Zee 1992; Weiser et al. 1989), and although suggestive of monocular encoding, these observations are not conclusive, nor do they it state the level where conjugacy and monocularity occurs within the oculomotor circuitry.

Evidence from plasticity studies for monocularity

When one eye muscle was surgically weakened in primates, both a monocular and conjugate encoding plasticity pathway were hypothesized to exist as the eyes were able to retrain monocularly towards normal behavior. However, in the most severe cases, only conjugate adaptation was observed (Viirre et al. 1988). One of the goals of this thesis was to determine whether eye movements are monocularly or conjugately encoded within the goldfish hindbrain.

Eye position holding

A basic understanding of the hindbrain eye movements pathways is required to asses whether eye movements are monocularly or conjugately controlled. In most animals, even in the absence of target motion, the visual environment is scanned and retinal slip must be actively minimized during the intervening eye fixations (Land 1999). The extraocular muscles and orbital connective tissue together generate a visco-elastic force when stretched, analogous to a spring and dashpot, that tries to re-center the eye back towards a null position. Miller and Robins utilizing transducers implanted in the extraocular muscles, measured the force produced during scanning. In monkeys, a “pulse, slide, step” pattern was observed in which the force exerted during the saccade was greater than that needed to maintain the eyes in the new position (Miller and Robins 1992). The muscle tension during fixation was proportional to the

eye position displacement, necessitating a central neural command to maintain the force and minimize eye drift (Miller and Robins 1992).

Role of afferent feedback in eye position holding

The central neural command needed to maintain the extraocular muscle force and minimize post-saccadic drift could be generated by proprioceptive afferent feedback from the extraocular muscles. Extraocular proprioceptive signals are known to be present in many animals (Donaldson 2000). Deafferentation in monkeys did not produce changes in post-saccadic drift indicative of modification in mechanisms responsible for generating the fixation time constant (Lewis et al. 1999). In addition, removal of proprioceptive afferent feedback did not prevent subsequent visually induced modification of the time constant (Lewis et al. 1999). These results suggest that a non-proprioceptive mechanism exists for maintaining eye position and modifying fixation time constants.

The horizontal eye velocity-to-position neural integrator

Based on the absence of any sensory or motor cues, stable eye position holding was proposed to be accomplished by a horizontal eye velocity-to-position neural integrator (PNI) that could convert transient velocity-related signals, saccadic or vestibular, into the appropriate eye position-related signals to maintain the eye muscle force (Skavenski and Robinson 1973). The time constant of fixation stability was employed as an indirect measure of the compensatory ability of the eye position neural integrator. As predicted in both mammals and goldfish, putative PNI neurons were noted to exhibit a burst-tonic firing rate pattern (Aksay et al. 2000; Baker et al. 1976; Lopez-Barneo et al. 1982). The sustained discharge was likened to a **persistent neural activity**

(Aksay et al. 2000; de Dios Navarro-Lopez et al. 2004). Currently, persistent neural activity is defined as the ability to maintain a sustained firing rate in the absence of additional input, and it is argued to be a mechanism underlying working memory (Brody et al. 2003; Deco and Rolls 2003; Major and Tank 2004).

The pattern of activity in a typical PNI neuron firing rate is shown in Fig. 1-6. Multiple levels of sustained firing rates were recorded during the intersaccadic intervals. The firing rate vs. eye position plot in Fig. 1-6, shows a linear relationship between the eye position and the firing rate in the absence of visual input. This is precisely the profile predicted necessary for an eye velocity-to-position integrator.

It has been commonly assumed that eye position stability occurs only when PNI neurons exhibit persistence and these terms are often used interchangeably (Cannon et al. 1983; Mensh et al. 2004; Seung 1996); however, this extrapolation may be incomplete, even incorrect, as alternative mechanisms for stability may exist, including saccadic dependent null shifts. A perfectly compensatory integrator would exhibit little drift at any eye position, similar to a circumstance with infinite null points shifts (Fig. 1-4 RE nulls 4&5). Without neuronal recordings, definitive conclusions are limited to fixation stability and, at most, suggestive of PNI neuronal activity.

During rotational VOR, hindbrain neurons and circuits must encode an eye position-related component to ensure proper phase and gain compensation (Skavenski and Robinson 1973). During head rotation at high frequency, this position signal phase is supplied by the physical dynamics of the oculomotor plant (Baker et al. 1981); however, at lower frequencies the “three neuron arc”

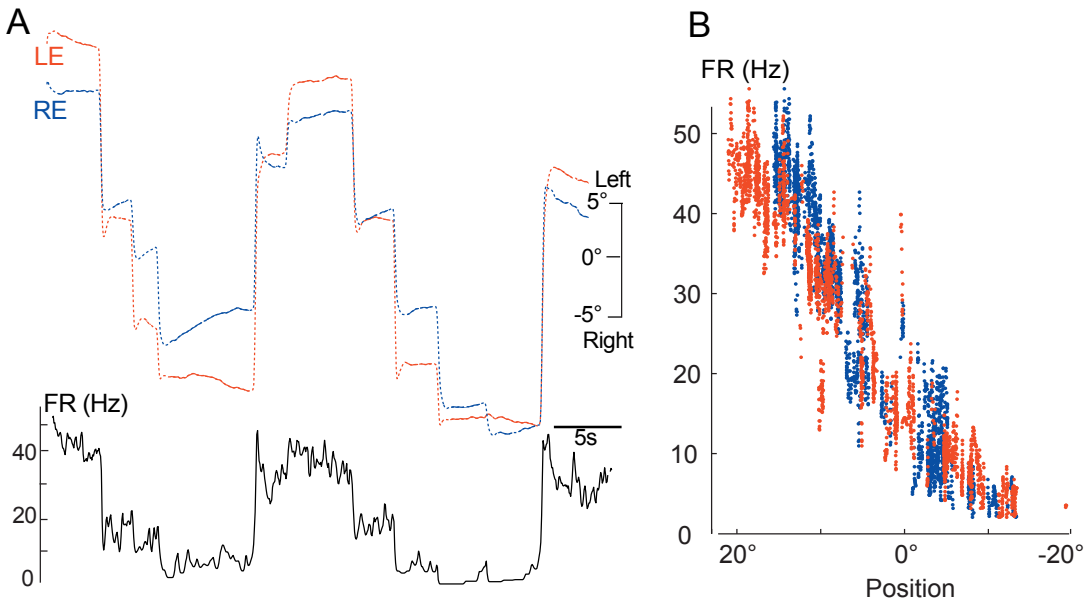


Figure 1-6: PNI activity during spontaneous scanning saccades and fixations. A: Eye position and firing rate during one minute of spontaneous behavior. Persistent activity is observed in the PNI neuronal firing rate during fixation. B: Firing rate (FR) versus eye position plot of the Left and Right eyes showing a position and velocity sensitivity (+ values leftward and – rightward) of 1.59 (sp/s)/° and -0.29 (sp/s)/°/s (r=0.96).

is insufficient to produce the proper compensatory signal. An additional eye position signal was proposed to originate in the PNI during the VOR (Skavenski and Robinson 1973). The theoretical ability of the integrator to extend the performance of the VOR is depicted in Fig. 1-2.

Hindbrain location of PNI in mammals

Until recently, the majority of the experimental work establishing the role, location, and mechanisms of horizontal eye velocity-to-position neural integration used mammals as the experimental animal model (Anastasio and Robinson 1991; Arts et al. 2000; Baker et al. 1976; Escudero et al. 1996; Godaux and Cheron 1996; Godaux et al. 1993; Lopez-Barneo et al. 1982; Mettens et al. 1994c). In the past decade, the goldfish has proven to be a better experimental model due to its simplicity. In addition, understanding the goldfish eye velocity-to-position integrator will extend to understanding the mammalian PNI because the basic hindbrain neural mechanisms, including the vestibular connections, are conserved in vertebrates (Baker and Gilland 1996; Gilland and Baker 1993, 2005; Graf et al. 1997).

Based upon direct stimulation of the vestibular nerve, along with known anatomical and electrophysiological established connections, the nucleus prepositus hypoglossi was hypothesized as a parallel vestibuloocular pathway in mammals (Baker and Berthoz 1975). Recordings from lightly anesthetized cats during vestibular rotation demonstrated that prepositus neurons responded similar to vestibular neurons except with a greater phase lag (Blanks et al. 1977). This response is consistent with the prepositus being the anatomical location of the horizontal eye velocity-to-position neural integrator. Electrophysiological recording from prepositus hyoglossi neurons in awake animals further

substantiated this claim as firing rates were related to eye position (Baker et al. 1976; Lopez-Barneo et al. 1982; Sylvestre et al. 2003). The aforementioned results, together with a process of eliminating possible contributions from other neurons, led to the conclusion that the location of the horizontal eye velocity–to-position integrator was the nucleus prepositus hypoglossi in mammals (Baker et al. 1981). Corroborating evidence for this conclusion has been provided by pharmacological inactivation and ablation studies, in which the time constant of eye position holding was reduced to ~200 ms in primates (Arnold et al. 1999; Cannon and Robinson 1987; Kaneko 1997) with similar observations in cats (Cheron et al. 1986a; Cheron et al. 1986b; Godaux et al. 1993; Mettens et al. 1994b). In addition, microstimulation of the nucleus prepositus hypoglossi caused changes in eye position and deficits in eye position holding (Godaux et al. 1989). Clinically, failures of gaze holding and vestibular function were observed after strokes involving the nucleus prepositus hypoglossi (Seo et al. 2004). Detailed reviews of the morphological and physiological evidence supporting the nucleus prepositus hypoglossi as the location of the PNI in mammals have been published recently (Fukushima and Kaneko 1995; Fukushima et al. 1992; McCrea and Horn 2005).

A number of studies also have implicated the medial vestibular nucleus, which lies adjacent to the prepositus hypoglossi, to be involved in horizontal eye velocity-to-position neural integration. In addition, the medial vestibular nucleus exhibits extensive reciprocal connections with the nucleus prepositus hyoglossi (McCrea and Horn 2005). Pharmacological microinjections in the medial vestibular nucleus have produced eye position drifts consistent with both failure (Mettens et al. 1994c) and overcompensation of the horizontal eye velocity-to-

position integrator (Arnold et al. 1999).

Selective ibotenic acid lesions of the nucleus prepositus hypoglossi resulted in a large decrease in the time constant; however, the time constant was ~ 2 seconds, an order of magnitude above the time constant of the oculomotor plant, suggesting other nuclei were involved in producing eye position stability (Kaneko 1997). Electrolytic lesions of either the NPH or MVN in cats caused changes in the phase and gain of low frequency VOR and a failure of post saccadic gaze holding, suggesting both nuclei are part of the integrator pathway (Cheron et al. 1986b). Definitive study of the integrator in mammals is also complicated because the medial vestibular nucleus is involved in the production of other oculomotor behaviors, such as velocity storage (Cannon and Robinson 1987; Moreno-Lopez et al. 1998).

Anatomical location of PNI in goldfish: Area I

By contrast in goldfish, eye velocity-to-position neural integration is associated with a distinct caudal nucleus, previously termed Area I (Aksay et al. 2000; Pastor et al. 1994b). Similar to previously published work, the effects of pharmacological inactivation are shown after bupivacaine application to the goldfish Area I in Fig. 1-7. Both the position holding and low frequency VOR were reversibly affected; however, the gain and phase of the VOR at 1 Hz was minimally changed corroborating the assertion that Area I is the location of the goldfish PNI.

Role of the cerebellum in eye velocity-to-position integration: mammals

In mammals, the cerebellum appears to have a role in elongation of the time constant, and has been considered part of the PNI. When the cerebellum was ablated in cats, the time constant was reduced from ~20 to 1.3 seconds,

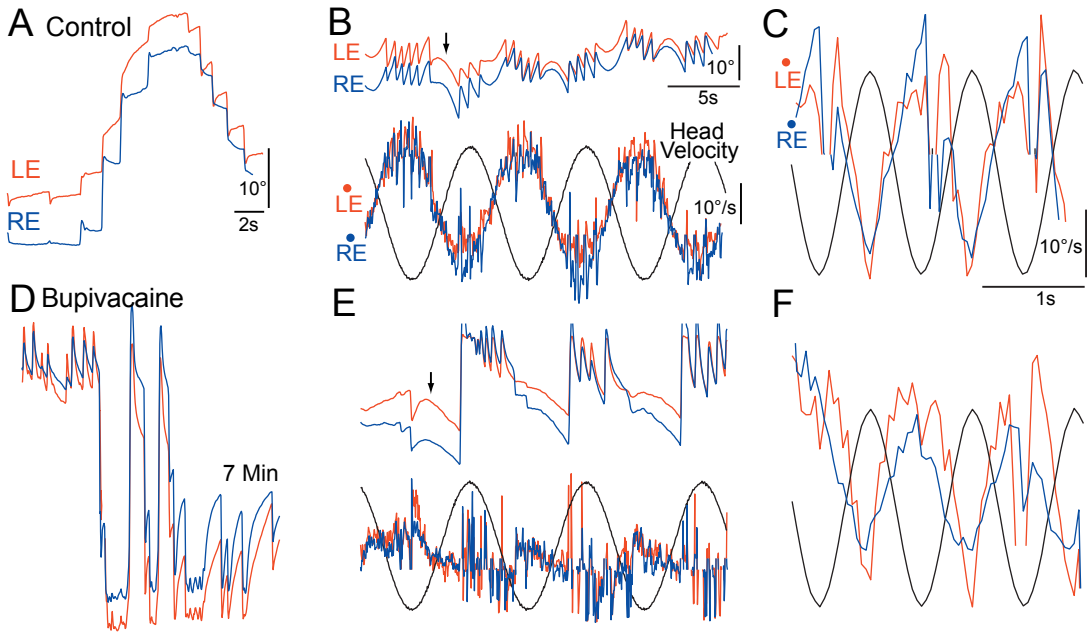


Figure 1-7: Fixation holding and VOR after PNI inactivation.

Control records are shown in A-C and 7-10 minutes after buvipicaine application in D-F. A: Spontaneous saccadic behavior was changed to a large centripetal drift (leak in D) that subsided by 60 minutes (not shown). B: Eye position and velocity traces during VOR were elicited at 0.125 Hz 15.7°/s before and (E) after inactivation. Both gain and phase were affected as indicated by the change in eye position inflection position (arrows). C: Eye velocity traces are shown during 1 Hz VOR before and (F) after anesthesia. At this higher frequency, phase and gain were not altered during PNI inactivation

and a wandering null position emerged (Godaux and Vanderkelen 1984; Robinson 1974). Similar reductions in the time constant were found in primates (Westheimer and Blair 1974). Clinically, patients with cerebellar lesions exhibit poor fixation time constants (Estanol et al. 1979; Hotson 1982; Leech et al. 1977; Zee et al. 1980). Subsequent work refined the anatomical location involved in eye velocity-to-position integration to the flocculus, since ablation of this area decreased the time constant to 1-2 seconds (Zee et al. 1981). The cerebellar cortex is not critical for PNI function as neonatal ablation did not diminish gaze holding ability in adult primates; however, similar to the results obtained by Zee *et al*, gaze stability was affected when the ablation included regions of the flocculus (Eckmiller and Westheimer 1983).

Role of the cerebellum in velocity-to-position integration: goldfish

In goldfish, the role of the cerebellum in time constant elongation has not been explicitly tested, but anecdotal evidence suggests that it is not involved (Marsh 1998). In contrast to mammals, Area I does not directly send efferents to or receive afferents from the cerebellum (Aksay et al. 2000; Straka et al. 2006). A second goal of this dissertation was to define the role of the cerebellum in horizontal eye velocity-to-position neural integration.

Intrinsic organization of the integrator

Although the mammalian hindbrain has been found to contain many populations of monocular neurons (McConville et al. 1994; Sylvestre et al. 2003; Zhou and King 1998), the issue of whether any of these neurons are specific to the integrator is unresolved. This is largely due to the limitation that monocularity was induced in primates by accommodative vergence, hence the monocularity could be attributed to, but certainly not distinguished from, the midbrain vergence

centers. *A priori,* it is assumed that the horizontal eye velocity-to-position integrator is conjugate in lateral eyed animals such as goldfish (McConville et al. 1994). However, anecdotal evidence of Area I firing rates during spontaneous disconjugate movements suggested the presence of an ipsilateral neuronal population, and hence the possibility of monocular organization (Pastor et al. 1994b). It is important to resolve the issue of monocularity in the goldfish integrator, because it can be experimentally accomplished using monocular versional stimuli and in turn the findings will comment on the assumptions underlying the structure/function of the mammalian horizontal eye velocity-to-position integrator.

Basic connectivity of PNI

The simple afferent and efferent PNI connections are shown in Fig. 1-8. In both mammals (Cazin et al. 1982; McCrea and Baker 1985; McCrea et al. 1979; McCrea and Horn 2005) and goldfish (Aksay et al. 2000) the afferent input to PNI originates primarily from vestibular neurons and saccadic burst neurons (Fig. 1-1). PNI has been documented to have efferent projections to the ipsilateral abducens motoneurons and internuclear interneurons in both mammals (Escudero et al. 1992; Graybiel and Hartwieg 1974; McCrea and Baker 1985; McCrea et al. 1979; McCrea and Horn 2005) and goldfish (Aksay et al. 2000). There are also contralateral projections from the presumed inhibitory PNI neurons to PNI and abducens nuclei (Aksay et al. 2000; McCrea et al. 1979).

Additional connections of nucleus prepositus hypoglossi in mammals

The prepositus hypoglossi is not as homogenous a structure in mammals as Area I is in goldfish. The prepositus hypoglossi is composed of many different

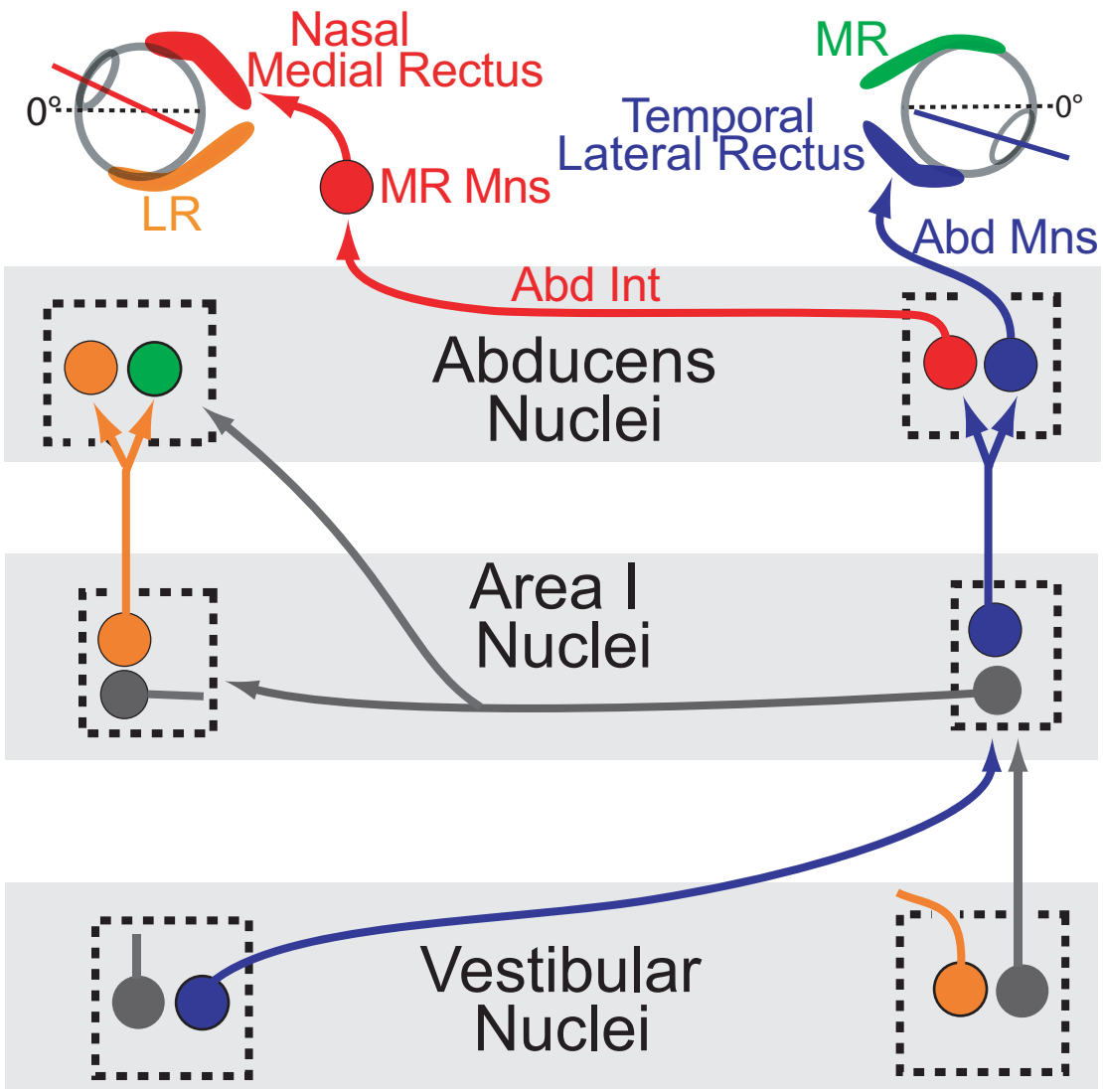


Figure 1-8: Efferent and afferent projections of the goldfish horizontal eye velocity-to-position neural integrator

Basic wiring diagram of the eye position integrator with afferent inputs from the second order vestibular neurons. Due to symmetry of connections, only those for the left-side vestibular neurons and right-side position integrator are shown. Pathways are color coded based on eye muscles. This pattern was followed in all other figures. Left Lateral Rectus Left Medial Rectus Right Lateral Rectus Right Medial Rectus

types of neurons with additional afferent and efferent connections (McCrea and Horn 2005). Synaptic inputs to the prepositus originate from the nucleus of the optic tract, caudal spinal trigeminal nucleus, the extraocular motor nuclei, the cerebral cortex, the fastigial nucleus and flocculus of the cerebellum and the superior colliculus (McCrea and Horn 2005). The efferent targets of prepositus neurons are also considerably more numerous than for the PNI neurons in goldfish. Retrograde and anterograde trace studies have shown projections to the cerebellum, inferior olive, vestibular nucleus, medial rectus motoneurons, superior colliculus, nucleus of the optic tract, and ventral lateral geniculate nucleus (McCrea and Horn 2005). Although all of the above neurons are localized within the prepositus, it is unknown which are part of the PNI circuitry, and which are involved in other oculomotor functions. Thus, extrapolating the role of the prepositus in elongation of the time constant is complicated in mammals, since theoretical models tend to utilize all connections deemed appropriate.

Advantages of goldfish as an experimental model

In contrast to mammals, structure/function studies of the goldfish PNI has found it more homogenous, as only one cell type has been described within Area I (Aksay et al. 2000). The limited efferent connections of the goldfish PNI allows more explicit interpretation and modeling of the behavior to understand the mechanism for eye velocity-to-position integration. A final advantage of the goldfish as a experimental model animal is that the intersaccadic interval can extend longer than a second in duration allowing analysis of the integrator time constant independent of the saccadic-related dynamics, e.g., saccadic overshoot and slide (Easter 1971).

In contrast to the many hundreds of neurons present in the prepositus

hypoglossi (Moreno-Lopez et al. 2001), the number of neurons believed to comprise the goldfish PNI is between 30-50 on each side (Aksay et al. 2000; Pastor et al. 1994b), Thus, local perturbations within the integrator are more interpretable, and neuronal recordings can analyze a larger fraction of the horizontal eye velocity-to-position integrator neurons allowing more critical evaluations of the proposed mechanisms underlying integration.

Eye velocity-to-position neural integration: Vertical eye movements

Eye velocity-to-position neural integration for vertical eye position is encoded by a different neuronal pathway than horizontal eye velocity-to-position integration. In mammals, the anatomical location of the hindbrain integrator for vertical eye movements is in the midbrain Interstitial Nucleus of Cajal (Fukushima and Kaneko 1995). No study has been carried out to locate the vertical eye velocity-to-position integrator in goldfish. This thesis was concerned with only the horizontal eye velocity-to-position neural integrator.

Theoretical mechanisms of neuronal persistence

As previously stated, the conversion of an eye velocity-related signal into an eye position-related signal is an example of neuronal persistence. The mechanism by which persistence is generated and sustained is unknown. It may be due to cellular properties of the eye velocity-to-position integrator neurons, including membrane conductance and ion channels, or alternatively due to network connectivity. Reviews of the theoretical mechanisms of persistent activity generation have been published (Brody et al. 2003; Brunel 2003; Durstewitz et al. 2000). More specifically, a recent review has addressed the evidence for either network or cellular explanations for persistence in the horizontal eye velocity-to-position neural integrator (Major and Tank 2004).

Synaptic potentiation

Persistence could be due to cellular mechanisms intrinsic to each of the PNI neurons, as depicted in Fig. 1-9A. Changes in either presynaptic channels, postsynaptic channels, or membrane properties such as synaptic potentiation, have been proposed as mechanisms of persistence generation and maintenance (Shen 1989). Experimentally, cholinergic synaptic potentiation in prepositus neurons evoked by activation of the burst neurons has been demonstrated *in vitro* (de Dios Navarro-Lopez et al. 2004; de Dios Navarro-Lopez et al. 2005).

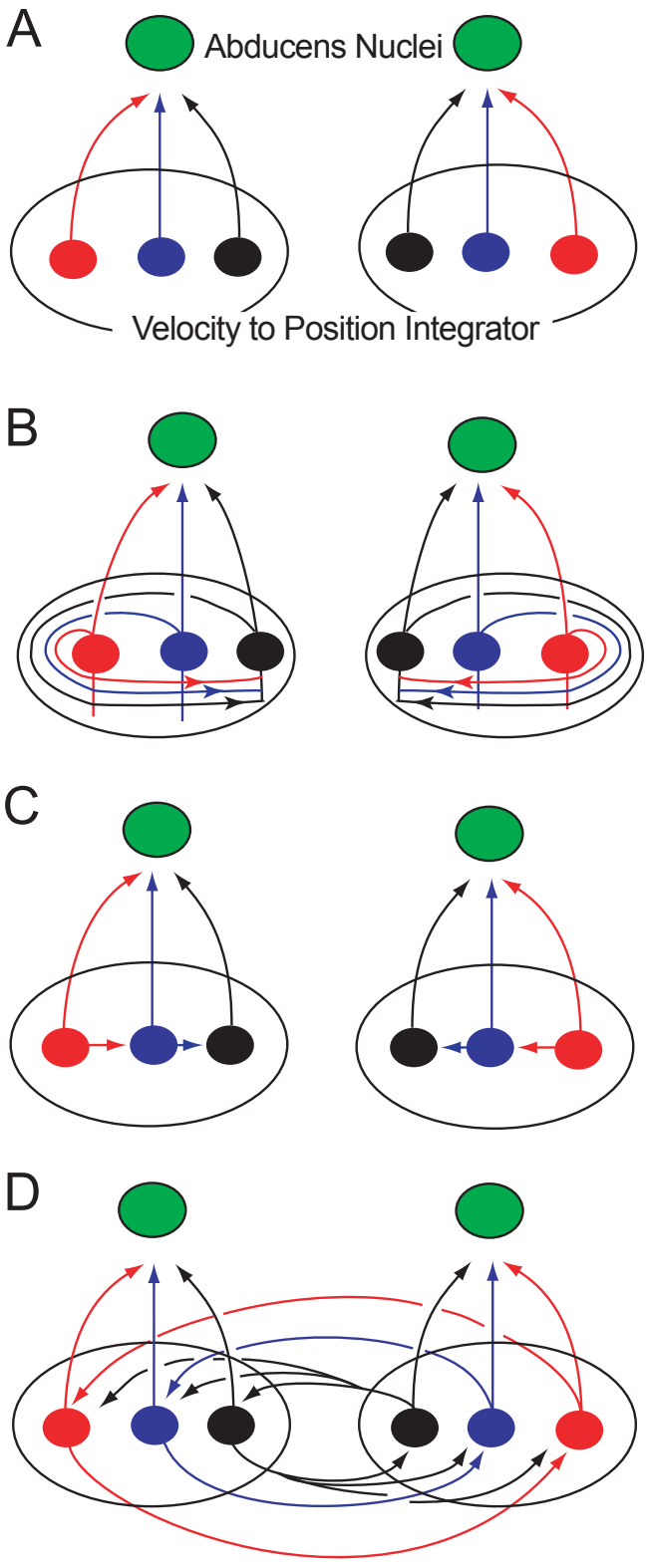
Nitric oxide

Nitric oxide signaling is another potential intracellular mechanism that could generate persistent activity. This gaseous neuromodulator affects intracellular signaling and has wide-ranging effects on a neuron's responses to neurotransmitters (Guix et al. 2005; Susswein et al. 2004). In support of such a role in integrator function, nitric oxide sensitive cells have been localized in the nucleus prepositus hypoglossi, and eye position holding is interrupted when nitric oxide donors are injected (Moreno-Lopez et al. 1996; Moreno-Lopez et al. 1998). However, pharmacological manipulation of nitric oxide synthase activity within the nucleus prepositus did not modify the integrator time constant, but rather eye velocity, indicating that the nitric oxide responsible for eye position integration was not synthesized within the PNI nucleus (Moreno-Lopez et al. 1998).

Membrane bistability

Multistable membrane potentials in either the soma and/or dendrites are another proposed mechanism to explain persistence observed in the eye velocity-to-position integrator neurons' firing rates (Goldman et al. 2003;

Figure 1-9: Proposed mechanisms underlying velocity-to- position integration. A: Intrinsic cellular properties. Each PNI neuron is envisioned to function as an autonomous unit in which persistence is generated by a cellular mechanism. B: Recurrent ipsilateral excitation. Proposed by Seung *et al.,* 2000 as a continuous line attractor model to explain persistence. Each neuron projects axon collaterals to all other neurons within PNI, providing a net excitatory feedback. C: Linear chain model. Similar to recurrent excitation except that each neuron's collateral projects to another neuron with a greater position, and less velocity, sensitivity. The first neuron is largely velocity sensitive and the last neuron in the chain is only eye position sensitive. A partial integration is envisioned to occur at each step as the signal progresses through the nucleus. Adapted from Escudero *et al.* 1992. D: Feedback inhibition. Each type of PNI neuron projects an inhibitory connection to its contralateral counterpart causing a net excitation of the neuronal population thereby producing persistence. Adapted from Arnold and Robinson 1997.


A
Abducens Nuclei
Velocity to Position Integrator
B
C
D

Koulakov et al. 2002; Loewenstein and Sompolinsky 2003). Perhaps the best known example is plateau potentials, comprised of sustained depolarizations in the absence of external input (Kiehn and Eken 1998). Experimentally, TTX sensitive plateau potentials have been demonstrated in the nucleus prepositus neurons *in vitro* implying the presence of voltage activated persistent sodium channels (Rekling and Laursen 1989). Recently, guinea pig prepositus neurons were also shown to exhibit TTX sensitive plateau potentials and some neurons displayed oscillatory behaviors, consistent with an intracellular mechanism for neuronal persistence (Idoux et al. 2006). Even though these findings support some role for intrinsic cellular properties, they were argued not to be robust enough to explain persistence without a network mechanism (Idoux et al. 2006). In goldfish, direct current injection failed to elicit an afterdischarge in PNI neurons, suggesting that plateau potentials are not a major mechanism responsible for persistence generation (Aksay et al. 2001).

Alternatively, multistability may be due to calcium currents, most likely through NMDA channels. This mechanism was employed by the Koulakov model, in which NMDA receptors within each cell caused membrane bistability, however the model is a network hybrid since all of the cells in the integrator are recurrently connected (Koulakov et al. 2002). Each neuron is argued to have a different threshold due to the synaptic weights; thus, when a saccade is initiated only those neurons near the NMDA threshold will be activated and exhibit a persistent firing rate (Koulakov et al. 2002). Mammalian velocity-to-position integration appears to include a NMDA mechanism, since selective NMDA antagonists cause a gaze holding failure, but non-NMDA glutamatergic antagonists do not (Mettens et al. 1994a, 1994b).

A similar theory utilizes calcium levels in the dendrites to produce persistence and velocity-to-position integration. The activity level causes “calcium wavefronts”, thereby the firing rate is determined by the concentration of calcium at the axon hillock (Loewenstein and Sompolinsky 2003). In contrast to the Koulakov theory, the Loewenstein model is network independent. Both Loewenstein and Koulakov claim that their models are more robust to noise and do not require fine tuning to prevent oscillations and integrator instability present in other models (Koulakov et al. 2002; Loewenstein and Sompolinsky 2003).

A different model has been proposed utilizing bistability to increase robustness of the integration in which the neurons may contain bistable or hysteretic dendrites (Goldman et al. 2003). Although no mechanism of bistability is proposed, the network is more robust to noise and mistuning. This model accurately accounts for the firing rate hysteresis associated with the previous saccade (Aksay et al. 2000) and the hysteretic cross correlations that exist between the firing rates of two integrator neurons (Aksay et al. 2003b).

A major weakness to all models in which a cellular mechanism generates persistence activity is that somatic current injection in Area I neurons could not introduce any non-linearity of cellular membrane potentials beyond the direct current injection (Aksay et al. 2001). The lack of inducible changes is inconsistent with an intrinsic mechanism of persistence activity generation and eye velocity-to-position integration in goldfish PNI, but did not test the distal dendritic membranes.

Local network models of persistence: feedback excitation and mutual inhibition

The geometry and synaptic connections within and/or between the PNI nuclei also may be considered as a mechanism responsible for eye velocity-

to-position integration. Most models utilizing network connectivity to produce persistent activity and eye velocity integration employ positive feedback connections. One frequently cited disadvantage to “pure” network models is that many rely on feedback and tuning that require extremely well adjusted inputs in which 1% mistuning causes the neuronal response to be unacceptable (Brody et al. 2003; Cannon et al. 1983). In one type of positive feedback model, the PNI neurons are arranged as inhibitory connections, such that a net positive feedback is developed (Fig. 1-9D). Since inhibition of inhibition is equivalent to net excitation, a transient input could cause net excitation of the same neuron, thereby generating persistence (Cannon et al. 1983). The authors stated that this model required a high degree of global tuning of the system (Cannon et al. 1983). In the Cannon model, all cells contained an eye position, but no eye velocity sensitivity (Cannon et al. 1983). This is inconsistent with experimental findings, as the largest fraction of eye velocity-to-position integrator neurons exhibit a velocity sensitivity (Fig. 3-8)(Sylvestre et al. 2003).

Subsequent models have employed mutual feedback inhibition located either in the commissure between the vestibular nuclei or the commissure between the PNI (Fig. 1-9) (Arnold and Robinson 1997; Galiana and Outerbridge 1984). The models have assumed stability to be generated by this positive feedback even though the intrinsic time constant of any single neuron was small, i.e., leaky integrator neurons (Arnold and Robinson 1997; Galiana and Outerbridge 1984). In a series of experiments to test the role of the contralateral projecting prepositus neurons, the midline was lesioned in three monkeys. In all experiments, an initial decrease was observed in the time constant; however, the time constant quickly improved over time (Anastasio and Robinson 1991;

Arnold and Robinson 1997). Electrical stimulation of the midline in cats (Godaux et al. 1989) and monkeys (Arnold and Robinson 1997) caused a shift in the null point and abolition of the time constant. Subsequent to these experimental findings, models of integrator function incorporated midline connections as vital assuming that the commissure was the source of the positive feedback excitation (Anastasio 1998; Cova and Galiana 1995; Cova and Galiana 1996).

By contrast, the role of the commissural PNI neurons was questioned in cats (Cheron et al. 1986b) and goldfish (Pastor et al. 1994b) since midline lesions did not abolish velocity-to-position integration. The time constants remained an order of magnitude above those of the oculomotor plant. Thus, models based upon a mechanism of integration requiring midline connections may be incorrect.

Local network models of persistence: neural chain and line attractors

Not all network models of integrator function require either midline connections or inhibitory neurons. Integration has been proposed to be generated through a neural chain in which each prepositus hypoglossi neuron projects an axon collateral that progressively converts velocity sensitivity into position sensitivity (Fig. 1-9C)(Escudero et al. 1992). Recurrent connection models based only on ipsilateral excitatory connections in which the network forms a line attractor have also been proposed (Seung 1996; Seung et al. 2000) (Fig. 1-9B). In mammals, axons of some prepositus neurons collateralize locally within the prepositus as well as the adjacent medial vestibular nuclei (McCrea and Horn 2005). However, it is unknown, by structure/function, if these neurons are part of the horizontal eye velocity-to-position neural integrator. There is a particular weakness to linear chain and recurrent excitatory models in goldfish

because intranuclear Area I axon collaterals have not been observed (Aksay et al. 2000).

Distributed network mechanisms

As mentioned, in mammals, removal of the cerebellar flocculus resulted in a time constant of ~ 2 seconds suggesting that the cerebellum was extensively involved in generation of persistence. Modeling has suggested the flocculus to be arranged as a parallel integrator pathway to the nucleus prepositus (Glasauer 2003). Although the time constant is ~2 seconds after total removal of either the cerebellum or flocculus, sequential hemi-cerebellectomy in juvenile monkeys only transiently affected eye position holding (Westheimer and Blair 1974). One week post hemi-cerebellectomy, eye position holding was nearly normal. If the intact half of the cerebellum was subsequently removed, eye position was only affected in the ipsilateral half of the oculomotor range (Westheimer and Blair 1974). Failure in eye position holding disappeared after an additional week, except in the extremes of the oculomotor range. This led to the hypothesis that the cerebellum modifies tonic activity of vestibular neurons suggesting, although not explicitly stated, that the cerebellum was not directly required for eye velocity-to-position integration (Westheimer and Blair 1974). When cerebellar removals occurred in adult monkeys, the deficits appeared more permanent implying that vestibular neurons might not be able to compensate for the loss of cerebellar activity (Westheimer and Blair 1974). A similar mechanism is unlikely in goldfish as there are no direct interactions occurring between the cerebellum and Area I (Straka et al. 2006). However, activity could be related indirectly since Area I receives innervation from vestibular neurons that are modulated by cerebellar inhibition (Straka et al. 2006).

Oculomotor plasticity

Oculomotor behaviors are expected to be compensatory during an animal's entire lifetime; however, during development, the size of the head and body are not constant, and the oculomotor system must adapt by either increasing neuronal number, their distribution, or modifying the strength of synaptic connections. Unexpected trauma could also cause loss of neurons responsible for a behavior or alter the dynamics of the oculomotor plant. Thus, it appears to be important that some mechanism of adjustment commonly called "plasticity" evolved in oculomotor behaviors. Indeed, experimentally eye movement behaviors can be modified over the course of hours to days (Gonshor and Jones 1976a, 1976b; Hopp and Fuchs 2004; Ito and Nagao 1991; Jones 1985; Marsh and Baker 1997).

Saccadic plasticity

The amplitude of saccades can be individually adapted for each eye after surgical muscle weakening (Viirre et al. 1988). Similar results have been observed after training with anisometropic spectacles (Erkelens et al. 1989; Lemij and Collewijn 1991a, 1992, 1991b). In addition to saccadic amplitude, post-saccadic drift can be modified by visual feedback (Kapoula et al. 1989; Optican and Miles 1985). After surgical muscle weakening, post saccadic drift could also be monocularly suppressed in most experiments (Viirre et al. 1988). This suggests that the eye velocity-to-position neural integrator can be monocularly modified.

By use of visual-vestibular stimuli the gain of the vestibuloocular reflex (VOR) can be either increased (Ito et al. 1979; Paige and Sargent 1991; Pastor et al. 1992; Schairer and Bennett 1986) or decreased (Godaux et al. 1983b; Ito

et al. 1979; Pastor et al. 1992; Schairer and Bennett 1986). In addition, VOR gain can be modified separately for each eye (McElligott and Wilson 2001; Viirre et al. 1988; Weiser et al. 1989). The phase of the VOR can be also be conjugately modified (Kramer et al. 1995; Tiliket et al. 1994). Since both VOR gain and phase can be changed at low frequency, the possibility exists that some plasticity occurred in the eye velocity-to-position integrator in agreement with the saccadic plasticity results.

Anatomical locations of oculomotor plasticity

The anatomical and electrophysiological substrates of oculomotor plasticity have been extensively investigated. One of the major roles ascribed to the cerebellum is motor plasticity (Marr 1969). Subsequent work showed that different locations were involved in the mammalian cerebellum, e.g., the flocculus was involved VOR adaptation, (Katoh et al. 1998; Lisberger et al. 1994a; Rambold et al. 2002) (Nagao and Kitazawa 2003) and the fastigial nuclei for adaptation of saccades (Optican and Robinson 1980; Robinson et al. 2002). Similar to mammals, the vestibulocerebellum appears important in VOR gain plasticity in goldfish, as ablation produced the same sinusoidal VOR gain regardless of the VOR gain prior to cerebellar removal (Michnovicz and Bennett 1987). This effect was also verified by lidocaine microdialysis, in which naïve performance of the VOR gain changes was unaffected, but acquisition and memory of the VOR adaptation was prevented (McElligott et al. 1998).

A brainstem site for the acquisition and retention of VOR modification has been suggested since midline lesion prevented plasticity of VOR gain changes in mammals (Cheron 1990). Using step stimuli in goldfish, it was demonstrated that the cerebellum was required for the acquisition of short latency dynamic and

longer latency sustained VOR responses, but since there was some retention of the short latency response, storage occurred outside the cerebellum in the brainstem (Pastor et al. 1994a). In mutant mice, the presence of LTD in the cerebellum correlated with the presence of short term VOR adaptation, but long term VOR adaptation occurred independent of LTD, although with a prolonged learning time course (van Alphen and De Zeeuw 2002). The correlation of LTD with short, but not long, term changes has been used to suggest that acute memory is encoded in the cerebellum, but chronic memory retention is within in the brainstem (Blazquez et al. 2004; van Alphen and De Zeeuw 2002). Changes in Purkinje cell firing rate were observed after VOR gain changes in primates suggesting the site of modification may be partially in the cerebellum (Blazquez et al. 2003). However, after large VOR gain changes in goldfish neither head or eye velocity sensitivity of Purkinje cells were different, supporting a brainstem site for neuronal plasticity (Pastor et al. 1997). Distinguishing the neuronal circuits and mechanism by which VOR and OKR learning and memory occur was not a goal of this thesis and many reviews of the topic exist (Blazquez et al. 2004; Kawato and Gomi 1992); however, these behaviors provide a basis for exploring the mechanisms and location of time constant plasticity.

Evidence for eye velocity to position neural integrator plasticity

Most models of PNI function assume that the eye velocity-to-position integrator is self-tuned for both short term (hrs) and long term (days) persistence (Major et al. 2000). Plasticity is assumed to be a manifestation or extension of the tuning behavior. Previous experiments have supported the hypothesis that the integrator time constant is optimally tuned because long-term visual deprivation in goldfish leads to “leaky” fixations (Mensh et al. 2004). Studies of

eye movements in blind humans have shown that previous visual experience improves the VOR response and fixation time constant stability (Leigh and Zee 1980). Notably, monocular blindness causes the stability to decay in the non-seeing eye to a greater extent than in controls with one eye blocked (Leigh et al. 1989). The presence of vision is not the only means to improve fixation holding, as blind patients can improve time constant stability by use of auditory feedback (Hall and Ciuffreda 2002).

The eye velocity-to-position neural integrator response has been shown to be modifiable by training. When the phase of the VOR was shifted to a phase lead during training for 1-2 hours, leaky post saccadic drifts occurred during scanning suggesting a modification possibly occurring within the integrator (Kramer et al. 1995; Tiliket et al. 1994). Similarly when the VOR included a phase lag, fixations became unstable as predicted by modeling (Kramer et al. 1995; Tiliket et al. 1994). In goldfish, post-saccadic fixations can be trained to either instability or leak using visual feedback (Major et al. 2004a). In addition, records from goldfish PNI neurons showed that the firing rates in most neurons were modified, consistent with a fixation plasticity encoded in PNI (Major et al. 2004b). The changes in firing rate behavior could have been a reflection of modifications occurring upstream within an afferent oculomotor pathway, or the plasticity might have been intrinsic to the integrator. Although determining the locus of the plasticity is a goal of this thesis, it is also important to determine whether the observed changes in time constant are monocular or conjugate.

