Transparent motion and compensatory eye movements in the rabbit

Transparante beweging en compensatoire oogbewegingen in het konijn

Proefschrift

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Omslag illustratie: de foto's geven een indruk van het retinale beeld bij bewegings paralax. Wanneer naar de boom gekeken wordt en men zich met een stap zijdelings verplaatst, verplaatsen de objecten voor de boom zich in tegengestelde richting en achter de boom in de richting van de verplaatsing. De achterste boom beweegt zich transparant ten opzichte van de boom waar naar gekeken wordt.

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Voor Zubin
en Anita
en mijn ouders.

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1

General Introduction

This thesis studies how the eye movements of the rabbit respond to transparent motion, and what the neural basis is of this behavior.The purpose of Chapter 1 is to provide insight in the most important aspects of the underlying literature and theories.

1.1 Eye movements in the rabbit

In order to stabilize the visual world on the retina, all vertebrate species are provided with oculomotor stabilization reflexes. The evoked eye movements, called nystagmus, consist of compensatory slow phases to stabilize the retinal image and fast resetting eye movements to keep the eyes in their oculomotor range. Compensatory eye movements are generated by the vestibular system and the optokinetic system (Baarsma and Collewijn, 1974).

The vestibular system generates the vestibulo ocular reflex (VOR), which consists of compensatory eye rotations opposite in direction to head movements. The VOR can be further subdivided into the linear VOR originating from the otoliths which sense linear acceleration, which is beyond the scope of this thesis, and the angular VOR originating from the semicircular canals which sense angular acceleration.
The optokinetic system activates the optokinetic reflex (OKR) which is triggered by *retinal slip* (movement of the visual world relative to the retina). It rotates the eye in the same direction as the moving visual world. Its function is to prevent the visual image from slipping across the retina, thus enabling detailed visual analysis.
Although in laboratory conditions the VOR is commonly measured in isolation -that is in darkness - under natural conditions head movements are usually generated in an illuminated environment. In this situation the VOR and OKR work in synergy resulting in the vestibulo optokinetic reflex (VOKR).
The rabbit is often used as a model to study these reflexes and the neuronal circuits involved since its reflexive oculomotor behavior in head fixed conditions is not contaminated by goal directed eye movements (saccades) or smooth pursuit of visual targets (Collewijn 1981). These voluntary eye movements which are present in more evolved species such as primates and humans, are controlled by higher order cerebral control systems. They direct the eyes such that the projection of a target is precisely on the fovea, a specialized central spot on the retina with high visual resolution.
The rabbit lacks a fovea. Instead, the retina of the rabbit has an area with elevated density of receptive neuronal elements, 'the visual streak'. This elongated receptive area which is aligned with the horizon together with the lateral position of the eyes in the head, provides the rabbit with panoramic vision (Hughes 1971), and eliminates the necessity to make precise scanning eye movements.

1.1.1 Compensatory eye movements and transparent optic flow

The seemingly simple organization of compensatory eye movement reflexes in the rabbit becomes more complicated when we take real world situations into account. In the real world all species are very often confronted with transparent optic flow, which is the situation when two or more visual patterns with different motion characteristics are simultaneously present on the retina. In daily life, such transparent motion is encountered when one looks at moving objects through a visual medium (e.g. snow, bushes, or a dirty window). Furthermore, in an environment where not all visual objects are at the same visual distance self-motion generates motion parallax, both during translation and rotation about an axis that does not intersect both eyes. This can easily be seen if you hold your hand at about 30 cm from your face and spread your fingers. If you rotate your head about the yaw-axis (shake "no"), the view of your hand moves with respect to the background. Thus, paralax also gives rise to transparent optic flow vectors on the retina (see cover of thesis).
During transparent visual stimulation, eye movements are not capable of compensating all retinal image motion, since the various motion components cannot all be compensated for simultaneously. So how do OKR and VOKR deal with this transparency problem? When transparent visual stimuli are presented to humans, they can by selective attention generate eye movements to (alternatingly) track one visual pattern, while ignoring the other (Kowler *et al.*, 1984; Howard and Gonzales, 1987; Niemann *et al.*, 1994). Although it is likely that foveal pursuit mechanisms play a role in this behavior, the distinction between the contribution of involuntary global OKR and voluntary foveal pursuit remains an issue (Niemann *et al.*, 1994; Mestre *et al.*, 1997).
Because the rabbit lacks a fovea and therefore voluntary pursuit mechanisms this animal is an interesting model to study global OKR and VOKR under transparent conditions, (*Chapter 2*).

To understand where and how transparent in the rabbit optic flow in OKR and VOKR may be processed, a short description of the most relevant characteristics of the VOR, OKR and their neuronal circuitry is described below.

1.1.2 How to quantify compensatory eye movements

To measure the VOR and or OKR, the rabbit can either be sinusoidally rotated or at a constant speed. In this thesis we exclusively used sinusoidal stimulation while recording the eye movements (vestibular nystagmus). After offline removal of the fast phases the slow phase compensatory eye movements are reconstructed and compared to the corresponding stimulus motion.

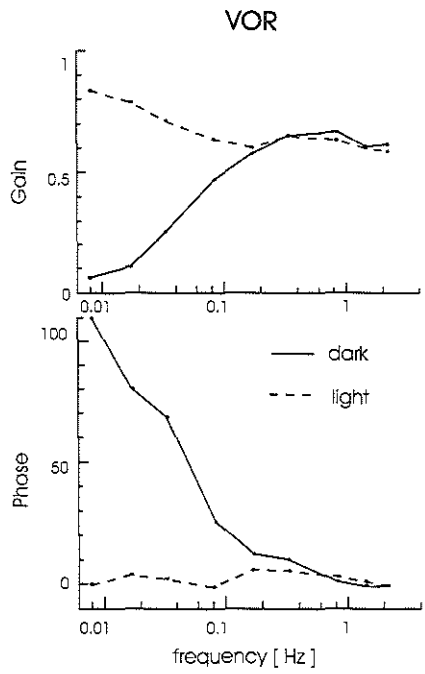

Figure 1.1. *Gain and phase of compensatory eye movements resulting from passive oscillations of the rabbit about the yaw axis as a function of frequency, measured in the light (interrupted lines) and in darkness (continuous lines). Oscillations were performed with amplitudes of 10°, except for the highest frequency for which it was 5°. Average values of three rabbits (Collewijn, 1981).*

A common method of quantifying the VOR/VOKR or OKR involves the use of bode plots that look at gain and phase as a function of frequency of head movement (see Fig 1.1). ***Gain*** is defined as the slow-phase eye velocity divided by head velocity or the ratio between the amplitude of the slow-phase eye movement and the amplitude of the head rotation. ***Phase*** is defined as the phase angle in degrees (°) between eye movements and head movements. If the VOR and/or OKR was completely compensatory, it would have a gain equal to 1.0 (unity) and no phase deviation.

1.2 Neuronal circuitry of the horizontal vestibulo ocular reflex (VOR)

The VOR has its origin in the hair cells of the vestibular apparatus. The hair cells send their afferent signals through neurons located in Scarpa's ganglion, which project to the vestibular nuclei (Fig 1.3). Neurons in the medial vestibular nucleus (MVN) receive the horizontal semi-circular canal afferents, which on their turn project directly to the oculomotor and abducens nuclei. These nuclei innervate the medial and lateral eye muscle, respectively. This pathway is the classic three-neuron-arc first described by Lorente de Nó (1933).
As a side path of the three-neuron-arc, secondary neurons in the MVN project through the mossy – parallel fiber (MPF) pathway on the dendritic tree of the Purkinje cells (P-cells) in the cortex of the flocculus. The P-cells relay back with inhibitory output (GABA) to a special group of neurons in the MVN , the so-called floccular receiving neurons (FTN's) (Stahl and Simpson, 1995).

1.2.1 Dynamic properties of the VOR

The semicircular canals mechanically integrate head acceleration and provide the brain with head velocity information (Wilson and Melvill-Jones,1979). When the stimulus frequency is higher than 0.1 Hz a velocity signal is generated, below that frequency it gradually becomes more a head acceleration signal. (Collewijn, 1981). The brain integrates this signal once more before an efferent position signal reaches the eye muscles.
When the frequency of head oscillation falls below 0.2 Hz the VOR gradually becomes less effective resulting in a gain decrease and increase in phase lead.

1.3 Neuronal circuitry of the horizontal Optokinetic reflex (OKR).

The origin of the optokinetic reflex is found in the on-type directional selective retinal ganglion cells(Oyster et al, 1980), which detect retinal slip (Barlow et al, 1964). These cells project through the optic nerve to the three (medial, lateral and dorsal) accessory optic terminal nuclei (AOSn) and the nucleus of the optic tract (NOT; Fig 1.3). The excitatory input from the NOT and the dorsal terminal nucleus of the AOS is relayed to the caudal dorsal cap of the IO (De Zeeuw et al, 1994, Tan et al, 1995, Nunes Cardoso and van der Want 1990, Simpson et al, 1979). The IO caudal dorsal cap sends projections to P-cells in the floccular vertical axis (VA) zones, which project to the oculomotor nuclei by way of the MVN. Electrical stimulation in these vertical axis (VA) zones revealed involvement in generation of horizontal eye movements (Van der Steen et al 1994).