Proposed mechanisms of eye velocity-to-position integrator plasticity

Since other oculomotor plasticity (VOR and OKR) appears to at least partially require the presence of the cerebellum, it is likely that fixation time

constant plasticity also may require the cerebellum. For example, little recovery was observed in the post saccadic drift after tectonomy of the eye muscles in floccular lesioned mammals (Optican and Robinson 1980). Straightforward interpretation of this result is difficult since floccular lesions alone in mammals severely affect gaze holding, thus plasticity may have been present in the integrator but not capable of expression. A locus of eye velocity-to-position integrator plasticity within the cerebellum is supported by observations that after its removal in cats, the low frequency VOR was more adversely affected than the high frequency rotation (Godaux and Vanderkelen 1984). As mentioned, low frequency VOR is largely produced by combining the direct “three neuron arc” with the parallel velocity-to-position neural integrator pathway and the eye velocity storage pathway (Skavenski and Robinson 1973). Thus changes in VOR response could be due to a modification in any of the above sites.

A consequence of suggesting that both learning and memory components of time constant plasticity require cerebellar involvement is that the integrator (Area I), may not exhibit local plasticity, hence tuning. Assuming the locus of memory encoding is cerebellar dependent and the integrator receives tonic and modulated input from the second order vestibular nuclei, then any changes in “spontaneous” vestibular neuron firing rate, caused by modified activity of Purkinje cells, precerebellar neurons or those within the vestibular nucleus would produce the appropriate changes in the PNI firing rate.

Alternatively, a memory of time constant changes may be cerebellar independent, with the necessary changes being encoded within a hindbrain nucleus. The cerebellum would only be necessary for the acquisition but not the expression of behavioral changes. Since all previous cerebellectomy studies

were carried out prior to considerations of time constant plasticity, it is unknown if the lack of modification of the time constant was due to a failure in acquisition of learning, memory or both.

In the goldfish, direct afferent and efferent pathways between the vestibulocerebellum and Area I do not exist. Cerebellar activity is relayed to PNI neurons through the vestibular nucleus. Cerebellar mossy fiber input originates from the precerebellar (Area II) and vestibular neurons with climbing fiber input arising from the inferior olive. Given the reduced circuitry, it can be easily determined if time constant changes are cerebellar pathway dependent in goldfish. The plasticity also must originate from or be reflected by the vestibular nucleus since this is the only source of neurons linking the cerebellar pathway to Area I.

Purpose of this thesis

The overall purpose of this thesis was to evaluate the proposed mechanisms for persistent neural activity and for time constant modification that are achieved in an eye velocity-to-position neural integrator located within a well delineated brainstem nucleus. This study determined the intrinsic organization of the integrator as related to either conjugate or monocular eye performance (Chapter 3). Based upon these results the role of the commissure in persistence and plasticity was explored, with emphasis on distinguishing whether either phenomena is cellular or network based (Chapter 4). Finally, the role of the cerebellum in integrator persistence and time constant plasticity was addressed (Chapter 5).

Chapter 2: Methods

Animal Care and Maintenance

One hundred and forty-four goldfish (*Carassius auratus)* 15-30 cm long were obtained from an institutionally approved supplier (Hunting Creek Fisheries, Thurmont, MD) for this thesis work that addressed the physiology and behavior of the horizontal eye velocity-to-position neural integrator. Animal preparation and experimental analysis were adopted from those previously described (Aksay et al. 2000; Marsh and Baker 1997; Pastor et al. 1994b). Prior to experimentation, all goldfish were acclimated and maintained in a temperature controlled (18°C) tank, pH 7.0-7.6, exposed to a 12:12 light/dark cycle. All protocols were approved by the NYU School of Medicine Institutional Animal Care and Use Committee (IACUC).

Surgical preparation

Goldfish were prepared in advance for experiments that required open cranial exposure. At least two days prior to either neuronal recording, injection or lesion, goldfish were anesthetized with buffered tricaine methanesulfonate solution (MS222; 1:5000 w/v; Sigma, St. Louis, MO) and placed on a surgical body-conforming-sponge that had been previously cooled, to provide additional anesthesia. Six self-tapping screws (Tx-#000-1/8” or Tx-#00-1/8”, Small Parts Inc., Miami Lakes, FL) were placed in the skull and two stabilizing bolts (#1-72) were anchored with Durabase dental acrylic (Reliance Dental Mfg. Co Worth, IL). A small (3-6 mm) section of the skull was removed over the vagal lobes covering the hindbrain and/or more rostrally over the cerebellum. The bone flap was reset and sealed using Vetabond tissue adhesive (3M, St. Paul, MN).

Goldfish were revived in anesthetic free water and then placed in an isolated temperature controlled recovery tank.

Eye movement measurements

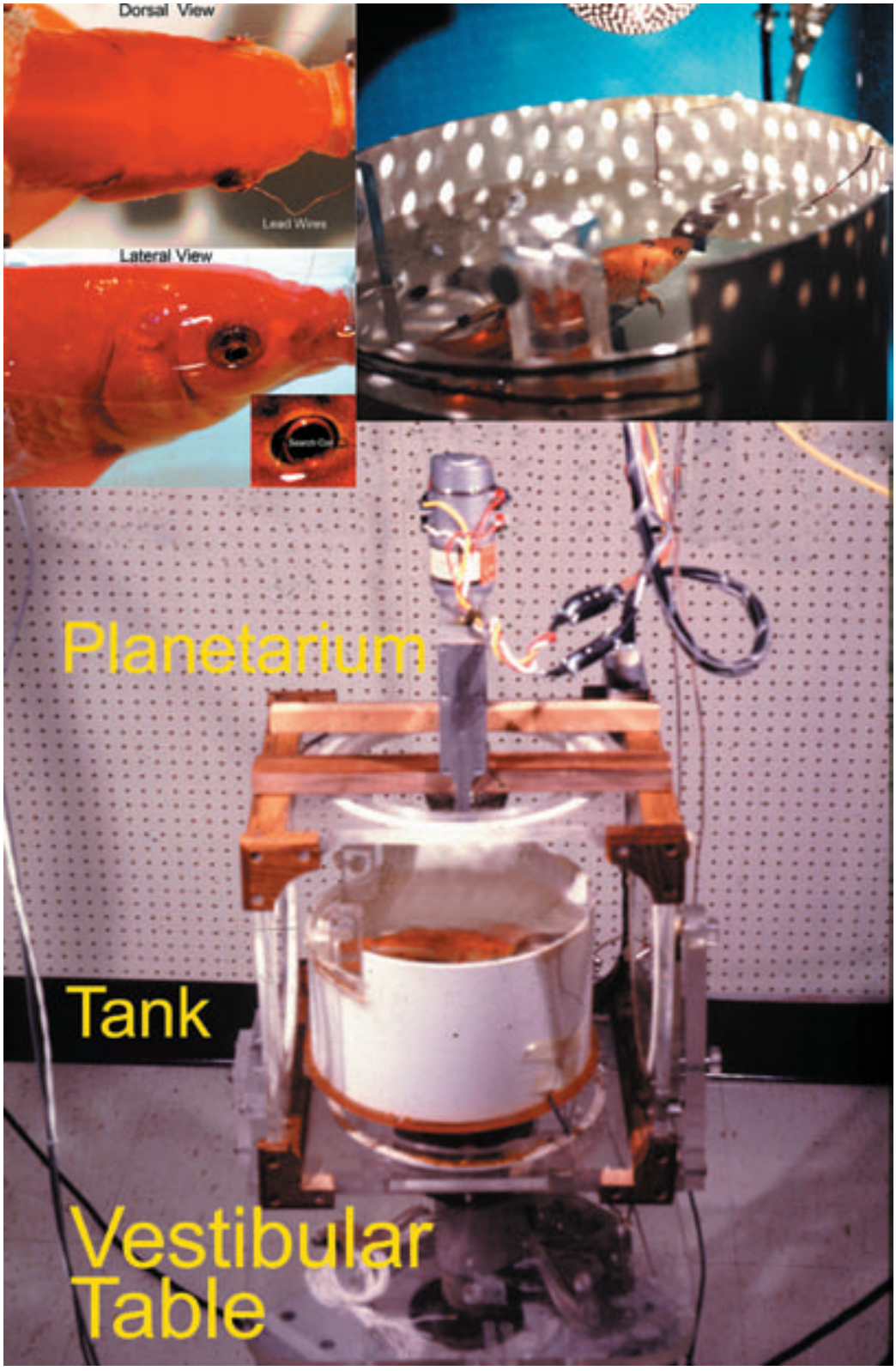
On the day of the experiment, a 4% topical xylocaine gel (Astra Pharmaceutical Products, Inc. Westborough, MA) was applied by cotton to the eyes, mouth, and skull. Goldfish were placed in a 38 cm diameter temperature-controlled (18°C) and aerated circular tank on top of a servo-controlled rate table. Goldfish were gently restrained in body-conforming acrylic holders that left the head and operculum uncovered. The animal's body was completely submerged with the water level maintained at the dorsal crest of the operculum. Water was continuously pumped through the gills to maximize respiration and indirectly minimize breathing artifacts. Additional immobilization of the head was accomplished by anchoring the previously attached stabilization bolts to a post on the experimental tank. Eye movements were measured utilizing the magnetic field search coil technique first described by Robinson (Robinson 1963). Eye movements were recorded by suturing (7-0 silk, Ethicon, Inc. Somerville, NJ) 80 turn 2.2 mm diameter copper coils (Sokymat, SA, Switzerland) to the eyes after topical application of 4% xylocaine (Fig. 2-1 right). The experimental tank was placed within a dual axis driven magnetic field (C-N-C Engineering, Seattle, WA). The eye position signals were 150 Hz lowpass filtered. During eye measurements in both light and dark, the experimental room was dimly illuminated and the entire setup was covered by a light proof cloth to eliminate external light sources.

Behavioral experiments

A photograph of the experimental tank, field coils, rate table and

Figure 2-1: Experimental apparatus.

Left side inset: Dorsal and lateral views of goldfish with a search coil sutured on the right eye. Higher magnification inset shows the coil centered around the pupil. Right side inset: Experimental tank with the planetarium displaying the visual stimulus on to the tank walls from above the goldfish. The 16” field coils surround the tank (top and bottom) and the eye signal detection preamplifiers are mounted below the vestibular rate table platform that rotates the entire setup about the vertical axis.


Dorsal View
Lead Wires
Lateral View
Planetarium
Tank
Vestibular
Table

planetarium is shown in Fig. 2-1. Vestibuloocular reflexes were elicited by utilizing a servo-controlled rate table (Biomedical Engineering, Thornwood, NY). Optokinetic stimulation was elicited utilizing either one or two servo-controlled planetariums (Biomedical Engineering, Thornwood, NY) mounted above the center of the head and projecting 3-5° red visual spots on a white background (Fig. 2-1 Left). Stimulus waveforms were generated by a computer controlled function generator (LabView, National Instruments, Austin, TX) in which the amplitude and phase of the vestibular and visual stimuli were individually controllable. To assess the condition of the animal and create a standard baseline prior to recording, goldfish were put through a battery of tests for 15-30 minutes with a combination of vestibular and/or visual stimuli. Goldfish were also placed in darkness before any experimental measurement to ensure that the visual threshold would be constant throughout the experiment (Bassi and Powers 1990a, 1990b).

A basic set of control data was recorded in every experiment that included 1) spontaneous scanning in both light and darkness, 2) sinusoidal VOR at 1/8 Hz 15.7°/s peak velocity, 3) VOR/OKR (gain=1.0) and 4) OKR at 1/8 Hz 15.7°/s peak velocity. Additional behavioral profiles such as VOR and/or OKR at other frequencies and amplitudes for Bode plots along with either VOR gain enhancement or suppression were also recorded when appropriate.

Monocular optokinetic behavior

For monocular experiments, two independent servo-controlled planetariums were utilized that projected visual spots to ~150° of the visual field (Fig. 2-2). The average binocular overlap in goldfish (central ~30°) was blocked from receiving any patterned visual stimuli (Trevarthen 1968). By necessity,

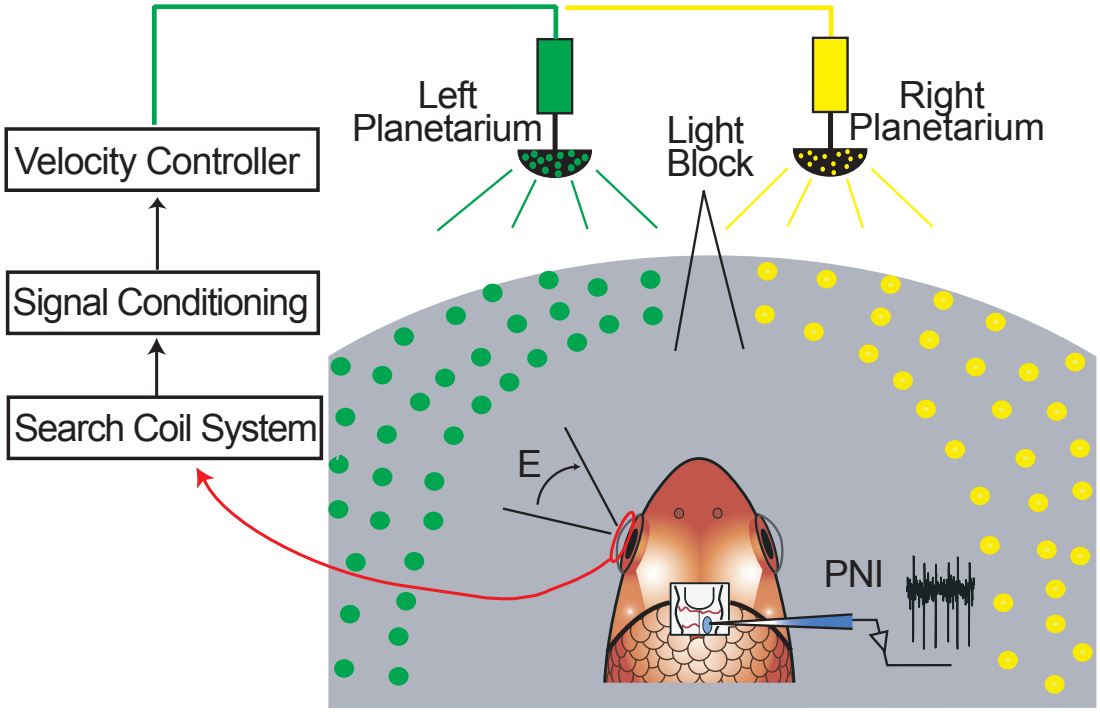


Figure 2-2: Monocular plasticity paradigm.

A schematic of the optokinetic arrangement used for producing monocular tracking and time constant plasticity. Two independent servo-controlled planetariums were used to produce visual patterns each subtending 150° with a 30° central stimulus free region. Adapted from Major *et al.* 2004a

each planetarium was slightly offset from table center with amplitude, and phase separately adjusted. Eye positions were calibrated with pseudo-wholefield conjugate stimulation, in which the planetariums moved in equal phase and amplitude. A set of conjugate OKR controls were recorded for each experiment with the light blockers in place during the course of the experiment to prevent any gain changes due to alteration of the optokinetic stimulus during the monocular paradigms. Disconjugate eye tracking was elicited when the planetariums differed in phase, frequency or amplitude (Fig. 3-3)

To distinguish pathways that are monocular from the visual periphery to the oculomotor output from those that include binocular active suppression, one eye was selectively occluded by a black painted hemi-sectioned ping-pong ball. Pattern blocking effectiveness was tested by the absence of eye movements in either eye during planetarium motion projecting to only the occluded eye as compared to the presence of eye movements during planetarium motion projecting only to the unblocked eye.

Conjugate visuomotor training and time constant plasticity

In order to modify eye position time constants, planetarium command signals induced visual slip by one of two experimental protocols. Eye position voltage was either directly provided as the input into the planetarium controller, to produce instability, with a maximal velocity of 30°/s, or it was inverted to produce leak (Major et al. 2004a). Alternatively, the voltage was fed into a custom built electronic device in which threshold could be adjusted for positive (left eye position) and negative (right eye position) voltage set points independently. Output voltage and voltage polarity were manually set for each half of the oculomotor range. For instability training, the planetarium was initially rotated at

20°/s. When eye velocity eventually matched planetarium velocity, the training velocity was increased up to a maximum of 40°/s.

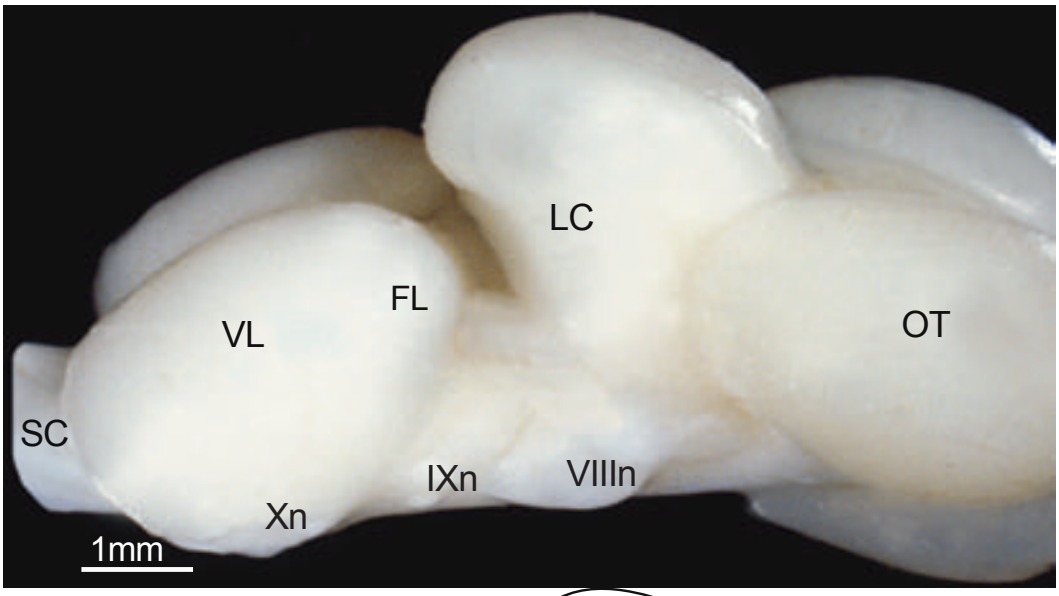
During leak training, lower initial planetarium training velocities were utilized (2-5°/s), with a large central region that was not entrained (null region) in order to prevent unidirectional leak training due to the saccadic pattern generator not resetting the eyes across the null position (Fig. 1-3C). This approach facilitated training by spontaneously switching between the two directions. As eye velocity improved over the course of the experiment to follow more closely the training paradigm, planetarium velocity was increased and null region reduced, until a maximal training stimulus of 40°/s was reached.

Neuronal Recording

Extracellular neuronal recordings from Area I neurons were obtained utilizing 3 mm O.D. glass electrodes pulled on a Narishige vertical puller with final tip diameters of 1-4 uM (resistances of 1-5 MΩ) filled with 2M NaCl, 50 mM LiCl and fast green. On the day of the experiment, a small surrounding dam of super strength denture adhesive (Rite Aid, Harrisburg PA) was constructed around the surgical window to prevent water from accidentally entering the brain vault during the experiment. Under local lidocaine anesthesia (2%), the surgical window was re-opened and a layer of flouroinert (FC75, 3M St. Paul, MN) placed above the hindbrain. To minimize body and head motion, the spinal cord between C1 and C2 was compressed with forceps in some cases. The anatomical location of PNI was approximated as described in previously published accounts (Aksay et al. 2000; Major et al. 2004b). A lateral whole brain view and sagittal diagram showing roughly the electrode placement is presented in Fig. 2-3. Area I neurons exhibiting eye position sensitivity were recorded ~400 microns from the midline,

Figure 2-3: Lateral view and sagittal schematic of goldfish brain.

The photograph shows the gross anatomical features of hindbrain structures including the cerebellum with relative relationships to the nuclei responsible for horizontal eye motion as illustrated in the drawing. OT: Optic tectum, FL: Facial Lobe, VL: Vagal Lobe, CC: Corpus Cerebelli, CR: Crus Cerebelli, egr: eminentia granularis of vesibulocerebellum, gr: granule cell layer of corpus cerebellum, LC: lobus caudalis of the vestibulocerebellum, III: Oculomotor Nucleus, IV: Trochlear Nucleus, VIII: Vestibular Nerve, IX: Glossopharyngeal Nerve, X: Vagal Nerve, Abd: Rostral and caudal abducens nuclei VN: Vestibular Nuclei, VNI: Velocity Neural Integrator (Area II), PNI: Position Neural Integrator (Area I) and IO: Inferior Olive. Adapted from Straka, 2006


LC
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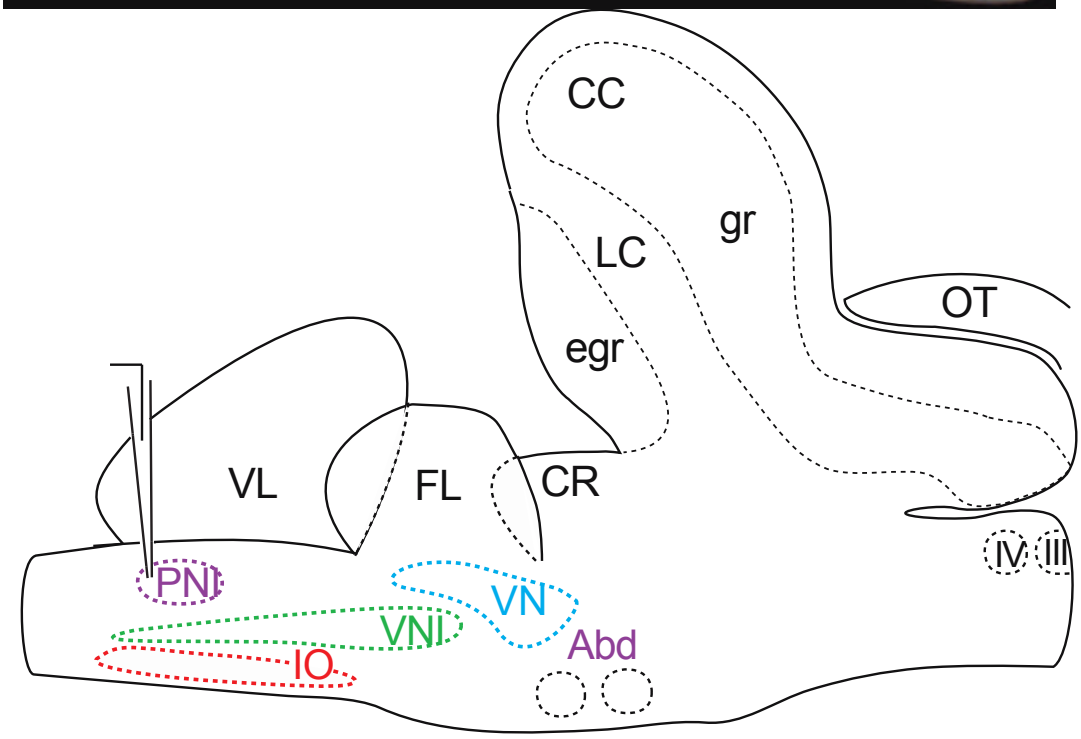
CC
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LC
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IV
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PNI
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VNI
Abd
IO

midway between the facial lobe and obex. Location of PNI was estimated by first identifying the midline and bilateral MLF boundaries followed by visualizing the facial lobe, obex and local vasculature. Electrical activity of PNI neurons was amplified by 10,000X and recorded with a bandpass of 100Hz-10kHz. The raw voltage records of the neurons and behavior were digitized at 15 kHz using a DigitaData 1320A A-D board (Axon instruments). The behavioral traces were subsampled offline to 300 Hz using a custom written algorithm (AxonSqueeze) provided courtesy of D.W. Tank. The minimum requirement for a recording of a particular behavioral set was thirty seconds; however, optimally a full set of conditions required ~ 10 minutes.

Firing rates were determined offline using previously described custom written algorithms that distinguished neuronal populations by both spike amplitude and half width time window (Aksay et al. 2000) (Fig. 2-4A). To aid in distinguishing neurons, a histogram of the peak-peak amplitude was also determined (Fig. 2-4B). To check that a single neuron was isolated, the voltage records were superimposed (Fig. 2-4D). Firing rates were computed from the interspike intervals (ISI) and smoothed by a 125 millisecond triangle moving average window (Aksay et al. 2000). A figure of the output of the spike sorting algorithm is shown in Fig. 2-4. This program was also provided courtesy of Drs. E. Aksay (Cornell University) and D.W. Tank (Princeton University).

Acute midline lesion methodology

On the day of the experiment, the surgical window was reopened under local 2% lidocaine anesthesia and the hindbrain visualized from roughly the facial lobe to the obex (Fig. 2-5 Schematic), and immersed under Flouroinert (FC75, 3M). Control behavioral sets were recorded and the midline cut from

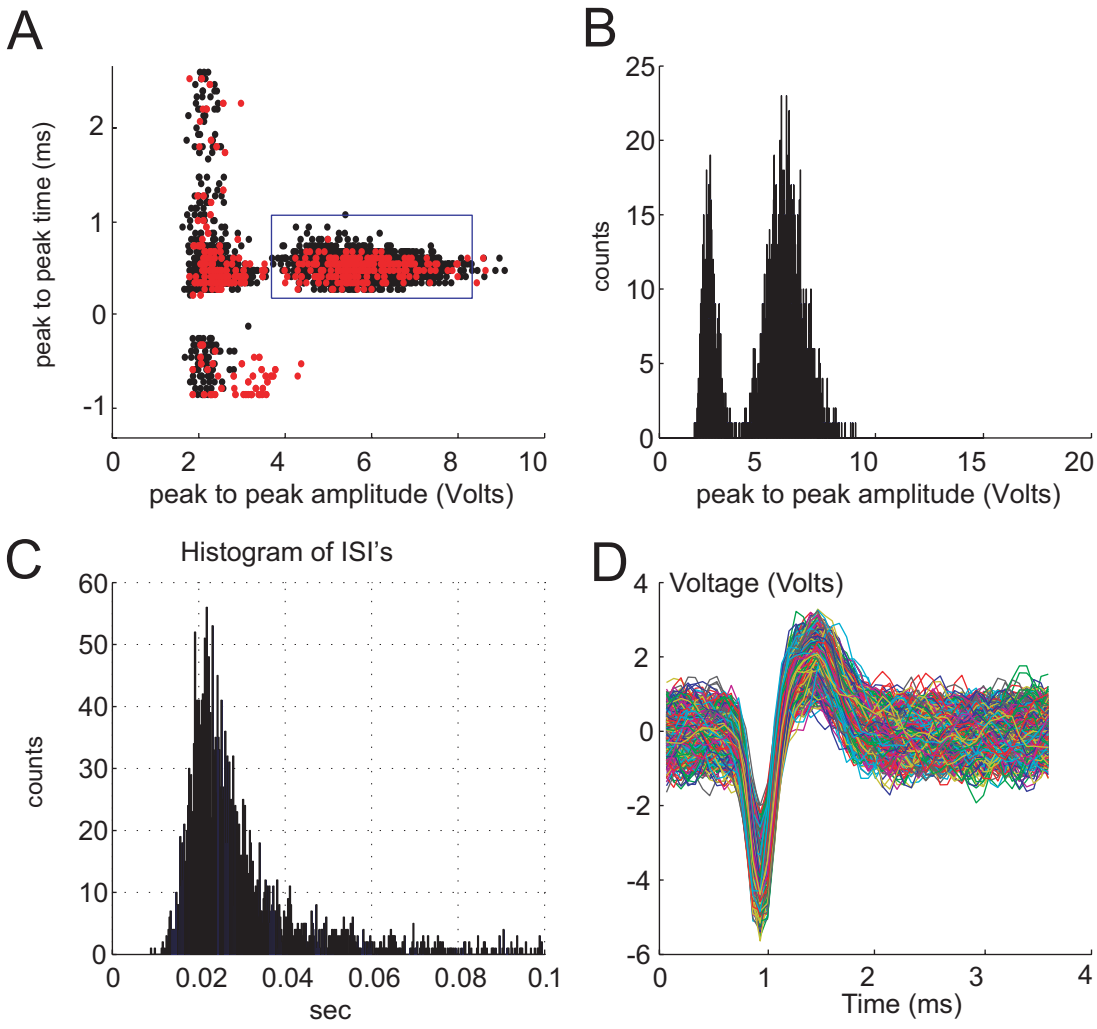


Figure 2-4:Firing rate discrimination of PNI neurons. A-D: Sample output of spike firing rate discriminator program illustrated for the PNI neuron in Fig. 3-1. A: Peak-to-peak amplitude versus half width plot. Red circles indicate excluded data points. Black circles are accepted spikes. B:Histogram of peak amplitudes. C: Histogram of interspike intervals D: Superimposition of extracellularly recorded spikes.

slightly caudal to the facial lobe through the obex either with a small glass shard #0 micro cover glass (Electron Microscopy Sciences, Washington PA) or iridectomy scissors (vannas type, Ted Pella, Redding, CA) (Fig. 2-5). Due to the proximity of PNI to major blood vessels in the hindbrain, immediately after lesion construction, small Kimwipe sponges were utilized to minimize bleeding and avoid behavioral deterioration caused by hemorrhagic effects. When surgical conditions were controlled (~3-15 minutes), the lightproof cloth was reattached, and eye position stability monitored in both the presence and absence of visual feedback, which on average, was 20 minutes post lesion. In 30% of the experiments the eye position time constant was markedly reduced in darkness. In these cases, the VOR or OKR also showed major effects due to perturbation and the eye position time constant was monitored in the presence of visual feedback for up to two hours and the changes in the time constant were periodically assessed.

In all experiments, a series of identical oculomotor behaviors were recorded after the lesion and compared to the control conditions. In 6/9 of these experiments, eye position drift was trained towards instability. In 3/6 cases, the bone flap was reattached over the surgical window and the animals returned to the home tank for chronic observation.

Chronic lesion methodology

To determine the long term effects of midline lesion on PNI function and to achieve greater control over the surgical procedure, a midline lesion was performed during attachment of the head restraint under general anesthesia (n=12). Including the successfully midline lesioned goldfish obtained from the above population of acute lesioned goldfish (n=3), eye position holding was

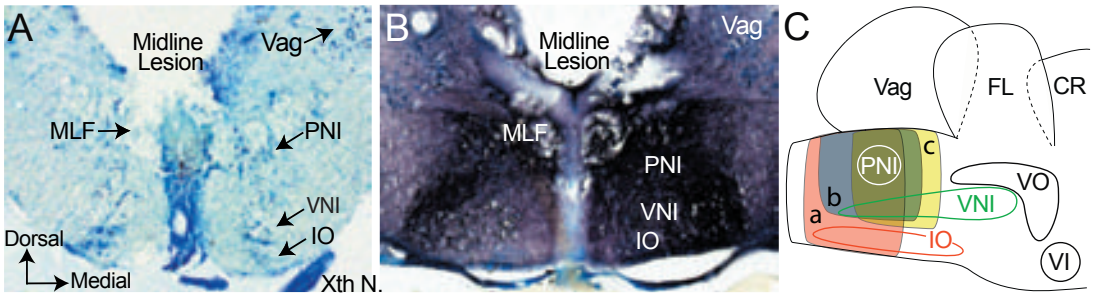


Figure 2-5: Summary of midline lesions between the bilateral PNI nuclei.

A: Photomicrograph of a coronal section showing a midline lesion encompassing about 75% of the dorsal-ventral depth. Behavioral records and P-V plots are shown before and after this lesion in Fig. 4-1A-E. B: Photomicrograph 15 days post midline lesion showing extensive biocytin labeled axons in the MLF and reticular formation after spinal cord label documenting that all commissural neurons were severed. In this example, P-V plots are shown in Fig. 4-3. C: Schematic of Anterior/Posterior and Dorsal/Ventral measurements in 3 out of 8 cases (a-c) in which data are illustrated in Figs. 4-1 to 4-5. All of the midline lesion experiments could be catergorized within one of these three groups. Abbreviations: PNI: Eye Velocity-to-Position Neural Integrator (Area I), VNI: Eye Velocity Storage Neural Integrator (Area II), MLF, Medial Longitudinal Fasiculus; FL, Facial Lobe; Vag, Vagal Lobe; VO, Descending Octaval Nucleus; Xth N., Vagus Nerve X and IO, Inferior Olive.

tested at various time intervals up to three months. Eye position drift was trained to both instability and leak in these experiments.

Histology

To verify that the lesion encompassed crossing midline projections of PNI, all animals were perfused with 4% formaldehyde, 0.5% glutaraldehyde and sectioned coronally at 75-100 μm with a freezing microtome followed by staining with crestyl violet. Histology was reconstructed for the three dimensional extent of the lesion. In several cases, two days prior to perfusion biocytin crystals (Molecular Probes) were placed in the spinal cord to better visualize hindbrain nuclei and pathway integrity for assessing completeness of commissure disruption. After sectioning, biocytin labeled slices were incubated in 0.3% hydrogen peroxide followed by three hours in a solution of 0.1M phosphate buffered saline solution containing 0.4% Triton X-100 and 1:100 dilution of avadin-biotin complex (Vector). The biocytin reacted with 0.04% 3'-3 diaminobenzidine, 0.4% ammonium nickel sulfate, and 0.02% hydrogen peroxide in a 0.05M tris buffer (pH 8.0) for eight minutes. For more detailed description of the method see Straka et al. (2006)

Acute cerebellar ablation

Similar to midline lesions, a circular dam of dental adhesive (Rite Aid, Harrisburg, PA) was constructed around a more rostrally located surgical window in a previously head bolted goldfish. Under local 2% lidocaine anesthesia, the bone flap was reopened. A series of control behaviors were recorded and the animals were then trained to an altered eye position time constant prior to cerebellar aspiration. The corpus cerebellum and caudal lobe were visualized utilizing a Zeiss dissecting microscope located above the tank. The cerebellum

was aspirated utilizing a 23 gauge needle to minimize bleeding. The aspiration completely removed the corpus and vestibulocerebellum, but left the crus cerebelli and vestibular nuclei intact in order to preserve the more ventral optokinetic fiber tracts. Variable amounts of the valvuli cerebellum were also left to avoid damaging the rostral midline structures. Profuse bleeding, when observed, was minimized by application of Kimwipe sponges. Fixation stability was monitored along with the VOR and OKR behaviors to assess brainstem integrity. Many of the animals were also trained to a different eye position time constant. After acute experiments were concluded, the bone was reset using Vetabond and the fish maintained in a recovery tank for chronic observation.

Evaluation after cerebellar ablation

The long-term effects on eye position time constant stability and plasticity were monitored periodically for up to one and a half years. The population of cerebellectomized fish were obtained from two different sources. Some of the acute cerebellectomized goldfish were monitored on subsequent days. Additional experimental animals were obtained by performing the cerebellectomy when the goldfish were under general MS-222 anesthesia during head stabilization attachment.

Data Analysis

All data were recorded by an A-D board (DigitaData 1320A, Axon Instruments, Union City, CA) either at 300 Hz (behavioral recordings) or 15 kHz (neuronal recordings) and saved to disk. The data was analyzed off-line using custom written algorithms in Matlab (The Mathworks, Inc; Natick, MA), courtesy of Drs. G. Major, J. Beck, E. Aksay, and D. Tank. Eye and head velocity were determined by digitally differentiating the position records and smoothed by a

moving average window of < 50 millisecond. Depending on the noise level in the record, the window size was varied between 10-50 ms. Since the goal was to analyze eye position independent of saccadic influences, the regions 200 ms before to 600 ms after each saccadic fast phase as determined by acceleration threshold were removed automatically. Movement artifacts were manually removed from the quantitative analysis. During monocular tracking experiments eye movements were more disconjugate and the excluded regions could differ in time or the number of saccades. When determining monocularity of Area I neurons, a combined fixation index was used to prevent false positives due to differences in the time periods and amount of data analyzed.

Calibration of velocity and positions

Head velocity was calibrated by sinusoidally rotating the table at 0.125 Hz and ± 20°. The differentiated table position voltage deflection was then assigned to be equal to a 31.4°/s peak-to-peak velocity. The planetarium was also then calibrated to 15.7°/s by adjusting the velocity and phase until the visual projection within the surrounding room was stationary during table rotation. These values were checked at the onset of nearly every experiments but rarely required adjustment.

Standardized eye coils (80 turn, 40 ohm, 2.2mm diameter, Sokymat SA), whose output voltages were measured on a mechanical model system before experimental use, were subsequently recalibrated during simultaneous visual-vestibular presentation of a sinusoidal stimulus at 0.125 Hz 15.7°/s peak velocity that assumed a VOR gain of 1.0 (eye/head velocity). The measured eye position voltages were differentiated into eye velocity. The desaccaded voltages of slow velocity eye movements were fit by a linear regression to the sinusoidal waveform

and a calibration constant was computed. Zero eye position within each orbit was determined as the mean eye position during 3-5 minutes of scanning a stationary background. Since eye velocity was determined as the derivative of eye position, both the position and velocity had the same calibrations. In this thesis all conclusions were based upon changes in oculomotor behavioral paradigms that occurred within the experiment, thus each goldfish served as its own control, and any slight miscalibration (absolute value) would not affect the qualitative results obtained.

Conjugate gain and phase measurements

The gain and phase of the VOR and OKR were computed by a least square linear regression of the desaccaded eye velocity as shown in Figure 4-5C. The gain was a ratio of the amplitude of the eye velocity versus the command stimulus (table or planetarium).

Disconjugate OKR gain and phase

Similar to conjugate gain and phase, a least squares regression of the desaccaded eye velocity was computed. However, gains during disconjugate eye movements were normalized to that during pseudo-whole field OKR at 0.125 Hz 15.7°/s to ascertain the percentage reduction in gain during monocular tracking.

Eye position stability

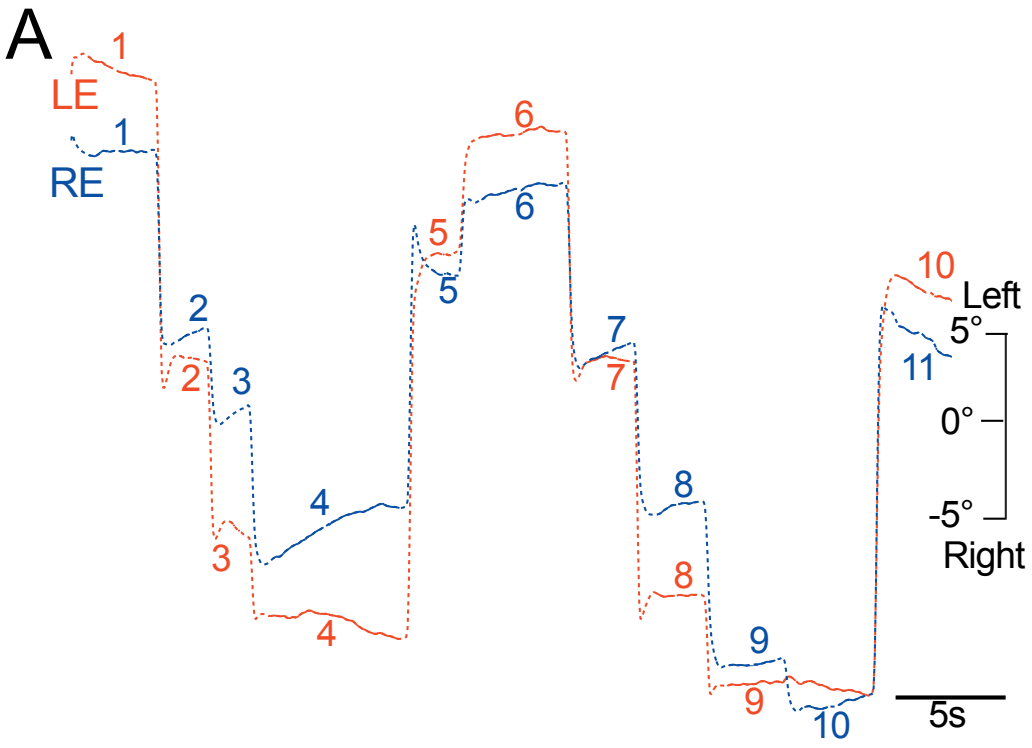
To determine the time constants of eye fixations, position-velocity (P-V) plots were constructed utilizing Matlab. A sample position-velocity plot is shown in Fig. 2-6, in which successive fixations are numbered in the eye position traces. The P-V plot consisted of mean eye position for each fixation lasting at least 150 ms set against average eye drift velocity determined as the slope of the linear

regression of eye position during the fixation. This yielded a scatter plot of eye velocity versus eye position (Fig. 2-6B). The slope of linear regression of the P-V plot is termed k, and is mathematically equal to the negative inverse of the time constant (τ=-1/k). This method is similar to that utilized in previous P-V plots showing stability of eye position holding and time constant plasticity (Major et al. 2004a; Major et al. 2004b; Mensh et al. 2004). One weakness of this approach is that all eye positions are assumed to have a single null point and time constant during the sampling period. In spite of this constraint, linear regressions of the P-V plots were used for ease of implementation and interpretation as well as to compare time constants with those from previous studies. However different slopes could be drawn for the nasal and temporal eye positions as illustrated in Fig. 3-6. Significance of the slopes between P-V plots covering the whole oculomotor range were determined by ACOVA with a post-hoc Tukey-Kramer analysis carried out in Matlab ($p<0.01$). Similar results were obtained if a Scheffe test correction or a Bonferroni correction were utilized rather than a Tukey-Kramer test. Due to multiple observation points within the training time period, the Tukey Kramer test corrected for a type 1 error that could occur due to multiple sequential t-tests. This method was especially useful in monocular time constant plasticity studies which also compared interocular measurements. Time constants could not be used directly for statistical analysis, since they are a non-continuous function. The “k” values were employed in all comparison tests. After analysis, “k” values were then reconverted into time constants with the consequence that standard deviations and confidence intervals became asymmetrical due to the mathematical transformation.

The average changes in time constants due to either experimental

Figure 2-6: Construction of eye position vs. velocity (P-V) plots.

A: Eye position traces of the Left and Right eyes were centered about 0° during spontaneous eye movements with each fixation sequentially numbered. B: Position-Velocity (P-V) plot of behavior shown in A, in which each fixation is plotted as a single point and subsequently fitted by a least squares regression line.


A
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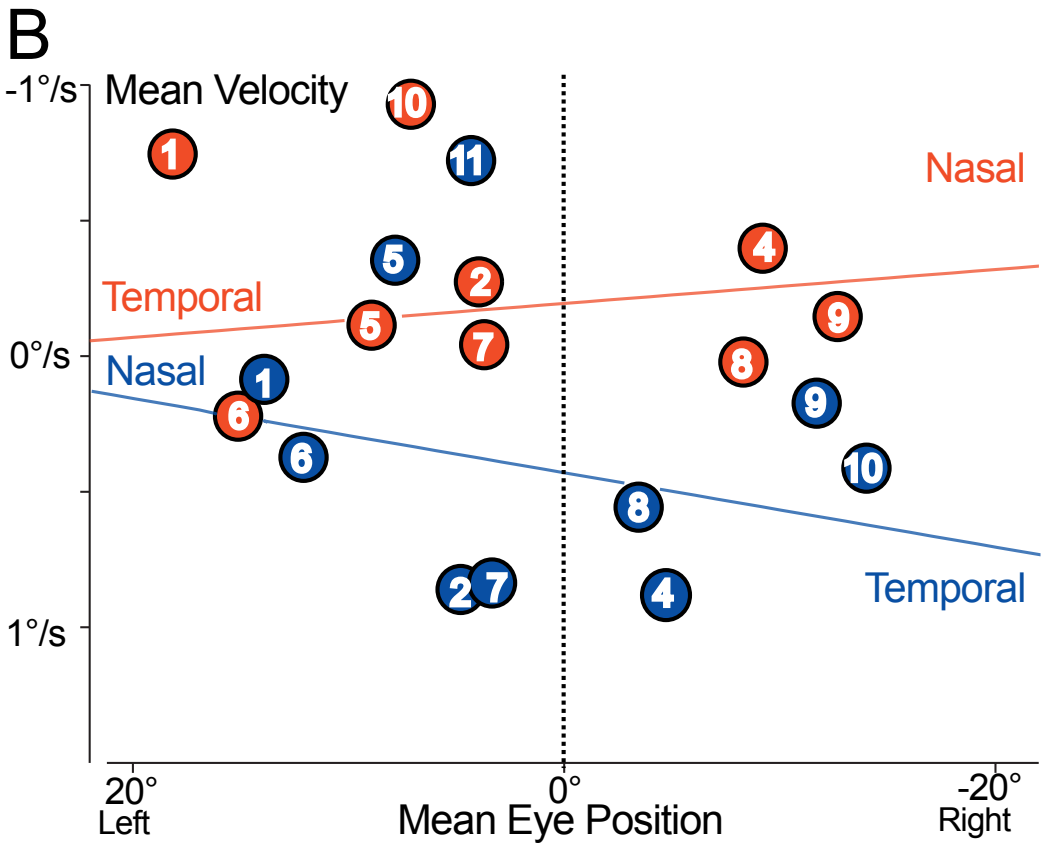
RE
Left
5°
0°
-5°
Right
5s


B
-1°/s
Mean Velocity
Nasal
Temporal
0°/s
Nasal
Temporal
1°/s
20°
Left
0°
-20°
Right
Mean Eye Position

perturbation or training were analyzed using paired t-tests in Microsoft Excel by comparing the initial to final observation. All average time constants are reported with either the standard deviations or range of values.