1.3.1 Dynamic properties of the OKR

The properties of pure OKR are illustrated in a bode plot for monkey data (Fig 2, adapted from Paige, 1985). It demonstrates that the OKR is most effective up to approximately 0.2 Hz, for higher frequencies gain and phase decrease. In rabbits the OKR gain saturates at a maximal stimulus velocity of about 2°/s (Collewijn, 1981).

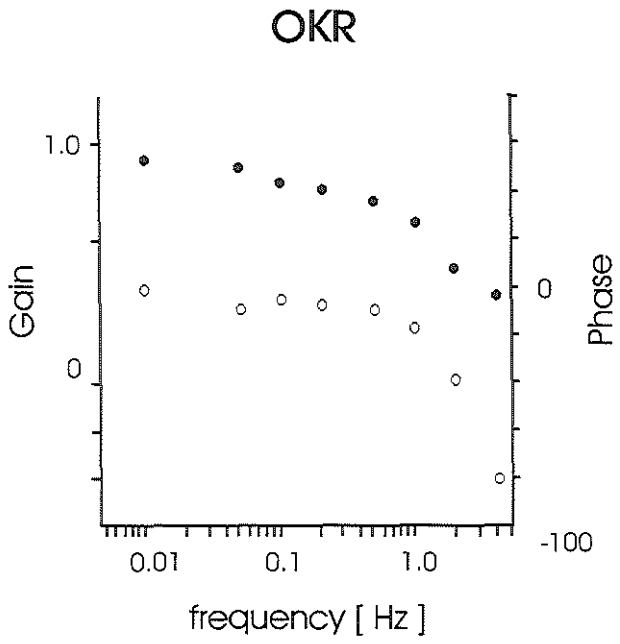

Figure 1.2. *Gain and phase of compensatory eye movements resulting from optokinetic stimulation about the yaw axis in monkeys as a function of frequency. The solid symbols correspond to the gains, the open symbols to phases. Amplitudes were adapted to the frequency to reach a constant maximal stimulus velocity (of 40°/s) (adapted from Paige, 1985).*

1.3.2 Dynamics of vestibulo-optokinetic interactions

The dynamics of the VOR and OKR are reciprocally organized. In the light the optokinetic component of the VOKR efficiently reduces the phase lead to almost zero and increases the gain to a higher level in the low frequency region which decreases with increasing stimulus frequency up to 0.2 Hz where the VOR component dominates.
For the frequencies and velocities tested in Fig1.1 it is reasonable to assume that for the VOKR gain, the VOR and OKR gain can be vectorially summed in a linear way, where the OKR deals with the slip that remains from an imperfect VOR (van Neerven, thesis)

$$G_{VOKR} = G_{VOR} + G_{OKR} (1 - G_{VOR})$$

Both OKR and VOR have adaptive properties. When the animal is subjected to prolonged optokinetic stimulation the gain of the OKR increases. In experiments by Collewijn and Kleinschmidt (1975) OKR gain increased from 0.65 to 0.8 after 4 hours of stimulation at a frequency of 1/6 Hz. This type of adaptability is, similarly to the VOR, frequency specific and was used in one of our experiments (*Chapter 5*).

1.4 Sites of VOR and OKR interaction and the cerebellar flocculus.

Visual and vestibular information can interact on several places in the brain. One of the sites where visual-vestibular interactions take place is the flocculus, which is part of the vestibulo-cerebellum. Electrophysiological (Graf et al., 1988; Leonard et al., 1988) and lesion studies (rabbit: Ito et al., 1982; Nagao, 1983; Barmack and Pettorossi, 1985; monkey: Zee et al., 1981, Waespe et al., 1983) have shown that the flocculus is intimately involved in the generation of the VOR and OKR. For this reason we focussed on the flocculus to study visuo-vestibular interactions during transparent motion (*Chapter 3 & 4*). Another advantage is that in this system all the major inputs and outputs related to VOR and OKR have been identified. Also the major characteristics of the response patterns of floccular P-cells to most natural visual and vestibular stimulation are known (Graff et al., 1988; De Zeeuw et al, 1994).

The rabbit flocculus consists of five zones (1,2,3,4 and C2) whose border can be delineated in the flocculus white matter using acetylcholinesterase staining (Tan *et al*, 1995, De Zeeuw *et al* 1994, Van der Steen *et al*, 1994). Two of these zones (2 and 4) receive CF projections from the IO caudal dorsal cap and have been electrophysiologically characterized as directionally sensitive to optokinetic stimulation about the yaw axis (Tan *et al*, 1993). The other zones are related to compensatory eye movements about the anterior and posterior canal planes except for the C2 zone which is involved in head movements (de Zeeuw 1997).

The Purkinje cells (P-cells) generate the sole output of the flocculus, which is an inhibitory efferent signal to the cerebellar nuclei. The input the P-cell receives is conveyed through two distinct pathways, the climbing fiber (CF) and the mossy – parallel fiber (MPF) pathway (Voogd et al., 1996). Both are excitatory inputs. Each P-cell receives a single CF projection, which exclusively originates from the neurons in inferior olive (IO). A CF produces a powerful excitation of the P-cell by way of 300 synapses, which always generates a complex spike (CS) (Eccles et al., 1966; Thach, 1967). Since the CS is an all-or-none response, it is a copy of the activity of neurons in the inferior olive. MPFs, have many sources within and outside of the cerebellum. In contrast to the CF projection, one P-cell receives about 100.000 – 200.000 projections from different MPFs. Many excitatory signals from MPFs lead to a simple spike (SS) discharge by the P-cell. SS occur spontaneously at about 50 spikes/sec in contrast to CS at 1 spike/sec.

Micro-injections of neurotransmitters and or neuromodulators in the flocculus can selectively modify the gain of VOR and OKR. Functional inactivation of

the floccular signal transmission by GABA agonists reduces the gain of the VOR and OKR (van Neerven et al., 1989). The opposite effect can also be achieved: Bilateral injections of cholinergic agonists (e.g. carbachol, an aselective cholinergic agonist) increase the OKR gain and to a lesser extent the VOR gain (Tan and Collewijn, 1991; *Chapter 3 & 5*).

P-cells in both VA zones send inhibitory projections to the MVN (Fig 1.3). This is one of the pathways through which VOR receives its is visual feedback. Potential sites for visual vestibular interaction in that scheme are the flocculus and the MVN. Other P-cells in these zones are part of a module (Groenewegen,1979, Voogd and Bigaré,1980) in which inhibitory projections are send to the nucleus prepositus hypoglossi (PH) which sends its inhibitory efferents to the caudal dorsal IO (De Zeeuw *et al* 1994).
MPF projections to the floccular HA-zone are derived from the nuceus reticularis tegmentum pontis (NRTP) which relays retinal slip velocity signals and head velocity signals as well (Kano et al 1991). Other major MPF projections to the flocculus are from the MVN and the nucleus prepositus hypoglosi (PH) (Tan et al 1992). In addition to optokinetic and vestibular information, proprioceptive information of the eye muscles reaches the flocculus as well (Maekawa and Kimura, 1980). In cats and monkeys an efferent copy of eye position information is derived from the PH (Nagao et al, 1997). Recent pilot experiments in rabbits have demonstrated that the PH provides the flocculus with horizontal eye position signals (Arts *et al,* 2000) direct anatomical pathways have not been demonstrated.

In this thesis stimulation versions of OKR, VOR and VOKR were confined to rotations about a vertical axis corresponding with optimal stimulation of the horizontal semi-circular canals, the 'yaw axis'. This earth vertical axis is centered on the midpoint between the eyes of the rabbit. One of the reasons for this restriction was that stimulation about any other axis would contaminate the isolated signal of the semicircular canals with signals from the otoliths, which are sensitive to gravity. The subsequent eye movement recordings were restricted to the principally horizontal directions.

1.5 Outline of this thesis

In chapter 2 we investigated how the OKR in response to a transparent visual stimulation depends on the luminance of the individual flow components, and in what way this relation is affected by concurrent vestibular stimulation.
In chapter 3 bilateral carbachol microinjections in the flocculus were used to study wether the flocculus is involved in processing of transparent optic flow in OKR and VOKR and if so wether and how this effect is affected by cholinergic neuromodulation.
In chapter 4 we investigated wether and how the elevated OKR gain after bilateral carbachol micro-injections in the flocculus is affected by natural OKR adaptation using prolonged non- transparent OKR stimulation.
In chapter 5 P-cell recordings were performed to study what the CS encode during transparent OKR, and how P-cell activity is affected by several relative luminances of the transparent patterns.

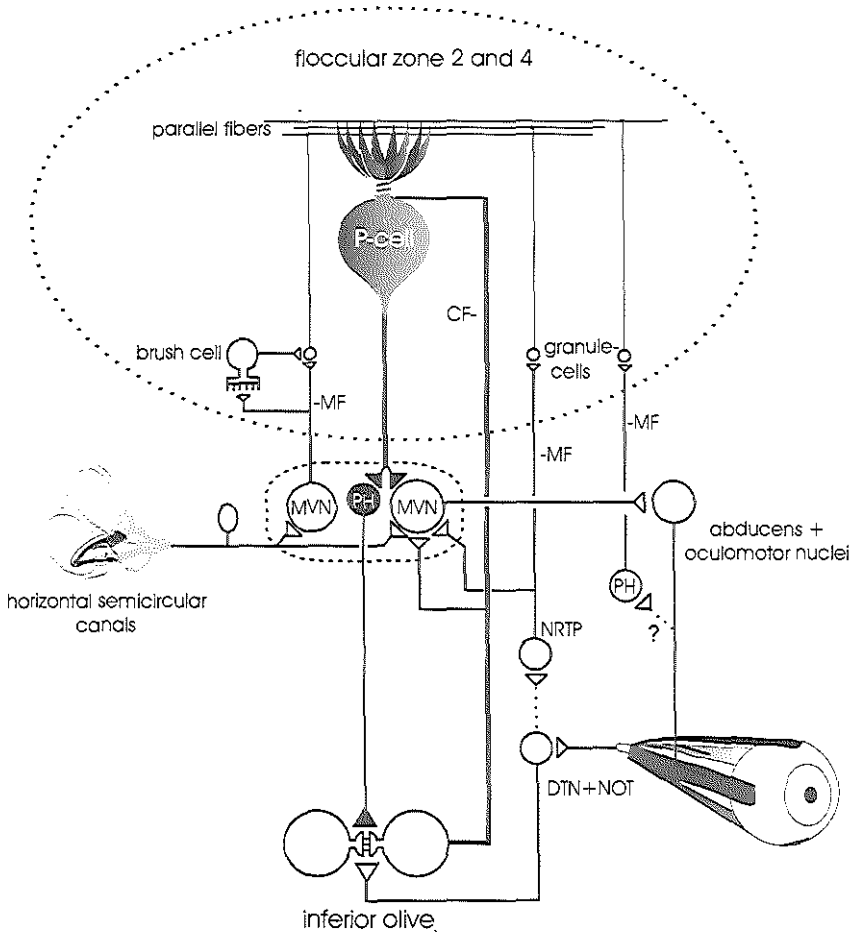

Fig 1.3 *This schematic model illustrates the neuronal connections of the horizontal VOR and OKR pathways and their interactions. For abbreviations see text. Olivary neurons are electronically coupled by gap junctions (Llinas et al 1974, De Zeeuw et al, 1989). This coupling is influenced the by inhibitory and excitatory inputs (De Zeeuw et al, 1989, Simpson et al 1996). Unipolar brush cells are inter-neurons which amplify the mossy fiber signal to granule cells. Note that the PH is drawn twice for clarity of presentation. Adapted from drawing by SKE Koekkoek (Koekkoek et al. 1997).*

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2

Visual-vestibular interaction during transparent optokinetic stimulation in the rabbit[1]

[1] A.L. Mathoera · M.A. Frens · J. van der Steen, Exp Brain Res (1999) 125:87–96

2.1 Introduction

During transparent optic flow, two or more visual motion patterns are simultaneously present on the retina. In daily life, such transparent motion is encountered when one looks at moving objects through a visual medium (e.g. snow, bushes, or a dirty window). Furthermore, in an environment where not all visual objects are at the same visual distance, self-motion generates motion parallax, during both translation and rotation about an axis that does not intersect both eyes. Parallax can also give rise to transparent optic flow vectors on the retina.

In order to stabilize the visual world on the retina, all vertebrate species are provided with oculomotor stabilization reflexes. The most important are the vestibulo-ocular reflex (VOR) and the optokinetic reflex (OKR; Baarsma and Collewijn 1974). The VOR originates in the semicircular canals whose output represents the angular velocity of the head. It elicits compensatory eye movements, which are opposite in direction relative to the head movements. The OKR is triggered by *retinal slip* (movement of the visual world relative to the retina), which elicits reflexive compensatory eye movements with a slow component that has the same direction as the moving image.

During transparent visual stimulation, eye movements are not capable of compensating all retinal image motion, since the various motion components cannot all be compensated for simultaneously. In humans, transparent visual stimuli cause eye movements to (alternatingly) follow one visual pattern while ignoring the other (Kowler et al. 1984; Howard and Gonzales 1987; Niemann et al. 1994). It is likely that foveal pursuit mechanisms play a role in this behavior. The distinction between the contribution of involuntary global OKR and voluntary foveal pursuit in the eye movement response remains an issue, even when the contribution of the pursuit system is minimized (Niemann et al. 1994; Mestre and Masson 1997). The rabbit does not have a fovea and does not possess a smooth pursuit system (Collewijn 1977). Therefore, since slow eye movements in the rabbit are generated as part of postural reflexes, the rabbit is an ideal model in which to study compensatory reflexive eye movements in response to transparent visual stimulation.In this chapter we describe how horizontal OKR and the combined horizontal vestibulo-optokinetic reflex (VOKR) of the rabbit are affected by fullfield transparent optokinetic stimulation. The purpose of this study is to investigate how the OKR in response to a transparent visual stimulus depends on the luminance of the individual flow components and in what way this relation is affected by concurrent vestibular stimulation. Preliminary results of these experiments have been published in abstract form (Mathoera et al. 1997).

2.2 Materials and methods

2.2.1 Animal preparation

Six young adult female pigmented Dutch belted rabbits were used. About 1 week prior to the experiments, the rabbits were implanted with permanent

scleral search coils in both eyes for eye movement recording. A coil of five turns of insulated stainless steel wire (Teflon- bioflex wire, type AS 632; Cooner Sales, Chatsworth, Calif.) was woven underneath the conjunctiva, the superior and inferior rectus muscles, and the inferior oblique muscle. Also skull screws were mounted for fixation of the head. Surgical procedures were carried out under general anesthesia, induced by ketamine (100 mg/ml Nimatek; AUV, Holland), 1% acepromazine (10 mg/ml Vetranquil; Sanofi, Holland), and 2% xylazine hydrochloride (22.3 mg/ml Rompun; Bayer Germany). Initial doses of 0.7 mg/kg of a mixture of ketamine and acepromazine (10:1 in proportion by volume) and, in separate injection, 0.25 ml/kg of xylazine hydrochloride were given intramuscularly. These initial doses, which maintained a good anesthesia for about 1 h, were supplemented as necessary. All surgical procedures, as well as the experimental protocols that are described below, were in accordance with guidelines set by the ethics committee of the medical faculty of Erasmus University and *Principles of laboratory animal care* (NIH publication no. 86–23, revised 1985).

2.2.2 Experimental procedure

The rabbit was restrained in a linen bag that was tied down on a small board. The head bolts were fastened to a head holder mounted on the board. The head was fixed with the nasal bone at an angle of about 35° off-vertical, which brings the horizontal semicircular canals perpendicular to the direction of gravity (Soodak and Simpson 1988). The rabbit on the board was placed on an earth-horizontal circular turntable (diameter 70 cm) with the middle of the interaural axis in the axis of rotation. Two experimental protocols were performed: the *intensity protocol* and the *frequency protocol*.

2.2.2.1 Intensity protocol

In this protocol two visual patterns were presented to the rabbit. Both patterns consisted of light spots on a dark background. One pattern was stationary relative to the head of the rabbit, whereas the other moved sinusoidally about the yaw axis with a frequency of 0.1 Hz and an amplitude of 2.5° for 11 cycles. To investigate the influence of the luminance of the two patterns, we varied the intensity of the stationary and the moving pattern independently. The five intensities, which were used in all combinations, were 0.0, 0.75, 1.5, 2.25, and 3.0 cd/m^2 , resulting in 25 conditions.

To investigate the effect of simultaneous vestibular stimulation, these 25 conditions were applied twice: once where the head of the rabbit did not move (optokinetic stimulation) and once where the rabbit moved with an amplitude and phase that was identical to the moving visual stimulus (vestibulo-optokinetic stimulation). Thus, in the latter condition, the pattern that oscillated relative to the world was stationary relative to the head of the rabbit, while the earthfixed stationary pattern moved relative to the head. Note that as a consequence the visual stimulation relative to the head in both vestibular conditions was identical. In the remainder of this thesis, the pattern that was

stationary relative to the rabbit will be referred to as the "S pattern" and the pattern that moved as the "M pattern" (Fig. 2.1).

The intensity protocol, consisting of 50 stimulus conditions (two vestibular and 5 times five spot luminance levels) was performed using three rabbits (animals C, F, and J). All stimuli were presented in random order. Between trials, it took less than 20 s to store the data on hard disc and to adjust stimulus parameters according to the protocol.