Behavioral measurements after perturbations

To determine if the average eye position changed after experimental manipulation, either a Kruskal-Wallis test of the median or ANOVA with Tukey-Kramer ad-hoc of the mean were used. Changes in the variance were analyzed by use of the Levene's test for homogeneity of variances as written for Matlab (Trujillo-Ortiz and Hernandez-Wallis 2003).

During the course of the thesis project, Matlab included the Levene test as part of the statistics toolbox. The results obtained utilizing either the prewritten or new program were similar. The average change in eye position was tested by paired t-test in Microsoft Excel.

Determination of neuronal monocularity

Neurons were analyzed for monocular eye position and eye velocity sensitivity. Firing rates were plotted along with both the velocity and position records from each eye, and the sequences visually inspected in all cases for rough correlation. Eye position and velocity for the RE and LE were regressed separately against the firing rate. The Pearson correlation coefficient of the right eye was compared for significance against the Pearson correlation coefficient for the left eye (Zar 1996). Neurons were determined to be correlated to eye position and eye velocity if the correlation coefficient (r) was greater than 0.7. In this study, correlation coefficients were used to determine monocularity rather than regression coefficients as used elsewhere (Sylvestre et al. 2003; Zhou and King 1996, 1998). The reasoning behind this strategy was similar to that pointed

out for abducens neurons in cats (Fig. 4 of Delgado Garcia *et. al* 1977). The eye position versus firing rate plot of the right and left eye had similar slopes however the scatter of the data points and hence the correlation was much better for one eye (Fig. 3-3C).

In the current study, the high degree of collinearity of eye movements produced unreliable regression coefficients in the combined left and right eye multiple linear regression as evidenced by reversed polarities and negligibly small numbers. Collinearity was avoided by regressing eye motions of the right and left eye separately. To test that large qualitative difference did not exist between the methods utilized in this work and in previous studies of neuronal monocularity, a position ratio was computed for the neurons as previously described (Zhou and King 1996). The position and velocity ratio is computed as the difference between the neuronal sensitivity divided by the sum of the sensitivities (i.e. $(K_r-K_l)/(K_r+K_l)$). Bootstrap analysis of the neuronal data was carried out for two PNI neurons as previously described (Sylvestre et al. 2003). Analysis of the neurons yielded similar results of monocularity versus conjugacy of the neurons regardless of the methodology employed.

Chapter 3: Monocularity of horizontal eye movements in the oculomotor hindbrain

Introduction

Spontaneous movements of both eyes in naïve goldfish are highly correlated due to conjugate timing and direction of saccades; however, it has been previously observed that the two eyes are not in corresponding positions after individual saccades and throughout the subsequent fixations. The vergence angle also continuously varies from saccade to saccade (Easter 1971; Mensh et al. 2004) (Fig. 3-1A).

The biological mechanism underlying stable disconjugate eye positions could be due to either 1) separate encoding of each eye position within a common premotor pathway (like PNI) (Von Helmholtz 1910) or 2) a combination of vergence and version signals each arising from different pre-motor pathways that conjointly encode the position of both eyes (Hering et al. 1977). It was long assumed that horizontal eye movements of both eyes was simultaneously encoded, due to the presence of abducens internuclear interneurons yoking the nasal motion, generated by the medial rectus of one eye, with the temporal motion, generated by the lateral rectus of the other eye (Fig. 3-1D) (Cabrera et al. 1992; Carpenter and Batton 1980; Highstein and Baker 1978).

However, a simultaneous version-vergence control of eye movements originating at the level the abducens internuclear interneurons has been challenged (Delgado-Garcia et al. 1977). Recently, ipsilateral and contralateral sensitive vestibular and prepositus hypoglossi neurons have been documented by use of the primate vergence system to change eye position alignment

(McConville et al. 1994; Sylvestre et al. 2003; Zhou and King 1996). However, the command for the vergence control system originates in the midbrain and may involve a separate eye position neural integrator (Mays and Gamlin 1995; Mays and Porter 1984). Thus although monocular neuronal sensitivity exists in, for example, the primate PNI, the observation is suggestive rather than conclusive of a separate versional control pathway for each eye.

Goldfish provide an attractive model to rigorously test neuronal monocularity within the eye velocity-to-position integrator using disconjugate eye movements. Anecdotal observations suggest some PNI neurons in goldfish encode ipsilateral monocular eye movements (Pastor et al. 1994b). Afferent inputs to PNI are largely, perhaps exclusively, from vestibular neurons and reticular burst neurons. Efferent targets of PNI consist of the abducens motoneurons, abducens internuclear interneurons and the neurons in the saccadic burst generator (Aksay et al. 2000). In contrast to the mammalian PNI, whose function may be distributed between two nuclei (vestibular and prepositus hypoglossi) as well as receive a direct cerebellar projection (McCrea and Horn 2005), the goldfish PNI appears as a single nucleus without direct cerebellar connectivity thereby allowing a clearer interpretation of monocular integrator performance and plasticity following experimental perturbations.

In many species, the direction and magnitude of saccades and the intersaccadic drift are highly modifiable by visuomotor training (Averbuch-Heller et al. 1999; Optican and Miles 1985; Tiliket et al. 1994). In the goldfish integrator, stability can be entrained to either overcompensate with an eccentric drift (Fig. 1-3B) or undercompensate with a central drift (Fig. 1-3C), in response to the long term presence of visual motion. Corresponding changes were found to be

reflected in PNI firing rates implying that eye position stability during fixation was a modifiable oculomotor behavior (Major et al. 2004a; Major et al. 2004b). By contrast, when monocular post-saccadic drift was trained by direct visual feedback in primates, only conjugate adaptations were observed (Kapoula et al. 1990).

Monocularity of eye velocity and position has important implications concerning internal organization of the integrator that indirectly address mechanisms of neuronal persistence. If eye position is conjugately encoded, then each PNI neuron potentially would control both the stability of nasal eye positions in the contralateral eye and temporal eye positions in the ipsilateral eye (Fig. 3-1D). Accordingly, if eye position is separately encoded for the ipsilateral temporal and contralateral nasal hemifield within each PNI, then two subpopulations of neurons should be co-localized in each integrator. Bilaterally this would be a total of four independent horizontal eye velocity-to-position integrators (Fig. 3-1D). In this case, inhibitory connections might then be envisioned to coordinate the nasal and temporal eye position integrator populations for each eye. This study was designed to determine the monocularity of the eye velocity-to-position integrator during performance and time constant plasticity.

Results

Scanning behavior and natural monocularity

A hallmark of Area I neuronal activity in goldfish is proportionality of the firing rate to eye position and persistence in the absence of sensory feedback, i.e., in the dark (Fig. 3-1A,C). Similar to previous work, scanning saccadic eye motions were conjugate in timing and direction (Fig. 3-1A); however the amplitude

differed between the right and left eye (Fig. 3-1A blue and red arrows) (Easter 1971; Mensh et al. 2004). Monocular saccades, although rare, were observed at the extremes of the oculomotor range (Fig. 3-1A fixation #9).

In contrast to the yoked behavior of saccades, large differences were observed in post saccadic drift when one eye (Fig. 3-1B; RE fixations #7,11) or both eyes were at nearly identical positions (Fig. 3-1B LE & RE fixation #4). During fixation #4 the eye position drift was ~0.5°/s in opposite directions. Analysis of the P-V plot (Fig. 3-1A RE fixations #2-7; LE fixations #1,4,10; 1B) revealed an offset in the regression lines from zero indicating a nasal bias with the left eye drifting right and the right eye drifting left. The presence of a nasal bias in eye velocity had also been previously observed (Easter 1971; Mensh et al. 2004). The nasal eye position drifts suggest two separate populations of neurons co-localized within PNI, one projecting to the abducens motoneurons to control ipsilateral temporal eye position and the other projecting to the abducens internuclear interneurons to control nasal contralateral eye position (Fig. 3-1D).

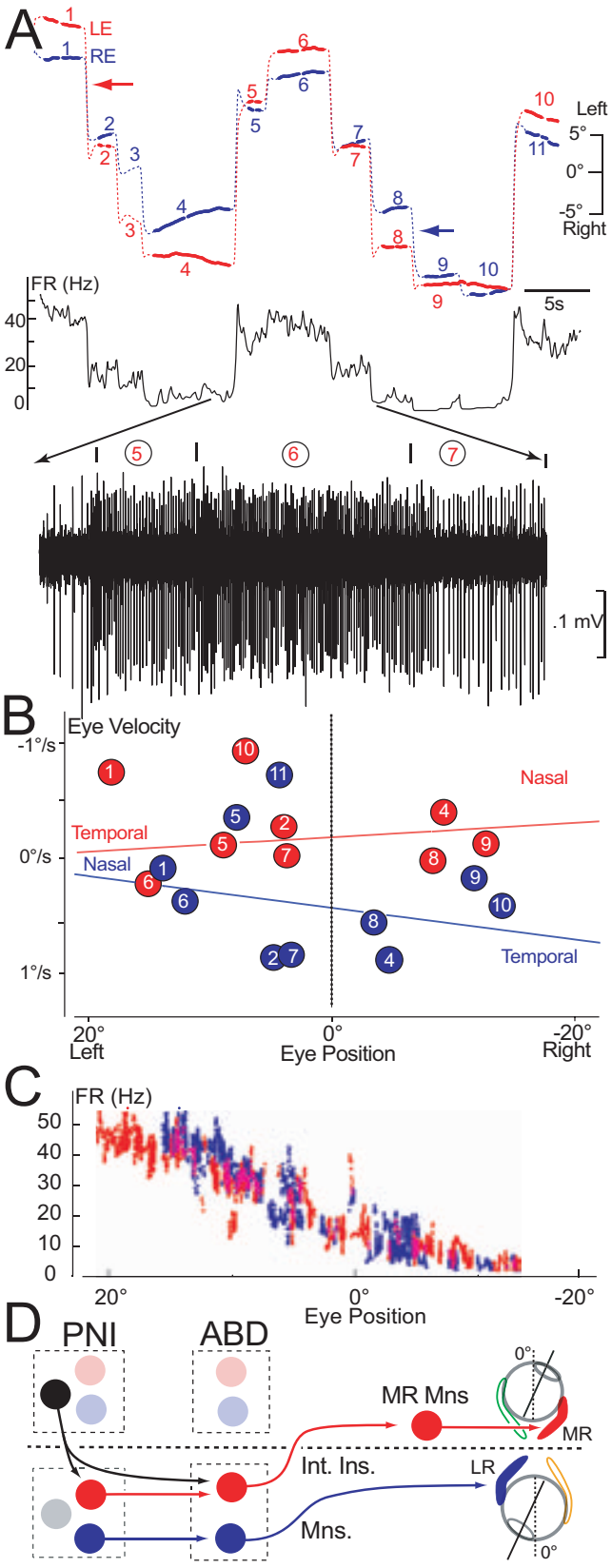
Monocular neurons in PNI

To test if PNI neurons preferentially encode for temporal positions of the ipsilateral eye or nasal positions of the contralateral eye, firing rates were recorded during naïve scanning. Two distinct populations of neurons were distinguished, one correlated better with ipsilateral temporal eye position (Fig. 3-2) and the other whose correlation was not significantly different for either eye and thus determined as conjugate (Fig. 3-3A). Conjugate neurons were recorded even when mild disconjugacy was observed in eye position records (Fig. 3-1).

In the right-side monocular PNI neuron shown in Fig. 3-2, ipsilateral (RE) preference was observed in fixation #5, since the left eye saccaded in the “off”

Figure 3-1: Quantification of eye position neural integrator (PNI) activity during spontaneous fixation behavior in darkness.

A-C: Firing rate (FR) of a conjugate left side PNI neuron. A: Left (LE) and right (RE) eye position was centered with each fixation sequentially numbered. Horizontal arrows denote saccades of different amplitude with neuronal records expanded for fixations 4-8. B: Eye Position-Velocity (P-V) plot of each numbered fixation (from A) in which eye velocity (ordinate) was calculated from regression of the eye position (filled part for each fixation). Time constant (τ) of the left eye was 159.7s and the right eye 72.9s. C: Plot of FR vs. eye position exhibiting a correlation with both left and right eye position. Averaged LE & RE position and velocity FR coefficients (+ values leftward and – rightward) were 1.59 (sp/s)/° and -0.29 (sp/s)/°/s (r=0.96). D: Color coded schematic showing separate PNI pathways to abducens (ABD) motoneurons (Mns) and internuclear Interneurons (Int Ins). Black indicates an inhibitory PNI neuron. LR and MR: lateral and medial rectus.


A
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RE
Left
5°
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Right
FR (Hz)
40
20
0
5s
.1 mV
B
Eye Velocity
-1°/s
0°/s
1°/s
Nasal
Temporal
Nasal
Temporal
20°
Left
0°
Eye Position
-20°
Right
C
FR (Hz)
50
40
30
20
10
0
20°
0°
Eye Position
-20°
D
PNI
ABD
MR Mns
MR
Int. Ins.
LR
Mns.
0°
0°

direction, but the neuronal firing rate and right eye position remained constant (Fig. 3-2A). Comparing the eye position and firing rates during fixations #1 and #6, showed that the firing rate increased (Fig. 3-2A grey line), when the right eye was further in the “on-direction” (Fig. 3-2A blue line), while the left eye was further in the “off-direction” (Fig. 3-2A red line) thereby indicating right eye position sensitivity. Combined eye position and eye velocity linear regression vs. firing rate was obtained separately for the right and left eye respectively (Table 3-1 Ipsi neuron). A graphical approximation of eye position versus firing rate was plotted in Fig. 3-2C. When the right eye position (blue) was regressed against firing rate, a clear linear band was observed. In contrast, the left eye (red) versus firing rate graph was far more diffuse, without a unique slope. When this neuron was analyzed using a combined left and right eye position and velocity and a ratio of the eye position/velocity regression coefficients calculated similar to previous studies (Zhou and King 1998), it would have been classified as ipsilateral-eye sensitive (see Table 3-1).

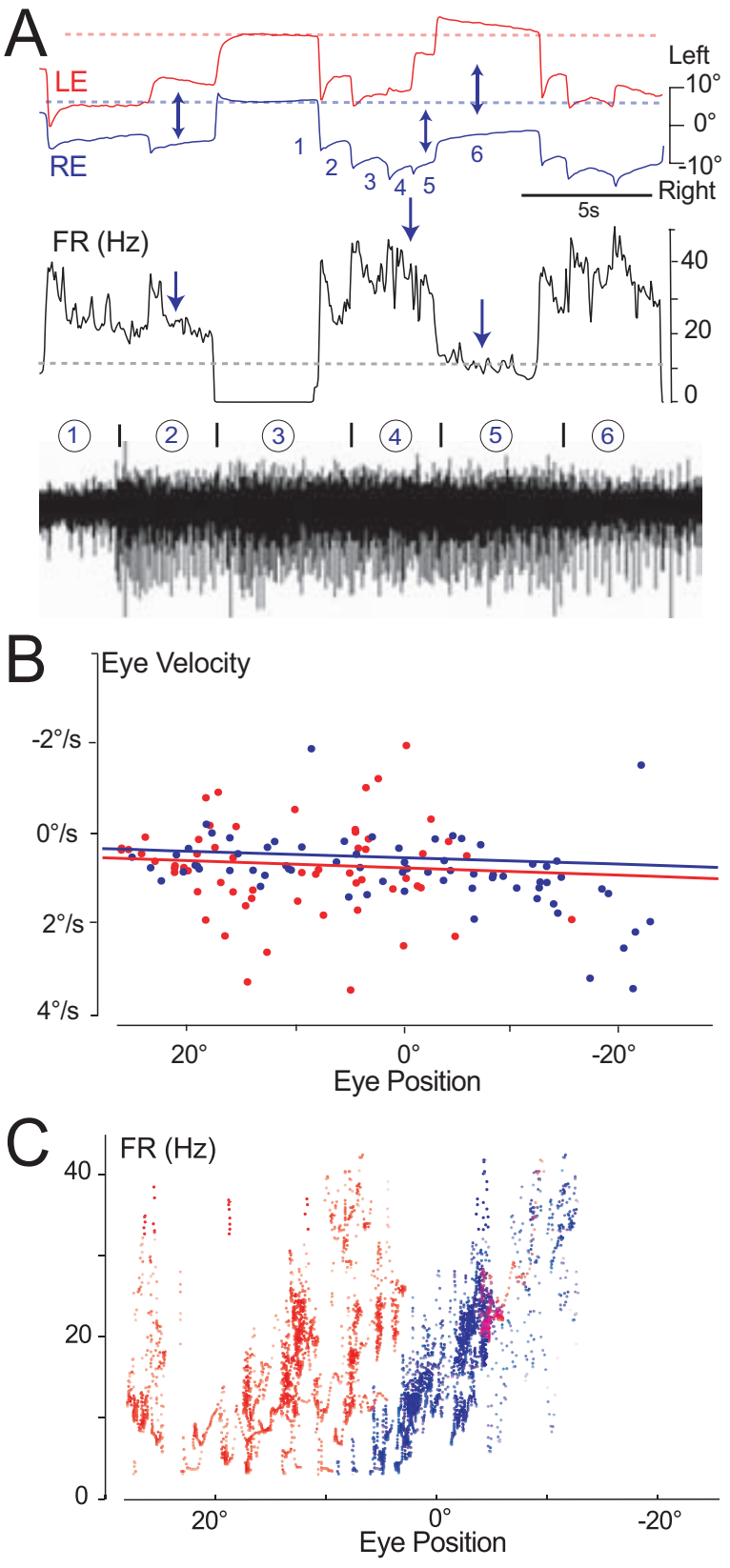
The conjugate neuron in Fig. 3-3A shows a large correlation between the right and left eye positions ($R^2 > 0.92$). No obvious ocular preference could be calculated by regressing the right and left eye positions separately due to the large overlap in eye position vs. firing rate plots (Fig. 3-3A). When analyzed utilizing the method of Zhou and King, a position ratio of 0.21 was obtained supporting a conjugate neuronal classification (Zhou and King 1996, 1998) (Table 3-1).

Conjugate and ipsilateral PNI neurons during scanning

Twenty-nine neurons, with correlation coefficients above 0.7 were recorded and analyzed during spontaneous scanning. Thirty-seven percent of

Figure 3-2: PNI neuron correlated with ipsilateral eye position during spontaneous fixation behavior.

A: Eye position and FR of a right-side (ipsilateral) PNI neuron with monocular eye position highlighted by arrows. Dashed lines show monocularity of LE and RE position. Neuronal activity is illustrated for 6 consecutive fixations (#1-6). B: P-V plot showing a LE τ of 111.5s and RE τ of 124.5s. C: Plot of FR vs. eye position in which the coefficients for the LE and RE were -0.52 (sp/s)/° & -0.41 (sp/s)/°/s (r=0.48) and -1.59 (sp/s)/° & -0.30 (sp/s)/°/s (r=0.83), respectively


A
LE
RE
Left
10°
0°
-10°
Right
5s
1
2
3
4
5
6
FR (Hz)
40
20
0
B
Eye Velocity
-2°/s
0°/s
2°/s
4°/s
20°
0°
-20°
Eye Position
C
FR (Hz)
40
20
0
20°
0°
-20°
Eye Position

Two Variable Regression

Neuron	LE Position sp/s/°	LE Velocity sp/s/°/s	LE r	RE Position sp/s/°	RE Velocity sp/s/°/s	RE r
Ipsi	-0.52 *[-0.55 -0.50]*	-0.41 *[-0.48 -0.34]*	0.48	-1.59 *[-1.62 -1.56]*	-0.30 *[-0.38 -0.23]*	0.83
Conj	1.06 *[1.04 1.08]*	0.21 *[0.097 0.32]*	0.77	1.33 *[1.30 1.36]*	-0.31 [-0.46 -0.16]	0.74
Contra	-0.64 *[-0.62 -0.66]*	-0.41 *[-0.52 -0.30]*	0.73	-0.22 *[-0.24 -0.20]*	-0.61 *[-0.73 -0.49]*	0.31

Four Variable Regression

Neuron	LE Position sp/s/°	LE Velocity sp/s/°/s	P Ratio	RE Position sp/s/°	RE Velocity sp/s/°/s	r	Vel. Ratio
Ipsi	-0.33	0.41	0.65	-1.58	-0.34	0.91	10.7
Conj	0.73	0.25	0.21	0.47	-0.04	0.77	1.38
Contra	-0.74	-0.53	1.62	0.18	-0.28	0.75	0.30

Table 3-1: Analysis of PNI neurons by separate and multiple linear eye regression.

Quantification of ipsilateral (Fig. 2), conjugate (Fig. 3) and contralateral PNI neurons by separate regression (2 variable) and multiple linear regression (4 variable). 99% confidence intervals are in brackets. Positive and negative numbers are leftward and rightward position and/or velocity sensitivity, respectively. r is the correlation coefficient and P is the position ratio.

Eye Preference During Scanning

Cell Type	Position *(sp/s/º)*	Velocity *(sp/s/º/s)*	% Oculomotor Range	Position Ratio
Ipsi (n=11)	1.67 ± *0.51*	0.95 ±*1.24*	63.4 ± *22.6*	0.70 ± *0.44*
Conj (n=17)	1.06 ± *0.44* *(p=0.004)*	0.79 ± *0.72* *(p=0.71)*	74.5 ± *22.4* *(p=0.22)*	0.21 ± *0.63* *(p=0.023)*

Table 3-2: Comparison of ipsilateral and conjugate PNI neuron ranges and sensitivity.

Eye position, eye velocity and the percentage (%) of oculomotor range (+/- SD) were compared during spontaneous fixation behavior. P values were obtained by student t-test.

the neurons (11/29) were ipsilateral-eye sensitive and 59% of the neurons (17/29) were conjugate (Table 3-2). The average eye position sensitivity of the ipsilateral related neurons was 63% greater than the conjugate neurons (p=0.004). However, the average velocity sensitivity during scanning was not significantly different. The thresholds of the conjugate neurons were slightly more nasal, although based on populations means not significantly different (p=0.22). Only a single PNI neuron was found to be correlated with the contralateral eye (Table 3-1). When the conjugate neurons were analyzed by position ratios, they were more likely to be categorized as either ipsilateral or contralateral eye sensitive. Twenty neurons (68%) exhibited a position ratio above 0.3 and were classified as ipsilateral eye sensitive. Four neurons (14%) exhibited a position ratio between 0.3 and -0.3 and were classified as conjugate. Five neurons (17%) exhibited a position ratio below -0.3 and were classified as contralateral eye sensitive.

Monocular optokinetic behavior

Due to the high degree of conjugacy between the eyes during scanning behavior, optokinetic stimuli were utilized to produce more disconjugate eye movements to better distinguish monocularity of PNI neurons. Although optokinetic stimuli most likely directly activated abducens and medial rectus motoneurons without involvement of the eye velocity-to-position neural integrator (Cochran et al. 1984; Uchiyama et al. 1988), PNI firing rates would be modulated through vestibular neuron activity (Allum et al. 1976; Green et al. 1997). Monocular behavior was recorded in 23 experiments and analyzed in 39 experimental conditions in which the pseudo-whole field optokinetic gain was above 0.2 for 0.125 Hz sinusoids with a peak velocity of 15.7°/s. The average gain during whole field optokinetic tracking was 0.47 ± *0.16* for the LE and 0.43 ± *0.16* for the RE. The phase lag was 13.3° ± *5.9* for the LE and 13.3° ± *5.6* for the RE. The slightly larger phase lag and lower gain compared to previous quantification of the OKR in goldfish was likely due to the 30° occlusion of the binocular overlap region for visual stimuli in the present study (Marsh and Baker 1997). To account for inter-experimental differences in optokinetic performance, the tracking gains were normalized to 1.0 for pseudo-whole field OKR at 0.125 Hz with a peak velocity of 15.7°/s.

Differences in tracking gain and phase were observed between the eyes, when the planetariums differed in amplitude (Fig. 3-3B), phase (Fig. 3-3C) or frequency (Fig. 3-3D). Together, these findings indicate that each eye could independently track the visual stimuli. Compared to pseudo-whole field stimulation, a gain decrease was always observed in both eyes when the stimulus differed between the planetariums, indicating the presence of some binocular rivalry in central visual pathways. Differences in planetarium amplitude

Monocular Optokinetic Reflex

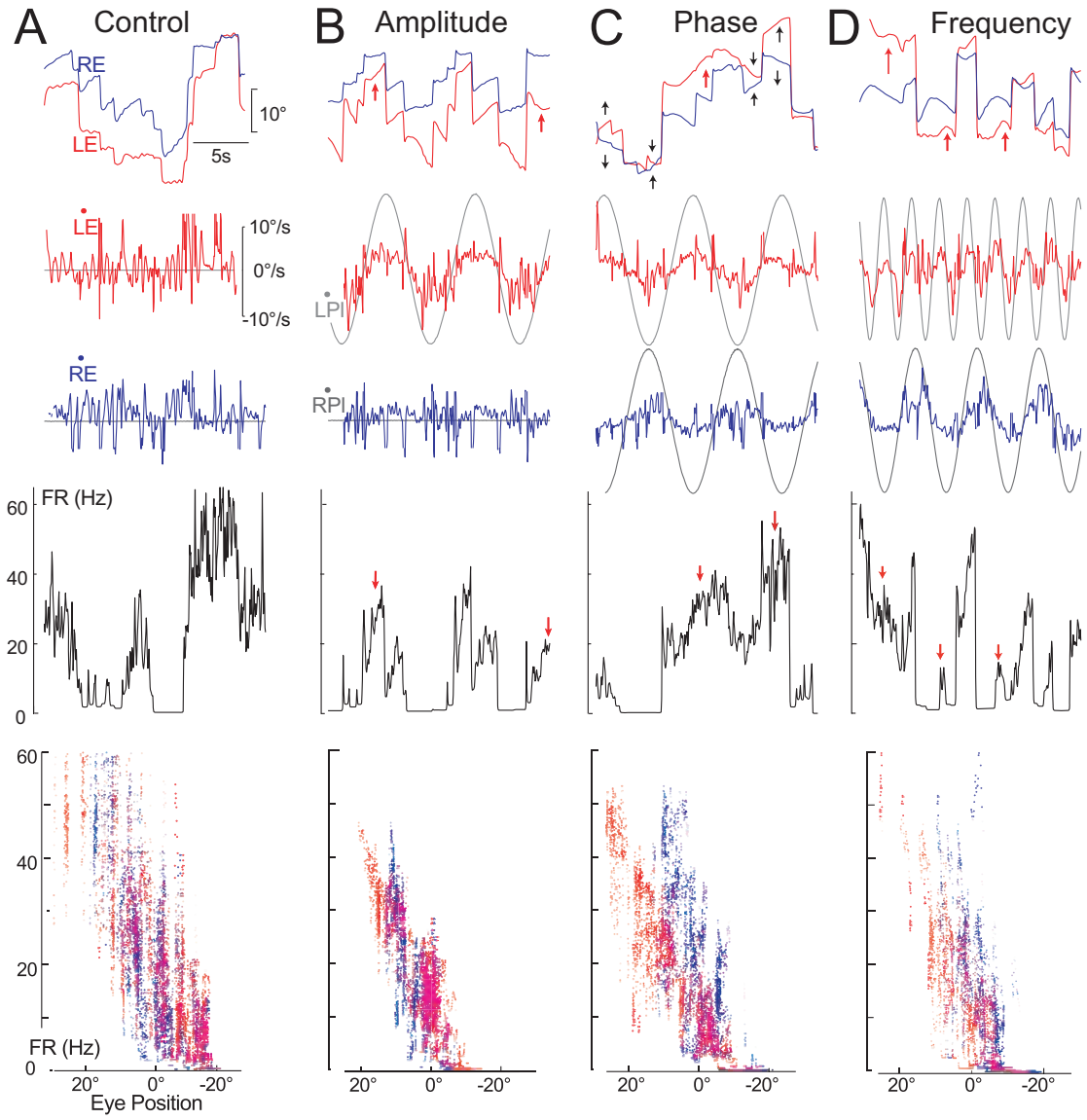
Stimulus	Gain *(Normalized)*	Phase
Amplitude (n=45)	0.66 ± *0.12*	9.5 ± *6.6°*
	0.15 ± *0.07* *(p<0.0001)*	16.6 ± *18.7°* *(p=0.003)*
Occulsion (n=4)	0.67 ± *0.10*	15.3 ± *4.9°*
	0.21 ± *0.13* *(p=0.01)*	28.0 ± *9.7°* *(p=0.11)*
Phase (n=20) (n=12)	0.41 ± *0.12*	109.5 ±*132.6°*
	0.36 ± *0.12* *(p>0.2)*	1.3 ± *12.9°* *(p=0.018)*
(n=8)	0.47 ± *0.20*	4.2 ± *6.5°*
	0.42 ± *0.10* *(p>0.2)*	1.4 ± *7.7°* *(p=0.19)*

Table 3-3: Quantification of monocular optokinetic performance. Normalized gain and phase of monocular OKR was measured during differences in amplitude, phase and eye occlusion (+/- SD). P values were determined by a paired t-test.

resulted in large differences in eye velocity between the stimulated (left) and stationary (right) eye (Fig. 3-3B). Significant differences were not observed in gain when either the left planetarium or the right planetarium was stationary ($p>0.05$) so the gain and phase data were pooled. The results of the monocular optokinetic stimulation experiments are summarized in Table 3-3. The disparity observed in eye velocity was due to independent optokinetic tracking and not

Figure 3-3: PNI activity during monocular OKR behaviors.

A-D: FR of a left-side (ipsilateral) PNI neuron during spontaneous (A) behavior and LE/RE differences in (B) OKR amplitude, (C) phase and (D) frequency. Red and black arrows show regions of monocularity and changes in vergence angle, respectively. A: FR sensitivity was found to be conjugate for the LE 1.06 (sp/s)/° & 0.21 (sp/s)/°/s (r=0.77) and RE 1.33 (sp/s)/° & -0.31 (sp/s)/°/s (r=0.74). B: Monocular planetarium (PI) amplitude differences (LPI, 15.7°/s and RPI, 0°/s) produced monocular LE and RE velocity gains of 0.31 and 0.05 with similar phase leads of 7° and 14°. FR sensitivity was LE 1.13 (sp/s)/° & 1.02 (sp/s)/°/s (r=0.92) and for the RE 1.57 (sp/s)/° & 1.40 (sp/s)/°/s (r=0.72). C: LPI and RPI were of similar amplitude (15.7°/s) but 180° out-of-phase. Gains were similar (0.16), but with a phase lag of 166.2° resulting in oppositely directed velocity sensitivity. LE FR correlation was 1.28 (sp/s)/° & 0.40 (sp/s)/°/s (r=0.92) and RE 1.47 (sp/s)/° & -2.18 (sp/s)/°/s (r=0.75). D: LPI and RPI were of similar amplitude (15.7°/s), but at different frequency (0.4 Hz and 0.18Hz). LE and RE gains were similar (0.20 & 0.23) with FR correlations of LE 1.32 (sp/s)/° & 1.42 (sp/s)/°/s (r=0.88) and RE 1.46 sp/s/° & 0.66 (sp/s)/°/s (r=0.66).


A
Control
B
Amplitude
C
Phase
D
Frequency
RE
LE
10°
5s
LE
10°/s
0°/s
-10°/s
LPI
RE
RPI
FR (Hz)
60
40
20
0
FR (Hz)
20°
0°
-20°
Eye Position

gain suppression in one eye, since contralateral eye occlusion exhibited similar eye velocity and phase differences (n=4).

In the majority of experiments (12/20), goldfish followed phase lagged stimuli moving in equal and opposite directions, with similar eye velocities (Fig. 3-3C), but with large phase differences during both convergence (arrows 2&3) and divergence (arrows 1&4) in either half of the oculomotor range. The eyes also independently followed planetariums rotated at different frequencies (n=3; Fig. 3-3D).

PNI activity during monocular OKR

PNI activity was recorded when the motion of each eye was driven by different optokinetic stimulation. Some PNI neurons that were considered conjugate due to their high collinearity during scanning (Fig. 3-3A), now clearly encoded the ipsilateral eye during monocular OKR due to the decrease in collinearity (Fig. 3-3B-D FR vs. Pos plots). During the experiments using phase lag, neuronal modulation correlated with slow phase movement of the left eye (Fig. 3-3C red arrow). Velocity dependence of the neuronal firing rate was clearly observed, but saccadic sensitivity was not found in the central region of the record, between the 2nd and 3rd black arrows (Fig. 3-3C FR). Monocularity was observed in the eye position versus firing rate plot with the left eye displaying a linear band and the right eye a cloud of points with greater vertical spread. Even in this monocular stimulus paradigm, a large covariation existed between eye positions due to the conjugacy of the saccades. The activity of this PNI neuron during variations in either stimulus amplitude (Fig. 3-3B) or frequency (Fig. 3-3D) was also ipsilateral-sensitive.

Twenty-three position sensitive neurons were recorded during monocular

OKR. Fifty-seven percent (13/23) of the neurons were ipsilateral-sensitive and 39% of the neurons (9/23) were conjugate-sensitive. Surprisingly, only one contralateral-sensitive neuron (4%) was found. When both scanning and stimulus evoked behaviors were recorded (n=16), some neurons that were conjugate during scanning became ipsilateral during monocular OKR. By contrast, neurons that were determined to be ipsilateral during scanning were never found to be conjugate during monocular OKR.

Conjugate vs. ipsilateral PNI neurons during OKR

The average position sensitivity of ipsilateral PNI neurons was larger than conjugate neurons (1.32 ± *0.58* (sp/s)/° vs. 1.02 ± *0.46* (sp/s)/° p=0.188). However, the velocity sensitivity of conjugate neurons was larger than ipsilateral neurons (1.98± *1.42* (sp/s)/°/s vs. 1.11± *0.96* (sp/s)/°/s, p=0.102). In neither case was a significant difference achieved in average sensitivity due to the large range of values within each population of neurons.

When analyzed by position ratios, the ipsilateral neuron average (0.822 ± *0.0246*) significantly differed from that of the conjugate neurons (0.147± *0.825* p=0.01). The nine PNI neurons determined by correlation analysis to be conjugate were then analyzed using position ratios. Three were found to be contralateral-sensitive (ratio < -0.3), four were ipsilateral-sensitive (ratio > 0.3) and two were conjugate-sensitive. Eleven of the thirteen neurons determined to be ipsilateral by correlation analysis had a position ratio above 0.6. The average velocity ratio was not significantly different between conjugate (0.45 ± *0.49*) and ipsilateral (0.70 ± *0.35* p=0.189) neurons.

Neurons were calculated to be either ipsilateral (69%), contralateral (17%), or conjugate (13%) using the multiple linear regression method of King

and Zhou. Of the sixteen neurons that were recorded during both scanning and monocular OKR, nine changed their monocular preference. Three of the neurons that correlated with one eye during scanning were correlated with the opposite eye during monocular optokinetic stimulation. Three of the neurons which were conjugate during spontaneous scanning were classified as monocular during monocular optokinetic evoked behaviors. In addition, three of the neurons that were monocular during spontaneous scanning were determined to be conjugate during optokinetically evoked behaviors. The loss of monocularity and change in eye preference observed when position and velocity ratios were used to determine monocularity raises doubt about the appropriateness of this method to delineate eye sensitivity preference of PNI neurons in goldfish.

Monocularity of time constant modification

In six of eight experiments, the time constant of fixation stability was successfully modified to monocular instability as measured in the absence of visual feedback. Monocular time constant plasticity was determined to have successfully occurred when either: 1) a statistical difference occurred in the time constant of the ipsilateral eye versus control while the contralateral eye was not significantly different from control or 2) when a significant difference occurred in the time constants of both eyes versus their respective controls and between the right versus left eye after instability plasticity (Fig. 3-4A). In five experiments, the time constant changes in the untrained eye (LE) were asymmetrical, as there was a greater drift on the nasal side of the range (right) as compared to the temporal side (left) (Fig. 3-4A, red). This difference was observed in the P-V plot, in which different slopes could be drawn for the left eye (red) nasal and temporal eye positions. The direction of conjugate changes

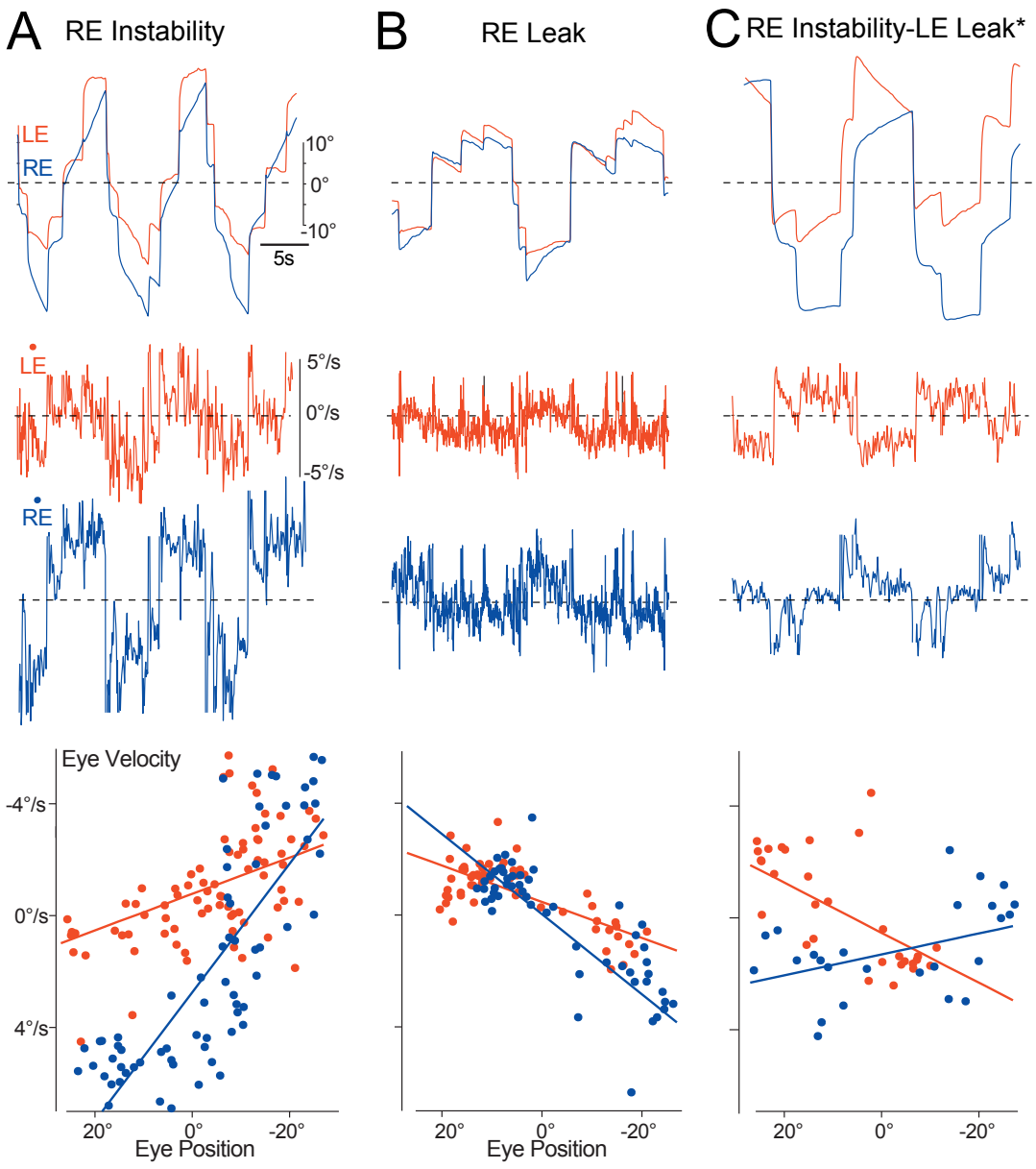
in time constant corresponded to the position and velocity “on” direction of the PNI neurons ipsilateral to the trained eye (Fig. 3-5A). The average changes in time constant were significant between the eyes and are summarized in Table 3-4. The time constant could be retrained to stability ($p \leq 0.01$; n=3) and after retraining no significant difference was observed in left eye versus right eye time constants. The average time constant after plasticity changed from –9.09 ± *5.3s* (trained) and -20.4 ± *12.6*s (untrained) to –35.9 ± 11.8s (trained) and -178.5 ± *123.0*s (untrained). In three experiments, one eye was trained to nasal and temporal instability and the contralateral eye occluded (Fig. 3-6A&D). The training paradigm was verified by the absence of OKR in either eye when the stimulus was only shown to the occluded eye. In two cases, the unoccluded eye trained to instability ($p<0.01$) implying that the changes in time constant were independent for each eye (both temporal and nasal). In addition, maintenance of stability in the untrained eye was not due to visual feedback (Fig. 3-6A&D).

Leak and instability-leak

In two cases, one eye was trained to both temporal and nasal leak and the time constant significantly differed from initial stability ($p<0.01$) without a statistical change in the contralateral eye when spontaneous scanning was monitored in the dark (Fig. 3-4B & 3-6B&E). However in a third experiment, both eyes exhibited a significant reduction in their time constants.

In contrast to monocular instability training, in which the non-trained eye’s drift occurred on the nasal side of the oculomotor range, after leak training, the drift was more likely to occur on the temporal half of the oculomotor range (Fig. 3-4B, Left Eye left). Nevertheless, the direction of visual slip for the asymmetrical changes in time constant was identical for instability and leak as

Figure 3-4: Monocular visual training changes in integrator time constants.
A-C: Behavioral records and P-V plots in darkness after 4-6 hrs of monocular training to instability (A), leak (B), and leak-instability (C). A: RE was trained towards nasal/temporal instability and the LE to stable fixation. Based on oculomotor ranges that were largely nasal for the LE and bidirectional for the RE, τ was -15.3 and -4.4s, respectively (+ and - values indicate leak and instability, respectively). B: RE was trained towards nasal/temporal leak and the LE to stable fixation. Based on oculomotor ranges that were observed to be bidirectional for the RE and temporal for LE (nasal drift), τ was 7.0s and 15.4s, respectively. C: Nasal/temporal training of RE instability and LE leak resulted in a significant change in the time constant for the nasal (left-side) oculomotor range in the RE and a bidirectional change in the LE with τ measurements of -26.7s and 11.2, respectively.


A
RE Instability
B
RE Leak
C
RE Instability-LE Leak*
LE
RE
10°
0°
-10°
5s
LE
RE
5°/s
0°/s
-5°/s
Eye Velocity
-4°/s
0°/s
4°/s
20°
0°
-20°
Eye Position
20°
0°
-20°
Eye Position
20°
0°
-20°

Time Constant Plasticity

Behavior	Control (s)	Training (s)	Memory *(p = LE vs RE)*	pτ
LE Instability	32.2 ± *14.8*	-3.7 ± *1.4*	-7.8 ± *5.5*	0.002
RE Stability (n=8)	31.2 ± *15.2*	30.0 ± *11.1*	-19.0 ± *18.0* *(p=0.005)*	0.03
LE Instability	96.8 ± *63.9*	-4.2 ± *1.9*	-8.1 ± *6.8*	0.02
RE Occlusion (n=3)	110 ± *62.5*	-20.8 ± *24.6*	-20.2 ± *11.2* *(p=0.14)*	>0.2
LE Leak	-77.5 ± *26.2*	1.96 ± *11.9*	7.9 ± *4.0*	0.04
RE Stability (n=3)	24.1 ± *9.6*	50.4 ± *10.4*	15.8 ± *6.8* *(p=0.03)*	>0.2
LE Instability	283 ± *228*	-12.2 ± *12.9*	-19.2 ± *9.7*	0.14
RE Leak (n=3)	-113 ± *85.2*	4.7 ± *10.2*	23.8 ± *13.0* *(p=0.04)*	0.18

Table 3-4: Quantification of monocular integrator time constant plasticity.

Measurements before, during and after monocular instability training of the LE when the RE was either stable or occluded (+/- SD). Leak training (LE) was compared with a stable RE. Column labeled pτ compares the initial and final time constant measurements with a paired t-test. P memory compares the right and left eye time constant during memory.

Figure 3-5: Schematic showing the results of monocular fixation plasticity along with a simplified set of significant neuronal connections.

A-D: Vignettes of eye position drift (e.g., monocular changes in time constant) during learning (L, dashed line) and during memory (M, solid line) of the visual training (at 4 hrs). Color coding associates each fixation with an active eye muscle (LE→MR; RE→LR;LE→LR;RE→MR). The same color coding indicates causally-related neurons in the vestibular nucleus (VN), position neural integrator (PNI) and abducens nuclei (ABD). Eye specific channels are hypothesized to exist in the vestibular nucleus in which one population encodes a monocular signal for Ipsilateral eye movements (RE→LR) and another population encodes conjugate eye movement (LE→MR). Note that excitation (instability) originates from the left vestibular nucleus and inhibition (leak) from the right vestibular nucleus. Based on this convention, vestibular signals control the activity of neurons in the right PNI nucleus that produce either instability or leak in the RE and LE as illustrated in A-D. The right inhibitory PNI internuclear interneuron (black) ensures coordination between the temporal (RE→LR) and nasal (RE→MR) position integrators. Note for simplification, connections are only shown to and from the right PNI with direct pathways from the vestibular neurons (VN) to MR and Abd Mns omitted. A: bidirectional instability for RE and either stability or occlusion for LE. B: bidirectional leak for RE and stability for LE C: RE trained to leak and LE to instability D**:** RE trained to instability and LE to leak. In C-D: the LE triggered the optokinetic stimulus for both eyes. Direction of visual training organized such that leftward drift is in the right column and rightward drift in the left column.

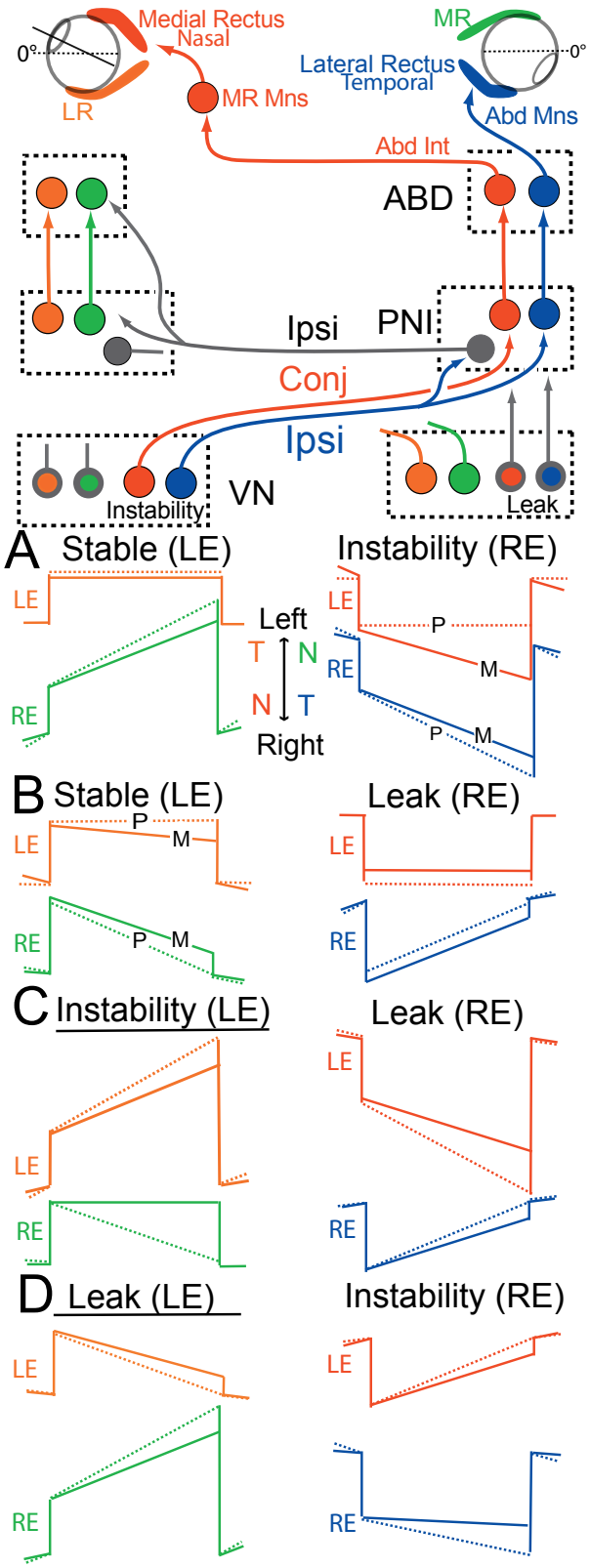
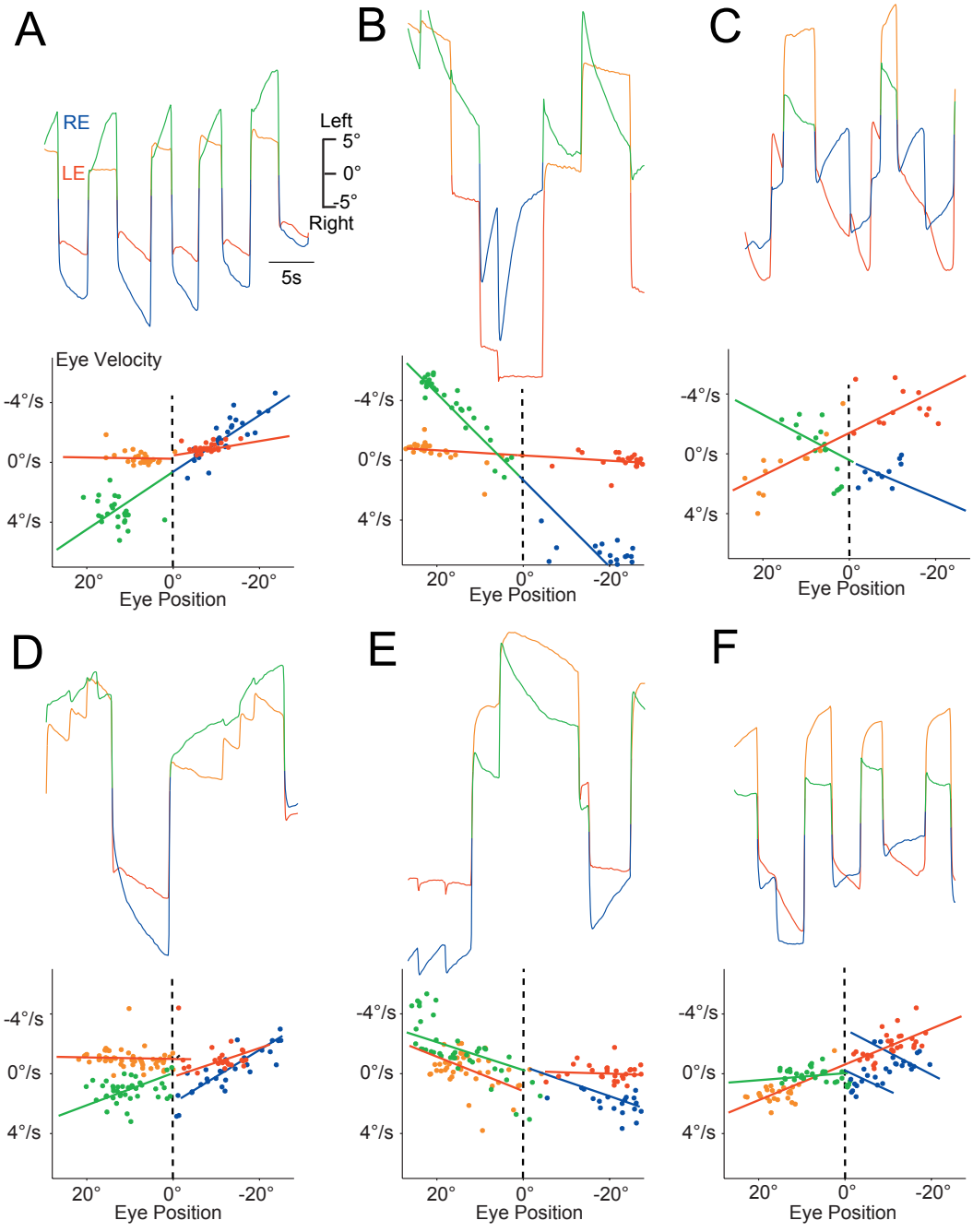

Medial Rectus
Nasal
0°
LR
MR Mns
MR
Lateral Rectus
Temporal
0°
Abd Mns
Abd Int
ABD
Ipsi
PNI
Conj
Ipsi
Instability
VN
Leak
A Stable (LE)
Instability (RE)
LE
RE
Left
T N
N T
Right
P
M
B Stable (LE)
Leak (RE)
C Instability (LE)
Leak (RE)
D Leak (LE)
Instability (RE)

Figure 3-6: Monocular modification of integrator time constants.

A-F: Eye position records and P-V plots during training (A-C) and memory (D-F) of monocular changes in the integrator time constant. A: RE instability performance at 6 hrs (τ -5.2s) with LE occluded (τ 27.0s). D: RE instability memory with τ of -14.5s and LE occlusion τ of 194.4s. Black circle in P-V plot highlights conjugate time constant memory in LE (nasal) and RE (temporal). B: RE leak performance at 4 hrs (τ 3.5s) with LE stability (τ 57.8s). E: RE leak memory with τ of 10.9s and LE stable τ of 107.2s. C: RE leak performance at 3hrs (τ 7.3) with LE instability performance (τ -8.1). F: RE memory leak τ was 43.2s and LE instability τ -8.4s.


A
B
C
RE
LE
Left
5°
0°
-5°
Right
5s
Eye Velocity
-4°/s
0°/s
4°/s
20°
0°
-20°
Eye Position
D
E
F

illustrated schematically in Fig. 3-5.

In a final series of plasticity experiments both the left and right eye time constants were trained in opposite directions. One eye was trained to instability in both the temporal and nasal half of the oculomotor range and the other eye to leak over a similar range (Fig. 3-4C & 3-6C&F). The time constant was modified to a greater degree in the eye triggering the training stimulus in two of the three experiments with some memory observed in the contralateral (right eye) when the drift direction was nasal (leftward). During performance of the learning task both eyes followed their respective stimuli (Fig. 3-6C), yet in all experiments, only one eye exhibited both temporal and nasal memory. As illustrated in Fig. 3-4C, the time constant in the eye triggering the training protocol (LE) significantly differed from control, thus demonstrating monocular leak plasticity (Fig. 3-4C behavior and P-V plot). A similar result was found during the instability training paradigm (Fig. 3-6). Thus monocular time constant plasticity appeared to be dependent on the direction of visual slip, rather than on the direction of time constant change. Nasal to temporal slip was conjugately encoded and temporal to nasal slip monocularly encoded as illustrated in Fig. 3-5. Combined results of the monocular fixation plasticity experiments are consistent with the presence of monocular and conjugate control pathways and is not consistent with the results expected from animals with a complete independence of eye movements such as chameleon or sandlance (Ott 2001; Pettigrew et al. 1999). It is assumed that since behavioral time constants were monocularly modified, parallel changes occurred in the firing rates of PNI neurons. As such, this work then corroborates separate monocularly encoding populations of neurons within PNI.

Discussion

PNI monocularity vs. conjugacy

The general issue addressed in the first part of this study was conjugate versus monocular signal processing in the goldfish eye position neural integrator during versional eye movements. Other behavioral studies have commented on monocular eye movement performance in fish, but none have simultaneously explored monocular learning, memory and neuronal activity (Dieringer et al. 1992; Easter 1972; Fritsches and Marshall 2002; Hermann and Constantine 1971). Seminal works by both Easter and Hermann showed that monocular and convergence-like eye movements occurred spontaneously (Easter 1972, 1971; Hermann and Constantine 1971). The nasally biased post-saccadic eye drift seen during scanning saccades and fixations (Fig. 3-2), might be expected from an 'undercompensating' leaky-temporal and 'overcompensating' unstable-nasal integrator. Since the eyes exhibit opposite drift directions, it raises the possibility that each integrator may be comprised of two independent populations of neurons. One set would control the ipsilateral eye in the temporal half of the oculomotor range (Fig. 3-5 LR) and the other would control the contralateral eye in the nasal half of the oculomotor range (Fig. 3-5 MR). However, since all previously recorded PNI neurons in the goldfish demonstrated a "leaky" persistent activity, without the appearance of unstable firing rates (Aksay et al. 2000; Pastor et al. 1994b), the nasal instability can not be explained by PNI activity. One parsimonious explanation for the eye position drift in naïve goldfish would be to assume the superimposition of a midbrain vergence signal on the abducens internuclear interneurons, or directly on the medial rectus motoneurons independent of PNI.

Previous studies have reported that convergence-like, but not divergence-

like versional eye movements can be optokinetically evoked in goldfish (Easter 1972). However, when alternating rather than constant linear motion was used in our experiments, both converging and diverging eye movements were equally robust in naïve goldfish implying the existence of monocular nasal-to-temporal and temporal-to-nasal optokinetic pathways (Fig. 3-3C). The ability to produce both converging and diverging eye movements with versional optokinetic stimuli were expected since horizontal visual directional sensitive neurons have been described in the fish pretectum that respond to both nasal-temporal and temporal-nasal retinal slip (Klar and Hoffmann 2002). Use of this behavioral paradigm allowed us to assess monocularity of individual PNI neurons and eye position holding time constant by exploring the nasal and temporal halves of the oculomotor range (Fig. 3-5).

Although the visual afferent pathways are monocular, the firing rate properties of PNI neurons makes determination of monocularity challenging. In the goldfish, PNI neuronal mean firing rates at any given eye position have been shown to vary even when hysteresis is accounted for in the eye position sensitivity (Aksay et al. 2003b). Since PNI neurons are irregular in firing rate (Fig. 3-1 & 3-2), the variation within each fixation is often greater than the small differences observed between the left and right eye since in most naïve animals individual fixations are generally stable (Aksay et al. 2003a). Even when small disconjugate eye motions or positions occur, many PNI neurons appear to be encoding a conjugate eye position (Fig. 3-1). Thus, it was not straightforward by statistical analysis to distinguish whether PNI neurons truly encoded conjugate eye position or alternatively reflected collinearization of saccades that tended to bring the eyes into a highly conjugate relationship. This study resolved the

issue of eye-position integrator conjugacy at both the behavioral and neural level by using monocular visual stimulation to bring about disconjugate eye movements in the naturally conjugate goldfish without involving viewing distance dependent vergence responses. These methods are in contrast to the methods employed in mammalian studies which utilized distance mediated vergence to induce monocular eye position and eye velocity changes (King and Zhou 2002; Sylvestre et al. 2003; Zhou and King 1996, 1998).

Monocular eye velocity-to-position integrator persistence

Initial observations of PNI neurons during monocular saccades reported that PNI firing rates tended to mimic ipsilateral eye position, suggesting that some PNI neurons were distinctly monocular (Pastor et al. 1994b). Herein systematically testing for monocularity utilizing disconjugate visual stimulation along with correlation analysis proved that assertion to be true, but somewhat surprisingly, a paucity of distinct contralateral neurons was found. Since over 50% of the PNI neurons were significantly related to the ipsilateral eye, a high degree of monocularity is present in at least one premotor pathway. The few contralateral PNI neurons (~4%) and many more conjugate PNI neurons (~36%) may be partially accounted for by the analysis method since 1) saccades were almost exclusively conjugate in direction and timing and 2) PNI firing rates were independently correlated with the left and right eye position and velocity.

Saccades and the ensuing fixations are generated by burst neurons projecting both directly to motoneurons and PNI neurons with minimal, if any, connections through the vestibular nuclei (Green et al. 1997). Optokinetic tracking is generated by the accessory optic pathway projections to both the motoneurons and largely to the vestibular nuclei. Thus the differences in the

percentage of ipsilateral PNI neurons during scanning saccadic and fixation behavior (Fig. 3-3A) compared to monocular OKR (Fig. 3-3B-D) could be due to a different extent of monocularity in each of the premotor pathways afferent to PNI. Alternatively, the Area I neurons may have encoded a conjugate eye position but monocular eye velocity sensitivity. Due to the lack of significant velocity during fixations, the velocity sensitivity of the PNI neurons would only have been revealed during monocular OKR. For example, PNI neurons with a conjugate eye position and ipsilateral eye velocity sensitivity would have been classified as conjugate during scanning, but classified as ipsilateral during monocular OKR. Although the neurons were classified as three discrete classes: ipsilateral, conjugate, and contralateral, the actual ranges of sensitivity formed a continuum from monocular to conjugate encoding. Given the conservative analytical methods used in this study, the percentage of conjugate neurons versus monocular ipsilateral PNI neurons was, if anything, overestimated in respect to both ipsilateral and contralateral neurons. In spite of this caveat, the somewhat unanticipated conclusion is that distinct populations of PNI neurons are present within PNI, one population is more closely related to the ipsilateral eye and a second population is more closely related to the contralateral eye albeit many of the neurons displayed a conjugate eye position signal.

Contralateral PNI neurons

To test if some of the conjugate PNI neuronal population might actually be contralateral eye sensitive neurons under conditions that better separate position and velocity between the eyes (Fig. 3-7A&B), monocular VOR gain changes were implemented as previously described in goldfish (McElligott and Wilson 2001; Weiser et al. 1989). After 3-4 hours of training, the modified neuronal

Figure 3-7: PNI neuronal firing rate during monocular OKR performance.

A, B: Eye position, eye velocity, FR vs. eye position plots and FR during monocular OKR at 0.125 Hz of a right-side PNI neuron. A: Monocular LPI 15.7°/s and RPI 0°/s stimuli produced normalized OKR velocity gains of LE 0.52 & RE 0.15. B: Monocular LPI 0°/s and RPI 15.7°/s gains produced OKR velocity of LE 0.21 & RE 0.54.

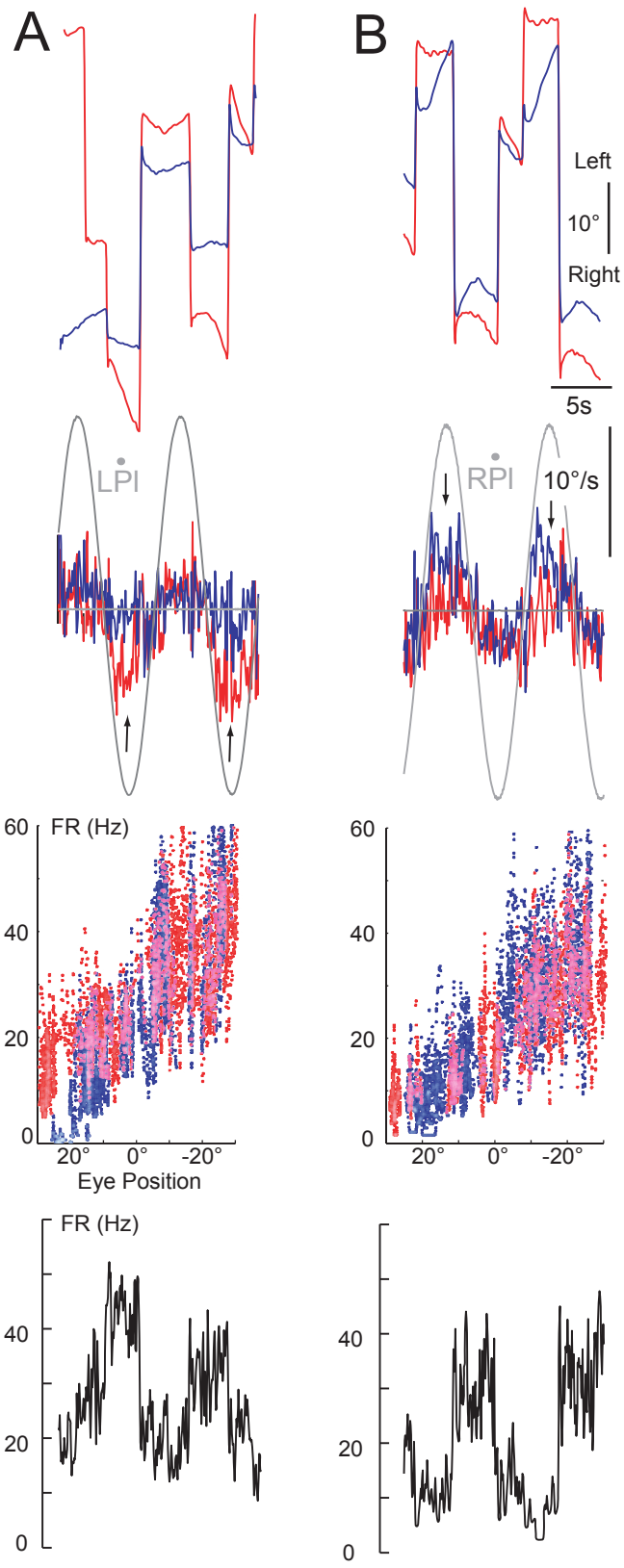
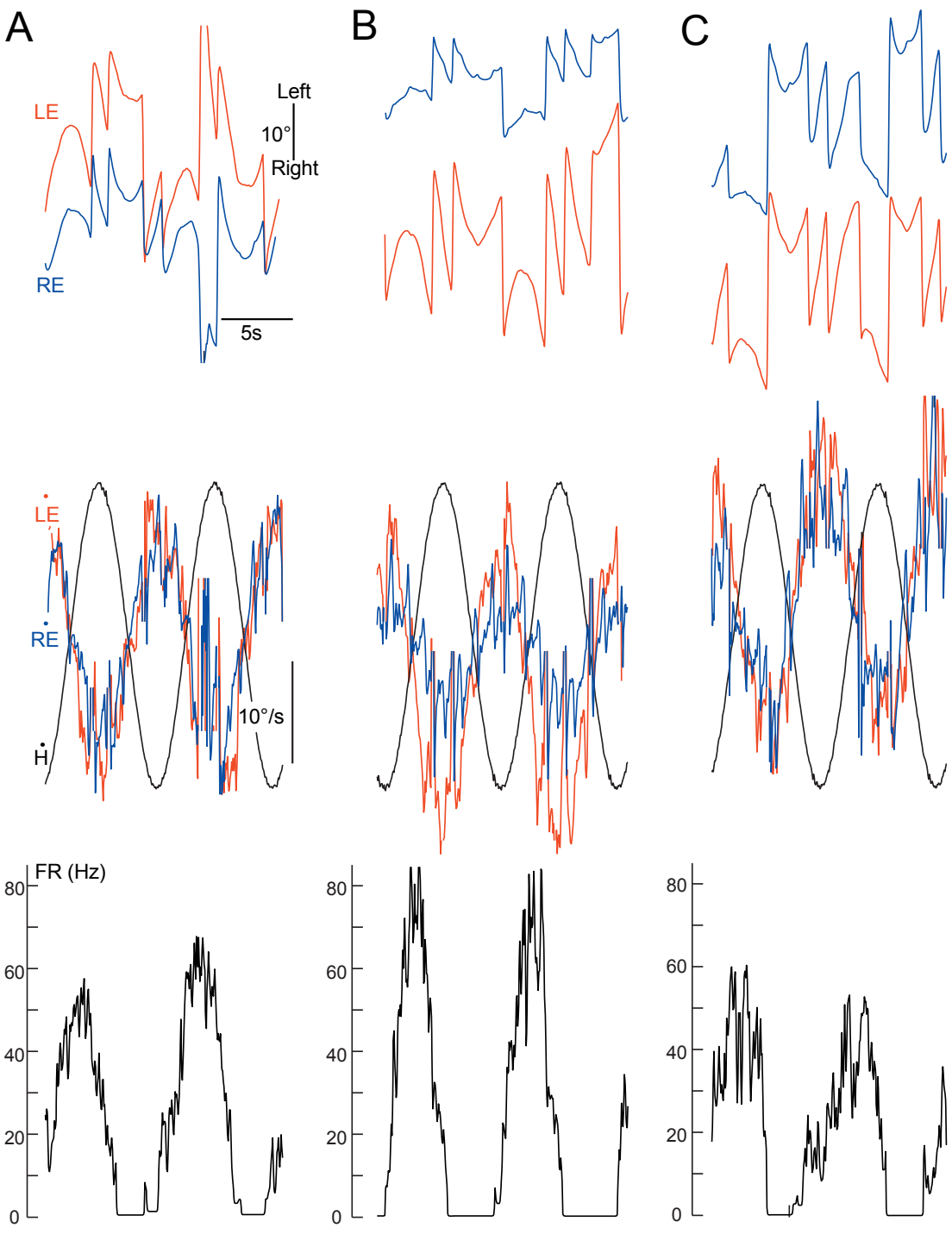

A
B
Left
10°
Right
5s
LPI
RPI
10°/s
60
40
20
0
FR (Hz)
20°
0°
-20°
Eye Position
FR (Hz)
40
20
0

Figure 3-8: PNI neuronal firing rate during VOR plasticity.

Same neuron as Fig. 3-7. A: Eye position, eye velocity and FR before training. B: 4 hrs after monocular training of eye velocity towards LE 2.0 and RE 0.0. C: 2hrs after training reversal of LE 0.0 and RE 2.0. VOR measurements at 0.125 Hz 15.7°/s were in the dark and from a right-side PNI neuron. Control LE and RE velocity gains were in (A) 0.86 & 0.73, (B) 0.94 & 0.33 and (C) 0.8 & 0.55.


A
B
C
LE
RE
Left
10°
Right
5s
LE
RE
H
10°/s
FR (Hz)
80
60
40
20
0

firing rates were significantly correlated to the contralateral and not ipsilateral eye gain. This finding was true for both VOR gain increases and decreases (Fig. 3-8A vs. B&C, respectively) suggesting that some of the conjugate PNI neurons are indeed “latent” contralateral eye neurons in the goldfish.

Comparison of analysis methods with mammals

The analytical methods used to determine monocularity of PNI neurons differed from that of other laboratories, which utilized regression coefficients (Sylvestre et al. 2003; Zhou and King 1996). Preliminary analysis of PNI neurons using multiple linear regression resulted in a greater percentage of ipsilateral (69%) and contralateral (16%) neurons than by correlation coefficient analysis. While such percentages of ipsilateral versus contralateral neurons is in better agreement with the primate literature, percentages in the latter case were gathered during smooth pursuit. In the latter case, the majority of monocular neurons were ipsilateral eye sensitive in contrast to analysis during monocular saccades and fixations, in which the ipsilateral and contralateral populations were found to be equally distributed (Sylvestre et al. 2003; Zhou and King 1996, 1998). In goldfish, the high degree of collinearity in eye position regression analysis during both spontaneous scanning and monocular OKR makes the ipsilateral and contralateral populations computed by the Zhou and King method suspect. These problems were in part minimized in mammals, since the spontaneous firing rates of the neurons were higher. Additionally in goldfish, thresholds for many PNI neurons were around the center of the oculomotor range, thus the oculomotor range over which neuron monocularity could be measured was reduced (Aksay et al. 2000).

Monocularity of time constant modification

The results of the monocular plasticity experiments changing the time constant of eye position holding are highly consistent with the presence of two distinct populations of Area I neurons (Fig. 3-4A-C and Fig. 3-6). When trained to oppositely directed eye position drifts (leak-instability) the monocular time constant measurements suggest that the PNI neurons within each position integrator are separately encoded for each eye (Fig. 3-6C&F). The finding is comparable and consistent with the results from either monocular surgical muscle weakening or retinal disparity training in primates (Lemij and Collewijn 1991a, 1991b; Oohira and Zee 1992; Viirre et al. 1988). This work thereby extends the phenomena of time constant plasticity across several species into a much wider evolutionary time scale.

The visually induced changes in time constant are currently believed to be relayed through the vestibular nuclei to the eye velocity-to-position neural integrator, since second order vestibular neurons in mammals (Barmack 2003; Henn et al. 1974) as well as in goldfish (Allum et al. 1976; Dichgans et al. 1973) have been found to be modulated by both vestibular and visual stimulation. Direct anatomical connections have been claimed between the accessory optic system and the horizontal extraocular motoneurons, medial rectus and abducens, so that during learning, the observed difference in time constant performance could be independent of PNI function (Cochran et al. 1984; Uchiyama et al. 1988). Thus, quantification of monocularity of eye position neural integrator plasticity can only be ascertained in the absence of feedback (i.e., memory).

Limitations of monocular plasticity

Since both the learning and memory phases of monocular instability were

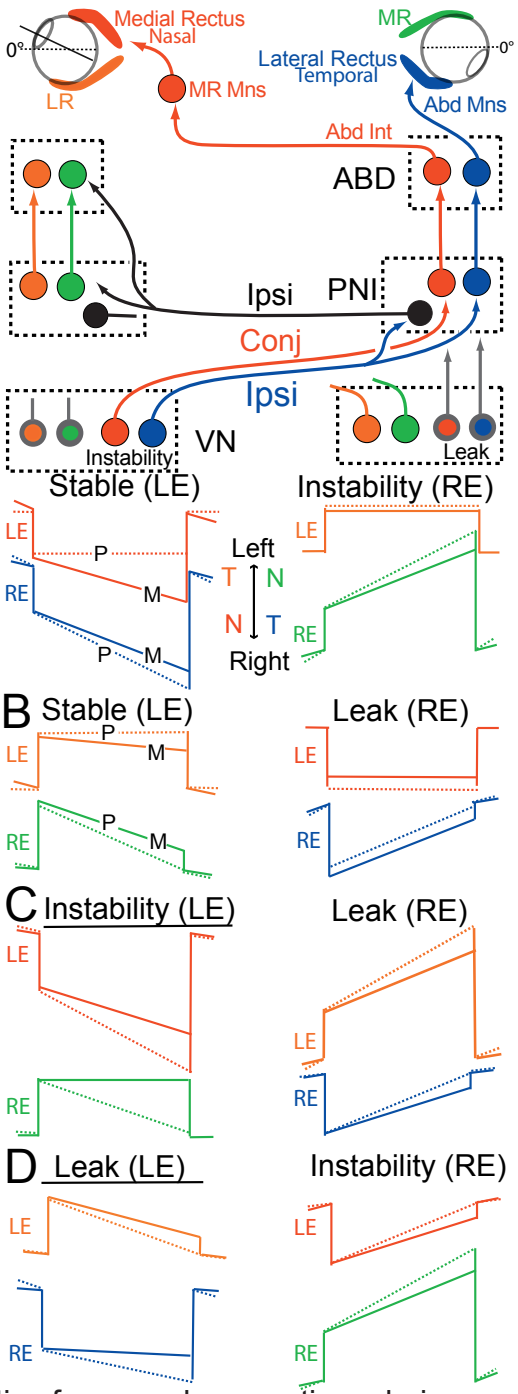


Figure 3-9: Schematic of neuronal connections during monocular time constant modifications. Similar to Figure 3-5; however this vignette is based on the the profile of activity within the left versus right PNI rather than vestibular nuclei.

similar when one eye was either occluded (Fig. 3-6D) or viewed a stationary pattern (Fig. 3-4A), the independent changes in right eye and left eye time constants were not due to an internal binocular suppression of plasticity, but rather the existence of eye-specific monocular visual and motor pathways within the hindbrain. Although clear differences could be observed in the time constants between the two eyes after monocular time constant plasticity, there were limitations to the extent which monocularity could be obtained. In agreement with the presence of conjugate PNI neurons, the time constant was detuned in the "untrained" eye (LE) to a greater extent, as tested during memory, than during training to either instability or leak (Fig. 3-6A & B vs. D & E). During training to oppositely directed time constants for each eye, bidirectional behavior was evident in both eyes, however memory was found to be bidirectional in only one eye (Fig. 3-4C; Fig. 3-6C vs. F). Since no error signal would be present during memory, the absence of monocular time constant modification could be due to constraints within AOS or the hindbrain motor control pathway. In the latter case this would occur via the vestibulo-cerebellar system during acquisition of the modified behavior which then becomes apparent as a conjugate plasticity.

The presence of both monocular and conjugate time constant modification seen during integrator plasticity is consistent with the presence of monocular (Fig. 3-2 and 3-3B-D) and conjugate PNI neurons (Fig. 3-1A and 3-3A). Conjugate time constant modification tended to be stronger in that part of the oculomotor range in which nasal-to-temporal drift was imposed in the "training" eye. Conjugate training was direction dependent rather than orbital position dependent (Fig. 3-4 & Fig. 3-6) suggesting that conjugate modifications occurred "upstream" of PNI. This finding is in agreement with the suggestion

that time constant plasticity includes pathways through the vestibular nucleus as well as the cerebellum (Beck et al. 2000). An alternative, but far less likely explanation, is that conjugate behaviors result from monocular training paradigms accentuating a natural nasal drift observed during goldfish fixation.

Based on the anatomy and physiology of the known vestibular connections to PNI, it can be suggested that instability is predominantly entrained through excitatory vestibular neurons projecting to the contralateral PNI, whereas leak is largely entrained by the ipsilateral inhibitory projections (Fig. 3-5). Conjugate behavior is believed to be mediated by the vestibular commissural neurons (Fig. 3-5).

Role of abducens internuclear interneurons in conjugate eye movements

Based on exclusive connections to medial rectus motoneurons, the role of abducens internuclear interneurons was envisioned as the essential neuronal link to ensure conjugacy between temporal movements of the ipsilateral eye and nasal movements of the contralateral eye. Afferent inputs from the vestibular and saccadic systems were envisioned to be shared equally between the abducens motoneurons and abducens internuclear interneurons (Baker and Highstein 1975; Delgado-Garcia et al. 1986b; Fuchs et al. 1988). This conjugate view was strengthened by the finding that firing rates of abducens internuclear interneurons were similar to those of the abducens motoneurons in primates during vergence-induced disconjugate eye movements (Gamlin et al. 1989). However, cat abducens internuclear interneurons were found to be also contralateral eye motion sensitive as they responded during monocular saccades/fixations of the contralateral eye (Delgado-Garcia et al. 1977; Delgado-Garcia et al. 1986b). In goldfish, abducens internuclear interneurons are a main efferent target of

both excitatory and inhibitory PNI neurons. Thus, any time constant change in nasal eye motion must be reflected and encoded through the abducens internuclear interneurons. If individual premotor neurons projected equally to the abducens motoneurons and internuclear interneurons, the latter would receive an inappropriate signal from a large percentage of monocular ipsilateral PNI neurons. Since time constant monocularity is stronger during nasal instability than during temporal instability, abducens internuclear interneurons should not be viewed as an inflexible part of an 'obligatory conjugacy' mechanism as they must encode monocular time constant changes.

In many mammalian species abducens motoneurons and internuclear interneurons are intimately intermingled suggestive of a structurally-determined conjugacy. (McCrea et al. 1986). By contrast, in goldfish the two nuclei are anatomically separated (Cabrera et al. 1992) making shared afferent inputs between the motoneurons and internuclear interneurons more unlikely. In addition this arrangement provides a good model system to easily test the hypothesis that abducens internuclear interneurons preferentially encode contralateral eye motion through use of monocular versional stimuli.

Function of PNI in oculomotor behaviors

The monocularity results in this thesis imply ipsilateral temporal and contralateral nasal eye movements are synchronous in timing and direction during version due to a common input of excitatory/inhibitory saccadic burst neurons, but downstream eye position holding as initiated by different amplitude of the saccades is separately encoded for the left and right eyes. This evidence suggests an important role for the PNI contralateral-projecting inhibitory internuclear interneurons (PNI Int) (Aksay et al. 2000). If the observation of a

small number of contralateral sensitive PNI neurons is correct, then the role of the PNI contralateral projecting neurons is severely limited. The implications of this evidence will be further explored in the next chapter.

Chapter 4: Role of PNI commissure in persistence and plasticity

Introduction:

As discussed in the introductory chapter (Chapter 1), most network models of eye velocity-to-position integrator function postulate feedback inhibition through contralaterally projecting PNI neurons as vital for production of neuronal persistence (Arnold and Robinson 1997; Cannon et al. 1983; Cova and Galiana 1995). According to these models, an interruption of this connection would cause a large decrease in the time constant of eye position holding. By contrast, midline lesions between the eye velocity-to-position integrator in mammals have yielded conflicting results, ranging from negligible effects (Cheron et al. 1986a; Cheron et al. 1986b) to a drastic reduction in eye time constant stability (Anastasio and Robinson 1991; Arnold and Robinson 1997). The majority of pharmacological studies support commissure mediated integrator stability since unilateral inactivation causes bilateral leak in the eye position time constants. (Arnold et al. 1999; Cheron and Godaux 1987; Mettens et al. 1994b, 1994c). Preliminary studies anecdotally noted that eye position holding was maintained in goldfish when either the midline was severed or PNI unilaterally inactivated (Pastor et al. 1994b).

The presence of monocular neuronal organization within the PNI raises the question as to the role of the contralaterally directed PNI interneurons (PNI Int) (Aksay et al. 2000). If ipsilateral related neurons projected to the contralateral PNI, their target would tend to be modulated with a conjugate firing rate. A consequence of this synaptic arrangement would be that only the conjugate cells could exert meaningful positive feedback to produce persistent neuronal activity

(Fig. 3-5). Alternatively, two independent PNI inhibitory neuron populations could be present in which one population is correlated with the ipsilateral eye and the other population is correlated with the contralateral eye. Due to the small number of PNI neurons in Area I, estimated to be ~ 60 (Pastor et al. 1994b), the presence of two independent populations of PNI Int is considered unlikely *a priori*. A more parsimonious hypothesis is that PNI Ints provide feedforward inhibition that adjusts the null point of the temporal and nasal eye position integrators.

To test if the PNI commissure was required for eye velocity-to-position integration, position holding was measured in the absence of the PNI midline. Although this perturbation does not provide direct evidence for the role of inhibitory crossing PNI neurons, the ability to observe oculomotor behaviors within minutes of the lesion allows inferences about the possible roles of the connection.

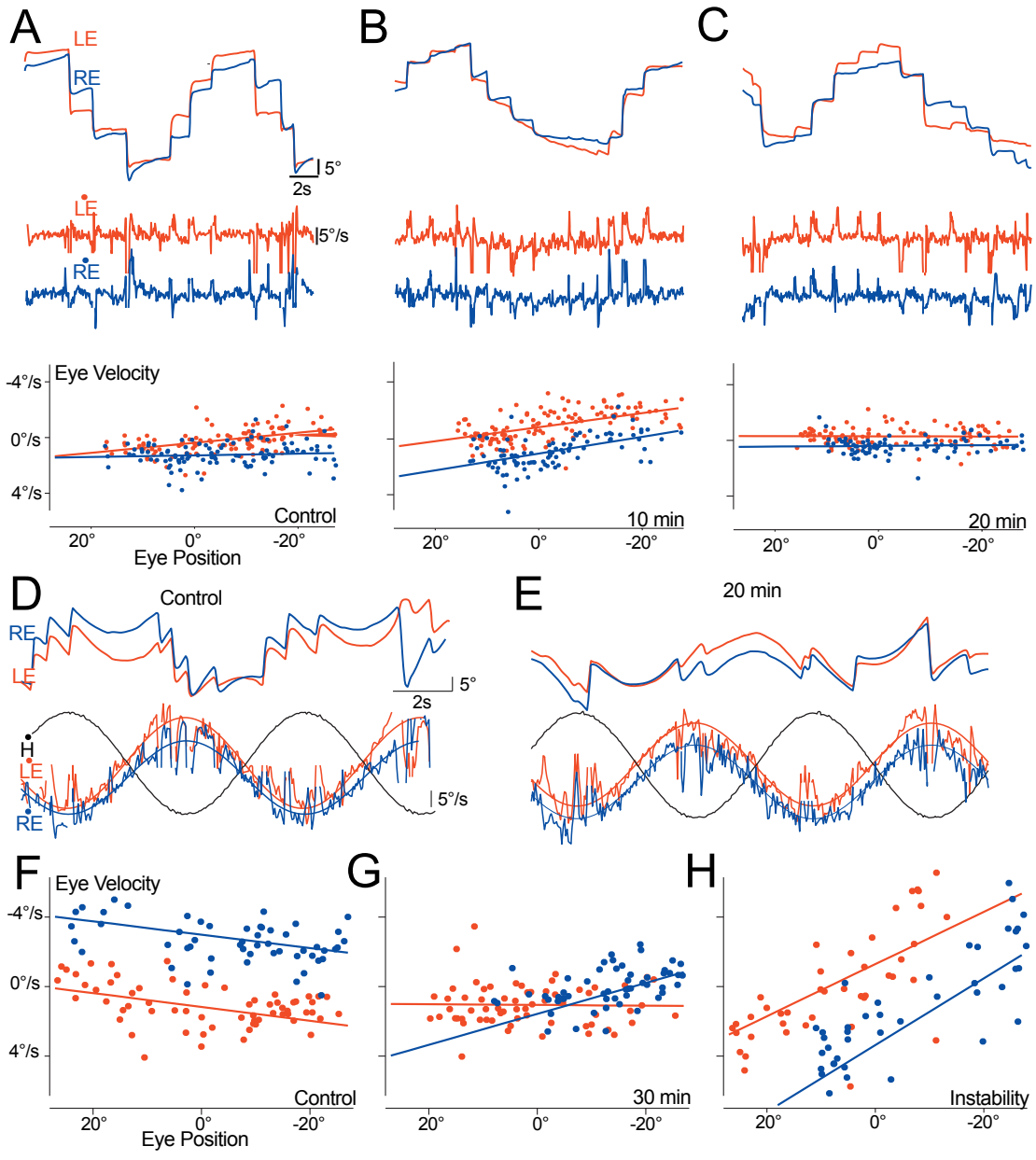
Results:

Role of the contralateral projecting PNI interneurons

Binocular time constant stability and plasticity was observed within twenty minutes after the lesion and when tested up to 3 months after midline lesion. Continued persistence and time constant plasticity indicates that midline connections are not necessary for these PNI functions (Fig. 4-1H). In nine goldfish, after acute midline lesion, the effects ranged from no change in stability (Fig. 4-1C) to a severe compromise in eye position holding thirty minutes after the lesion (Fig. 4-2B). In the majority of cases (7/9), a slight decrease in stability towards leak was observed, with the time constant remaining above 10s, when measured 20-30 minutes after lesioning the midline. Pharmacological inactivation of the integrator demonstrated that the time constant of the oculomotor plant

Figure 4-1: VOR behavior and integrator time constant plasticity after midline lesion.