2.2.2.2 Frequency protocol

All six rabbits were exposed to 11 luminance levels (76.0, 36.0, 30.0, 16.0, 9.3, 6.0, 4.0, 2.7, 1.7, 1.0, 0.4, and 0.0 cd/m^2) of the M pattern, while the luminance of the S pattern was set at a fixed level (4.0 cd/m^2). As in the previous protocol, each visual stimulus was also presented in combination with vestibular sinusoidal stimulation with the same frequency, amplitude, phase, and direction as the moving component of the transparent visual stimulus. The 22 conditions were tested for three stimulus frequencies: 0.05, 0.1, and 0.2 Hz for 11 cycles. Amplitudes were chosen in such a way that the maximum velocity that was obtained was 1.7°/s for all three frequencies. These amplitudes were 5°, 2.5°, and 1.25°, respectively. Again all 66 conditions were presented in random order.

2.2.3 Eye position recording

Horizontal components of eye position were measured with the magnetic induction method, with ocular sensor coils in an earth-fixed rotating magnetic field (1300 Hz), based on phase detection. (Collewijn 1977). The position of the left eye and the right eye were recorded simultaneously. Eye position data were gathered by a data acquisition unit (CED 1401 PLUS) operated by a Pentium PC at a sample frequency of 250 Hz.

2.2.4 Stimulus generation

2.2.4.1 Vestibular stimulation

The platform on which the rabbit was placed was oscillated about the yaw axis, driven by a servomotor (Mavilor-DC motor 80). The driving signal, which specified the required position, was computed and delivered by the CED unit. Actual position of the platform was measured by an angular displacement transducer (Trans-Tek 600) whose output was recorded by the CED unit and stored along with the eye position and visual stimulus position data for off-line analysis.

2.2.4.2 Transparent optokinetic stimulation

The rabbit has an almost panoramic visual field, with an optokinetic responsive area for each eye that extends from about 100° anterior to 75°

posterior, and 10° inferior to 50° superior (Dubois and Collewijn 1979). Therefore a panoramic visual stimulus was used (Fig. 2.1) for optimal visual stimulation. The visual stimulus consisted of a translucent dome (90 cm in diameter) with a plain white inner surface, which was centered around the head of the rabbit. In the top of the dome a planetarium was mounted, which projected light spots on the inside of the dome. The outer surface of the dome was painted black except for a number of spots at random locations. Because the dome was made of opaque white Perspex, the unpainted spots on the outer surface were only visible on the inside when the dome was illuminated from outside. These light spots matched in dimensions with the ones produced by the planetarium. The outer surface was adjustably illuminated with 12 halogen lamps, which were fixed to the exterior of the dome. A servomotor with position feedback coupling could rotate the planetarium and the dome independently about the yaw axis, thus generating two transparent horizontal retinal flow patterns. Similarly to the turntable, the position of the visual stimulus was measured with an angular displacement transducer and controlled by a driving signal from the CED unit.

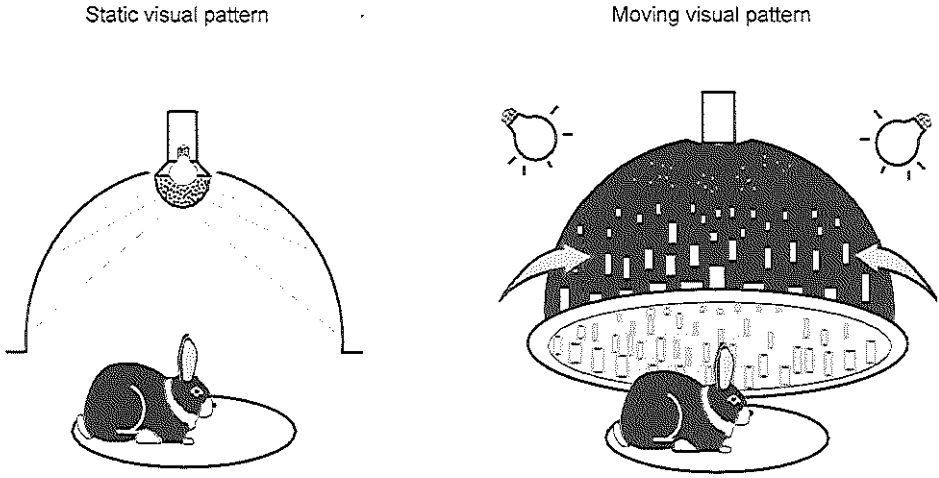

Fig. 2.1 *Schematic layout of the optokinetic stimuli. The static visual pattern was generated by projecting light spots from a planetarium on the inner surface of a dome. Illumination of the outer surface of the dome created the moving pattern. Random spots left open in the painted surface of the dome appeared on the inner surface as light spots. Spots of both pat-terns were matched in size*

The transparent stimulus consisted of a pattern that was stationary relative to the rabbit combined with a pattern, which moved sinusoidally about the vertical axis. Even though both patterns were similar in structure, the

effectiveness in eliciting OKR turned out to be slightly different for the individual patterns. Therefore the pattern produced by the planetarium was always kept stationary (the S pattern) and the pattern produced by external illumination of the dome was always moving relative to the rabbit (the M pattern). Thus the transparent motion was in all respects identical for optokinetic stimulation and for combined vestibulo- optokinetic stimulation.

The mean light spot luminance of both visual patterns was calibrated independently by measuring the luminance (with a Minolta LS100 luminance meter) in the center of 20 individual light spots, while only one pattern was turned on. The selected light spots appeared at an elevation of about 3° above the rabbit's eye level. This corresponds with the area projected on the visual streak of the rabbit's retina (Hughes 1971). The mean luminance value of the light spots was calculated for each pattern. Subsequently a light spot on the 3° meridian whose luminance came nearest to the mean value was selected. Next, the luminance in this particular light spot was measured while the voltage to the light source of the pattern was varied. In this way conversion of voltage to mean light spot luminance was obtained for both patterns. The contrast of the M-pattern with respect to the background was 0.49 and of the S-pattern was 0.73, both remained the same when the spot luminance of either pattern was changed. The visual stimulations were always binocular.

2.2.5 Data analysis

From the eye position data, the gain and phase relative to the stimulus were determined off-line. The eye-in-head position signal was determined by subtracting the platform position signal from the original eye position data. Next we removed occasional fast phases and saccadic eye movements from the eye-in-head position, based on a velocity criterion (5°/s during at least 10 ms). In the eye velocity trace, the resulting gaps were linearly interpolated by averaging the eye velocity directly before and after the gap. Subsequently this velocity trace was integrated, and this was taken as the slow-phase position signal. Through both this slow-phase position signal and the stimulus position signal, a sinusoid curve was fitted, with a frequency that was identical to the stimulus, leaving only the amplitude (A) and phase (F) to be fitted. In order to avoid artifacts caused by the onset of stimulation, we discarded the 1st cycle of the stimulus and the response from the fitting procedure, so 10 of the 11 cycles were eventually used. The gain (G) of the response was defined as A_{eye}/A_{stim}, and the phase difference the stimulus and the response ($\delta\phi$) as $\phi_{stim} - \phi_{eye}$.

2.3 Results

In all rabbits, the stimuli that were used in our experiments proved to be capable of eliciting compensatory eye movements with a substantial amplitude. Pure optokinetic stimulation (a moving pattern without presentation of either a stationary visual stimulus or vestibular stimulation) resulted in eye

movements with a mean gain of 0.64 (SD 0.15), whereas vestibular stimulation in the dark produced a mean VOR gain of 0.52 (SD 0.12; both gains measured with a stimulus amplitude of 2.5° at a frequency of 0.1 Hz). In none of the experiments described in this chapter was a systematic relation found between stimulus parameters and the phase of the response. Under all experimental conditions, saccades or nystagmus fast phases were occasionally made. An average of 1.05 (SD 2.63) saccades per trial was found. In the remainder of this chapter, we focus on the gain of the eye movements and its relation to the stimulus parameters.

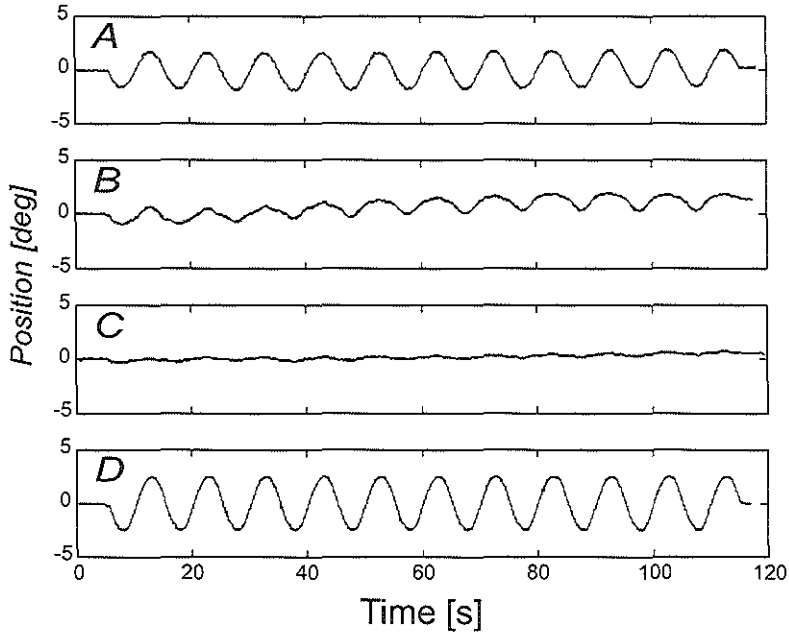

Fig. 2.2 *Eye movements in response to transparent visual stimulation (rabbit C) A, B, and C are typical examples of monocular eye position traces obtained during optokinetic stimulation. The position signal of the visual pattern that moved in space is shown in D.* ***A*** *Response to a nontransparent optokinetic reflex (OKR) stimulus with a luminance of 3.0 cd/m^2 ; the gain was 0.84 with a phase lag of –0.02°.* ***B*** *Here both the S- and the M pattern had a luminance of 2.25 cd/m^2 (gain 0.31, phase lead 0.19°).* ***C*** *The S pattern had a luminance of 3.0 cd/m^2 and the M pattern 0.75 cd/m^2 (gain 0.07, phase lag 12.20°)*

Figure 2.2 shows typical examples of eye position data, which were obtained during optokinetic stimulation with three different luminance combinations of the two visual patterns. As can be seen from this figure, the responses of the rabbits under transparent conditions were relatively constant but depended on the luminance of the two patterns presented. Eye movements were generated to compensate for the moving stimulus with gains that depended on the luminance of both patterns. The intensity protocol (see Materials and methods) was designed to investigate this phenomenon systematically.