A-E: P-V plots of eye position holding (A) before, (B) 10 min and (C) 30 min after the midline lesion illustrated in Fig. 2-4A & Cb. Control time constants in A were LE -27.8s and RE -170.3s, in B -19.8s and -16.6s and in C 1428.5s and -384.3s,. D: Vestibuloocular reflex (VOR) at 0.125 Hz and 15.7°/s before and (E) after the lesion with least square regression fits of eye velocity. Head velocity in black. Gains changed minimally from (LE) 0.90 to 0.80 and (RE) 0.73 to 0.71 with negligible shifts in phase from (LE) 3.2 ° to 2.3° and (RE) 0.2° to 3.0°. F-H: P-V plots showing (F) initial stability, (G) 30 min after the midline lesion illustrated in Fig. 2-4Cc and (H) memory in dark after 4hrs of instability training. LE and RE τ was (F) 25.2s & 26.1s, (G) 476s & -11.2s and (H) -6.7s & -5.2s.


A
LE
RE
5°
2s
LE
RE
5°/s
Eye Velocity
-4°/s
0°/s
4°/s
Control
20°
0°
-20°
Eye Position
B
10 min
C
20 min
D
Control
RE
LE
5°
2s
H
LE
RE
5°/s
E
20 min
F
Eye Velocity
-4°/s
0°/s
4°/s
Control
Eye Position
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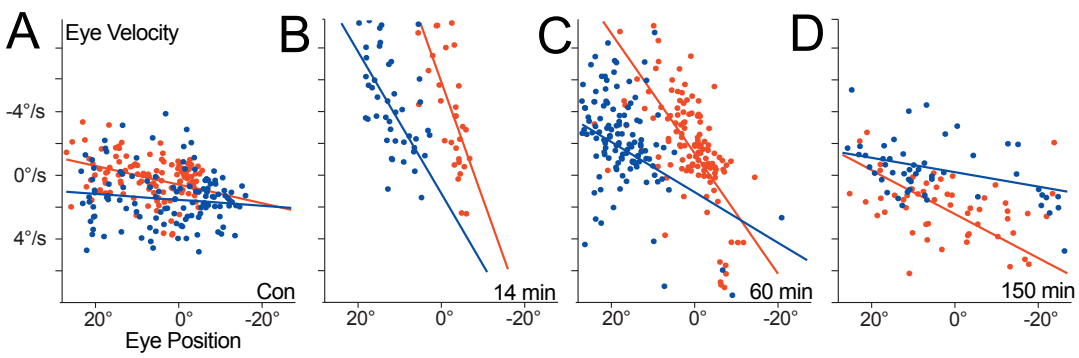


Figure 4-2: Time course of changes in the eye position holding time constant after midline lesion.

A-D: P-V plots (A) before and (B) 15 min, (C)1 hr and (D) 2.5 hrs after midline lesion. LE and RE τ were (A) 17.1s & 53.4s , (B) 4.2s & 5.5s , (C) 2.6s & 6.3s and (D) 12.7s & 16.4s. Description in text.

was less than 1-2s (Aksay 2001; Pastor et al. 1994b). After midline lesion, the time constant of eye position holding remained an order of magnitude above the upper limit of the plant time constant (10s vs. 1s). Since long time constants are assumed, *a priori*, to be a behavioral indicator of persistence in the eye position integrator (Seung 1996), it can be concluded that midline connections contribute minimally, if at all, to fixation stability and neuronal persistence.

Behavioral records (Fig. 4-1A-E) and histology (Fig. 2-5A) are shown following an acute midline lesion completely severing bilateral PNI. The naïve time constant of over 20s (Fig. 4-1A) remained unaltered twenty minutes after the lesion (Fig. 4-1C); however, a slight instability was observed ten minutes after the lesion (Fig. 4-1B vs. A). The oculomotor range shifted nasally and decreased, as observed in a P-V plot of the left eye (red) (Fig. 4-1C). The scanning pattern became more bimodal with small amplitude saccades. Low

frequency VOR, 0.125 Hz 15.7°/s peak velocity, showed a small gain decrease and negligible phase shift of < 3° at 20 minutes after the lesion (Fig. 4-1D&E). The major VOR characteristic affected by the lesion was an altered saccadic beating field, as shown by the inflection points in the eye position records (Fig. 4-1D&E). In a few experiments, larger changes occurred in the time constants that were similar to the effects of eye velocity-to-position integrator inactivation by lidocaine (Fig. 4-2A). However the effects were transient and disappeared over the course of hours much like after lidocaine inactivation. These results indicate that a functionally impaired integrator had the ability to recover in the absence of PNI inhibitory neurons (Fig. 4-2B-D). In six of eight experiments, a significant ($p<0.01$) nasal shift occurred in mean eye position measured in the dark in either one or both eyes after the lesion. The variance of mean eye position demonstrated a significant decrease in one or both eyes (8/9, $p \leq 0.01$), suggesting a decrease in the oculomotor range.

Time constant modification after midline lesion

Time constant plasticity was tested (Fig. 4-1F-H) with training to instability in six cases when time constant stability of greater than 9s was maintained 30 minutes after lesion (Fig. 4-1G). In 5/6 experiments, the eye position triggering the planetarium was successfully trained to instability. The non-triggered eye position time constant was more unstable in only 2/5 experiments ($p \leq 0.01$). The average time constant changed from 16.4 ± *7.5s* to -5.1 ± *41.0*s (n=5, p=0.09 LE) and 13.1 ± *8.6*s to -5.3 ± *24.8*s (n=6, p=0.02 RE). These results indicate that the ability to modify the time constant is not effected by lesion of the PNI commissure, implying that the commissure is not involved in producing the mechanism for instability modification of the time constant.

Long term recovery after midline lesion

Chronic effects after the midline lesion were analyzed in 15 cases in which the lesion was determined adequate by histological criteria. Eye position holding remained an order of magnitude above the oculomotor plant time constant of 1s, two weeks after lesion (Fig. 4-3B,E,F) (Pastor et al. 1994b) with large time constants (>20 seconds) observed up to ninety days (Fig. 4-3C).

Seven fish comprised a longitudinal group that were tested on multiple occasions. In one case, an initially leaky time constant lasting a week after the lesion (Fig. 4-3A), improved over the subsequent weeks towards a more naïve-like stable time constant (Fig. 4-3B $p<0.01$). The histology of this lesion is shown in Fig. 2-5B, in which the entire midline was severed, including that between the inferior olivary nuclei. In other cases, time constants were observed to change towards instability over 2-3 weeks (Fig. 4-3E,F). Bimodal scanning and large eccentric drifts were observed in the eye position records, which also showed rhythmic oscillations (Fig. 4-3E) suspected to be due to the severing of the olivary connections (Marsh 1998). These results indicate that the PNI commissure is not involved in the long-term maintenance of stable time constants.

Long term effects of midline lesion on time constant modification

Using binocular stimulation, integrator time constants could be successfully modified to either instability or leak during the first week as well as anytime examined thereafter (Fig. 4-4). Average stability during the first week after lesion was found to be 22.9 ± *16.0*s (LE n=8); 40.2 ± *28.4*s (RE n=9). Plasticity towards instability (n=4) altered time constants to an average of -4.6±*34*s; -5.5±*30.1* (Fig. 4-4B). When time constants were modified towards leak by visual feedback, the average time constants were 13.9±*16.1*s (n=3) and

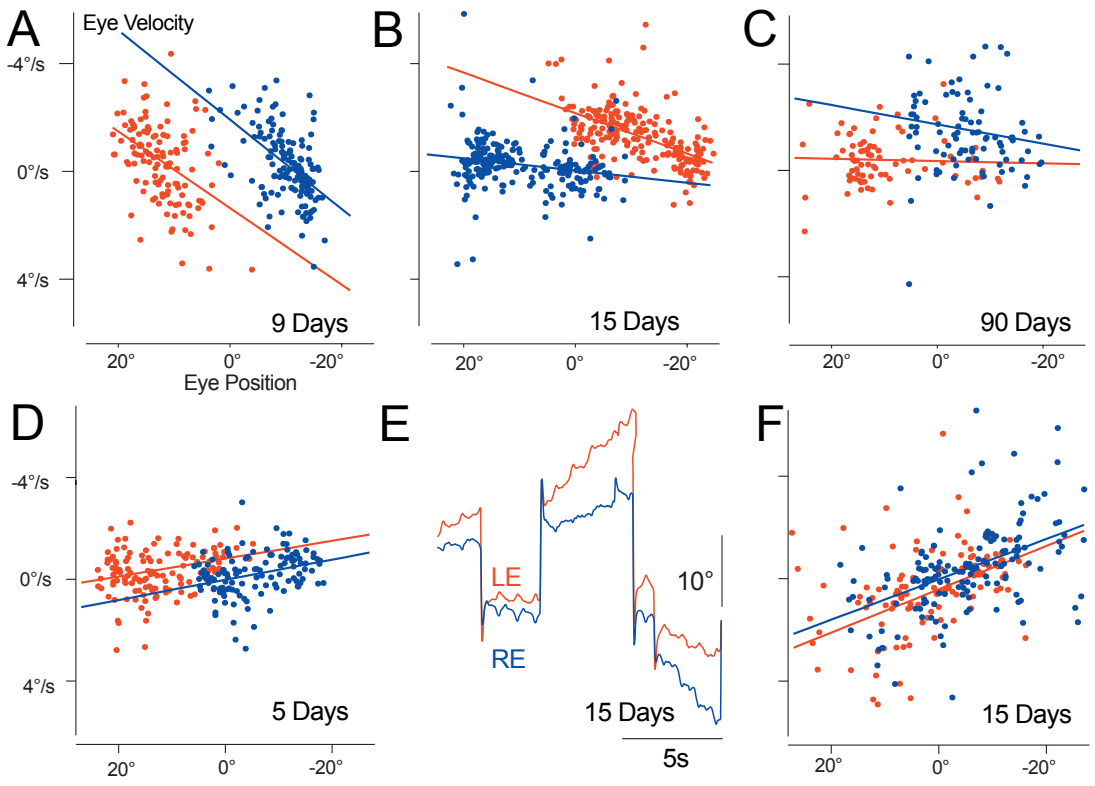


Figure 4-3: Long term maintenance of integrator time constant.

A: P-V plots at 9 days and (B) 15 days after midline lesion depicted in Fig. 2-4B. LE and RE τ was (A) 7.0s & 6.0s and (B) 13.3s & 44.1s. C: P-V plot at 90 days with LE and RE τ of 232.2s & 27.6s. D: P-V plots at 5 days and (F) 15 days post lesion with eye position shown in E. LE and RE τ were (D) 28.6s & 25.2s and (F) -11.8s & -12.7s. Eye velocity oscillations are shown in E and midline lesion in Figs. 2-4Ca

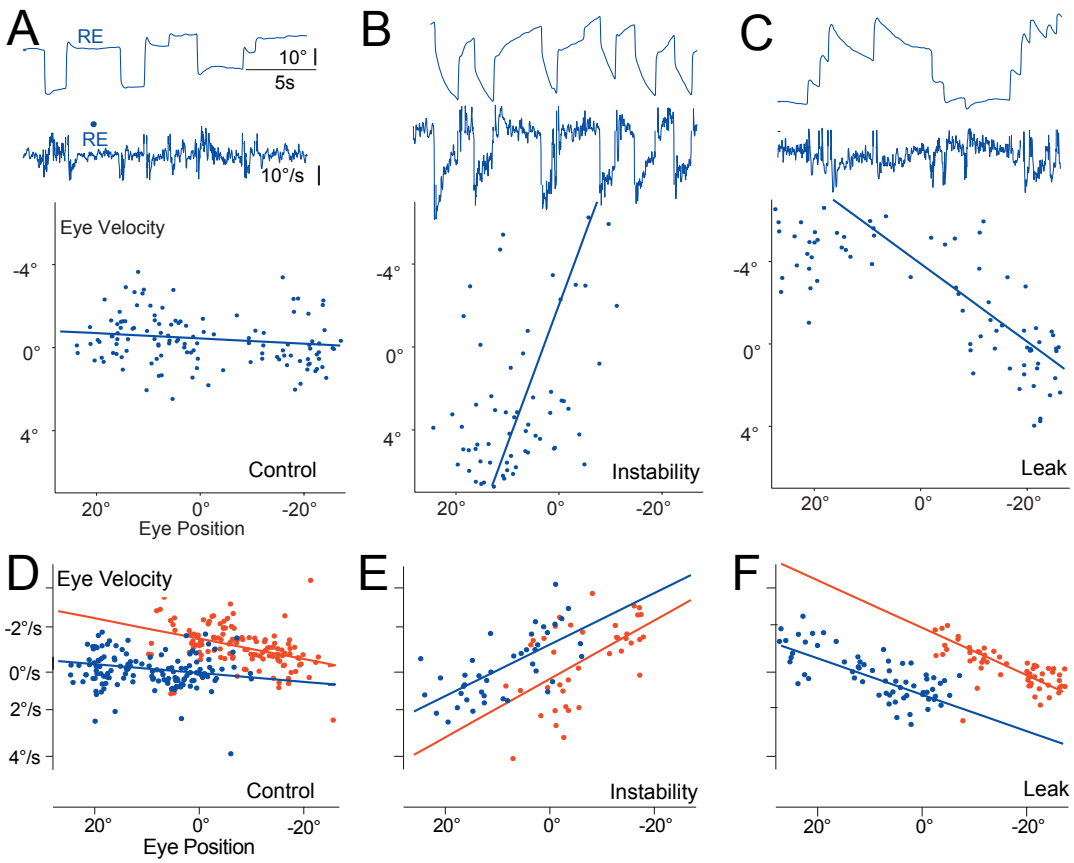


Figure 4-4: Bidirectional time constant plasticity.

A-C: Behavior and P-V plots of (A) RE in darkness, (B) 4hrs after instability training and (C) 2 hrs after leak training 5 days after a midline lesion as illustrated in Fig. 2-4Cc. RE τ was (A) 79.4s, (B) -1.5s and (C) 5.3s. D-F: P-V plots of time constant plasticity 15 days after complete midline bisection measured in (D) darkness, (E) following 4hrs of instability training and (F) 2 hrs of leak training. LE and RE τ was (D) 13.3s (LE) and 44.1s (RE), (E) -7.2s & -8.2s and (F) 8.9s & 11.6s.

7.4±*3.4s* (n=5; Fig. 4-4C).

In five fish the average time constants two to three weeks after lesion were LE 95.2 ± *81.5*s and RE 434.8 ± *411.5*s. Four animals were trained towards instability, and a robust performance was observed in all cases (Fig. 4-4E). In three experiments the time constant during memory was significant ($p \leq 0.01$) when compared to initial stability (Fig. 4-4D). The average time constant during memory was -5.4± *43.4*s for the left eye and -4.1 ± *22.6*s for the right eye. When subsequently re-trained towards leak (n=3), the average time constants were modified from -4.2 ± *8.1*s to 14.2 ± *42.7* s (LE p=0.018) and -3.3 ± *7.4*s to 13.4 ± *21.5*s (RE p=0.6; Fig. 4-4F). Although the eye position record controlling the planetarium motion tended to have a greater change in the time constant than the eye position record of the contralateral eye, the eye position time constants between the left and right eye were never significantly different from each other when tested in darkness. The ability to change the fixation time constant in the nasal and temporal oculomotor ranges for both eyes implies that midline inhibitory pathways are not necessary for time constant plasticity.

When time constant stability was analyzed regardless of recovery time, in cases when the initial eye position time constant post-lesion was less than 7s, time constant plasticity could not be observed (n=3). This suggests that a minimal functional level of integrator performance is necessary for time constant plasticity.

Since in many oculomotor plasticities such as VOR gain, the mechanism responsible for increases are postulated to be different than the mechanisms responsible for decreases, the reversibility of the time constant modifications was tested (n=5). In all cases, the time constant could be reversibly modified,

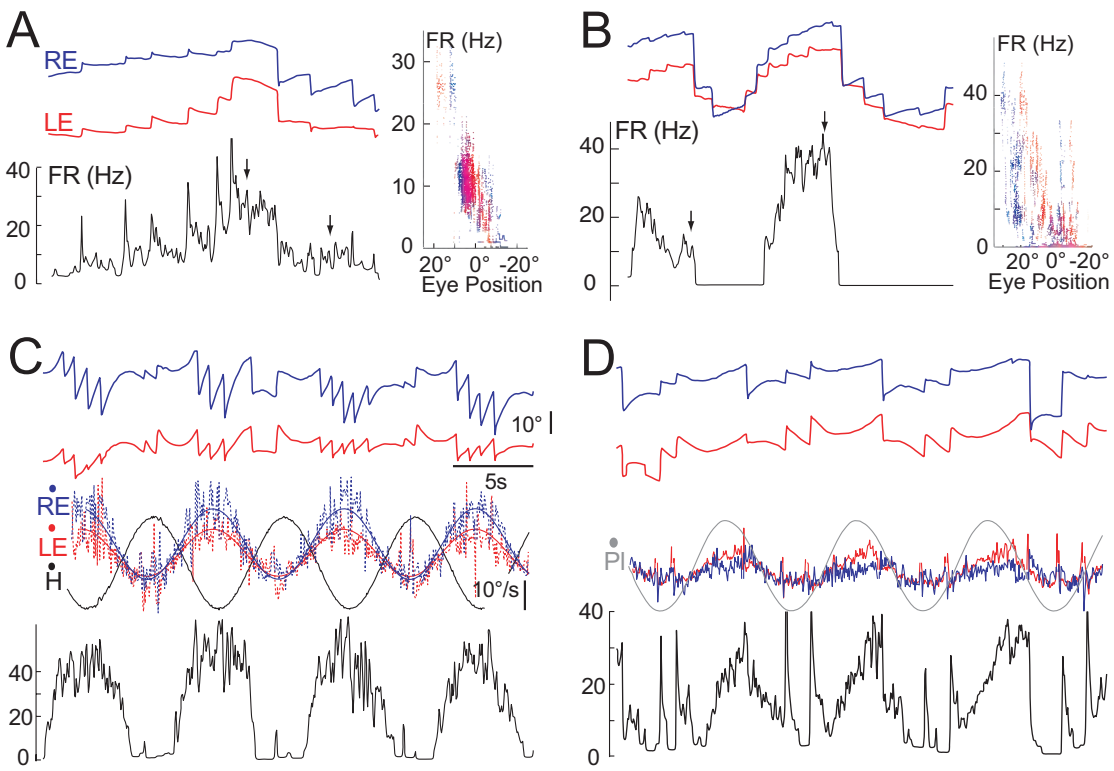


Figure 4-5: Persistence of PNI neuronal activity without commissure.

Eye position records, FR and FR vs. eye position plots during spontaneous fixation in darkness for two ipsilateral-eye related PNI neurons (A & B). A: LE FR coefficients were 0.84 (sp/s)/° & 0.23 (sp/s)/°/s (r=0.81). RE FR constants were 0.72 (sp/s)/° & 0.33 sp/s/°/s (r=0.58). B: LE FR 1.45 (sp/s)/° & 0.63 (sp/s)/°/s (r=0.76) and RE FR 1.18 (sp/s)/° & 0.17 (sp/s)/°/s (r=0.49). C-D: Modulation of neuron shown in A by eye/head velocity (0.125 Hz) during (C) VOR and (D) OKR.

and in 40% of the cases, the time constant after training to the opposite time constant stability was significantly different than the initial control time constant ($p \leq 0.01$).

PNI neuronal activity after midline lesion

The presence of continued stability at all times after midline lesion implied but did not prove, that PNI was functional. PNI neurons were recorded after midline lesion (n=9 fish) to determine if their firing rates remained correlated with the behavioral effects observed after midline lesion. If so, then they could be inferred to be the cause of the changes in behavioral time constants. Five neurons exhibited correlation coefficients above 0.7, and were concluded to be position sensitive. PNI neuronal activity could be observed after midline lesion in the absence of visual feedback as shown in Fig. 4-5. The left-sided PNI neurons in Fig. 4-5A&B demonstrated multi-stable persistent activity and in the absence of visual feedback their firing rates were clearly correlated with the ipsilateral eye. Activity of the neuron shown in Fig. 4-5A was modulated during VOR (B) and OKR (C), but the firing rate appeared to be dominated by eye/head velocity. The velocity dominance of the firing rates was largely due to the limited eye position range available to produce the compensatory response. Combined with similar results from other PNI neurons, it can be concluded that contralateral projecting PNI neuron are not necessary for either the generation of long integrator time constants and thus persistent activity or modulation of PNI firing rates occurring during all other oculomotor behaviors.

Discussion:

A limitation of lesion experiments is that it provides information of the response of the system in the absence of a neuronal structure, and does not

provide direct evidence of the role of the ablated neurons. Thus the results of the commissurectomy are at best suggestive of the role of the PNI commissure. If the PNI Int connections provided positive feedback, then post-lesion there would be less sustained neural activity in PNI and the eye position holding time constant would be compromised. Insignificant changes in the time constant within twenty minutes after the lesion (Fig. 4-1C &G), strongly suggests that the PNI Int connections are unnecessary for eye position integration and thus neuronal persistence. Due to the short time of observation and the absence of visual feedback continued eye position stability could not be due to short-term time constant plasticity. The phase and gain of the low frequency VOR were also unaffected suggesting, indirectly, that PNI Int are not essential for the mechanism of eye velocity-to-position integration (Fig. 4-1E). Alternatively eye position holding could be encoded by a single lateral integrator, but this would require eye position to be limited to one half of the oculomotor range (either nasal or temporal). The alternative explanation is unlikely since although the orbital eye position were more restricted after PNI commissurectomy, the eyes continued to scan in both the nasal and temporal halves of the oculomotor range (Fig. 4-1E).

In some experiments, there were significant decrements in the eye position time constant immediately after lesion (Fig. 4-2). The decrease in time constant could have been due to either the removal of the Area I internuclear interneurons or could have been due to mechanical effects of the lesion. Within a few hours, particularly in the presence of visual feedback (Fig. 4-2D), nasal and temporal time constants of both eyes improved, suggesting that eye velocity-to-position integration and plasticity mechanisms were intact. These observations serve

to re-enforce the conclusion that PNI internuclear interneurons, including their connections to the contralateral PNI and abducens nuclei, are not necessary for either integrator time constant stability or plasticity.

Occasionally time constant instability was observed after PNI Int Ins lesion (Fig. 4-1B) providing additional evidence against PNI feedback inhibition producing persistence. An increase in the firing rate, and thus behavioral instability, would be expected if a simple feedforward inhibition were interrupted, due to the release of the contralateral neurons from inhibition. The ability to produce instability plasticity was clearly evident after PNI Int lesion in both the temporal-to-nasal and nasal-to-temporal directions, (Fig. 4-1H) demonstrating that PNI Int inhibitory connections are not likely essential for retuning eye position holding (Fig. 4-1H).

Role of PNI Ints in null position and oculomotor range

Based upon their putative inhibitory connections and the effects of unilateral inactivation of Area I on PNI neuronal firing rates (Aksay 2001), commissure interruption was expected to increase activity of PNI neurons at low firing rates and thus affect the mean eye position and oculomotor range. Since ipsilateral excitatory Area I neurons project to the abducens motoneurons and internuclear interneurons, eye position could potentially shift in either direction, temporally or nasally, depending on the major targets of Area I Ints within the contralateral Area I and abducens nuclei. If Area I Ints provided positive feedback to all neurons, then random directional shifts would have been more likely as both abducens and medial rectus motoneurons would have decreased their firing rates. The observed nasal shift in eye position is much more consistent with interruption of a feedforward inhibition of Area I neurons that projects to conjugate Area

I neurons and then onto the contralateral abducens internuclear interneurons (Fig. 3-1D and Fig. 3-5). Release of inhibition would increase activity in MR Mns to shift eye position nasally. In addition, greater MR tonic rates would increase the opposing antagonistic muscle force to be overcome by the lateral rectus. Such a medial bias might then prevent the eye position and/or central pattern generator neurons from reaching comparable temporal extremes, thereby decreasing the oculomotor range. If this hypothesized Area I Int connectivity is correct, then abducens internuclear interneuron firing rates should exhibit an entirely conjugate and/or contralateral eye sensitivity in goldfish.

Corroborating evidence for a role in null position coordination is provided by correlation studies of Area I firing rates. Bilateral pairs had a negative correlation suggesting inhibition; however, for ipsilateral pairs the firing rate correlations were highest around the center (±5º) of the oculomotor range, corresponding to the region in which the crossing fibers should be most effective (Aksay et al. 2003a). In a feedforward model, there should be little activity in the contralateral Area I when the eyes are in the extreme temporal part of the orbit (>10º), thus extinguishing the Area I Int common inhibitory input. This decrease should be correlated with decreasing the synchrony of neurons located ipsilateral to each other. In a feedback model, the opposite circumstance would be expected as synchrony should increase at more temporal eye positions, due to an increase in a common net excitation. Theoretical studies have shown that two neurons receiving random excitatory input have an increased cross-correlation when inhibited by a common neuron (Lytton and Sejnowski 1991). Inhibition is believed to enhance synchrony through hyperpolarization thereby restricting the firing times and phase locking the neurons. The effect of common input on synchrony

has been tested *in vitro*, where it was shown that both excitatory and inhibitory common input can induce synchrony. (Turker and Powers 2001)

An additional role of the Area I Int neurons may be to facilitate ipsilateral eye movements. Since it is argued that Area I Ints project to the contralateral Abd Int. Ins (Fig. 3-5), they should augment ipsilateral temporal eye motion by inhibiting the contralateral abducens internuclear interneurons either directly, or indirectly This dual level of inhibition would ensure the absence of ipsilateral MR motoneuron activity, thereby allowing, possibly more rapid temporal eye movements. More importantly, such Area I Int activity might help prevent oscillatory eye velocity drifts due to differences in firing rates of Area I neurons in the ipsilateral and contralateral nuclei.

Midline Area I Int axons, in addition, appear to have a role in shaping the multistable saccadic pattern seen during spontaneous conjugate scanning eye movements. Frequently after a lesion, the saccadic pattern shifted to many (~5-10) small (1-3°) saccades in the extremes of the oculomotor range (Fig. 4-1) and larger saccades appeared to become more bimodal (Fig. 4-1). Both the crossed Area I Ints and ipsilateral excitatory Area I neurons project more anterior to the rostral abducens subnucleus and terminate in reticular nuclei where the saccadic central pattern generator is presumed to be located (Henn and Cohen 1976). PNI Ints would provide a negative eye position signal feedback to the saccadic generator neurons (Aksay et al. 2000) and obviously the presumed imbalance with the ipsilateral Area I neurons might lead to positive feedback, hence the high frequency repetitive saccades. However, the saccadic generator appears to have the intrinsic ability to compensate, as both the bimodality of large saccades and smaller eccentric saccades were minimized over time.

In fact they are absent in nearly all longer term conditions spanning weeks to months.

Long term stability (persistence) and time constant plasticity

Lesions of the PNI Int pathway centrally did not interfere with long term maintenance of either eye position holding or plasticity. Time constants varied widely between animals but when measured longitudinally in individual cases, time constants showed both decreases and increases (Fig. 4-3). Longitudinal changes in mean eye positions and oculomotor ranges were not evaluated as eye coils were not chronically implanted to link essential calibrations between separate recording sessions.

Time constants were modifiable in either direction, i.e., instability or leak, suggesting that Area I Ints are not required for plasticity, and by inference, the day-to-day tuning of the integrator towards a prolonged stable eye position. Time constant plasticity was not dependent on post-lesion recovery time; however, while performance of the training paradigm was intact, little memory was observed when the initial time constant was <7s. Perhaps this implies that a minimal level of eye position 'integration' is required for the expression of the learning component of integrator plasticity (Fig. 4-4).

Interruption of PNI Ints in mammals

The effects of bisecting the PNI Int are not similar in all species tested. In primates, a major diminution of the time constant was reported. (Anastasio and Robinson 1991; Arnold and Robinson 1997). However, similar to this study, bisection of PNI caused a shift in eye position but as only one eye was monitored the result cannot be interpreted in the context of nasal versus temporal eye movements (Anastasio and Robinson 1991). In the primate experiments (n=3)

time constants were already reduced to 30-50% of expected control values prior to the hindbrain lesion due to utilization of the monkeys in previous experiments. Yet recovery was noted in all cases (n=3) and in one instance >50 %, when tested at later dates (Anastasio and Robinson 1991; Arnold and Robinson 1997). The most significant observation in the primate studies was an improvement and maintenance of eye position holding, implying that “persistence”, i.e. velocity-to-position integration, and eye velocity-to-position plasticity mechanisms were intact. While these results were viewed as due to incomplete lesions, an alternative explanation is that the initial time constant decrement was trauma-related. When allowed to recover (τ 3-4s), the eye position time constants was an order of magnitude above that of the oculomotor plant. Similar experiments in cats first measured eye position time constants on days 4-7, thereby avoiding considerations of any immediate trauma effects (Cheron et al. 1986b).

Inferior olivary role in eye position time constant, PNI persistence, and plasticity

In addition to removing the Area I Int crossing fibers, many of the deeper lesions bisected the ventral decussation of inferior olivary (IO) neurons (Fig. 2-5B). This would abolish all climbing fiber activity in Purkinje cells (Demer et al. 1985; Sugihara et al. 1999). But it did not affect the spontaneous saccadic pattern along with stable eye position holding (Fig. 4-3A&B). However, a distinctive oscillation in instantaneous eye velocity was observed during scanning (Fig. 4-3E) (Marsh 1998). This variation was initially termed “an eye instability” but eye velocity oscillation is a better term since instability is likely due to eccentric drift of eye position and thus reported as a negative time constant measurement (Marsh 1998). Since eye position holding is maintained in the absence of IO,

cerebellar complex spike activity is not necessary for either eye position stability or for time constant modification. This result extends findings showing that VOR and OKR gain changes as well as period tuning were unaffected by inferior olive lesions (Marsh 1998).

Abolishing complex spike activity has significant implications for the role of the cerebellum in motor plasticity. Here the presence of plasticity without olivary pathways suggests that the motor learning is predominantly controlled by simple spike activity and not by complex spike activity (see Chapter 5). Although these findings do not preclude a role of cerebellar long term depression (LTD) in integrator plasticity, they do exclude conjunctive LTD due to interaction of the complex spike with the simple spike activity as currently hypothesized (Ito 2001, 2000; Winkelman and Frens 2006).

PNI neuronal response after PNI Int lesion

Continuation of both stable eye position holding and plasticity after midline lesion, although highly suggestive of 'appropriate' Area I neuronal activity, did not provide direct evidence that PNI neurons exhibited eye position sensitivity and/or 'persistence' well correlated with the measured time constants. When Area Ineurons were recorded after the lesion, ipsilateral eye position-related neurons were still present. These results suggest that the behaviors previously discussed are due to Area I activity, not a reflection of new compensatory neuronal pathways or mechanisms. As expected, neuronal firing rates exhibited a high correlation with eye position and showed persistent activity in both the presence of, and more importantly, the absence of, visual feedback. Continuation of persistence in the absence of visual feedback was an important distinction. Area I activity might have been compromised by the midline lesion, but continued to exhibit

well correlated eye position related activity in light due to affferent input from second order vestibular neurons. On the other hand, when vestibular function was tested, Area I neurons were also modulated demonstrating intact vestibular nuclei connections after lesion. These observations are in contrast to mammalian lesion experiments testing integrator function, which in addition to severing Area I internuclear interneurons compromised commissural vestibular fibers. Continued modulation shown during optokinetic stimulation in the representative neuron of Fig. 4-5 demonstrates that the signals necessary, but not sufficient, for eye position holding plasticity are present after Area I Int axotomy.

Chapter 5: Role of the vestibulocerebellum in eye position holding and time constant plasticity

Introduction

Classical views suggests an intimate relationship between the eye velocity-to-position integrator and the cerebellum. In mammals, neurons within the nucleus prepositus hypoglossi have been shown anatomically and electrophysiologically to project to the floccular lobe of the cerebellum (Escudero et al. 1996; McCrea and Baker 1985). Physiological recordings of floccular Purkinje cells signals described a static eye position sensitivity supporting connectivity with the horizontal eye velocity-to-position neural integrator (Noda and Suzuki 1979). Although it is still proposed that prepositus hypoglossi neurons receive a direct afferent input from the flocculus (McCrea and Horn 2005), no direct correlated inhibition has been demonstrated by electrophysiology (Escudero et al. 1996; Lisberger et al. 1994b).

Regardless of whether the flocculus directly projects to the prepositus, let alone PNI neurons, the above anatomical and electrophysiological evidence led to the hypothesis that the cerebellum is vital for the generation and maintenance of neuronal persistence underlying eye position signals within the prepositus hypoglossi (Fukushima 2003). Strengthening this claim, acute inactivation of the flocculus, or the entire cerebellum, reduced the time constant of eye position holding to 1-2 seconds in mammals (Eckmiller and Westheimer 1983; Godaux and Vanderkelen 1984; Optican and Robinson 1980; Robinson 1974; Westheimer and Blair 1974). Separate electrophysiological evidence supported an interactive role between the flocculus and the horizontal eye

velocity-to-position neural integrator as changes in eye position sensitivity of floccular Purkinje cells paralleled changes in VOR gain (Blazquez et al. 2003). Theoretical models of integrator function have also proposed the cerebellum to act as a parallel feedback loop in order to lengthen the eye position holding time constant (Glasauer 2003).

Relationship of PNI and the cerebellum in goldfish

Anatomical studies of the vestibulolateral lobe of the goldfish cerebellum and the horizontal eye velocity-to-position neural integrator are in contrast to that in mammals because no direct afferent or efferent connection exist between these two structures (Fig. 1-8) (Aksay et al. 2000; Straka et al. 2006). The lack of a direct cerebellar-integrator interaction suggests that the cerebellum either indirectly, or perhaps not at all, lengthens the eye position time constant. A minimal cerebellar role is suggested by analysis of goldfish Purkinje cell firing rates which lack an eye position sensitivity (Pastor et al. 1997). It also has been observed that cerebellar removal does not affect the saccadic pattern, or eye position holding, of spontaneous fixations in goldfish (Marsh 1998).

Role of the cerebellum in time constant modification

In both mammals and goldfish, the vestibulo-cerebellum appears to play a major role in the acquisition and retention of other oculomotor-related plasticity as well as suppression of post saccadic drift (McElligott et al. 1998; Michnovicz and Bennett 1987; Nagao and Kitazawa 2003; Optican and Robinson 1980; Pastor et al. 1994a; Rambold et al. 2002). VOR gain changes and post saccadic drift suppression are both alterations of eye velocity, and the same neuronal pathways would be envisioned to be involved in both behaviors. The role of the cerebellum in gain adaptation has been recently reviewed and the work

concluded that both a brainstem and cerebellar site of memory exists (Broussard and Kassardjian 2004). Although complete cerebellar removal in adult monkeys reduced the time constant of fixation holding to 1-2 seconds, sequential hemi-cerebellectomy separated by two weeks in juvenile monkeys only reduced gaze holding in the extremes of the oculomotor range (Westheimer and Blair 1974). Since suppression of post saccadic drift is similar, but not equivalent, to the paradigm used to modify the time constant of the eye position neural integrator (Major et al. 2004a), the cerebellum may be important for eye position time constant changes induced by short term visual feedback training. By contrast, the cerebellum may not be necessary for the normally observed eye position time constant or eye velocity plasticity seen during OKR and VOR.

Cerebellar contribution to VOR eye velocity plasticity

A non-cerebellar hindbrain VOR gain adaptation pathway has been hypothesized since after acute cerebellar removal in goldfish, there was a significant retention of VOR gain changes (Pastor et al. 1994a). However, the acquisition of gain changes in chronic cerebellectomized animals was substantially reduced (~30%) compared to that observed in cerebellar intact animals which could exhibit a two hundred percent change in VOR gain (Pastor et al. 1992). Thus, VOR gain adaptation is largely cerebellar dependent but a cerebellar-independent 'adaptable' hindbrain pathway also exists.

This thesis tried to further delineate the role of the goldfish vestibulo-cerebellum in both time constant stability and plasticity by performing both reversible and irreversible cerebellar inactivations after modifying the time constant either to leak or to instability with visual drift training. The role of the cerebellum in maintaining the new level of fixation stability was explored

by measuring the time constant of eye position holding utilizing P-V plots immediately after cerebellar removal. The experimentally measured time constants were then compared to those in naïve goldfish (Fig. 5-2). These measurements were contrasted with those of the oculomotor plant and after Area I inactivation. Retention of changes in fixation time constant as well as subsequent modification was tested acutely (Fig. 5-4 & 5-5) and three months after cerebellar removal (Fig. 5-9) to determine the integrity and effectiveness of the hindbrain pathways.

Results

Fixation stability and time constant modification after cerebellectomy

The vestibulo-cerebellum (Fig. 5-1A&B) was removed by aspiration either prior to or after modifying the time constant for four hours (n=12). After cerebellectomy, a velocity bias or nystagmus was frequently observed when the animal was in darkness; however, the eye velocity drift was nearly always suppressed by visual feedback. The velocity bias varied, but ranged between 1-10°/s.

The eye position time constant was analyzed in nine goldfish. As shown in Fig. 5-2, a reduction was observed in fixation stability compared to naïve goldfish, both 5 minutes (Fig. 5-2B) and 30 minutes (Fig. 5-2C) after complete cerebellum removal (Fig. 5-1A). However, the time constant although reduced after naïve removal was still an order of magnitude greater than the time constant of the oculomotor plant (10 vs. 1s). A time constant of over 10 seconds implies that the mechanisms underlying horizontal eye velocity-to-position integration were still functional. The average eye position holding time constant after cerebellar removal regardless of training direction is summarized in Table 5-1. The average

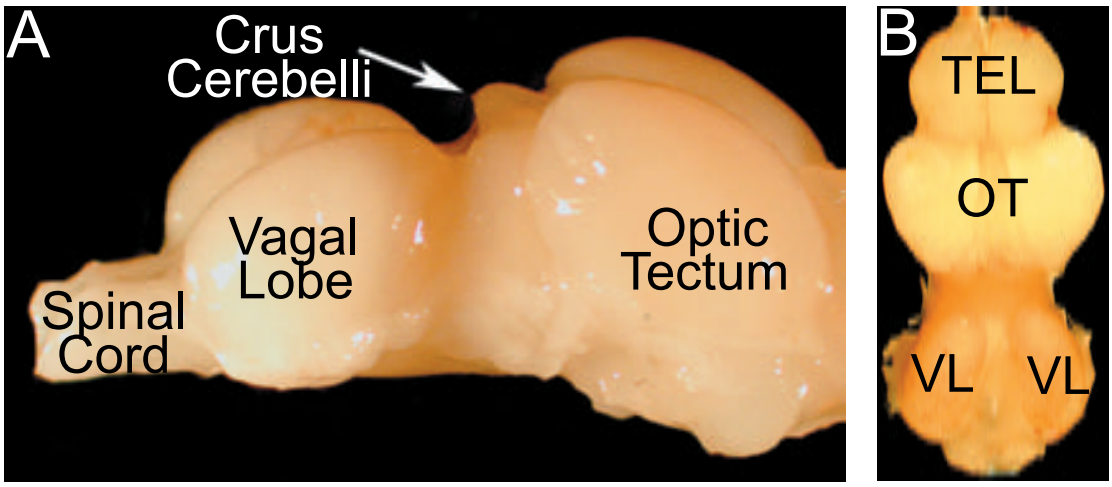
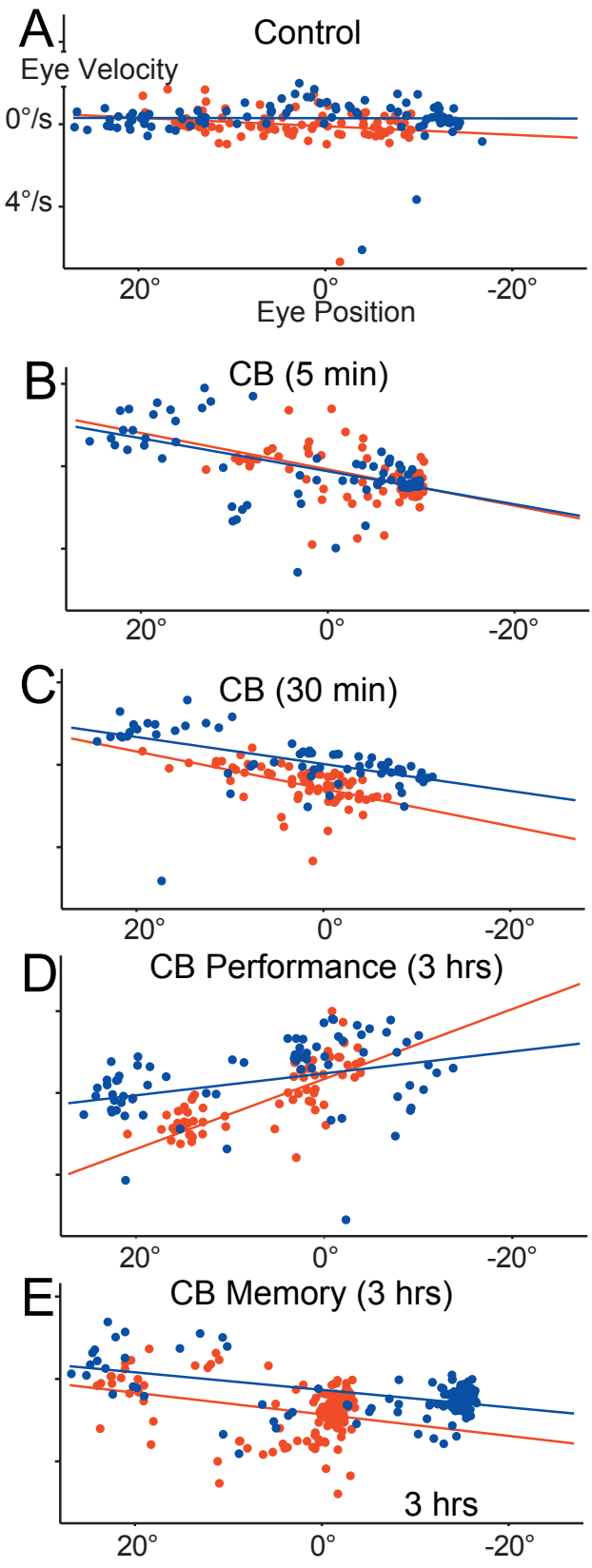


Figure 5-1: Hindbrain morphology after cerebellar ablation.

(A) Lateral and (B) dorsal views of the brainstem after two separate complete cerebellar ablations (excluding the valvula cerebelli). Behavioral records in these two cases are shown in Figures 5-2 and 5-4, respectively. Tel: Telencephalon OT:Optic tectum VL: Vagal lobe.

Figure 5-2: Eye fixation time constant after acute cerebellar (CB) removal. A-C: P-V plots of fixation time constant (A) prior to (control), (B) 5 min and (C) 30 min after a cerebellar removal as illustrated in Fig. 5-1A. LE and RE τ was (A) 48.5s and 1821.6s, (B) 16.5s & 13.0s and (C) 11.0s & 15.3s. D-E P-V plots after (D) 3rd hour of instability performance and (E) memory in darkness. LE and RE τ was (D) -5.6s & -18.1s , (E) 19.1s & 23.5s.


A
Control
Eye Velocity
0°/s
4°/s
20°
0°
-20°
Eye Position
B
CB (5 min)
20°
0°
-20°
C
CB (30 min)
20°
0°
-20°
D
CB Performance (3 hrs)
20°
0°
-20°
E
CB Memory (3 hrs)
3 hrs
20°
0°
-20°

Eye Position Time Constants after CB

Control *(SD)*	CB (5-10 Min)	CB (15-30 min)
-81.1s ± *38.4* n=16	11.3s ± *15.8* n=11 *p≤0.0001*	32.3s ± *21.1* n=14 *p=0.006*

Table 5-1: Eye position time constants after cerebellar removal.

Eye position time constant 5-10 minutes and 15-30 minutes after cerebellar aspiration. All statistics were computed by paired t-tests.

time constant 15-30 minutes after cerebellar removal was significantly leakier than in naïve animals (p=0.006 paired t-test), but over ten times longer than the 1-2 seconds reported in mammals following comparable ablations. In contrast to the maintenance of eye position time constant stability, the ability to modify the time constant after cerebellar removal was severely affected (Fig. 5-2E), however performance of the learning paradigms after cerebellar ablation was maintained (Fig. 5-2D).