2.3.1 Intensity protocol

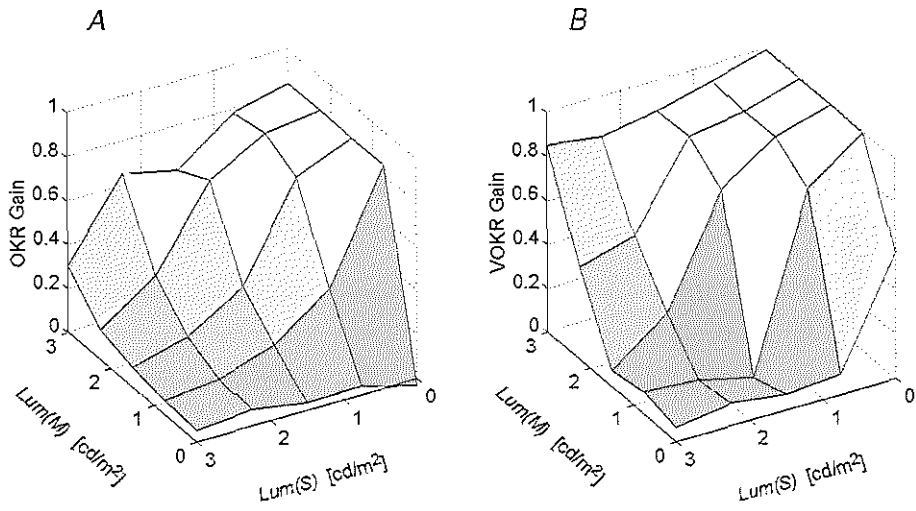

Fig. 2.3 *OKR and combined horizontal vestibulo-optokinet-ic reflex (VOKR) gain during transparent visual stimulation (rabbit C)* ***A*** *Three-dimensional plot of the OKR gain as a func-tion of both the luminance of the S pattern [Lum (S)] and the*
M pattern [Lum(M)]. Pure (nontransparent) optokinetic condition is represented by the most rightward curve [Lum(S)=0]. ***B*** *Similar plot for the VOKR gain of the same rabbit. Vestibular suppression stimuli (see text) are represent-ed by the front row [Lum(M)=0], vestibular stimulation with pure optokinetic stimulation is represented by the most rightward curve [Lum(S)=0]*

Figure 2.3A shows a typical example of how the gain of the optokinetic responses behaves under transparent conditions. In this figure, eye position gain is plotted as a function of the luminance of the moving and the stationary pattern. Within the range that was tested, the responses to pure optokinetic stimulation (represented by the outer right curve, Lum(S)=0) proved to be *independent* of the luminance of the pattern. However, when a stationary pattern was added to our stimulus, the gains varied with M pattern luminance. Increasing the luminance of the stationary pattern usually decreased the optokinetic response to a certain M pattern. A similar behavior was observed when the optokinetic stimulation was combined with a vestibular stimulus (Fig. 2.3B). However, note that in this figure VOKR gain rises to higher levels at lower luminances of the M pattern when compared with the OKR gain in similar visual conditions. The data presented in Fig. 2.3 suggest that there is a tradeoff between the intensities of the moving and the stationary pattern that determines the size of the oculomotor response under transparent conditions. We therefore calculated the normalized luminance (NL) of the moving pattern and plotted the gains of the eye movement responses as the function of NL (Fig. 2.4). NL is defined as:

$$NL = \frac{Lum(M) - Lum(S)}{Lum(M) + Lum(S)} \qquad (1)$$

where Lum(M) and Lum(S), are the luminances of the M pattern and the S pattern, respectively. NL=—1 indicates a purely stationary stimulus (of any intensity), whereas NL=1 indicates nontransparent optokinetic stimulation. Equal luminances for the M- and the S pattern result in an NL of zero.

In Fig. 2.4, we replotted the data of Fig. 2.3 as a function of NL. As one can see, the values of the different curves of Fig. 2.3A (symbolized by the size of the data points) are now distributed around one sigmoidal curve (closed symbols), indicating a straightforward relationship between NL and the oculomotor responses. The same is true for the VOKR data (open symbols). In none of the rabbits could a significant modulation by absolute stimulus intensity on the NL-gain relation be demonstrated for both OKR and VOKR gains (P>0.05, mixed-model ANOVA). Another salient phenomenon in Fig. 2.4 is that the data points of the VOKR-curve are shifted to the left with respect to the OKR curve. In other words, adding a vestibular stimulus changes the relation between NL and the ensuing oculomotor response. Since vestibular stimulation increases the gain at low values of NL, this modulation can be described as an increase in the weight of the M pattern in the trade-off between the moving and the stationary stimulus. In order to quantify this effect of vestibular stimulation, we fitted sigmoid curves through both data sets. From both the OKR and the VOKR curve the transitional NL-value (tNL) was determined. The tNL of a curve is defined as the NL-value where the gain equals half of the saturation level of that curve ("the maximum gain"). The difference between the two tNL-values (ΔtNL $= tNL_{OKR} - tNL_{VOKR}$) was taken as a measure for the leftward shift of the NL-gain relation, caused by vestibular stimulation. In the example in Fig. 2.4, the mean ΔtNL is 0.19 (SE 0.03).

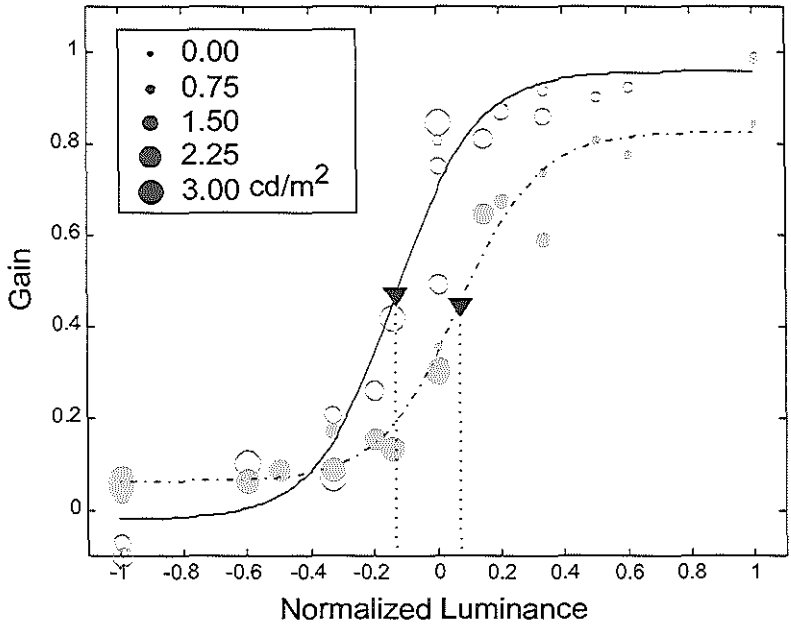

Fig. 2.4 *Relation between normalized luminance (NL) and eye movement gain (rabbit C) Same eye position data as in Fig. 3 as a function of the NL (see text). Note the leftward shift of VOKR (empty circles) curve relative to the OKR (filled circles) curve. Symbol size is indicative for the abso-lute intensity of the S pattern .Triangles indicate the transi-tional normalized luminance (tNL; see text). tNL VOKR is –0.13 (SE 0.019), tNL OKR is 0.07 (SE 0.0264).* ΔtNL *=1.36, which is statistically signifi-cantly different from zero (P<0.001, Student t-test). The* r^2 *of the OKR curve (dotted line) is 0.98 and of the VOKR curve, 0.96*

The histogram in Fig. 2.5 shows ΔtNL of all three rabbits that were tested in the intensity protocol. All ΔtNL values were positive and significantly different from zero (P<0.01). The mean ΔtNL was 0.25 (SE 0.04). A mixed-model ANOVA did not reveal a significant effect of absolute visual stimulus luminances on the ΔtNL values that were measured for each rabbit, indicating

that the effect of vestibular stimulation did not correlate with the absolute intensities of the visual stimuli.

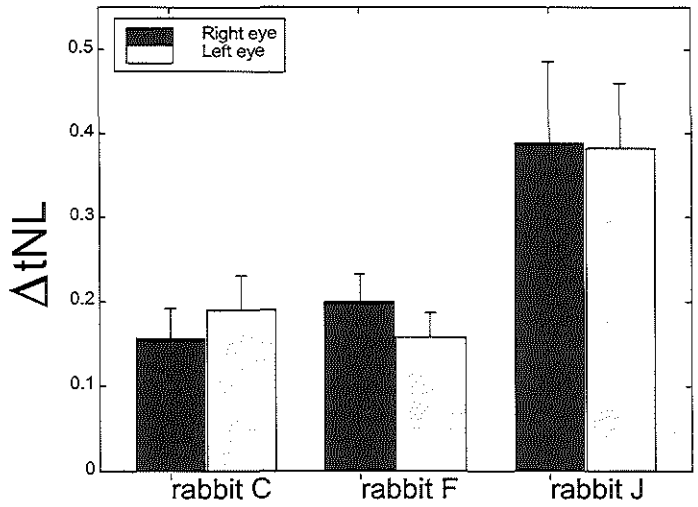

Fig. 2.5 *Overview of ΔtNL -values of all rabbits that were tested in the intensity protocol. In all animals, ΔtNL is more than zero (P<0.01, Student's t-test). No significant differences were found between both eyes, indicating that the implantation of the eye coils did not hamper the eye movements in any way*

2.3.2 Frequency protocol

Since the gain of the VOR and OKR depend on the frequency of stimulation (Baarsma and Collewijn 1974), three test frequencies were chosen to explore how the frequency of stimulation contributes to the behavior of both in transparent conditions. The data that were measured in the intensity protocol demonstrated that the absolute luminance of the S pattern did not have systematic effects on the OKR and VOKR gain as A function of NL. Therefore 11 NL levels ranging from –1 to 0.9, with increments of 0.2, were created by setting the luminance of the S pattern at a fixed level while the luminance of the M pattern was altered (see Materials and Methods).

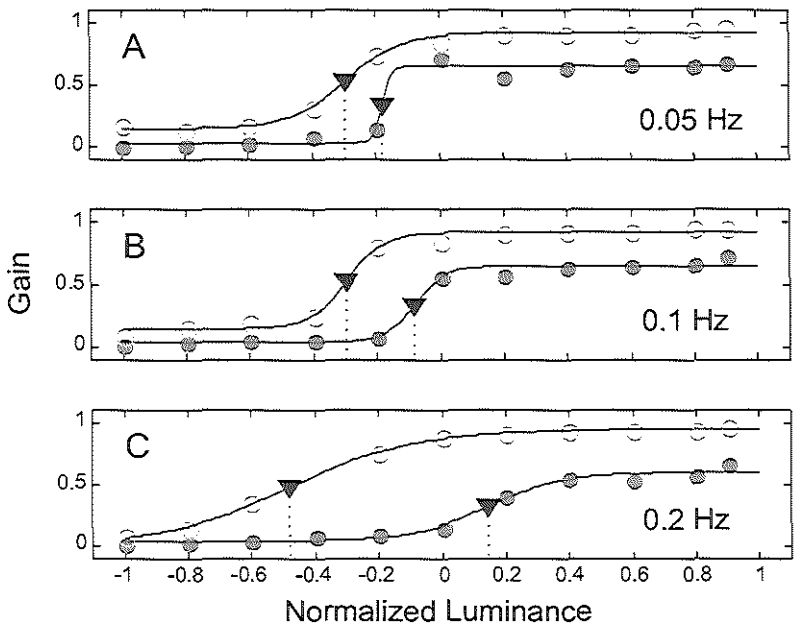

Fig. 2.6 *NL-gain relations at various stimulus frequencies (rabbit J). The relation between the relative luminance and the eye movement responses at various stimulus frequencies. Symbols are identical to those in Fig. 4. Note the increase in* ΔtNL *with increasing stimulus frequency, and the rightward shift of* tNL_{OKR}

Figure 2.6 shows an example of the OKR and VOKR gain plotted as a function of NL in separate panels for the different stimulation frequencies in ascending order. Sigmoid curves were fitted for tNL estimation to the OKR and VOKR data set for each frequency. As can be observed, the tNLs of the OKR and VOKR curve tend to move further apart from each other as the frequency increases. In 4 out of 36 cases, the sigmoid fit resulted in tNLvalues estimated beyond the NL range that was tested. These values were particularly obtained in OKR conditions when stimulated at 0.2 Hz. In these

cases, we took a tNL-value of 0.8 as a low estimate (note that tNL must always be smaller than 1) in order to have a realistic estimation of the vestibular-visual effect.

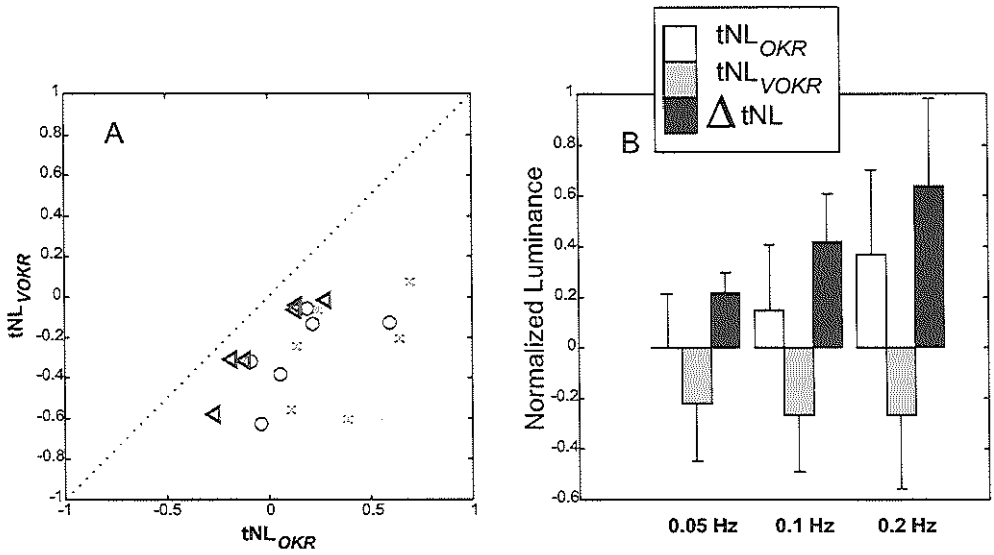

*Fig. 2.7 Overview of the tNL values estimated in the fre-quency protocol. **A** tNL VOKR is plotted as a function of tNL_{OKR} . Each point represents the response of a rabbit at a certain frequency, averaged for both eyes. The symbols repres-ent the stimulus frequencies: triangles 0.05 Hz, circles 0.1 Hz, crosses 0.2 Hz. Note that all points lie below the unity line, which means that in all cases tNL_{OKR} is larger than tNL_{VOKR} . **B** Mean values of tNL_{OKR}, tNL_{VOKR} , and ΔtNL, averaged over seven animals. Note the increase in both tNL_{OKR} and ΔtNL with increasing stimulation frequency.*

Figure 2.7A shows the tNL values for the rabbits tested in this protocol (n=6 rabbits) for all frequencies of stimulation. In this figure the tNL estimations from OKR gains (tNL OKR) are plotted against tNL from VOKR gains (tNL VOKR) averaged for the left and right eye. Since all points lie below the unity line, this figure shows that for all frequencies of stimulation tNL OKR is larger than the tNL VOKR . The distance of the points from the unity line gives an indication of ΔtNL, which enlarges as the stimulation frequency increases. Figure 2.7B shows the mean values of the tNL OKR , tNL VOKR , and ΔtNL for all stimulation frequencies. Here one can see that the increase in ΔtNL as a function of stimulus frequency is due to both a rightward shift of the OKR-curve and a small leftward shift of the VOKR curve.

2.4 Discussion

Transparently moving patterns impose a conflict situation on the main retinal stabilization reflexes (the OKR and VOR). These eye movements stabilize the visual image on the retina, in order to minimize blur caused by retinal slip. An ideal OKR or VOR would therefore cancel all movement on the retina, which is of course impossible when several moving patterns are simultaneously present in the stimulus.

So far, the eye movements that are evoked by transparent motion have only been investigated in humans (Kowler et al. 1984; Howard and Gonzales 1987; Niemann et al. 1994; Mestre and Masson 1997). However, humans have a fovea and may therefore resort to a response strategy of *foveal* stabilization. In other words, in a conflict situation, humans may track one stimulus element on the fovea while ignoring all others. Smooth pursuit, rather than reflexive eye movements may be responsible for at least part of their behavior. In this respect it is noteworthy that, when instructed to attend to one particular pattern, human subjects most dominantly respond to this pattern (Kowler et al. 1984; Mestre and Masson 1997).