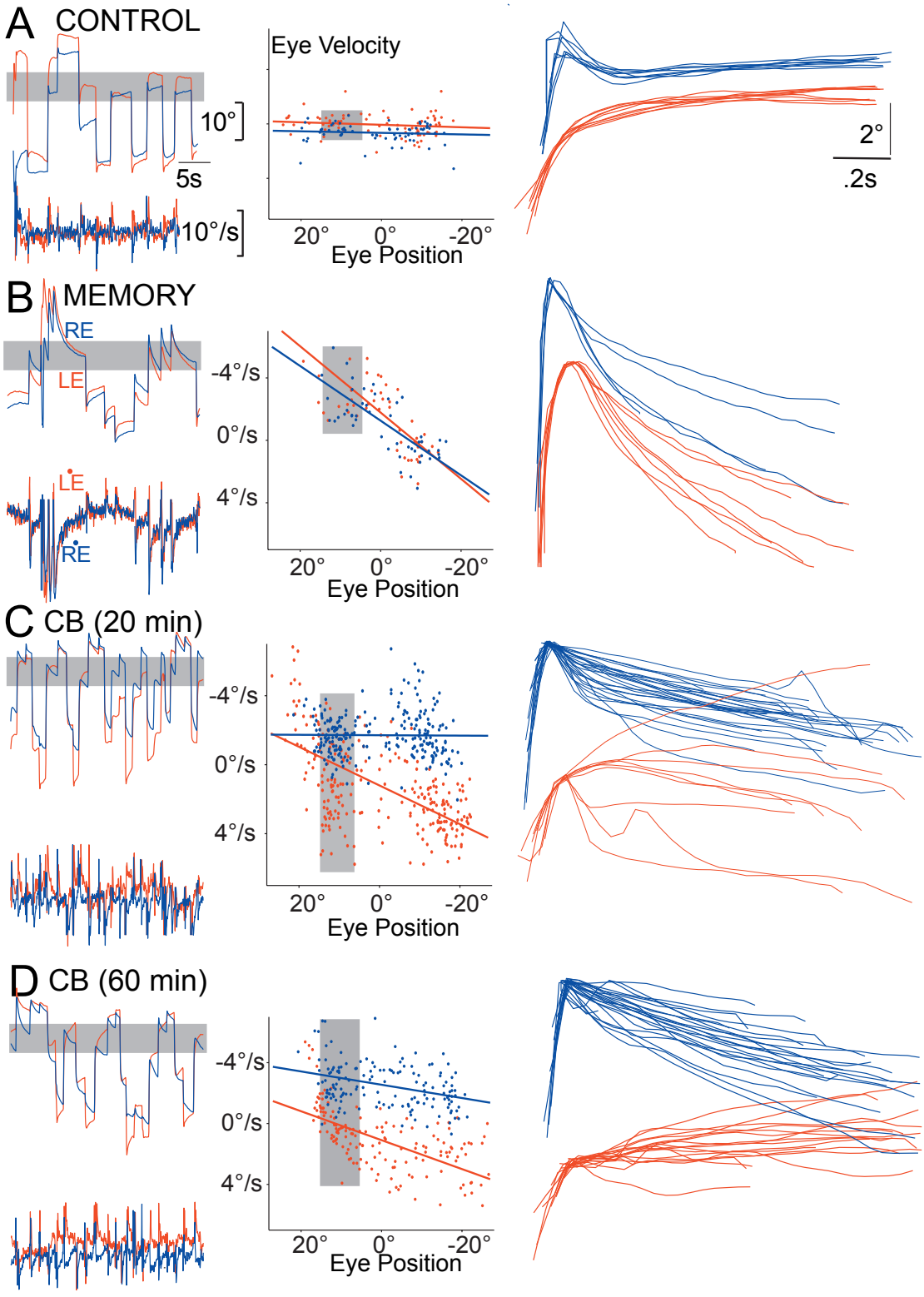
In general, after cerebellar ablations variability of the time constant for individual fixations increased, as demonstrated by the superimposition of fixations in Fig. 5-3 and the larger vertical spread of the P-V plots (Fig. 5-3C&D vs. A).

Cerebellectomy after instability training

In four of the nine acute cerebellar ablations, the time constants were modified to instability prior to cerebellar removal. Since PNI was shown to be monocular (Chapter 3), the time constants for each eye were measured and compared separately. The average time constants of eye position holding are

Figure 5-3: Eye fixation drift variability after cerebellectomy.

A-D: Eye position, eye velocity, P-V plots and superimposed post saccadic drifts (A) control before, (B) after 4 hrs leak training, (C) 20 minutes after CB and (D) 60 minutes after CB. Superimposed LE and RE fixation drifts after saccades with eye positions between 5° and 15° (P-V plot shaded box) to the left over three minutes. τ was (A) 93.3s, (B) 5.7s , (C) 842.1s and (D) 22.9s.


A CONTROL
10°
5s
10°/s
Eye Velocity
20°
0°
-20°
Eye Position
2°
.2s
B MEMORY
RE
LE
LE
RE
-4°/s
0°/s
4°/s
20°
0°
-20°
Eye Position
C CB (20 min)
-4°/s
0°/s
4°/s
20°
0°
-20°
Eye Position
D CB (60 min)
-4°/s
0°/s
4°/s
20°
0°
-20°
Eye Position

summarized in Table 5-2. In three of five measurements the eye position time constants were significantly more leaky seven minutes after cerebellar removal ($p \leq 0.01$) than in either the pre-trained naïve condition or after instability training (Fig. 5-4 A-C; morphology in Fig. 5-1B). Fifteen to thirty minutes after cerebellar ablation, the average fixation time constant improved, and was similar to pre-trained naïve goldfish. In 6 of the 7 observations, a significant difference was not observed between the naïve and post-cerebellar removal time constants. The disappearance of the memory after instability training (Fig. 5-4C) was not due to the inability to perform the behavior as visuomotor performance towards instability was still robust, although performance was always reduced compared to during the fourth hour of training prior to cerebellar ablation (Fig. 5-4D). After cerebellar removal, there was no significant change in time constant with additional training for 1-2 hrs (n=3 goldfish). These results indicate that the cerebellum is not necessary for maintaining fixation time constants, however it is important for time constant plasticity, in these examples towards instability. More specifically, since there was no memory of the time constant modification after cerebellar removal, while the requisite training behavior was still present, the cerebellum is required for the expression, or perhaps retention, of the memory.

C*erebellar removal after leak*

Four animals were initially trained to leak (n=7 measurements) (Fig. 5-5) and the cerebellum subsequently removed with the average eye position time constant comparisons summarized in Table 5-3. Five to seven minutes after cerebellar removal, the average time constant determined by P-V plots was not reduced but significantly larger than in the trained condition ($p<0.01$;Fig. 5-5C solid lines vs. 5-5B). By contrast, the time constant after ablation was

Instability Memory after CB

Control *[range]*	Memory (4 hrs)	CB (5-10 min)	CB (20-30 min)
43.4s *[-20.3 40.8]* n=7	-2.1s *[-1.7 -3.2]* n=7	12.9s *[322.0 5.0s]* n=5	27.7s *[-42.2 13.4]* n=7
vs. control *vs. memory*	*p≤0.001*	*p=0.02* *p≤0.001*	*p=0.02* *p≤0.001*

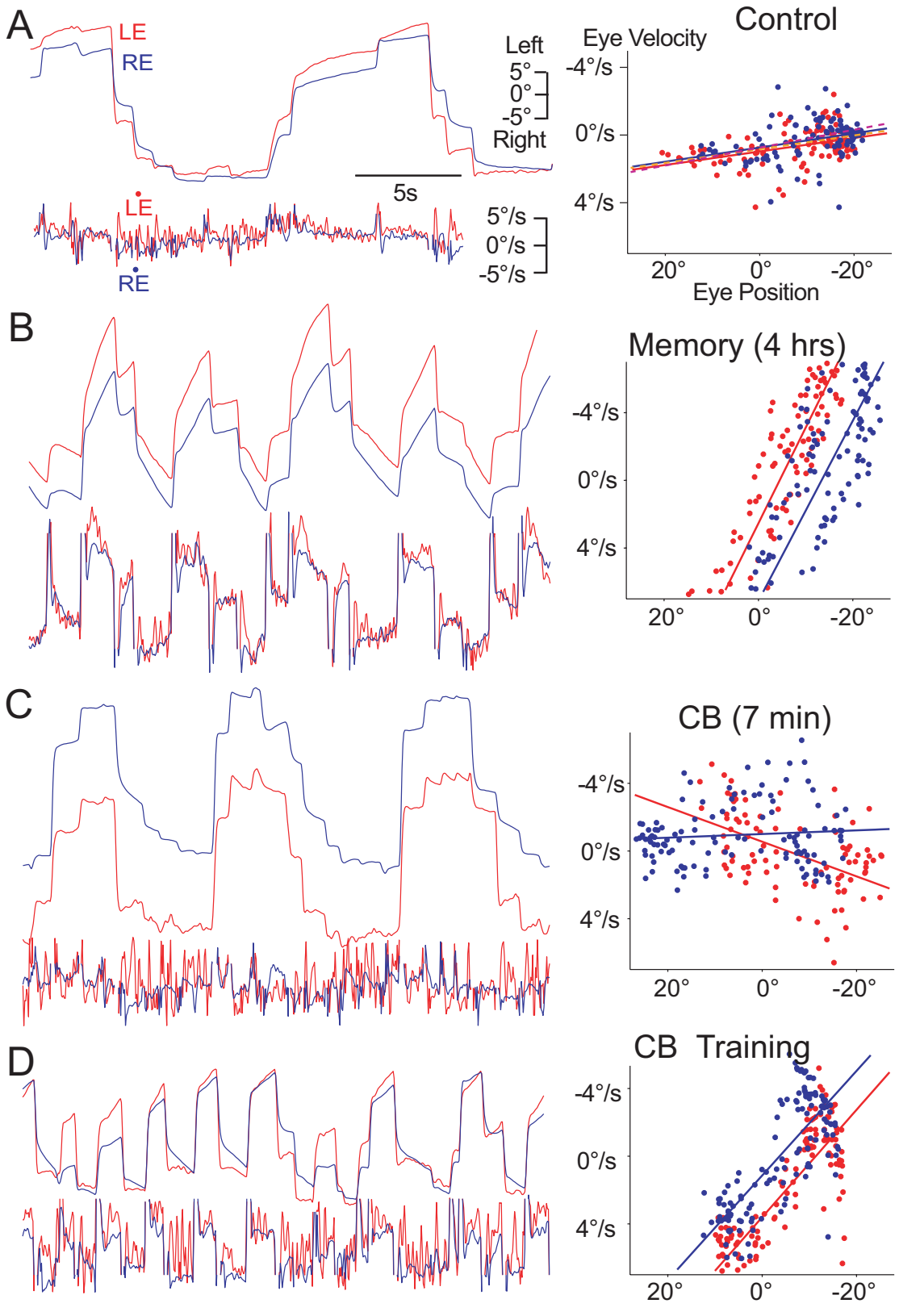
Performance (4 hrs)	Performance (CB)	Memory (CB)
-0.86s *[-0.68 -1.4]* n=7	-3.64s *[-1.66 -10.5]* n=7	11.7s *[22.7 5.1]* n=5
vs. performance *vs. CB (20-30 min)*	*p≤0.001* *p=0.006*	*p=0.10*

Table 5-2: Acute cerebellar removal and the time constant memory after 4 hrs of instability training.

Eye position time constant 5-10 minutes and 20-30 minutes after cerebellar aspiration (upper panel). Goldfish were trained for 4 hrs towards instability with a 10-40 °/s visual drift (Performance 4 hrs) and the time constant was measured in darkness (Memory 4 hrs). The cerebellum was aspirated and the time constant measured. Cerebellectomized goldfish were trained for 1-2 hrs towards instability (Performance CB) and the time constant was re-assessed (Memory CB).

Figure 5-4: Memory of time constant plasticity after instability training following CB removal

A-D: Eye position, eye velocity and P-V plot of (A) initial time constant (control), (B) after 4 hrs instability training, (C) 7 min after CB removal and (D) instability performance after CB removal. Morphology shown in Fig. 5-1B. LE and RE τ was (A) -33.5s & -23.9s , (B) -1.9s & -1.9s , (C) 17.0s & -318.9s , (D) -3.0 & -3.4s


A
LE
RE
Left
5°
0°
-5°
Right
5s
LE
RE
5°/s
0°/s
-5°/s
Control
Eye Velocity
-4°/s
0°/s
4°/s
20°
0°
-20°
Eye Position
B
Memory (4 hrs)
-4°/s
0°/s
4°/s
20°
0°
-20°
C
CB (7 min)
-4°/s
0°/s
4°/s
20°
0°
-20°
D
CB Training
-4°/s
0°/s
4°/s
20°
0°
-20°

significantly reduced when compared to that of the naïve pre-trained goldfish ($p<0.01$;Fig. 5-5C solid lines vs. 5-5A).

When monitored 20-30 minutes after perturbation (Fig. 5-5B dashed lines) the average time constant lengthened, such that in 3 of the observations no significant difference was detected by ACOVA analysis with the naïve time constant. In an additional case, the time constant was unstable 20 minutes after cerebellar removal.

When the time constants were trained towards leak after cerebellar removal (n=3), performance of training was robust (Fig. 5-5D); however, no significant changes in time constants were detected by ACOVA analysis after prolonged training (Fig. 5-5E). The increased time constant after cerebellar removal in goldfish trained to leak, is an important finding. If the cerebellum was vital for eye position time constant stability, removal would cause a large leak. Unlike the experiments in which the time constant was trained to instability, the more stable time constant after ablation of goldfish trained towards leak clearly indicates that the memory of the time constant modification was removed but the mechanism producing neuronal persistence was still intact. The inability to subsequently reduce the time constant to a more leaky value, is similar to the result obtained for modification of the time constant to instability. Thus, the cerebellum is required for the expression of both an **increase** and a **decrease** in the time constant.

VOR after cerebellectomy

In addition to maintaining fixation stability, the eye velocity-to-position neural integrator supports VOR gain and phase at low frequency rotations in the goldfish. To explore whether this aspect of integrator function was affected by

Retention of leak fixation plasticity

Control [range]	Memory (4 hrs)	CB 5-10 Min	CB 20-30 Min
-122.2 [--24.9 23.2] n=7	4.8 [3.4 10.9] n=7	8.8 [7.5 10.9] n=4	18.0 [-7.9 8.9] n=5
vs. control *vs. memory*	*p≤0.001*	*p=0.007* *p=0.007*	*p=0.08* *p=0.05*

Performance (4 hrs)	CB Performance	CB Memory
1.1s [0.45 3.3] n=5	0.82 [0.40 5.3] n=5	75.7s [-9.7 8.1] n=5
vs. performance *vs CB (20-30 min)*	*p=0.56* *p=0.06*	*p=0.18* *p=0.05*

Table 5-3: Acute cerebellar removal after training the fixation time constant to leak.

Eye position time constant 5-10 minutes and 20-30 minutes after cerebellar aspiration (upper panel). Goldfish were trained for 4 hrs towards leak with 2-30 °/s visual drift and the time constant was meaured at 4 hrs in light (Performance 4 hrs) and darkness (Memory 4 hrs). After cerebellar aspiration, the goldfish were trained towards leak for additional 1-2 hrs (CB performance) and the time constants measured again (CB memory).

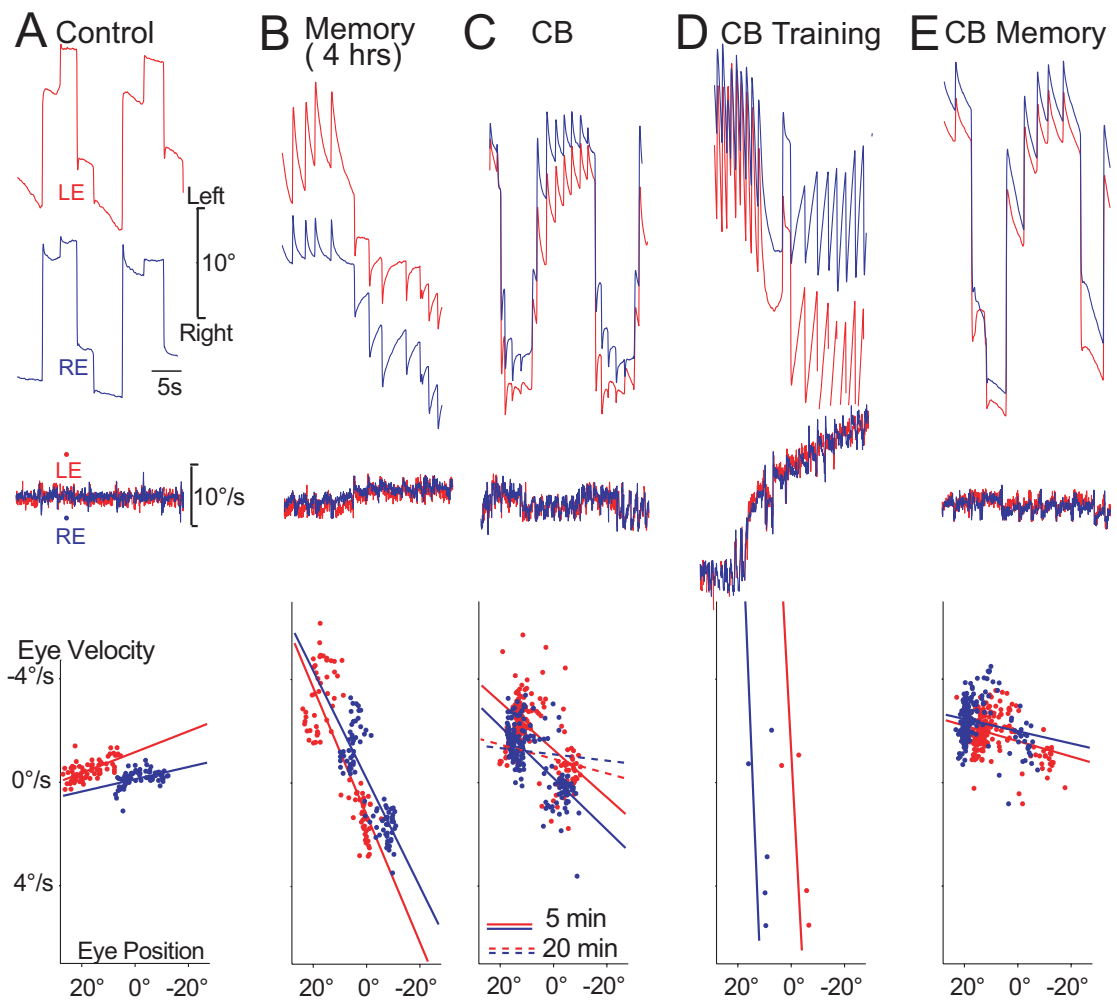


Figure 5-5: Cerebellar removal after fixation time constant training to leak.

A-E: Eye position, eye velocity and P-V plots at six different times (A) initial stability, (B) after 4 hrs of leak training, (C) 5 (solid) & 20 (dashed) min after CB removal, (D) leak performance after CB removal, and (E) memory after 1 hr training. LE and RE τ was (A) -24.9 & -42.0s, (B) 4.1s & 4.8s, (7 min, C solid lines) 10.8s & 10.0s, (20 min, C dashed) 38.6s & 88.5s, (D) 0.52s & 0.40s and (E) 33.5s & 42.3s.

cerebellar removal, gain and phase of the VOR was monitored before and after cerebellectomy at 0.125 Hz (n=4) and also at 1 Hz (n=3). Similar to other studies, the VOR gain at 0.125 Hz increased after cerebellar inactivation (Michnovicz and Bennett 1987). This increase occurred regardless of whether the initial fixation time constant was trained towards instability (Fig. 5-6B, animal from Fig. 5-4) or leak (Fig. 5-7, animal from Fig. 5-5). Changes in gain and phase of the VOR are summarized in Table 5-4. The average gain changes of 25% and 66% at 0.125 and 1 Hz were significant. Phase did not significantly change at either frequency with an average shift of 1.2° at 0.125 Hz and 7.9° at 1 Hz stimulation. By contrast, noticeable changes were observed in the multistable saccadic pattern after cerebellar removal (Figures 5-6 and 5-7). This included increases in both the nasal and temporal components of the oculomotor range, suggesting alterations within the saccadic pattern generator. These results corroborate the observations on studies of post-saccadic drift showing that eye velocity-to-position integration in goldfish is minimally affected after cerebellar removal (Figs. 5-2, 5-4 and 5-5) (Marsh 1998).

Frequency	Naive Gain (SD)	CB Gain *(paired t test)*	Naive Phase	CB Phase *(paired t test)*
0.125 Hz (n=8)	0.70 ± *0.07*	0.87 ± *0.11* *p<0.001*	182.8° ±2.2	184.0º ± *5.3* *p=0.47*
1 Hz (n=5)	0.66 ± *0.15*	1.1 ± *0.24* *p=0.006*	180.5° ± 3.3	188.4º ± *10.5* *p=0.27*

Table 5-4: Vestibulo-ocular reflex gain and phase after cerebellar ablation. Gain and phase changes of VOR at 0.125 Hz and 1 Hz after cerebellar ablation.

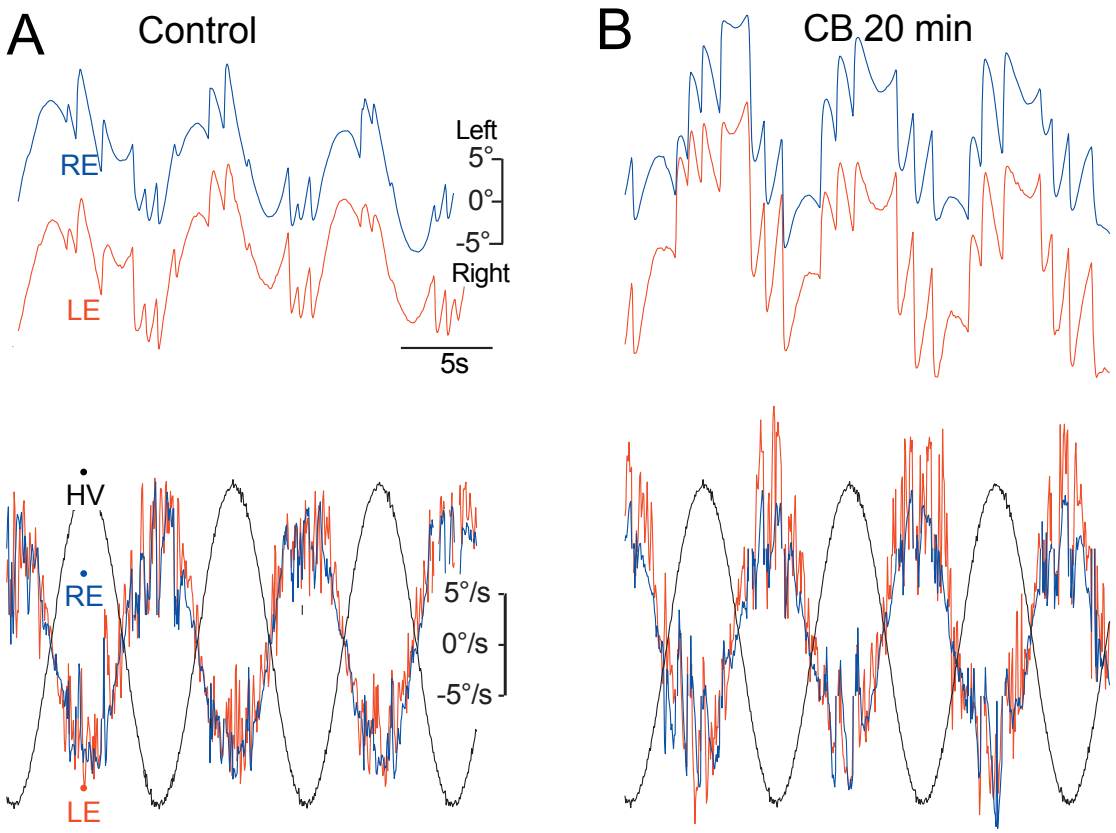


Figure 5-6: VOR after cerebellar removal at 20 minutes.

A,B: Eye position and eye velocity VOR at 0.125 Hz (A) before and (B) 20 min after cerebellar removal. Effects on fixation time constant stability are illustrated in Fig. 5-4, with morphology in Fig. 5-1B. LE and RE VOR gains were (A) 0.78 and 0.74 and (B) 0.99 and 0.88. Phase changed from (A) 183.5° and 180.3° to (B) 191.9° and 186.4°.

Figure 5-7: VOR after fixation plasticity and cerebellar removal.

A-C: Eye position and velocity of VOR at 0.125 Hz (A) before, (B) after leak memory and (C) 20 minutes after cerebellar removal. Effects on fixation stability are shown in Fig. 5-5. LE and RE eye velocity gains were (A) 0.68 & 0.56, (B) 0.58 & 0.46, (C) 0.78 & 0.72. Eye velocity phases were (A) 181.2° & 180.6, (B) 184.0° & 183.7° and (C) 179.0° & 180.2°. D-F: Eye position and velocity of VOR at 1 Hz (D) before, (E) after leak memory, and (F) 20 min after cerebellar removal. LE and RE velocity gains were (D) 0.63 & 0.47, (E) 0.66 & 0.50, and (F) 0.94 & 0.69. Eye velocity phases were (D) 181.5° & 182.4°, (E) 190.4° & 192.2°, and (F) 184.5° & 181.1°.

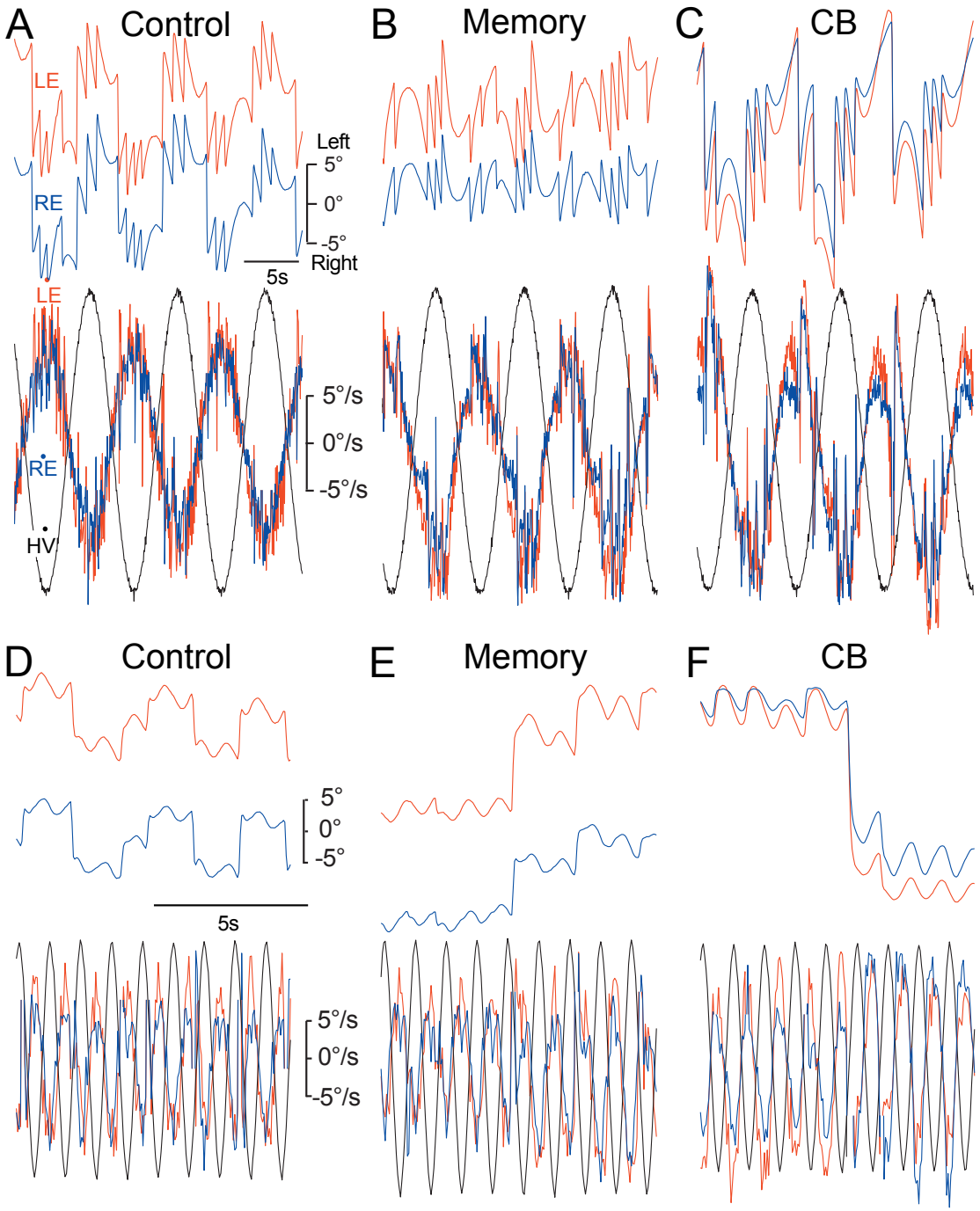
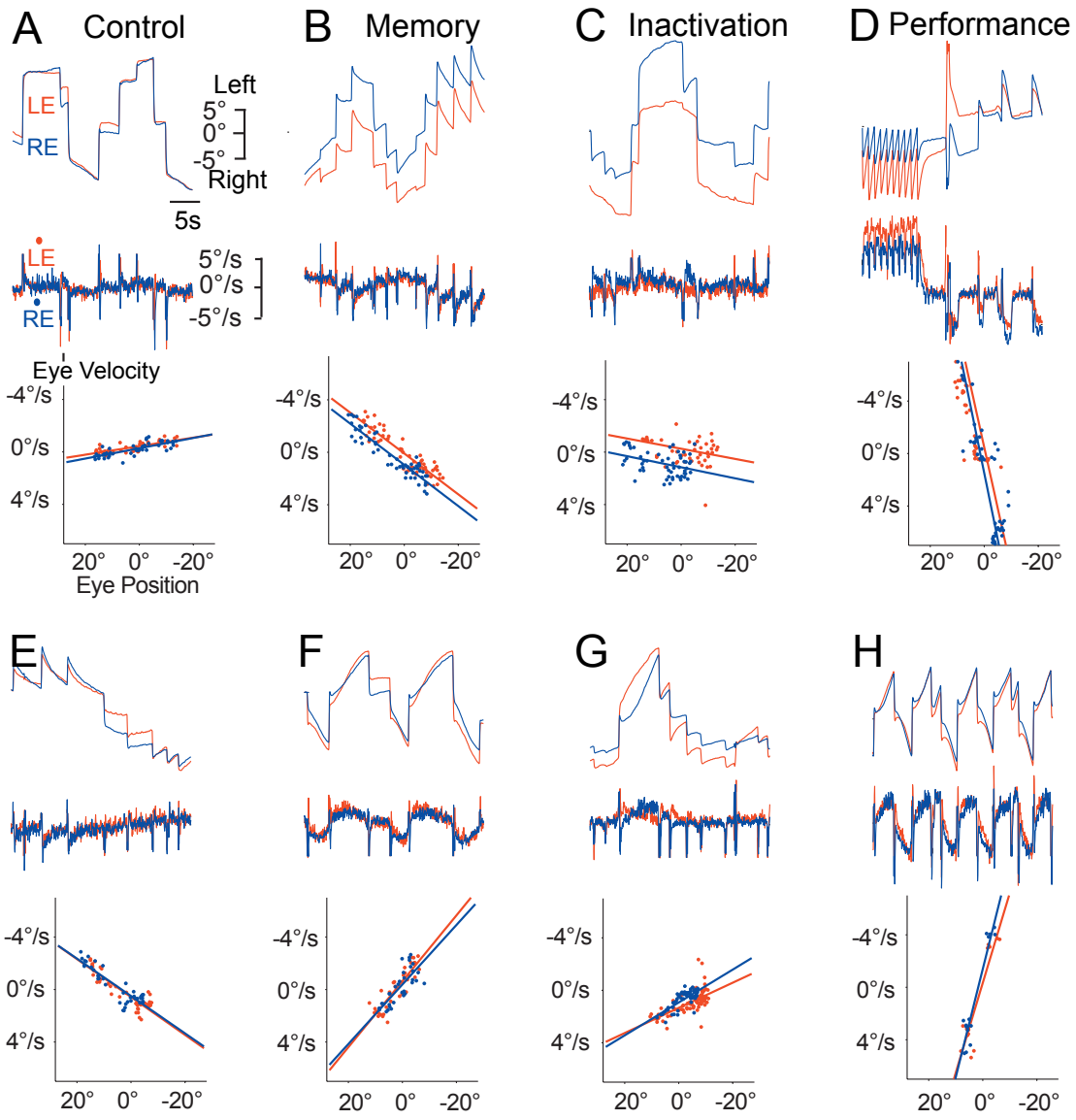

A
Control
B
Memory
C
CB
LE
RE
Left
5°
0°
-5°
Right
5s
LE
RE
HV
5°/s
0°/s
-5°/s
D
Control
E
Memory
F
CB
5°
0°
-5°
5s
5°/s
0°/s
-5°/s

Figure 5-8: Modification of fixation time constant and reversible cerebellar inactivation.

A-H: Eye position, velocity and P-V plots during (A) initial stability, (B) memory after 4 hrs training to leak, (C) 5 minutes after cerebellar inactivation, (D) performance to leak 20 minutes after injection, (E) memory 4 hrs after training to leak, (F) memory after training to instability, (G) 5 min after cerebellar inactivation and (H) performance to instability. LE and RE τ were (A) -30.7 & -25.4, (B) 6.4s & 6.4s, (C) 25.6s & 23.7s (5 min;solid), 45.4s & 28.8s (20 min) 37.3s & 36.3s (1 hr), (D) 1.1s & 0.99s, (E) 6.9s & 7.0s, (F) -4.0s & -4.4s, (G) -10.4 & -8.0s and (H) -1.5s & -1.2s.


A
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B
Memory
C
Inactivation
D
Performance
LE
RE
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5°
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5s
5°/s
0°/s
-5°/s
Eye Velocity
-4°/s
0°/s
4°/s
20°
0°
-20°
Eye Position
E
F
G
H

Change in fixation time constant after reversible cerebellar inactivation

Pharmacological inactivation was used to minimize the mechanical trauma accompanying cerebellar ablation. After training of the fixation time constant to either instability or leak, the cerebellum was inactivated (13 trials; 9 goldfish) by either 4% lidocaine and/or 1% bupivacaine. The VOR was monitored to ensure that inactivation was limited to the cerebellum and did not spread to the hindbrain. Immediately after anesthetic injection, a nystagmus of variable amplitude was observed that usually subsided within 5-10 minutes. The occurrence of velocity bias and nystagmus after cerebellar inactivation by lidocaine has been previously reported where it was considered to be due to asymmetrical cerebellar inactivation (McElligott et al. 1998).

The inactivation results were quantified in 10 trials. In experiments in which the time constant was modified to leak prior to inactivation (5 trials; 10 measurements) (Fig. 5-8A-D), anesthesia increased the time constant in 8/10 cases (Fig. 5-8C) with 3/10 cases determined statistically significant by ACOVA analysis ($p<0.01$) (Fig. 5-8B). In 7/10 observations, time constant differences were not statistically significant, although they tended to be smaller than control time constants of the naive goldfish. The time constants never returned to the trained value during extended observation of over an hour after anesthetic injection. The more stable time constant observed after cerebellar inactivation compared to the memory time constant after 4 hrs of training to leak (Fig. 5-8B), was not due to the inability to perform the leak training paradigm (Fig. 5-8D). Thus, all the effects observed were concluded to be due to cerebellar inactivation rather than non-specific damage caused by the pressure injection particularly since time constant training for three to four hours after anesthetic

injection could produce robust memory of either instability or leak (Fig. 5-8E). These results are in agreement with the changes in time constant after cerebellar ablation, in which the time constant increased to a value between the trained leak state and the naïve animal.

Similar results were observed when the time constant training was to instability (n=5 trials). Cerebellar inactivation made the eye position holding more stable by increasing the time constant (Fig. 5-8F vs. G). In 6/9 cases, the changes were significant ($p \leq 0.01$). In 8/9 observations, the time constants returned close to naïve-like values as determined by ACOVA analysis, although a slight instability was a frequent observation (Fig. 5-8G). The reversible inactivation experiments are consistent with the interpretation of the cerebellar role based on the ablation studies. In short, eye position time constant maintenance is cerebellar pathway independent. When the time constant is altered to either instability or leak, the cerebellum is assumed to be required for acquisition and, equally important, for the retention of post saccadic eye velocity changes. Observations of continued instability or leak after pharmacological inactivation, implies a 'minor' brainstem expression of the time constant modification in addition to the cerebellar dependent pathway.

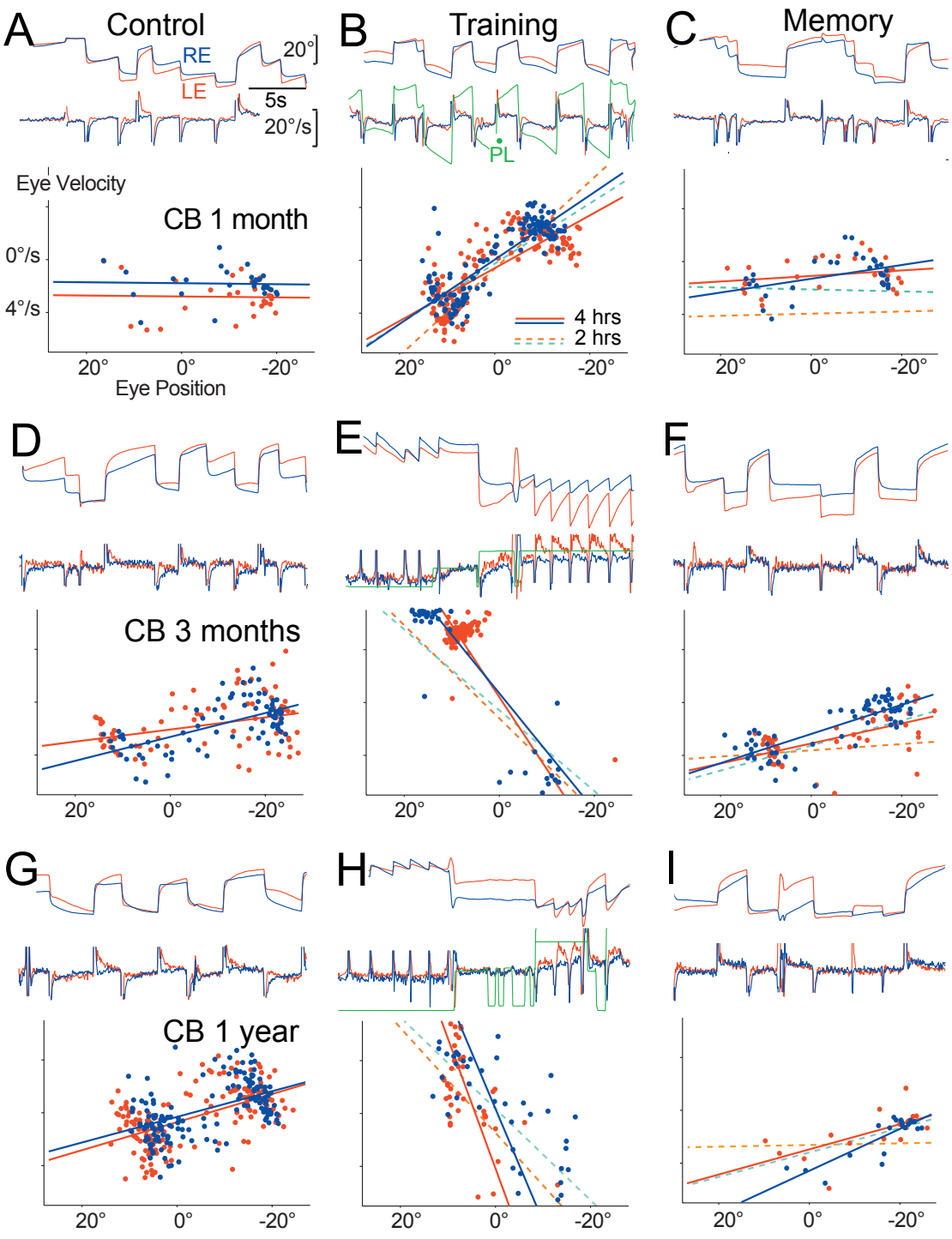
Long term time constant maintenance after cerebellar ablation

Stability of fixation time constants were monitored at various time intervals from one day to a year and a half in 15 goldfish. The change in fixation time constant over prolonged recovery time is demonstrated by the stability shown at 1 month, 3 months and 1 year, in which the eyes became more unstable with time (significant at the $p<0.01$ level at 1.5 years) (Fig. 5-9A,D,G).

The general trend observed was that the time constant became larger;

Figure 5-9: Long term time constant stability and modification after cerebellar removal.

Morphology of cerebellar ablation is shown in Fig. 5-1B. A-C: Eye position, velocity and P-V plot of (A) initial stability, (B) performance to instability at 2 hrs (orange and cyan, dashed) and 4 hrs (red and blue, solid), (C) memory of time constant modification 1 month after cerebellar removal. LE and RE τ were (A) 287.4s & 276.7s, (B) -3.0 & -4.4s (2hrs, dashed), -5.0s & -4.1s (4hrs) and (C) 124.1s & 136.3s (2 hrs), 47.7s & 19.2s (4 hrs,). D-F: Eye position, velocity and P-V plot of (D) initial stability, (E) performance to instability at 2 hrs (orange and cyan, dashed) and 4 hrs (red and blue, solid) and (F), memory of time constant modification 3 month after cerebellar removal. LE and RE τ were (D) -21.8s & -11.1s, (E) 2.8 & 3.3s (2hrs, dashed), 1.8s & 2.3s (4hrs), (F) -41.9s & -10.5s (2 hrs), -13.2s & -9.1s (4 hrs). G-I: Eye position, velocity and P-V plot of (G) initial stability, (H) performance to instability at 2 hrs (orange and cyan, dashed) and 4 hrs (red and blue, solid) and (I) memory of time constant modification 1 year after cerebellar removal. LE and RE τ were (G) -9.4s & -10.3s, (H) 2.5s & 2.9s (2hrs, dashed), 1.0s & 1.2s (4hrs), (I) -159.8s & -10.9s (2 hrs), -15.5s & -7.5s (4 hrs).


A
Control
RE
LE
20°
5s
20°/s
Eye Velocity
CB 1 month
0°/s
4°/s
20°
0°
-20°
Eye Position
B
Training
PL
4 hrs
2 hrs
C
Memory
D
CB 3 months
E
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CB 1 year
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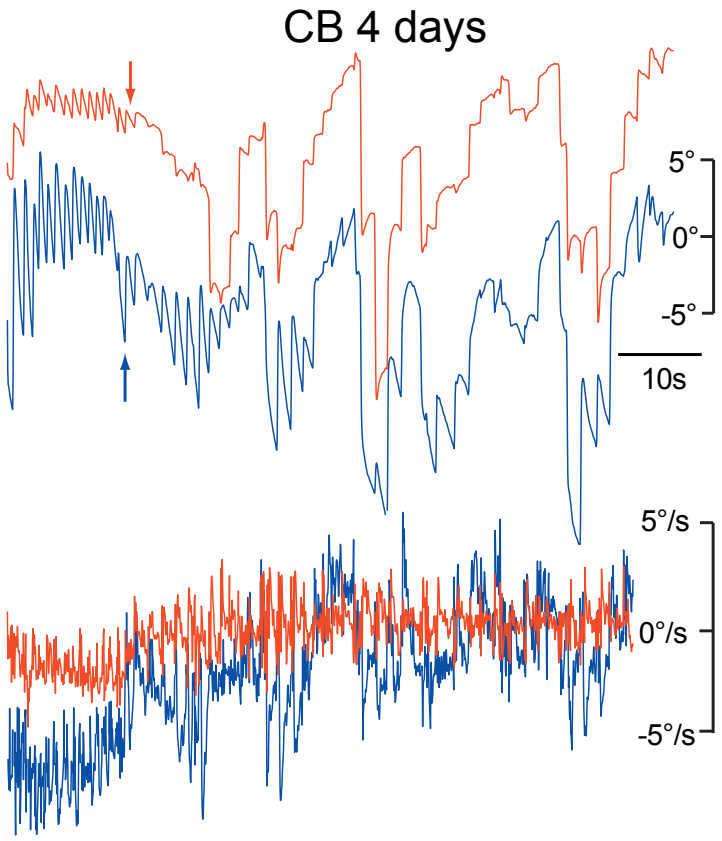


Figure 5-10: Nystagmus and saccadic scanning after cerebellar ablation.