We are to our knowledge, the first to describe the effect of transparent motion on the eye movements of an *afoveate* species, the rabbit. Since pursuit in the rabbit is absent (Collewijn 1977), this animal is an ideal model to study the retinal stabilization reflexes in isolation during visual conflict situations. The influence of stimulus intensity on the OKR Our first paradigm (the intensity protocol) was designed to systematically study the influence of stimulus intensity on optokinetic responses. In advance it was to be expected that some tradeoff would occur between both patterns: patterns with a high intensity are likely to evoke a more robust response than lowintensity patterns. However, it is not insignificant that this trade-off is identical at different levels of absolute stimulus intensity.

Moreover, our data show that the gain of the oculomotor response is invariant, when a "pure" (nontransparent) optokinetic stimulus is used, irrespective of the stimulus intensity that was used (range 0.75–3.0 cd/m^2 ; Fig. 2.3A). Thus, the "robustness" of the response cannot be measured using such stimuli. When we plot the gain of the oculomotor response as a function of the stimulus intensities (Fig. 2.3A), a trade-off is apparent. A high-intensity M pattern evokes eye movements with a gain that is similar to pure optokinetic stimulation, irrespective of the intensity of the S pattern. Likewise, a high-intensity S pattern inhibits virtually all movement. From the shape of the relation in Fig. 2.3A, one can see that, at increasingly lower intensities of the S pattern, an increasingly lower M pattern luminance is required to evoke the same optokinetic response. Figure 2.4 (closed symbols) shows that the oculomotor response is purely determined by the normalized lumi-nance of both patterns. There is a straightforward relationship between NL and the gain of the response. This relationship is not significantly modified by the absolute intensities of either visual pattern.

2.4.1 Influence of vestibular stimulation

During the optokinetic stimulation described above, no vestibular stimulation was applied. Thus, the input from the vestibular system was in accordance with the static visual input. In order to investigate the role of vestibular input, we presented identical transparent flow patterns to the animal, while it was rotated about the vertical axis in phase with the visual stimulation (Figs. 2.3B and 2.4, open symbols). By doing so, the vestibular input was now in accordance with the moving visual input. During nontransparent visual stimulation (only an S-or an M pattern), the influence of vestibular stimulation was according to the literature (Baarsma and Collewijn 1974; van der Steen and Collewijn 1984). Vestibular stimulation combined with the M pattern resulted in an increase in gain, whereas the combination with the S pattern (the "suppression paradigm") completely suppressed the response, irrespective of the luminance of the S pattern. During transparent visual stimulation, a trade-off occurred that was similar to the optokinetic situation without vestibular input. However, the NL-gain relationship (Fig. 2.4) was altered. The leftward shift of the curve with respect to the optokinetic situation indicates that the responses now followed the M pattern more effectively. In other words, the effect of vestibular stimulation was that it increased the relative contribution of the visual pattern that was in accordance with the vestibular input.

The magnitude of the vestibular influence (expressed as the shift of tNL; Fig. 2.5) did not depend on the absolute intensity of the visual patterns. This suggests that the trade-off between the two visual inputs is made before the visual input interacts with vestibular information. Thus, at the subsequent stage of processing, the vestibular signals interact with a relative visual motion signal. Since the vestibular nuclei and the cerebellar flocculus have been identified as the sites where visual-vestibular interaction takes place (Waespe and Henn 1977; Collewijn and van der Steen 1987; Stahl and Simpson 1995), we hypothesize that the visual trade-off takes place upstream of these nuclei, most likely in the assessory optic system (AOS) and/or the nucleus of the optic tract (NOT). In the AOS, neurons have been identified that respond to large flow fields (Soodak and Simpson 1988).

The AOS, which receives input from optic nerve ganglion cells in the retina (Oyster et al. 1980), projects directly to the vestibular nuclei and is considered to be the main source of visual information for the OKR. Responses of neurons in both the AOS and the NOT of anesthetized monkeys were inhibited when an oppositely moving pattern was added to a pattern that moved in the preferred direction (Hoffman and Distler 1989), which is in accordance with our hypothesis.

2.4.2 Influence of stimulus frequency

Both OKR and the VOR are frequency-dependent. The OKR responds optimally to low frequencies, whereas the VOR is predominantly sensitive to high-frequency input (Baarsma and Collewijn 1974; van der Steen and

Collewijn 1984). We therefore investigated the visual trade-off and the visual-vestibular interaction at various frequencies. Figure 2.7B summarizes the results. An increase in stimulation frequency results in an increasing tNL value of the NL-OKR relationship. In other words, as the OKR gets less effective, the trade-off of visual signals is increasingly dominated by the static pattern. On the other hand, the influence of the vestibular stimulation in-creases with increasing stimulus frequency, which is shown as a larger shift of ΔtNL. Thus, both the optokinetic and vestibular components during transparent stimulation behave in line with what can be expected on the basis of their properties in isolation.

2.4.3 Comparison with human studies

Several studies that have investigated OKR responses in humans report that the subjects alternatingly respond to one of the patterns (Howard and Gonzales 1987; Niemann et al. 1994). Thus, attention seems to play a role under these conditions. Such phenomena were never observed in our study of the rabbit. The gain of the eye response was more or less constant throughout the trials (see Fig. 2.2 for some examples). We therefore conclude that the responses in rabbits are only determined by the stimulus parameters. In this respect it is noteworthy that very early responses in humans, at the onset of transparent stimulation, equal the mean motion vector of the stimulus (Mestre and Masson 1997) and only after some time do the eyes follow one of the patterns. This initial phase may be an analog to the responses found in rabbits.

2.4.3 Model

Based on our results, we have made a conceptual model (Fig. 2.8A). The model has three input sites: one for vestibular input (V) and one for each visual pattern (M and S). The strength of the vestibular input is simply set to zero when the rabbit is stationary, and to 1 when the animal moves. The strength of both visual inputs scale linearly with the intensity of the pattern and are subsequently normalized for the total visual input by dividing each input by the sum of the visual inputs. This represents the stage where the trade-off between the visual inputs is effectuated (putatively the AOS/NOT).

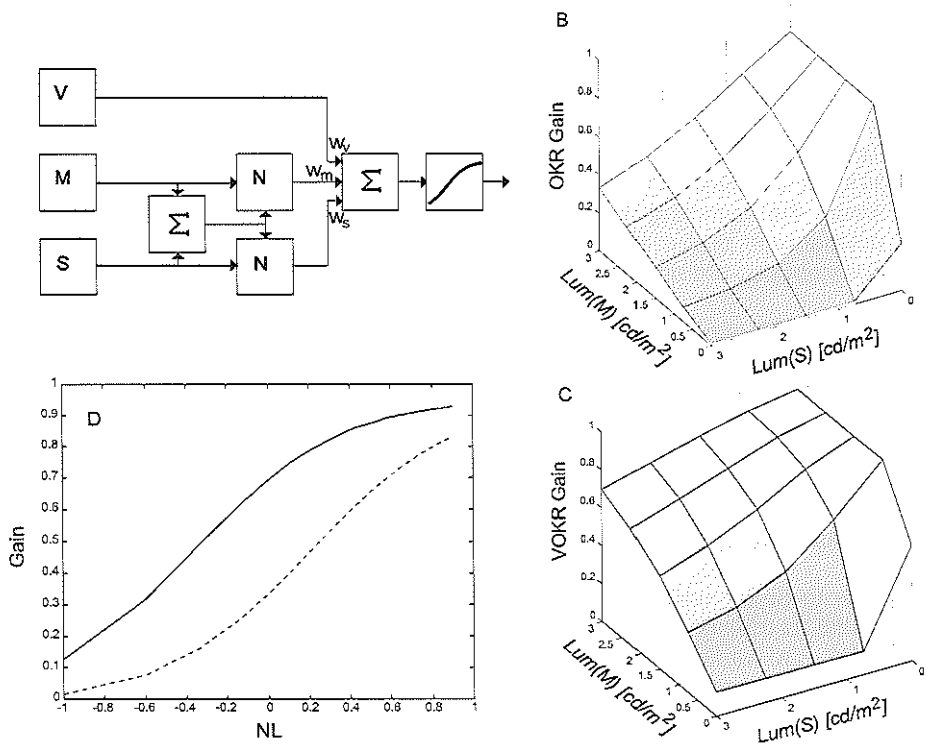

Fig. 2.8 *Conceptual model* ***A*** *Layout of the model. V, M, and S represent the input sites of vestibular information, and the moving and stationary visual input, respectively. The visual inputs are summed (left Σ box). This summed visual input is used to normalize the M and S input (N boxes). All inputs are subsequently weighted and summed (right Σ box). The output of the summation is fed to a sigmoidal function.* ***B, C, D*** *Model results, presented in the format of Figs. 3 and 4.* ***B*** *The OKR gain as a function of the luminances of both visual inputs.* ***C*** *The VOKR gain as a function of the luminances of both visual inputs. In* ***D*** *the gain of the OKR (dashed line) and the VOKR (solid line) are plotted as a function of normalized luminance. For these panels, the weights were estimated according to the data of rabbit F (W_M =0.4; W_S= –0.3; W_V =0.2; offset=–0.25; see Appendix)*

The vestibular and the normalized visual signals are weighted and subsequently summed. This linear procedure represents the visual vestibular-interaction stage (possibly the flocculus or the vestibular nuclei). The output of this summation is fed to a sigmoidal function, whose output equals the gain of the oculomotor response. This sigmoid serves to prevent gains smaller than zero as well as gains larger than 1. This scheme has several properties that agree with our data:

1. When two visual patterns are present, the oculomotor response depends on the relative light intensities of both patterns (Fig. 2.4).