Eye position and velocity during scanning saccades in darkness four days after cerebellar ablation. The rightward bias (nystagmus) observed at the beginning of the record in both eyes ceased (arrows). RE scanning pattern showed a unstable time constant bidirectionally, while LE time constant was stable with a minor leftward bias.

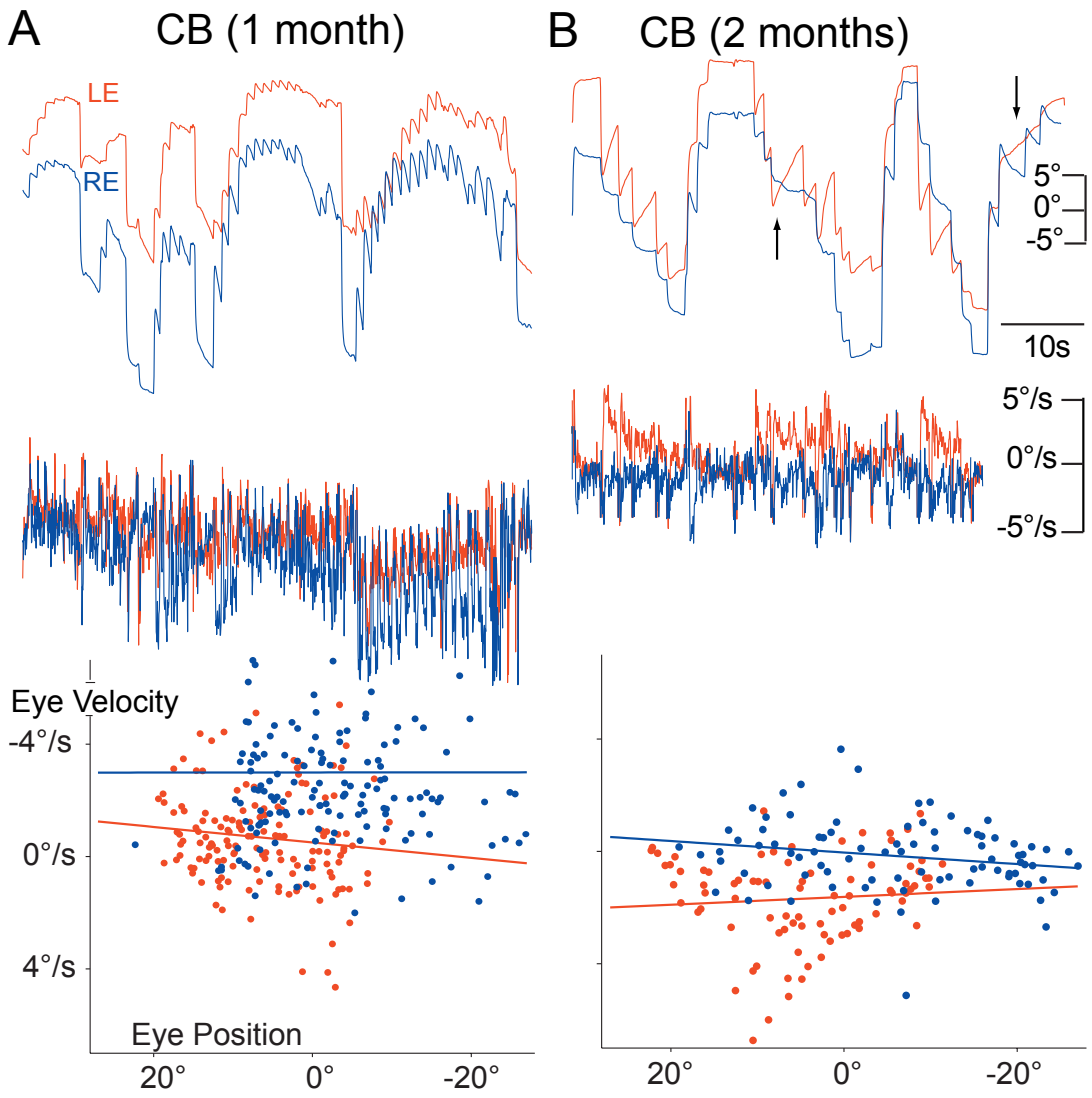


Figure 5-11: Fixation time constants and saccadic pattern after cerebellar ablation.

Eye position, eye velocity and P-V plots of the LE and RE (A)1 month and (B) 2 months after cerebellar removal. A large eccentric nystagmus was observed at one month with the rightward bias absent at two months. Arrows indicate large saccadic amplitude differences between the eyes. LE and RE τ were (A) 36.3 & 5000 and (B) -71.6 & 48.6s.

however statistically there was no change in the average time constant (ANOVA p=0.16). Time constants of nine fish were quantified on multiple occasions. Due to the variability in P-V plots, 7/9 cases observed at multiple time periods showed no statistical difference in time constant when compared by ACOVA analysis although in 6/9 measurements, time constants became more unstable with longer recovery time. Time constants could change from leak to stability or from the latter to instability. In one of the three cases in which the time constant did not become progressively more unstable, the initial post lesion measurement showed an instability that was maintained when observed subsequently. In 1/9 cases, the time constant progressively became leakier between observations. Thus the cerebellum is not required for long term maintenance of fixation time constant, however it does appear to be required for extremely stable time constants (<100s) observed in naïve goldfish.

Nystagmus could be observed months after cerebellar ablation; however, both nystagmus and velocity biases would frequently resolve spontaneously into a normal scanning pattern without any predictable reason (Fig. 5-10 & 5-11A). As shown in Fig. 5-10, 4 days after cerebellectomy a rightward bias at the onset of the record switched to a normal saccadic pattern with characteristic eccentrically drifting fixations (Fig. 5-10).

Improvement of the time constants with post-ablation recovery time, was paralleled by an improvement in saccadic pattern (Fig. 5-11A vs. B). In addition, large monocular eye velocity drift differences and monocular saccades could be observed after long-term cerebellar removal as highlighted in Fig. 5-11B (2 months), in which both eyes exhibited a noticeable temporal, rather than nasal bias as previously described in naïve goldfish (Easter 1971; Mensh et al. 2004).

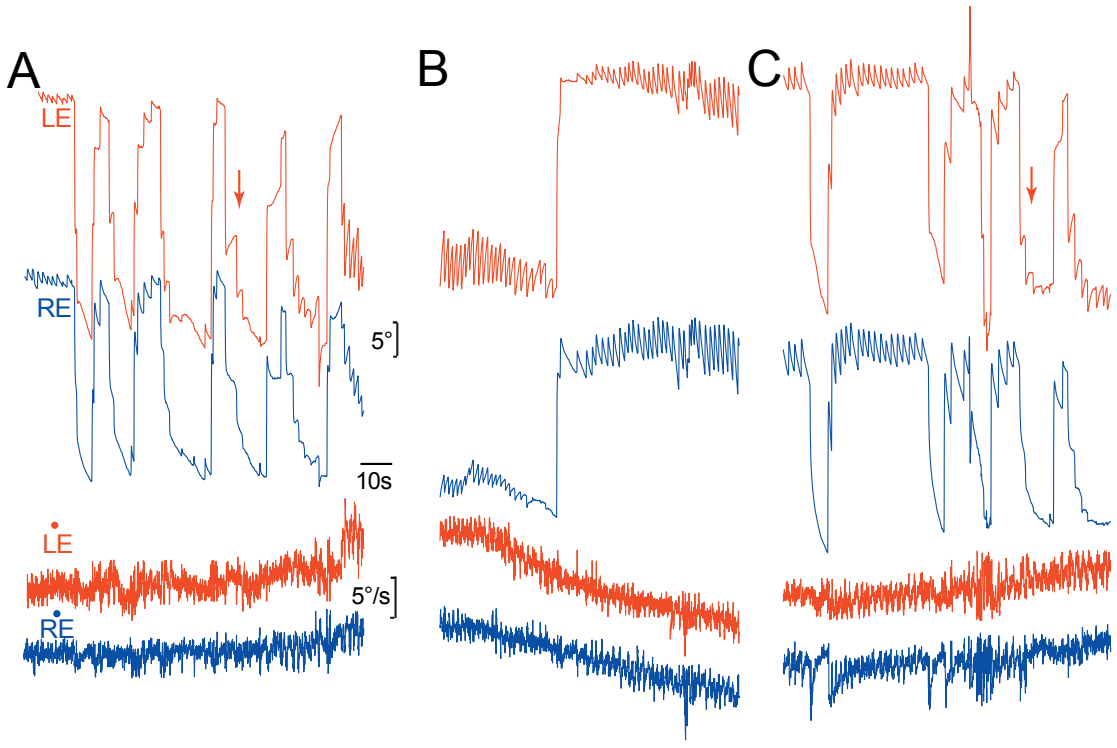


Figure 5-12: Null position changes after cerebellar ablation.

A-C: Eye position and velocity during spontaneous scanning in darkness 3 months after cerebellar removal. Nystagmus was observed in the eccentric parts of the oculomotor range. The scanning pattern shown could shift rapidly as illustrated in B & C. Null shifts (red arrows) are shown in A & C. Eccentric nystagmus is observed in B and in LE of C.

One consequence of a highly variable null position was that time constants and saccadic pattern could differ markedly within minutes (Fig. 5-12) in contrast to naïve fish in which the scanning pattern and time constants gradually changed over the course of an hour (Major et al. 2004a). The rapid shifts in scanning pattern and null points were still present three months after cerebellar removal (Fig. 5-12; naïve fish Figure 5-5). Similar to acute inactivation, a general characteristic of the scanning pattern observed during long term observation was a larger schlagfeld, in which eye position remained in either the nasal or temporal extremes with a corresponding “leak” nystagmus (Fig. 5-12B). Eye position tended to be more stable throughout the rest of the oculomotor range (~ +/- 15°) (Fig. 5-12A&C).

Fixation time constant learning and memory after long term recovery

A robust optokinetic response was maintained days to weeks after cerebellar removal as was performance of time constant training to either instability (Fig. 5-9B) or leak (Fig. 5-9E&G). When time constants of the initial performance were compared to those of performance at 2 and 4 hrs, in 9/24 (38%) plasticity experiments the time constant of at least one eye was significant ($p<0.05$) at the later time intervals. This plasticity suggests a non-cerebellar learning pathway which was probably underestimated in the percentage comparison due to the robust initial performance of the training paradigm. In one experiment, a significant difference was observed in the time constant after training compared to control ($p<0.05$). Time constant learning is qualitatively similar to the results obtained after VOR gain change plasticity, in which small but significant changes occurred after training; but the plasticity was greatly reduced compared to that possible in an intact animal.

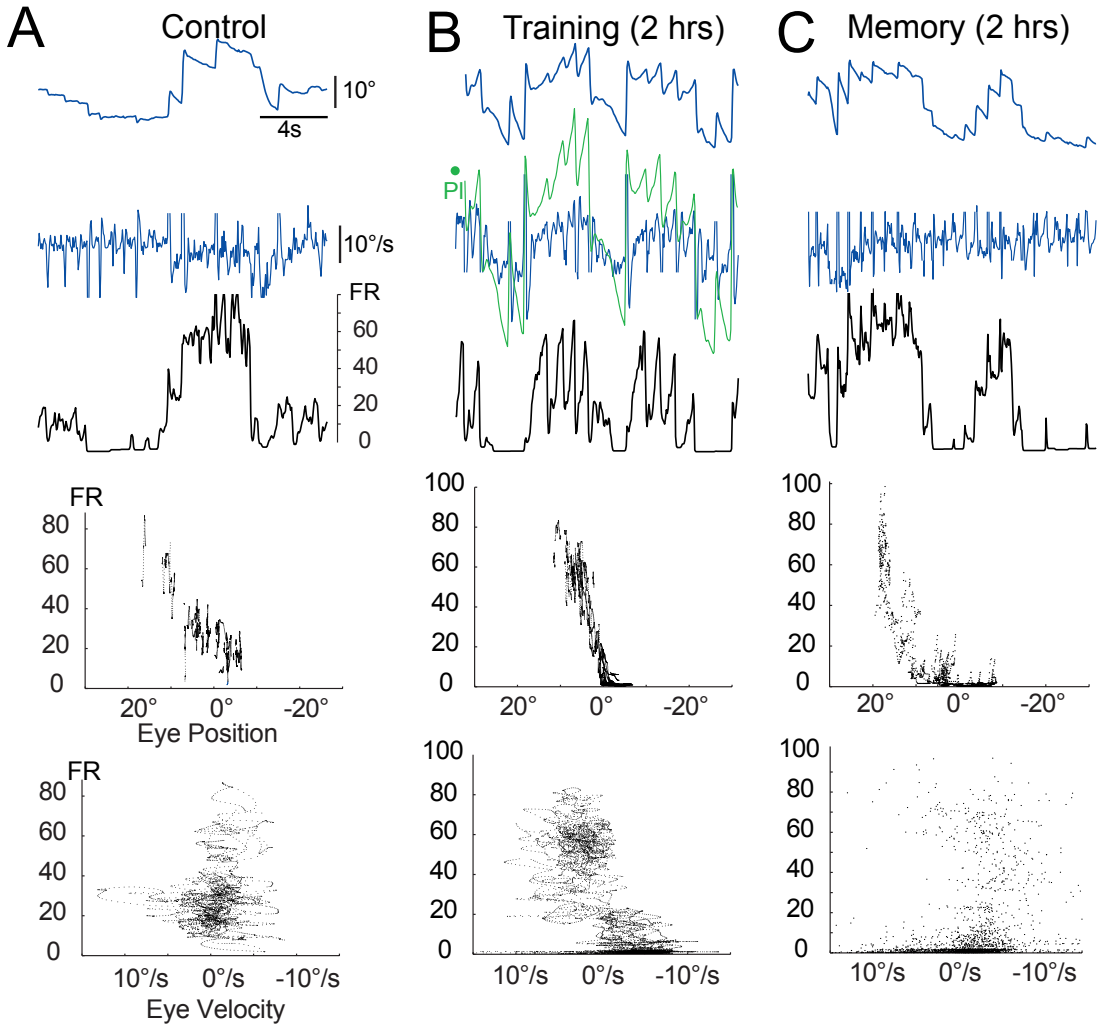


Figure 5-13: PNI neuronal activity after acute cerebellar removal.

A-C: RE position, velocity, firing rate and FR vs. position and velocity plot (A) before, (B) during and (C) after 2 hrs of instability training subsequent to cerebellar removal. Both the position sensitivity and correlation increased in relation to the eye position, however no evidence of memory was observed. Eye position and velocity sensitivities were (A) 1.6 sp/s/° & 0.33 sp/s/°/s r=0.58, (B) 4.5 sp/s/° & 1.1 sp/s/°/s r=0.80 and (C) 5.2 sp/s/° & 0.56 sp/s/°/s r=0.84.

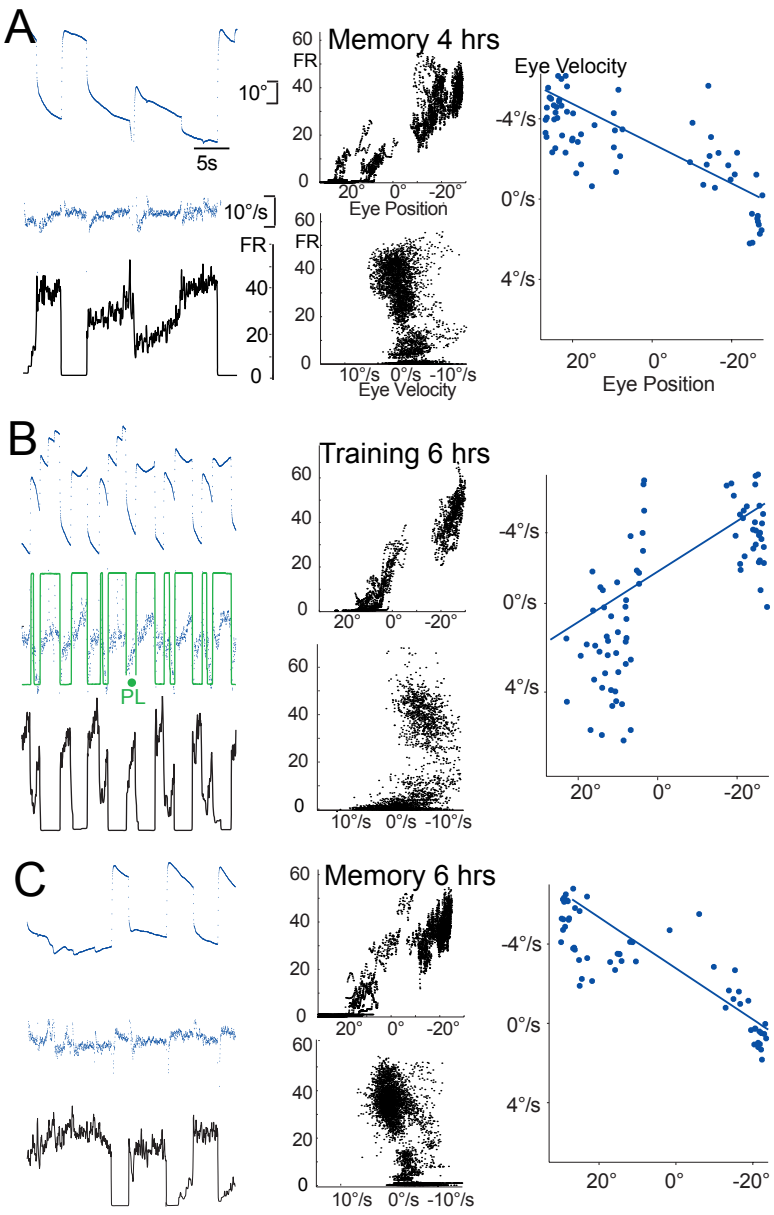


Figure 5-14: PNI neuronal activity 10 days after cerebellar removal.

A-C: RE position, velocity, firing rate, FR vs. Pos, FR vs. Vel and P-V plots after (A) 4 hrs, (B) performance at 4 hrs, and (C) after 6 hrs training to instability 10 days after cerebellar removal The time constants were (A) 10.0s, (B) -8.8s and (C) 7.7s. The eye position, velocity and correlation coefficient were (A) -1.04 sp/s/° -1.07 sp/s/°/s r=0.88, (B) -1.08 sp/s/° -0.22 sp/s/°/s r=0.88 and (C) -1.00 sp/s/° -1.23 sp/s/°/s r=0.86.

Neuronal activity of PNI after cerebellar removal

To ascertain if the absence of fixation plasticity was due to a lack of the appropriate neuronal signal reaching the eye velocity-to-position neural integrator, the activity of Area I neurons were recorded after cerebellar removal (n=8). In six animals, a total of twenty cells were recorded in Area I as determined by anatomical location. Five neurons were recorded ($r \geq 0.6$) that showed position sensitivities that ranged from 0.99 to 5.2 (sp/s)/° and velocity sensitivities from 0.03 to 1.4 (sp/s)/°/s. As observed in Fig. 5-13 & 5-14, when the activity was recorded before, during and after fixation time constant plasticity, the firing rate reflected eye position and eye velocity in all conditions, implying that the visually-mediated training signals were present in Area I. Thus, the lack of time constant plasticity was due to the absence of cerebellar pathways not due to the absence of Area I neuronal modulation.

Discussion

The vestibulo-cerebellum has been thought to play an important role in horizontal eye velocity-to-position integration because mammalian experimental work demonstrated a very large decrement in fixation time constant after cerebellum ablation (Robinson 1974). This finding, in turn, led to the theory that the cerebellum acted as an amplifier that in this case elongated hindbrain PNI persistence and therefore the time constant (Robinson 1974). The flocculus was specifically implied to have a role in this behavior as well as in time constant plasticity, since after surgical weakening of an eye muscle, animals whose cerebellar lesions spared the flocculus were still able to suppress fixational drift (Optican and Robinson 1980). This suggested that the locations for learning and memory aspects of fixation time constant plasticity were co-localized in

the pathway responsible for producing the long time constant. However, in light of the morphology and electrophysiology in goldfish, in particular a lack of direct cerebellar connections to PNI or the precerebellar nuclei (Area II), this hypothesis requires re-evaluation at least in this species. The anatomy of the goldfish hindbrain circuitry, suggests that all cerebellar related effects on fixation stability are mediated through the vestibular nuclei. The overall conclusion from this study is that cerebellar pathways are not directly involved in the principal mechanism responsible for persistence generation or fixation time constant stability since ablation reduced the time constant to ~ 10 seconds. Thus, the cerebellum in the goldfish does provide a positive feedback role to lengthen the time constant; however, this role is likely independent of the principal mechanism that determines the time constant.

In contrast to the proposed role of the Area I interneurons, the cerebellar pathways appear to be important for the acquisition and expression of changes in other oculomotor behaviors, VOR and OKR, as well as fixation time constant plasticity. Previous studies have shown that cerebellar inactivation immediately, and severely, impaired acquisition and expression of period tuning, and changes in eye velocity during either OKR or VOR gain adaptation (Marsh 1998; Pastor et al. 1994a). Therefore it can be concluded that the cerebellum appears vital for the expression of all ‘eye velocity memory’, as previously learned fixation time constant plasticity is either attenuated or abolished when the cerebellum is inactivated. Thus it is most likely that the Area II-cerebello-vestibular pathways modify an intrinsic default persistence state generated within Area I. Since small changes occurred in learning and memory components of fixation time constant after cerebellectomy in some animals, it is hypothesized that the hindbrain Area

II-vestibular reciprocally-related pathways are sufficient to induce some degree of plasticity similar to the results reported for VOR gain adaptation (Pastor et al. 1994a). This observation is further bolstered by the general trend for eye position time constants to become unstable over the course of 3 months to a year after cerebellar removal.

Removal of the cerebellum eliminates a common inhibitory drive to the neurons located in the vestibular nucleus. Neuronal firing rates would be expected to increase along with variability due to a decrease in synchrony, causing PNI neurons in Area I to receive an equally variable afferent activity that in turn might allow different drifts and time constants for identical positions. The increase in vestibular neuronal firing rate has been partially corroborated by the increase in VOR gain after cerebellar removal. In addition, the average spontaneous activity of vestibular neurons after flocculus removal in monkeys increased to 53 sp/s from an average firing rate of 38 sp/s in a naïve monkey (Waespe and Cohen 1983; Waespe and Henn 1977). Increased tonic vestibular activity also would be conveyed to Area II neurons which exhibit a positive feedback pathway with the vestibular neurons (Straka et al. 2006). Over the course of several months, changes of synaptic efficacy within the Area II-vestibular neuron pathways might occur. Since the major afferent input to Area I is from vestibular neurons, elevated vestibular activity would produce a larger eye velocity, and hence eye position signal possibly contributing to the observed mild instability. Alternatively, cerebellar removal could indirectly alter the intrinsic properties of Area I neurons over the course of the months.

Neurons in the left and right vestibular nuclei are arranged in a ‘push-pull’ relationship, such that increases in activity in one subset of neurons decreases

the activity in the contralateral subset (Straka and Dieringer 2004). Although not proven by any particular cellular structure/function study, the two vestibular nuclei have been frequently modeled as a crossed inhibitory feedback excitation pathway (Galiana and Outerbridge 1984). Thus an increase in the activity of one side, would increase inhibition of the contralateral commissural inhibitory neurons, effectively causing feedback excitation leading to a potential instability and oscillatory firing rates that contribute to the observed velocity biases, null shifts, and in extreme cases, nystagmus. Cerebellar Purkinje cell inhibition clearly helps to prevent these effects as velocity bias or nystagmus in the absence of visual feedback was always more frequent after cerebellectomy.

Effects of cerebellar removal on memory expression

Previous experiments investigating oculomotor learning and memory in goldfish after cerebellum removal indicated an involvement in retention of changes in eye velocity, but not necessarily in maintenance of fixation time constant (Marsh 1998). Cerebellar removal after modifying the fixation time constant to instability, abolished the eccentric post saccadic eye velocity drift, but not the ability to maintain a constant eye position (Fig. 5-4). In the majority of cases when the cerebellum was aspirated after training to instability, five minutes after the ablation, the time constant was reduced and a centripetal eye velocity drift larger than exhibited in naïve goldfish occurred. In the majority of cases by twenty minutes after the lesion, the time constant was not significantly different than that of the naïve animal. This result suggests that the initial decrease may have included mechanical effects of spreading depression caused by the aspiration. It was previously observed that after cerebellar removal, increases in eye velocity during OKR were abolished, and this was interpreted as abolition

of OKR memory (Marsh 1998). The slight post-cerebellectomy decrease in time constant of performance after cerebellar lesion might be explained as a removal, in part, of OKR 'memory'. Thus the default performance after a saccade was smaller because the retinal slip signal was not amplified by the cerebellar loop, which caused a decrease in eye velocity.

Cerebellar inactivation after training centripetal post saccadic drift (leak), caused an abolition of the 'memory' as the time constant lengthened indicating less velocity drift after lesion. The overall stability of the fixation time constant after leak training followed by cerebellar ablation was similar to that obtained after instability training, suggesting that the eye fixation time constant might exhibit a default value. The long-term unstable eye fixation time constant observed after cerebellar removal, suggests that the cerebellum, itself, cannot be involved in the principal mechanism for fixation time constant stability and the underlying neuronal persistence. Since instability is theoretically an overcompensation, this condition would not be envisioned as possible by simple removal of the mechanism for persistence generation.

Cerebellar ablation and VOR gain and phase

In cats, complete cerebellar removal caused large VOR gain decreases and phase leads at low frequency rotation, but not at high frequency rotation, indicative of horizontal eye velocity-to-position neuronal integrator failure (Carpenter 1972; Godaux and Vanderkelen 1984). In previous studies ablation of the goldfish cerebellum and inactivation of the climbing fibers in cats resulted in an increase in VOR gain, with negligible changes in phase suggestive, in this case, retention of normal horizontal eye velocity-to-position integrator function (Demer and Robinson 1982; Marsh 1998; Michnovicz and Bennett 1987).

The VOR gain increase and minimal change in phase observed after cerebellar removal in goldfish was expected since removal of feedback inhibition should cause an increased neuronal excitability. The effect should be independent of rotational frequency as it occurs within the vestibular nuclei and not the PNI. Phase changes would only have been seen when the eye position time constant was less than the frequency of head rotation. Since the time constant as determined by eye position fixation was longer than the period of the stimulus frequencies used in this study, no major changes occurred in phase. If the integration mechanism was directly affected by cerebellar removal, a smaller gain and larger phase lead should have been seen at 0.125 Hz, but not at 1 Hz stimulation. The occurrence of a gain increase, if anything, would indicate an unstable eye velocity-to-position integrator.

During sinusoidal stimulation the eye position turn around points (Fig. 5-6&5-7) are independent of cerebellar influences on the vestibular nuclei, as goldfish vestibular neurons, in contrast to mammalian vestibular neurons, exhibit little eye position sensitivity. Instead a shift in eye position response dynamics are indicative of changes in the saccadic central pattern generator neurons. Although goldfish vestibular neurons lack eye position sensitivity, a schlagfeld sensitivity has been described (Green et al. 1997). The removal of cerebellar inhibition may have altered vestibular firing rates sufficiently to change eye position inflection points. One current hypothesis is that vestibular activity is forwarded through both the excitatory and inhibitory PNI neurons to medium lead burst neurons in the saccadic generator and alters their dynamics. This modification of saccadic pattern is similar to that presented in Chapter 4 to explain inflection point changes after interrupting the PNI interneuronal pathway.

Reversible cerebellar inactivation

The results of reversibly inactivating the vestibulo-cerebellum after modification of time constants are consistent with the ablation studies. Inactivation of Purkinje cells increased time constant stability in the majority of cases. The effects differed subtly from those of ablation in that a greater degree of time constant memory was retained after inactivation. Two reasonable explanations may underlie this occurrence. Irrespective of how careful the ablation, cerebellectomy is still a traumatic perturbation that causes short-term effects throughout the hindbrain. A spreading depression, which affects vestibular neurons, would cause a greater degree of leak. This condition would mask the minor degree of memory retention that may well be cerebellar independent and entrained within the Area II-vestibular nuclei circuitry.

A second explanation for a slight memory retention of both leak and instability training may have been incomplete inactivation of all the cerebellar circuitry by the injected anesthetic. Small amounts of lidocaine or bupivicaine were used to avoid diffusion throughout the hindbrain inactivating vestibular neurons. Although unlikely, the observed increase in time constant stability over the initial twenty minutes might have resulted from “training” to stability in the first few minutes of stationary visual feedback that occurred during injection of the anesthetic. Since half-life of changes in fixation time constant in the presence of stationary visual feedback is about 18 minutes (Major et al. 2004a), the increase in stability is believed due to the anesthetic effects on the Purkinje cell output.

The effects of anesthetics were not analyzed on naïve fixation time constants since a lack of changes could be interpreted as either the cerebellum is not involved in producing fixation stability or injection-related failure of the

electrode. Inactivation of the cerebellum permanently abolished time constant 'memory' since hours after injection the entrained memory did not return to the time constant prior to inactivation. This finding indicates that expression of the plasticity during memory requires a functional hindbrain-cerebellar circuitry. This conclusion is consistent with the observation that memory of both instability and leak decrease with time in darkness. When visual training signals are no longer actively modulating vestibular neurons, adaptive changes begin to deteriorate (Major et al. 2004a). In addition, the half-life of the memory observed in these experiments, is consistent with the idea that the proposed default PNI stability mechanism is modified by the vestibulo-cerebellar pathways (Major et al. 2004a). To maintain time constant values above or below the default value, modifications must be constantly entrained such that learning and memory are always occurring simultaneously.

Unfortunately, learning and memory of time constants could not be tested for a long enough time period with the local anesthetics utilized. Bupivicaine while a longer acting anesthetic than lidocaine, exhibited a half-life ~ 1 hr as determined by Area I injections. This is too brief to inactivate the cerebellum during learning, but not for testing memory. Future experiments could include utilizing an even longer acting tertiary amine anesthetic, or dialysis, to distinguish cerebellar roles during entrainment of fixation time constant plasticity and the expression of the memory. Since, time constant plasticity became robust after the effects of lidocaine disappeared, the behaviors observed were due to inactivation of the cerebellum and not permanent cerebellar damage due to the pressure injection methodology.

Long term time constant maintenance

Continuation of eye position holding time constants of minimally one, but close to two, orders of magnitude above that of the oculomotor plant months after ablation implies that the cerebellum is not necessary for functional activity within the PNI. Since goldfish were allowed to swim freely in the aquarium, constant visual stimuli were present over the course of recovery after cerebellectomy for self-tuning PNI time constants to stability. Thus all eye movement measurements are equivalent to a long duration plasticity experiment. In most cases, following cerebellar ablation eye position time constants increased towards instability. This observation implies that the eye position integrator exhibited a modicum of learning and memory, although reduced, from the long duration training apparent in naïve circumstances. An alternative hypothesis is that increases in the time constants was due to an innate mistuning of the principal mechanisms for the integrator time constant generation due absence of the visual slip feedback signal, i.e., complex spikes (shown in Fig. 5-14). If the increase in time constant was due to simple short-term plasticity mechanism, then the time constant should have changed to train asymptotically towards stability and never overcompensate.

Non-cerebellar plasticity mechanisms

The hypothesis of eye velocity plasticity in a hindbrain pathway is supported by the fixation time constant plasticity experiments in chronic cerebellectomized animals. In 38% of the trials, some evidence for learning was observed as eye velocity significantly improved during time constant entrainment. Although a robust memory of eye fixation drift to either instability or leak was absent after four hours of training in cerebellectomized animals, these results are in agreement

with the reduction of VOR plasticity when eye velocity gain changes were at most 30% of naïve values (Pastor et al. 1994a). It is conceivable that if time constant plasticity training had continued for extended periods of time, a significant memory component might have emerged. Regardless of this fact, the small amount of plasticity after four hours of training points out that latent mechanisms in the hindbrain cannot compensate for the loss of cerebellar pathways. Thus, expression of short-term post saccadic fixational drift modification requires the cerebellum.

Location of post saccadic eye velocity drift memory

The requirement of the cerebellum for robust expression of eye velocity memory, does not specify the cerebellum to be the location of the synaptic changes required for memory expression. Since qualitatively similar results were observed during fixation time constant and VOR gain plasticity in the goldfish, it is assumed that the same Purkinje cells pathways were utilized. During VOR gain plasticity, Purkinje cells do not change in either eye velocity or head velocity sensitivity, strongly suggesting that the actual site of modification is located within the brainstem (Pastor et al. 1997). This hypothesis is also likely to be true in post saccadic drift plasticity, since direct cerebellar-Area I connections do not exist. Diminished acquisition of time constant plasticity or VOR plasticity in the absence of cerebellar pathways is thus more due to loss of modulation than disappearance of the location of synaptic change. Since the vestibular nuclei are central elements in both cases, the parsimonious conjecture is that the time constant plasticity is not encoded within the cerebellum.

Eye position nystagmus

The effects of cerebellar removal on eye position drift is similar in

mammals and goldfish. In a classic study, it was observed that removal of the cerebellum caused both a decrease in the time constant and a wandering null position (Robinson 1974). There is some evidence in mammals indicating the cerebellum may not be absolutely necessary for eye position time constant stability. In neonatal hemi-cerebellectomy involving the flocculus and cerebellar cortex, long term eye position stability was maintained in some cases similar to findings in goldfish. Gaze holding failure only occurred in the extremes of the oculomotor range (Eckmiller and Westheimer 1983). Although goldfish eye position stability was found to be maintained in the majority of the oculomotor range, nystagmus was often present but largely occurred in the extremes of the oculomotor range. In these cases, drift direction was similar to large leaks in the PNI time constant. The changes in the saccadic pattern that were also observed in the VOR are the most likely explanation for the nystagmus observed. In the presence of a larger oculomotor range, eye position displacement from the null position in the orbit is greater and requires a larger maintenance force in the oculomotor plant. Anecdotal evidence suggests that the oculomotor range may exceed the functional range of PNI activity, since naïve goldfish trained to instability exhibit P-V plots that are somewhat sigmoidal in shape (Debowy et al. Unpublished; Major Unpublished). Thus at the extremes there appears a saturation phenomenon. As a result, the large time constant leak seen in the extremes of the oculomotor range could be due to eye position exceeding the physiological limits of PNI compensation. As a result, eye position would drift back to a null that is set at the level of PNI saturation. Additionally, due to excessive eye velocity, the saccadic generator induces a nystagmus.

Null position shifts

A persistent velocity bias was frequently observed throughout the oculomotor range as previously described in cats (Robinson 1974). Similar to the original logic used therein to explain null shifts, it can be suggested that occurrence of null shifts may be due to vestibular neuron firing rate fluctuations that are released by cerebellectomy (Robinson 1974). The occurrence of a large nystagmus, due to both a velocity bias extending throughout the entire oculomotor range along with shifting of the null position are a direct consequence of removing cerebellar inhibition. As mentioned previously, removal of the cerebellum has been shown to elevate the resting firing rate of vestibular neurons. Since the vestibular commissures presumably provide reciprocal feedback inhibition, the initial elevation in firing rate should not induce a bias, as the two nuclei remained balanced. However, any slight elevation and/or depression of the firing rates in a set of neurons will lead to the opposite response in the contralateral nucleus that will be reinforced by the feedback inhibition. This network phenomena would be integrated by the eye velocity-to-position integrator and shift the balance set point between the nasal and temporal integrator neurons and thus shift the observed null position. Large velocity biases leading to nystagmus can be treated as null positions that lie beyond the limits of oculomotor range (Robinson 1974). In the absence of external feedback provided by the visual system, the altered system dynamics in the vestibular nuclei will lead to a large velocity bias. Imposing visual feedback can re-balance the signaling between the two nuclei, and thus extinguish the velocity bias.

Horizontal eye velocity-to-position integration function

To determine if the lack of integrator plasticity was due to mechanisms

extrinsic to the PNI or whether the cerebellectomy simply prevented the appropriate training signals from reaching PNI, firing rates of Area I neurons were recorded during training. Since optokinetically driven time constant learning paradigms were paralleled by changes in PNI firing rates, adequate learning signals were conveyed to PNI. Hence, fixation plasticity is not likely to be encoded directly within the PNI as was previously parsimoniously inferred (Major et al. 2004b). Since the firing rate of PNI neurons remained position sensitive after entrainment to instability in either acute or chronic cerebellectomized goldfish, time constant memory is not located within the PNI nucleus. PNI neurons appear to encode eye position regardless of the memory state or experimental perturbation, hence the more global role of the PNI within the oculomotor system needs to be revisited. Area I does not appear to have a mechanism to alter the degree of integration to a given velocity signal as was previously implied (Major et al. 2004b). Since eye position time constant stability appears independent of time constant plasticity, it is conjectured that the principal mechanism of neuronal persistence is 'not' tuned within Area I. Instead, PNI appears to act more as a fixed unmodifiable integrator, whose time constant output is based upon the input eye velocity. One corollary is that if Area I utilizes a network mechanism to achieve persistence, then this network is not tunable by short-term (hrs) plasticity.

Preliminary experiments have shown that the velocity signal responsible for fixation time constant plasticity is reflected in the firing rates of Area II and Purkinje cells. Thus, it is assumed that this signal is also reflected within the firing rates of the vestibular neurons (Bassett et al. 2005; Beck et al. 2000). After time constant training to either instability or leak, a higher or lower tonic firing rate will be signaled to Area I during the memory component, which when

integrated by Area I, will cause a ramp in firing rate and a fixational drift.

Eye velocity-to-position persistent neural activity

The maintenance of eye position sensitivity and visually induced firing rate modulation was found immediately after cerebellar removal (Fig. 5-13) as well as weeks later (Fig. 5-14). This observation corroborates the idea that both learning and memory of fixation time constant plasticity are extrinsic to the eye velocity-to-position integrator. A more important consequence of this result is that since PNI neurons are still modulated during fixation time constant training after cerebellar removal, PNI neuronal persistence cannot be the simple consequence of integrator self-tuning. Since neither the learning or memory components appear to be intrinsic to PNI and time constant stability is maintained without the cerebellum, the primary mechanism which causes the persistence, and thereby the long time constant, must be independent of any short-term feedback control. If the PNI integrator persistence needed constant re-enforcement to maintain persistence over the long term, then eye position sensitivity and clear persistence of neuronal firing rates in the dark would not have been present days to months after cerebellar removal.

The principal mechanism by which time constant stability is achieved is still not resolved. A recurrent network mechanism producing eye position time constant stability and persistent neural activity appears unlikely for a variety of reasons. As stated in the introductory chapter each Area I contains ~ 50 neurons (Aksay et al. 2000; Pastor et al. 1994b) that were shown in Chapter 3 to be subdivided into two functional classes. Temporal and nasal eye velocity-to-position integrators are independent of each other and eye position time constant stability is independent of the PNI commissure (Chapter 4). As a result,

two local recurrent networks, nasal and temporal, would have be present within each Area I. Eye position time constant stability appears independent of the mechanism of short-term visual feedback and consistent with the absence of recurrent axon collaterals within the goldfish Area I nuclei (Aksay et al. 2000). Almost all network models of eye velocity-to-position integration that have relied upon the network are dependent on plasticity to maintain stable persistence. Due to the limited size of the neuronal network and the independence of time constant stability from time constant plasticity, it is most parsimonious to assume that each Area I neuron acts as an autonomous partial cellular integrator to generate neuronal persistence (Fig. 1-9A). The motor output that determines the time constant of the eye position holding is encoded by the Area I population as a whole projecting onto the target neurons.

Chapter 6: Conclusions

Monocular organization of oculomotor system

Animals with highly conjugate eye motions have long been envisioned to have independent, binocular versional and vergence eye movement control pathways (Hering et al. 1977). Due to this philosophy, theoretical models of eye motion were often simplified to represent the two eyes as a “single” eye (Robinson 1989; Seung 1996). Although some models of the horizontal eye velocity-to-position neural integrator exist for separately controlling the eye motions of each eye, the simulations were extensions of binocular based models (Cova and Galiana 1995; Cova and Galiana 1996). Representation of both eyes as a single, structured unit was frequently justified by the logic that the abducens internuclear interneurons yoked the temporal motion of one eye (e.g., left) with the nasal motion of the other eye (e.g., right) (Carpenter and Batton 1980; Highstein and Baker 1978). This reductionism led to the common experimental practice of recording eye motion from only one eye, and assuming that the motion in both were nearly identical. If the versional system was organized with binocular eye controllers, then the firing rates of the neurons in the eye velocity-to-position neural integrator (Area I) should all be equally well related with either eye. However, this was found not to be the case in goldfish (Chapter 3).

These results showed two clearly distinct populations of neurons localized within Area I, namely those related best to the ipsilateral eye position and those that statistically related equally well with either eye. The presence of this monocularity in Area I neurons suggests that vestibular and medium lead saccadic burst neurons must also be monocularly organized as they provide the

afferent input to Area I. Combined with similar findings in mammals, the overall conclusion is that versional oculomotor control is structurally monocular and the convergence between the two separate pathways produces a species specific variable extent of conjugacy as shown in Chapter 3 (Sylvestre et al. 2003).

The results of the cerebellar inactivation studies in Chapter 5 demonstrated that acquisition of time constant modification required intact hindbrain-cerebellar pathways. In addition, it was also shown in Chapter 3 that modification of the fixation time constant could be encoded separately for nasal and temporal eye positions of the two eyes, suggesting, indirectly, separate nasal and temporal plasticity pathways. Since other cerebellar-dependent oculomotor plasticities, such as OKR and VOR gain changes, also exhibited monocularity, distinct right eye and left eye pathways must exist in the brainstem and cerebellum (McElligott and Wilson 2001; Michnovicz and Bennett 1987; Pastor et al. 1994a; Weiser et al. 1989). The presence of ipsilateral and conjugate Area I neurons implies that at least some of the second order vestibular neurons must also encode monocularly-related eye velocity produced by putative monocularly-related Purkinje cells. Preliminary observations of the firing rates in pre-cerebellar Area II neurons supports this contention (Debowy and Baker 2006). Thus, the entire horizontal hindbrain oculomotor circuitry, possibly in all vertebrates, may be monocularly organized, but with the caveat that species-specific behavioral requirements determine the ratio of conjugate and contralateral eye sensitive neurons.

Mechanism of eye velocity-to-position neural integration

The theoretical mechanisms, described in the introduction, for eye velocity-to-position integration can be divided into four main classes. Velocity-to- position

integration could be generated solely or by a combination of 1) extrinsic-PNI networks, 2) excitatory intra-PNI networks, 3) crossed feedback inhibition, or 4) intrinsic cellular integration. In contrast to mammals, it is clear from the inactivation studies in Chapter 5 that post saccadic fixation time constant stability is much less cerebellar dependent in goldfish. Since the principal mechanism producing neuronal persistence and fixation stability does not require cerebellar pathways, the only other possible extrinsic-PNI connections would arise from the vestibular nuclei and/or from Area II. Both are considered unlikely since vestibular neurons and Area II neurons exhibit negligible eye position sensitivity in goldfish (Beck et al. 2006; Green et al. 1997). Since an external PNI network mechanism for persistence generation is excluded, then the persistence responsible for eye fixation time constants must be generated either within or between the Area I nuclei.

The results of Chapter 3 led to the conclusion that eye velocity-to-position integration for each eye is separately encoded by two sets of neurons within PNI. Since, as shown in Chapter 4, an average bidirectional eye position time constant of ~10s was maintained after midline lesion, then both temporal and nasal eye positions are independently controlled for each eye. These findings clearly suggest an organization within each Area I nucleus in which two separate populations of ipsilateral projecting neurons are co-localized. One population provides integration for ipsilateral temporal eye positions and the other for contralateral eye nasal positions. The contralateral Area I nucleus encodes the opposite half (nasal) of the oculomotor range for each eye. Thus, the bilateral Area I nuclei contain a minimum of four “seemingly” semi-autonomous integrators.

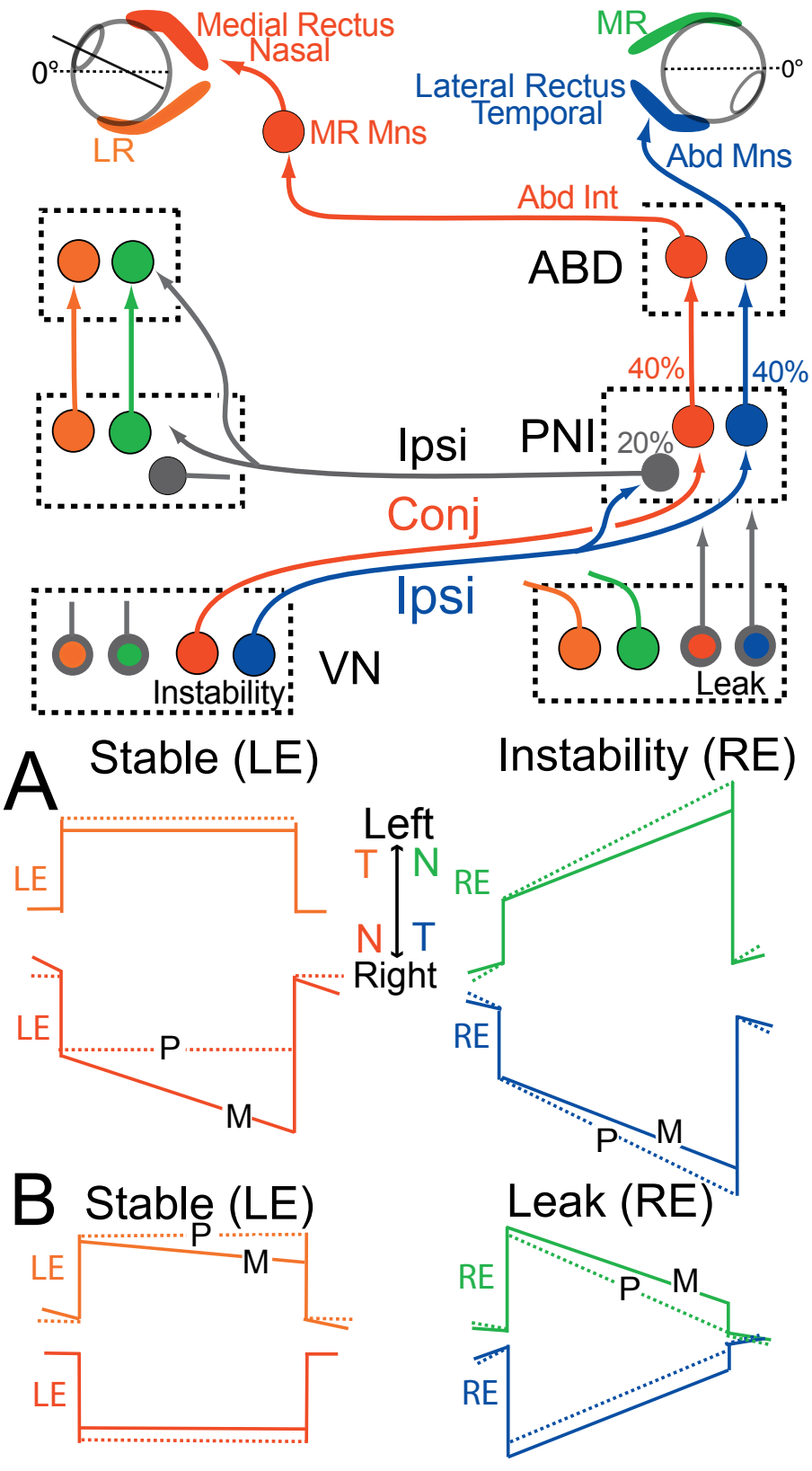
In addition to the ipsilateral projecting excitatory Area I neurons, there

exists a population of contralateral projecting Area I neurons. Based upon behavioral and physiological rationale (Fig. 6-1), these neurons are assumed to be inhibitory (Aksay et al. 2000). In Chapter 3, it was shown that during monocular optokinetic tracking ~ 60% of Area I neurons were ipsilateral-sensitive and ~ 40% were conjugate-sensitive. After acute midline lesion, a nasal shift in eye position was frequently observed and occurred in at least one eye, suggesting that Area I internuclear interneurons are part of the ipsilateral temporal eye position population. Since each Area I provides eye velocity-to-position integration for both nasal and temporal eye positions, it is assumed that the number of neurons are approximately equal for that purpose in the nasal and temporal integrator subgroups. Thus, a reasonable surmise is that ~40% of the neurons comprise the temporal integrator, another ~40% comprise the nasal integrator and ~20% are the inhibitory contralateral interneurons. This subdivision is in rough agreement with previous findings that ~ 1/3 (2/6) of Area I neurons intracellularly injected with biocytin projected contralaterally (Aksay et al. 2000). Thus the hypothesized 40-60 Area I neurons in each Area I nuclei is at minimum divided into three distinct functional cell types (Fig. 6-1).

The third potential mechanism of neuronal persistence generation, after excluding feedback inhibition and cerebellar pathways is an intra-Area I recurrent synaptic network. However, if this were to be the “principal” mechanism for eye velocity-to-position neural integration, then monocular encoding of eye positions by an intra-Area I network consisting of between 16-20 neurons would be a computational constraint. A ‘traditional’ intranuclear feedback network is considered an unlikely mechanism of neuronal persistence generation since no intra-Area I axon collaterals have been found after intracellular biocytin injections

Figure 6-1: Schematic of proposed vestibular (VN) and integrator (PNI) connectivity during monocular time constant plasticity.

Similar to Figure 3-5, but with this case the performance and memory paradigms are shown using the convention of left versus right eye. A: Bidirectional instability training for RE and either stability or occlusion for the LE. B: Bidirectional leak training for the RE and stability for the LE.


Medial Rectus
Nasal
0°
LR
MR Mns
MR
Lateral Rectus
Temporal
0°
Abd Mns
Abd Int
ABD
40%
40%
PNI
20%
Ipsi
Conj
Ipsi
VN
Instability
Leak
A
Stable (LE)
Instability (RE)
Left
T
N
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Right
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RE
P
M
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Stable (LE)
Leak (RE)
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LE
LE
RE
P
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RE

(Aksay et al. 2000). Thus, of the four major model types proposed, by default, the one most likely to form the major, possibly only, mechanism responsible for eye velocity-to-position integration is that of **intrinsic cellular properties**.

Role of PNI inhibitory neurons

The results of Chapter 4 in which midline connections between Area I were severed, provides some insight into the multiple roles of PNI interneurons. Based on the average of all experiments there was a slight diminution of the average fixation time constant from >30 to ~ 10s. This decrease is not surprising due to the mechanical effects of the lesions that completely bisected the midline. In light of the fact that the time constant remained >20s in many experiments as well as the occasional instability that could occur after midline lesion provides convincing evidence against a feedback “inhibition” mechanism of persistence generation. This view is reinforced, since instability is equivalent to more, rather than less, feedback (Goldman et al. 2003; Seung et al. 2000).

The mean eye position frequently shifted nasally in one, if not both eyes after midline lesion. Given the monocular pathway organization as determined by behavioral performance and plasticity, the PNI interneurons may largely, perhaps only, project to the conjugate PNI neurons and the abducens internuclear neurons controlling nasal eye position, thereby largely bypassing the “temporal” PNI neurons and abducens motoneurons (Fig. 6-1). Since PNI interneurons are presumed to be inhibitory, the lesion would be expected to disinhibit the abducens internuclear neurons directly, and then indirectly, by disinhibiting the conjugate PNI neurons. As a result, the higher firing rate of nasal controlling PNI neurons would shift the horizontal oculomotor null position and range nasally.

The saccadic pattern was notably affected by midline lesion as might

be predicted from the morphology of ipsilateral and contralateral projecting PNI neurons which extend rostral to the abducens nucleus into the location of the 'presumed' saccadic pattern generator (Aksay et al. 2000). Since saccadic amplitudes determine eye position and do not affect velocity-to-position integration per se, the change in scanning pattern is believed due to disrupting a balance between excitatory and inhibitory inputs to the saccadic pattern generator, in particular from the nasal eye velocity-to-position integrator. A net excitation, due to the lack of direct inhibition, in the central pattern generator as well as the disinhibition of the conjugate PNI neurons, evidently causes the saccadic generator to become active at smaller displacements from the zero eye position.

During low frequency VOR (< 0.125 Hz), the eye position at which eye velocity reverses direction, i.e. the mathematical inflection points, were shifted as shown in Fig. 4-1. This shift reduced the oculomotor range and could be argued to be caused by a reduction in inhibitory input to the saccadic pattern generator. Interestingly, the acute effects of removing PNI interneuronal inhibition appears to be compensated for in the saccadic central pattern generator, since scanning pattern was more like naive goldfish one week after the lesion.

Role of abducens internuclear interneurons in eye velocity-to-position neural integration

A consequence of the monocular structural and behavioral organization of hindbrain circuitry in goldfish is that maintenance of the fixation time constant stability for nasal eye position is equally as important a role for the abducens internuclear interneurons as that of "yoking" eye motion between the left and right eye. A direct projection of Area I neurons to medial rectus motoneuron has

not been found in goldfish (Aksay et al. 2000). Since the Ascending Tract of Dieters (ATD) vestibular neurons lack any significant saccadic and eye position sensitivity, the appropriate eye position signal for the medial rectus motoneurons must be relayed through the abducens internuclear interneurons (Green et al. 1997). This conclusion is strengthened by a comparison of animals with highly independent eye movements and comparable hindbrain circuitry such as flatfish (Graf and Baker 1985a, 1985b; Graf et al. 2001). Since eye motion is independent in saccadic direction, amplitude and timing, the nasal and temporal eye position-to-velocity integrators must be completely independent for each eye. In this species, pure contralateral and ipsilateral eye Area I populations must exist. Similar to all other vertebrates, abducens internuclear interneurons have been identified in flounder (Graf and Baker unpublished) suggesting that their evolutionary specialization may not have been to achieve conjugacy between the two eyes, as much as providing an essential conduit from the hindbrain vestibular and saccadic neurons to the medial rectus motoneurons. Investigation of PNI behavior in flounder would be a valuable future direction of study, since it would provide further insight as to the intrinsic organization of PNI in vertebrates. If inhibitory PNI interneurons are present, then they could only be arranged as a feed-forward pathway as hypothesized in Chapter 4 of this thesis.

ATD vestibular neurons exhibit a distinct embryological origin in rhombomere 3 as opposed to those projecting to the Abd Mns and Abd Int Ins that originate from rhombomere 6, although both subgroups lie within the descending octaval nucleus (Baker 1998). This difference together with the findings in this thesis have led to the speculation that early oculomotor hindbrain circuitry may have evolved with two purely structurally monocular pathways, one to control

temporal eye movements through Abd Mns and the other to control nasal eye movements through the ATD. However, given the evolutionary acquisition of binocular visual overlap (depth and motion cues), it became advantageous to move the eyes in a conjugate fashion during the majority of versional tasks. The emergence of Abd Int Ins in the abducens nucleus provided a structural substrate by which the monocular visual and to a large extent vestibular systems could take advantage in order to produce conjugate horizontal eye movements. The degree of species-specific monocularity or conjugacy of the Abd Int Ins could be determined by either vestibular commissural connections and/or cerebellar projections. Since the vast majority of naturally induced eye movements in goldfish are conjugate, the response of Abd Int Ins and PNI Ns logically appear conjugate under most experimental conditions (Fig. 6-1).

Role of the cerebellum in fixation stability and adaptation

Disrupting the midline between Area I and removal of the cerebellum noticeably, and somewhat similarly, affected the average fixation stability, saccadic pattern, and oculomotor range. However, the neural basis for the behavioral changes differed between the two perturbations, and a comparison of the two populations helps to better delineate the roles of the cerebellum and midline connections in PNI function. The direction of changes in time constant stability were similar after disrupting either the midline or cerebellum as an average time constant of ≥10s was more than an order of magnitude above the reported time constant of ~ 1s after Area I inactivation (Pastor et al. 1994b). In Chapter 5, a post-cerebellectomy time constant of ~10s was argued to represent a “default” value for the principal mechanism producing the time constant, especially since this eye position holding was found regardless of

initial time constant stability (leak or instability) before cerebellar removal. It is hypothesized, therefore, that the long time constant observed in 'naïve' goldfish is comprised of two mechanisms. The first component is the intrinsic 'fixed' time constant produced within PNI that represents the average persistence values for individual PNI neurons. The second component of a 'naïve' time constant is more likely the result of goldfish living and moving about in a normal visual environment that constantly self-trains the integrator time constant towards a greater stability measured as over a hundred second long time constant. This continual training is expressed in the eye position time constant by activity within the vestibulo-cerebellar pathways.

The hypothesis that naïve eye fixation time constant stability is composed of both an intrinsic and extrinsic mechanism is well supported by previous experimental findings. It was observed that naïve goldfish left in the dark for over an hour exhibited time constants that became leakier with an average stability of ~12s (Mensh et al. 2004). Second, goldfish left in darkness after time constant modification exhibited a recovery towards naïve stability regardless of training direction; however, the recovery half-life was over four times longer when the training was towards leak (Major et al. 2004a). In either case, when exposed to a visual surround, the time constant quickly adapted in less than thirty minutes to a naïve stability (Major et al. 2004a). Plasticity experiments strongly supports a 'dual component' Area I time constant hypothesis because cerebellar inactivation abolishes all memory of time constant changes (Fig. 5-3 & 5-4). More significantly, cerebellectomy prevents significant time constant modification by subsequent training. In contrast to cerebellar inactivation, midline lesions did not prevent learning and memory of time constant training,

thus the reduction of the naïve time constant after cerebellar ablation occurs by a different mechanism than after disruption of PNI inhibitory interneurons.

The optokinetic training stimulus producing eye tracking is reflected within Area I neurons after cerebellar removal. This modulation suggests that the lack of time constant plasticity is not due to the lack of an adequate 'learning' stimulus being conveyed to Area I neurons. Rather the result strongly supports the conclusion that the principal mechanism for time constant persistence is not modifiable by short term visual feedback. Thus Area I is not likely the site of plasticity for any of the observed changes in eye fixation time constants.

Although short-term modification of the integrator time constant did not occur after acute cerebellar removal, over the course of weeks to months the time constant tended to increase towards stability and some time constants became unstable as described in Chapter 5. This instability appeared in spite of visual feedback, as daily visual tuning of the integrator should have resulted in time constant stability. This suggests that increases in time constants might have been due to the principal "integrative" mechanism itself becoming unstable rather than a long-term functional recovery to stability. It is possible that cerebellar removal altered neuronal excitability in vestibular neurons which when superimposed on Area I resulted in the unstable time constants. Alternatively, cerebellar removal may have indirectly changed intrinsic excitability within Area I neurons and led to fixation instability. Either argument would strengthen the view that instability was due to modifications of intrinsic excitability of Area I neurons, and hence the time constant. As observed in Chapter 4, midline lesions occasionally resulted in unstable fixation time constants; however, in contrast to cerebellectomy, time constant plasticity elicited by visual feedback

training was still robust. In the clearest examples of time constants becoming unstable oscillations occurred within the fixations which are characteristic of interrupting inferior olivary pathways (Fig. 4-4D-F). Thus when instability was observed, climbing fiber activity would also have been disrupted, as also would be the case after cerebellar removal. These observations suggest that alteration of inferior olive activity mediated by complex spike activity of Purkinje cells may lead to instability. These result strongly suggest that the mechanisms of short-term modification of the time constant and the long-term modification of persistent neural activity within Area I are implemented by different cerebellar mechanisms. Thus, the mossy fiber system is more involved in producing the short-term visually-induced time constant plasticity. The inferior olive-climbing fiber pathway appears to affect the intrinsic excitability of Area I neurons, and is responsible for the long-term readjustment of the time constant.

Cerebellar removal also affected the saccadic central pattern generator. After cerebellar removal small saccades tended to occur at the maximal nasal and temporal eye positions (Fig. 5-10 & 5-11). However, in contrast to the saccades at extreme eye positions after midline lesion, the post saccadic fixations after cerebellar ablation tended to exhibit large drifts towards a centered eye position in a nystagmus-like pattern. The eye position drift after saccades at smaller deviations from zero tended to exhibit more stable time constants. The presence of more frequent saccades after cerebellar removal may have been due to changes in firing rate of either vestibular neurons projecting directly to the central pattern generator, or from Area I neurons after vestibular neuron disinhibition.

During low frequency VOR (< 0.125 Hz) the saccadic pattern was altered

after cerebellar removal such that the inflection points shifted and the oculomotor range increased. In contrast, after midline lesion, oculomotor ranges decreased suggesting the two perturbations affected the hindbrain circuitry at different sites. In contrast to midline lesion, cerebellectomy resulted in no consistent shifts in either mean eye position or range of eye deviations as well as no marked difference in nasal and temporal integrators.

Null eye position determination after cerebellar removal

Null eye position can be viewed as the location at which the agonist and antagonist forces on the eye plant are perfectly counterbalanced. Altering the balance between lateral rectus and medial rectus motoneuronal activity at a given eye position would shift null positions. Although cerebellar removal caused frequent shifts of the null position during spontaneous scanning that were similar to those after lesioning the midline, the mechanisms responsible for the shifts are likely quite different. The excitability and thus firing rates of both excitatory and inhibitory vestibular neurons should increase after cerebellar removal along with reduced synchrony within the vestibular nucleus, due to loss of Purkinje cell inhibition. Any fluctuation in the firing rate of a specific vestibular neuronal subgroup would be reflected by direct excitation of extraocular motoneurons and indirectly by activity of Area I neurons. Depending upon the efferent targets, either nasal or temporal, PNI activity would be elevated and accordingly result in an eye position null shift. This scenario is in contrast to midline lesions, in which the null shifts were likely generated entirely within Area I by de-coupling of the nasal and temporal integrators for each eye. This suggestion also explains why the velocity bias present after cerebellar removal frequently shifts direction within minutes, whereas the velocity bias after hindbrain lesion tended to be

unidirectional over long intervals (hours).

Mechanism of fixation time constant modification

Since the mechanisms responsible for the time constant plasticity are located within the hindbrain-cerebellar circuitry, not within PNI, a new structural model has been assembled to facilitate understanding of the neuronal pathways responsible for producing time constant modifications (Figs. 6-2 & 6-3) The results from Chapter 3 indicate that the neurons within these pathways must be monocularly encoding, since nasal and temporal changes in fixation time constants occurred separately for both training to leak and to instability. Although all time constant modifications were enabled through use of visual pathways, understanding the anatomy and physiology of the accessory optic system is not essential for considering the hindbrain mechanisms of time constant plasticity. Neither the 'learning' or 'memory' phases of plasticity could be encoded by direct pretectal AOS connections to the motoneurons, especially since time constant memory was tested in the absence of visual stimulation. Rather, it is more parsimonious to assume that memory is established through and/or within the vestibular nucleus, since goldfish vestibular neurons have been shown to exhibit an optokinetic eye velocity sensitivity (Allum et al. 1976; Dichgans et al. 1973; Green et al. 1997).

In Chapter 3, monocular time constant plasticity was demonstrated to be drift direction dependent, such that nasal drifts (ipsilateral vestibular nucleus) tended to be more monocular than temporally directed drifts (contralateral vestibular nucleus). This result is somewhat of an oversimplification however, since overtraining at large stimulus (>20°/s) velocities for extended periods (>4hrs) often resulted in bidirectional binocular time constant modifications.

In these cases, the eye viewing the training stimulus exhibited a more robust memory of the time constant changes. Given the lack of a significant role for PNI internuclear interneurons in plasticity along with the fact that monocularity was not dependent on nasal vs. temporal eye position, Area I cannot be the location of time constant plasticity. Since monocularity was dependent on drift direction, the pathway encoding the plasticity also should be responsible for eye velocity. Thus the memory of the time constant changes must be encoded by, and lie within, vestibulo-cerebellar pathways. Accordingly, a new model of neuronal connectivity was constructed that is built on previous diagrams in the literature, with the noticeable exemption that the current model is structurally monocular (Beck et al. 2006). Since the anatomical wiring connections and neurophysiological signals of nearly all of the neurons involved in hindbrain vestibulo-cerebellar pathways are known, the model is fairly well constrained. The connections least well established are those between the bilateral vestibular nucleus.

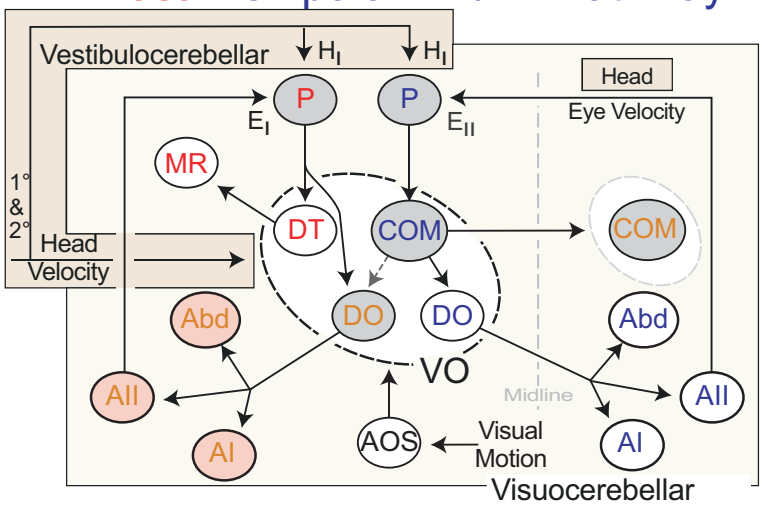
Extraocular motoneurons organization in vertebrates

Medial rectus motoneurons receive innervation from both ipsilateral ATD Ns and contralateral Abd Int Ins in all vertebrates (Nguyen et al. 1999; Reisine et al. 1981; Suwa and Baker 1996). However in contrast to Abd Int Ins, ATD Ns do not exhibit significant eye position signals but only those correlated with head/eye velocity (Reisine et al. 1981; Suwa and Baker 1996). The lack of a significant ATD eye position signal suggests that the eye velocity-to-position integrator does not project to the ATD Ns which agrees with the observed PNI morphology in goldfish (Aksay et al. 2000). MR Mns are also the only extraocular motoneuronal subgroup that does not receive a second order inhibitory pathway

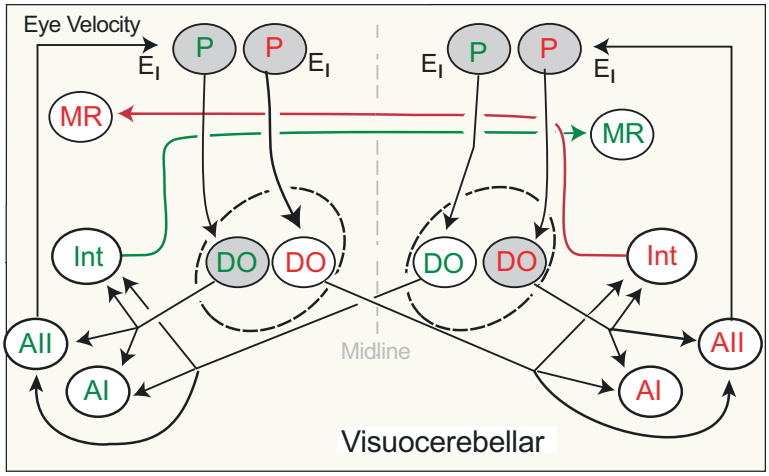
Figure 6-2: Proposed organization of nasal and temporal brainstem/cerebellar pathways.

Purkinje cells (P) with distinct head (H) and eye (E) velocity sensitivities innervate different subgroups of vestibular neurons in the descending octaval (DO) nucleus. Based upon the results from monocular fixation and VOR plasticity experiments separate DO neurons are hypothesized to project to the abducens motoneurons (**Abd**) and Internuclear interneurons (**Int**). An $H_{II}E_{I}$ inhibitory commissure interneuron (COM) in the vestibular nucleus is the hypothesized target of $H_{I}E_{II}$ and E_{II} Purkinje cells. The COM neuron projects to DO excitatory and inhibitory vestibular neurons projecting to the ipsi- and contralateral ABD, Area II (AII) and Area I (AI) nuclei, respectively. The Ascending Tract of Deiters (DT) and DO neurons projecting to AII and AI nuclei are the targets of $H_{I}E_{I}$ and E_{I} Int Purkinje cells. Area II axon collaterals to vestibular neurons are omitted. By convention the neurons shown are responsible for rightward eye velocity during either leftward head velocity and/or rightward visual motion. Inhibitory neurons are colored gray and excitatory neurons are white. AOS, accessory optic system. AII, Precerebellar Area II, AI, Eye velocity-to-position neural integrator Area I. Adapted from Beck *et al* 2006.

Nasal Temporal MR/LR Pathway



Nasal Internuclear MR/MR Pathway



Nasal Temporal MR/LR Pathway

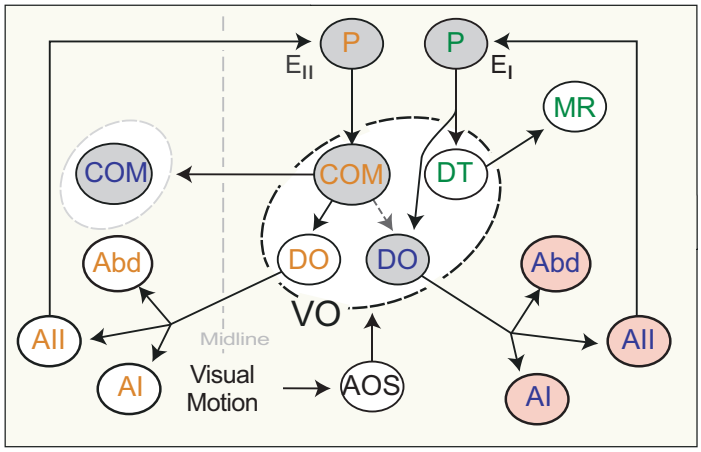
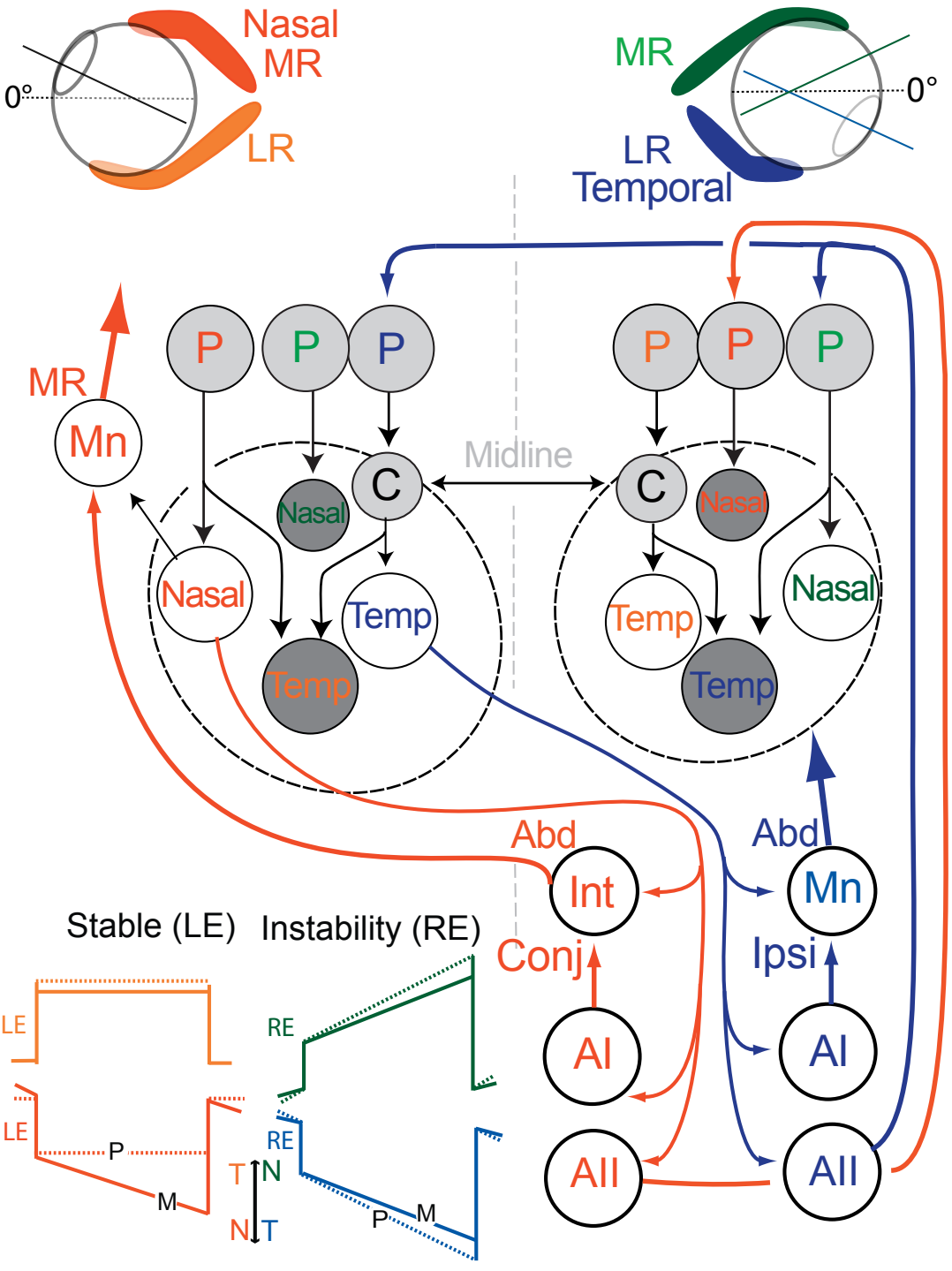


Figure 6-3: Simplified functional diagram of monocular brainstem/cerebellar pathways.

Nasal and temporal eye controllers are depicted to describe the signal flow producing monocular eye position to the right as illustrated in the behavioral inset. Left Eye Nasal vestibular neurons are regulated by E_I Purkinje cells and their signals diverge to contralateral Abd internuclear interneurons (Int), Area I (AI) and precerebellar Area II (AII) neurons. Ipsilateral $H_{II}E_I$ AII neurons regulate E_I Purkinje cells to control Left eye Nasal vestibular neurons. Right Eye Temporal vestibular neurons diverge to contralateral Abd motoneurons (Mn), AI and AII neuronal activity is regulated by E_{II} Purkinje cells. Accordingly, these AII neurons likely signal contralateral E_{II} and ipsilateral E_I Purkinje cells to regulate the Right Nasal and Left Temporal controllers. During monocular instability, cross-training occured when the eyes moved to the right (inset). The required convergence of signals are illustrated by the pathways shown to the Left Temporal and Right Temporal controllers. Inhibition between the bilateral temporal controllers is illustrated by the $H_{II}E_I$ commissural (C) pathway. For illustration clarity, LE Temporal and RE Nasal connections as well as all inhibitory vestibular signalling are not shown, but without doubt they are required to fully explain the illustrated monocular plasticity.


Nasal
MR
0°
LR
MR
0°
LR
Temporal
MR
Mn
P
P
P
P
P
P
Midline
C
Nasal
Temp
Temp
Nasal
C
Nasal
Temp
Temp
Nasal
Abd
Int
Abd
Mn
Conj
Ipsi
AI
AI
AII
AII
Stable (LE)
Instability (RE)
LE
LE
P
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RE
RE
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P
M

indicating that ATD Ns are only excitatory (Baker and Highstein 1978). In contrast, Abd Mns and Abd Int Ins in both mammals and goldfish receive a second order contralateral excitatory and an ipsilateral inhibitory vestibular connection (Fig. 6-1) (Graf et al. 1997; McCrea et al. 1980).

Eye and head velocity sensitivity of Purkinje cells and hindbrain neurons

Purkinje cells with eye velocity sensitivity that respond to ipsilateral or contralateral directed visual stimuli have been termed E_I and E_{II} respectively (Pastor et al. 1997). In goldfish Purkinje cell vestibular sensitivity was always to head rotation in the ipsilateral direction and termed H_I (Pastor et al. 1997). Five categories of Purkinje cells have been described in goldfish: 1) E_IH_I, 2) $E_{II}H_I$, 3) E_I, 4) E_{II}, 5) H_I (Pastor et al. 1997). When the distributions of Purkinje cell subgroups were determined it was found that about 60% were E_I sensitive, whereas only ~30% were E_{II} sensitive with the remaining 10% only head velocity sensitive (Pastor et al. 1997).

Interestingly, by contrast all precerebellar Area II neurons exhibited an E_IH_{II} sensitivity, with a small fraction exhibiting only an eye velocity sensitivity (E_I) (Beck et al. 2006; Pastor et al. 1994b). Area II neurons were found to project largely (75%) to both ipsilateral and contralateral granule cell layers through a cerebellar decussation (Straka et al. 2006). For comparison, Area I neurons also exhibit an E_I velocity sensitivity but with the addition of an eye position sensitivity (Aksay et al. 2000; Pastor et al. 1994b).

Eye velocity-to-position neural integrator and precerebellar neurons

Area I, Area II, and Abd Mns (Fig. 6-2 Top) and Area I, Area II and Abd Int Ins (Fig. 6-2 middle) represent two target populations that receive inputs from different vestibular neurons. The excitatory projection of Area I neurons is to the

ipsilateral abducens nucleus (Aksay et al. 2000). The inhibitory Area I neurons project to the contralateral abducens nucleus and Area I (Aksay et al. 2000). Study of the distribution and projections of Area II neurons has concluded that the majority of Area II neurons axons target both the ipsilateral and contralateral vestibulocerebellum. Retrograde tracing from the vestibulocerebellum has indicated that the vast majority (90%) of Area II neurons are located ipsilaterally (Straka et al. 2006). These observations suggest that the majority of Area II neurons likely target ipsilateral E_I Purkinje cells, and a smaller fraction, the contralateral E_{II} Purkinje cells (Fig. 6-2 & 6-3).

Purkinje cell projections to the vestibular nucleus

Vestibulocerebellar Purkinje cells only project to the ipsilateral vestibular nucleus (Straka et al. 2006). Direct inhibition has been demonstrated for ATD neurons and inhibitory vestibular neurons (DO) that project to the Abd motoneuronal pathway in mammals (Highstein 1973; Ito 1982; Sato and Kawasaki 1991) and goldfish (Baker Unpublished). In contrast, Purkinje cells do not directly inhibit excitatory vestibular (DO) neurons that target contralateral Abd Mns (Ito et al. 1977; Sato and Kawasaki 1991). Based on electrophysiological criteria in goldfish, the excitatory vestibular neurons that project to contralateral Abd motoneurons are not directly inhibited by cerebellar stimulation (Fig. 6-2) (Baker Unpublished).

Neurons in the vestibular nuclei are arranged in a push-pull reciprocal fashion with commissural neurons that are believed to be inhibitory. The vestibular interneurons most likely to be the target of E_{II} Purkinje cell inhibition are commissural neurons (Fig. 6-2 & 6-3). The commissures between the bilateral vestibular nuclei have been modeled as feedback inhibition, thus causing a

net functional excitation (Galiana and Outerbridge 1984). A current structural model of these vestibular neuronal connections is illustrated in Fig. 6-2. One extrapolation of this arrangement is that cerebellar pathways function in parallel with the VOR circuitry and in so doing amplify specific behavioral signals.

Based on monocular plasticity results, the model in Fig. 6-2 predicts three separate monocular vestibular pathways, in which conjugacy of eye movement is achieved by commissural vestibular connections. One monocular pathway controls the ipsilateral eye in the nasal half of the oculomotor range (Fig. 6-2 MR pathway). A second pathway controls the contralateral eye in the temporal half of the oculomotor range (Fig. 6-2 LR pathway). These two pathways are then combined in Fig. 6-2, as nasal temporal MR/LR pathways. The third pathway controls the abducens internuclear interneurons, as depicted in Fig. 6-2 (Int MR pathway). As mentioned earlier, due to monocular neuronal signaling of Area I neurons and monocular plasticity, vestibular neurons must also be assumed to be monocular encoding. This assertion is supported by the observation that Area I neurons can exhibit monocular VOR gain changes in which the control was vestibular rotation producing conjugate eye movements. Since VOR gain changes are predominantly acquired by cerebellar pathways, then Purkinje cells must also be monocular. Monocular pre-cerebellar Area II neurons also have been recorded during monocular optokinetic stimulation (Debowy and Baker 2006).

Nasal medial rectus pathway

Ascending Tract of Dieters vestibular neurons exhibit an E_{II} sensitivity and only signal nasal movements of the ipsilateral eye as shown in Fig. 6-2 (DT neurons in red). Purkinje neurons modulating DT vestibular neurons should have

an E_IH_I sensitivity. In addition, E_IH_I Purkinje cells directly inhibit the inhibitory vestibular neurons that project to the ipsilateral Abd Mns (DO). Since the red DT neuron and the orange inhibitory DO neuron control the medial rectus and lateral rectus of the left eye, respectively, they likely receive innervation from the same Purkinje cells because the two muscles should always be reciprocally related. It is also known that vestibular neurons that project to abducens motoneurons are the same neurons that project to Area I and Area II neurons. Since Area II does not receive a connection from or project to ATD vestibular neurons, the red E_I Purkinje cells are modulated by activity of the orange Area II neurons.

Temporal lateral rectus pathway

The temporal contralateral eye pathway is regulated by activity of $E_{II}H_I$ Purkinje cells. However, since excitatory vestibular DO neurons must exhibit an $E_{II}H_I$ sensitivity and do not receive direct cerebellar inhibition, the $E_{II}H_I$ Purkinje cells must inhibit E_IH_{II} vestibular interneurons that also are hypothesized to be commissural neurons between the bilateral vestibular nucleus (Com). The E_IH_{II} commissural vestibular neurons project to the contralateral vestibular nucleus and establish connections with the corresponding commissural neurons. Thus the neuronal signaling pathway that causes excitation of ipsilateral Abd Mns should indirectly modulate inhibition of contralateral Abd Mns. In addition, the Com neurons are also hypothesized to contact inhibitory DO neurons, thus yoking nasal motion of the ipsilateral eye with temporal motion of the contralateral eye through the activity of E_I Purkinje cells. However, Com projections to the inhibitory DO neurons are not necessarily required as yoking of the eye motion may be achieved solely by the commissural connection between the vestibular nuclei.

Conjugate abducens internuclear interneuron pathway

Due to the observed monocular behaviors in goldfish, Abd Mns and Int Ins are required to receive different sources of vestibular excitation. The internuclear interneurons should receive vestibular excitation from conjugate eye velocity encoding vestibular neurons, since conjugate Area I neurons have been recorded during monocular OKR. However, such a pathway alone would be considered unlikely, since contralateral-sensitive Area I neurons have been recorded after monocular VOR gain changes, indicating that at least some vestibular neurons are exclusively monocular. Excitatory DO vestibular neurons projecting to the Abd Int Ins should receive an identical cerebellar modulation as the ATD neurons. However, since no direct vestibular inhibition exists to MR Mns in vertebrates, inhibitory DO neurons that project to the Abd Int Ins must receive a unique E_I Purkinje cell modulation. The E_I Purkinje cells, shown in green, directly inhibit inhibitory DO neurons projection to ipsilateral Abd Int Ins, thus these Purkinje cells control motion of the contralateral eye in the nasal half of the oculomotor range. Since activation of the ipsilateral and contralateral medial rectus motoneurons/muscles are not obligatorily related, green E_I Purkinje cells do not project to DT neurons. In contrast to the nasal/temporal pathway in which the Area II neurons potentially targets both ipsilateral and contralateral Purkinje cells, Area II neurons in the Abd internuclear MR pathway can only have an ipsilateral cerebellar projection.

Combined results and predictions of the structural model

The wiring diagram overlain with head and eye velocity sensitivity is consistent with predictions of the types and relative numbers of Area II neurons and Purkinje cells. In agreement with experimental data, the model predicts

roughly twice as many E_I Purkinje cells as E_{II} Purkinje cells (Pastor et al. 1997). The model also predicts that at least half of the Area II neurons should have bilateral cerebellar projections; however, unilateral Area II cerebellar projections should also be present. These calculations are in rough agreement with the experimental results (Straka et al. 2006). The only major issue is that the model as drawn does not provide sufficient Area II neurons which target only E_{II} Purkinje cells, however some Area II neurons may only project contralaterally, and not bilaterally, to the cerebellum.

Simplified theoretical model centered on nasal/temporal control

A simplified version of the model shown in Fig. 6-3 was constructed to show how nasal and temporal control of eye movements might be coordinated by hindbrain/cerebellar pathways. Due to the eye velocity sensitivity of Purkinje cells, ipsilateral projecting Area II neurons regulate activity of nasal Purkinje cells and contralateral Area II neurons, temporal Purkinje cells. The potential key sites for time constant plasticity are between the Purkinje cells and the three vestibular subgroups. Thus three distinct classes of Purkinje cells based on targets must exist in each vestibulocerebellum. The ipsilateral nasal Purkinje cell that has an E_I sensitivity projects to DT neurons, either excitatory DO neurons to Abd Int Ins and inhibitory DO neurons to ipsilateral Abd Mns. A separate set of E_I Purkinje cells project to inhibitory DO neurons controlling the Abd Int Ins. A third class consisting of E_{II} Purkinje cells inhibit the commissural inhibitory vestibular neurons. Since monocularity and conjugacy of the training paradigms were based upon drift direction and <u>not</u> eye position, the sites of time constant plasticity must be encoded within vestibulocerebellar pathways and <u>not</u> in Area I. Since visual pathways to the pretectum completely decussate within the midbrain, the hindbrain vestibular commissures must be the pathway

responsible for “cross-training” conjugacy observed during memory (Fig. 6-3 inset). Clearly time constant cross-training was more robust when eye position drift was in the E_{II} direction.

Monocular conjecture and contemplation

From the above simplified working scenario, some interesting suppositions can be drawn from this thesis work. First, the observation of robust monocular behavior in goldfish is surprising since the vast majority of ‘learned oculomotor experience’ occurs in a conjugate visual environment. Due to the absence of a fovea, goldfish are entrained for conjugate eye movements. The experimentally observed monocularity, both behavior and plasticity, suggests that the hindbrain pathways controlling eye movements were formed from predetermined ‘monocular’ embryological origins. Altogether, these findings strongly support a ‘learned’ conjugacy much like that first proposed by Von Helmholtz. By contrast, based on developmental origin of the above hindbrain monocularity, it is highly likely that ‘monocularity’ is operationally acquired by the cerebellum.

Another extrapolation from the simplified circuit model is that a primary role of the abducens internuclear interneurons is to exclusively provide the eye position signal essential for the nasal half of the oculomotor range. This conclusion is strengthened by the observation that ATD neurons in all vertebrates provide, at most, a minimal eye position signal. In consequence, the abducens internuclear interneurons ensure conjugacy of the eyes as argued by the Von Helmoltz scheme rather than the obligate structural conjugacy envisioned by Hering.

Visual motion detection was discernibly monocular from evolutionary appearance of eyes with temporal and nasal pathways to extraocular motoneurons arising as soon as eye muscles appeared in jawless vertebrates

(Baker and Gilland 1996). When these visual pathways expanded through the hindbrain, vestibular neurons were co-opted to augment optokinetic reflexes, thus vestibular neurons became even more ‘obligatorily’ monocular. Thus the visual sensory system itself promulgated hindbrain monocular organization. Evolutionary rationale also suggests that Abd Int Ins co-evolved with the hindbrain gaze system allowing saccadic resetting of the eye position in the orbit and centrally coordinating hindbrain nasal and temporal movement of the two eyes. Requirements related to monocular development of the gaze system in addition to those of the visual pathways enhanced the monocularity of Abd Int Ins.

Therefore, It is more than likely, and prudent to accept that eye movements were monocular from early evolutionary onset due to selective pressure existing from both the sensory and motor systems. The inevitable conclusion is: Binocular vision required monocular eye movements.

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