2. When only a moving visual pattern is present, the gain of the eye is independent of the intensity of this pattern, since it is normalized by only itself. In themodel, under these circumstances the value of the normalized M input is always 1.
3. Since the summing junction between visual and vestibular signals is downstream of the visual normalization process, the vestibular influence does not depend on the absolute intensities of the visual patterns.

Figure 2.8B–D shows the output of the model (see the Appendix for a choice of the model parameters). One can see that the model output is similar to the data presented in Figs. 2.3 and 2.4. The model suggests that visual-vestibular interaction during transparent stimulation can be considered as a linear process, since the visual and the vestibular inputs are simply summed. This is in agreement with findings in nontransparent situations (Baarsma and Collewijn 1974; Batini et al. 1979). However, the normalization of visual inputs that takes place upstream of the visualvestibular interaction site is nonlinear.

In order to better understand the processes that determine the response of the rabbit to transparent visual stimulation, electrophysiological recordings in the AOS, the vestibular nuclei, and the flocculus are necessary. The latter data are presented in chapter 4.

2.5 Appendix

The model that is depicted in Fig. 2.8A, requires six parameters.These are the weights of the three inputs to the visual-vestibular interaction stage (W_M ,W_S , and W_V , respectively) and three parameters that determine the shape of the sigmoidal function. In order to choose parameters that mimic the response of a certain rabbit at a given stimulus frequency, we took for the model sigmoid the parameters of the function that was fitted through the NL gain relation during combined visual-vestibular stimulation (e.g., Fig. 2.4, open symbols). This relation is likely to mimic the model sigmoid needed, since it saturates at the minimum (zero) and maximum gains that can be obtained by the animal at a certain frequency. To compensate for the fact that the sigmoid in the model is a relation between "summed weighted sensory input" and gain, rather than "normalized luminance" and gain, we summed an extra offset to the input of the sigmoid.

We then estimated the values of the three weights of the three inputs to the visualvestibular summation (W_M ,W_S , and W_V ; Fig. 2.8A). We did this by measuring the gain of the response during four conditions: vestibuloocular reflex (VOR) in the dark, nontransparent optokinetic reflex (OKR) and stimulation with two visual patterns of equal luminance with and without vestibular stimulation. Since "gain" is the output of the sigmoidal stage, we can straightforwardly look up what input is needed for the sigmoid to result in the desired gain. The following relations are valid:

1. During vestibular stimulation in the dark, the vestibular signal is 1 and the visual signals are both zero. Thus, this input (I_{VOR}) is W_V +offset.

2. Similarly, during pure optokinetic stimulation, the normalized moving visual signal is 1 and the others are zero. Thus, I_{OKR} is W_m +offset.
3. When the S pattern and the M pattern have an equal luminance, and there is no vestibular input, then both normalized visual signals have a value of 0.5 and the vestibular signal is zero. As a result, $I_{M=S} = 0.5W_m + 0.5W_s$ +offset.
4. The same visual patterns as above, but in combination with vestibular stimulation, correspond to an input of the chosen sigmoid of zero. Consequently, $0 = W_v + 0.5W_m + 0.5W_s$ +offset.

From these equations it follows that:
$W_M = I_{OKR} - I_{VOR} - I_{M=S}$
$W_S = I_{M=S} - I_{OKR} - I_{VOR}$
$W_V = I_{M=S}$
offset$= I_{VOR} + I_{M=S}$

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3

Effects of cholinergic neuromodulation in cerebellar flocculus on transparent motion processing in the rabbit[2]

[2] A.L. Mathoera · M.A. Frens · J. van der Steen, (2000) Exp Brain Res 134: 255-260

3.1 Introduction

Transparent motion is a natural phenomenon, which is for instance encountered when one looks at moving objects through a visual medium (e.g. a dirty window or a fence), but it also occurs as part of motion parallax. In lateral eyed species, such as the rabbit, motion parallax is especially important, since both translation and rotation of the head result in linear flow patterns on the retina that relate directly to the distance of objects. During transparent motion there is more than one visual motion vector on the retina. Therefore, no eye movement can entirely compensate for all retinal slip. Thus, retinal motion signals potentially give ambiguous information to the ocular stabilizing reflexes [the optokinetic reflex (OKR) and the vestibulo-optokinetic reflex in the light (VOKR)].

In chapter 2 we reported on the gain of these compensatory eye movements in response to two transparent patterns. We found that the gain was fully determined by the difference between the luminances of the patterns, weighted by their sum. We refer to this process as 'visual normalisation'.

Oscillation of the animals about the yaw axis in the presence of the same transparent visual stimuli (VOKR) enhanced the response to the visual pattern that encoded the same head movement as the vestibular stimulation. The magnitude of this vestibular effect did not depend on the absolute intensities of the visual patterns, which suggests that the weighting process of the transparent visual patterns occurs upstream from the site of the visual-vestibular integration. A simple model showed that a linear addition of vestibular and normalised visual signals was sufficient to explain the visual-vestibular interaction.

The flocculus is one of the sites where visual-vestibular interactions take place. Electrophysiological (Graf et al., 1988; Leonard et al., 1988) and lesion studies (rabbit: Ito et al., 1982; Nagao, 1983; Barmack and Pettorossi, 1985; monkey: Zee et al., 1981, Waespe et al., 1983) have shown that the flocculus is intimately involved in the generation the VOR and OKR.

Because the flocculus is one of the sites where visual-vestibular interaction takes place, we pharmacologically manipulated the flocculus by placing bilateral micro-injections of the non-selective cholinergic agonist Carbachol. Recently it has been shown that such injections can selectively modify the gain of compensatory eye movement responses. They predominantly increase the OKR gain and to a lesser extent the VOR gain. This effect takes place almost directly after injection, and lasts at a constant level for several hours (Tan and Collewijn, 1991).

The different effects of Carbachol on the OKR and VOR can be used as a tool to test specific predictions about information processing in the flocculus. The scheme for information processing of transparent motion that we have proposed in chapter 2 roughly consists of three stages (see also Fig. 2.8): A:

before visual normalisation; B: between the normalisation and visuo-vestibular interaction; C: after visuo-vestibular interaction.

If the flocculus is active before the normalisation stage (A), it is expected that both visual patterns become more salient. If the effect on both patterns is equally strong, these effects cancel in the normalisation. Thus, no change in the relation between the luminance of the stimulus and the OKR-gain is expected. The effect of concomitant vestibular stimulation should be *larger*, since a somewhat enhanced vestibular signal is added to the normalised signal.

If the floccular involvement is in stage B, the normalised visual motion signal is enhanced. Consequently, higher OKR-gains are expected at lower intensities of a moving optokinetic stimulus. Furthermore, a *reduced* difference between OKR and VOKR is expected, since the enhancement of the normalised visual signal should exceed the enhancement of the vestibular input.

An effect below the visual-vestibular interaction site (C) is not to be expected, since this is incompatible with the notion that there are different effects on 'pure' VOR and OKR.

3.2 Materials and Methods

3.2.1 Animal preparation

Six young adult female pigmented Dutch belted rabbits were used. The absence of a fovea makes the rabbit in particularly suitable to study the effects of transparent motion stimuli on compensatory eye movements. In this animal, the OKR and VOKR can be analyzed without intervening spontaneous eye movements (Collewijn, 1969, 1977).

Surgical procedures were as described in chapter 2. In short, general anaesthesia was induced and maintained by intra-muscular injection of a mixture of ketamine (100 *mg/ml*) acepromazine 1 % (Vetranquil, 10 *mg/ml*) and xylazine-HCl (Rompun 2%, 22.3 *mg/ml*). Scleral search coils were permanently implanted for eye movement recording. For electrophysiological recordings and implantation of guide cannulae a bilateral craniotomy was made over the paramedian lobes of the cerebellum and a recording chamber was placed over the opening.

All surgical procedures and experimental protocols were in accordance with guidelines set by the ethical committee of the medical faculty of the Erasmus University and the principles of laboratory animal care (NIH publication No.~86-23, revised 1985).

3.2.2 Implantation of the guide cannulae

About one week after surgery, electrophysiological recordings were made in the alert animal to localise the vertical axis (VA) zone in the flocculus (Van der Steen et al., 1994). The flocculus was localised on guidance of electrophysiological recordings using a glass micropipette with a 4 µm tip, filled with 4.0 M NaCl. Floccular Purkinje cells sensitive for optokinetic stimulation about the vertical axis were identified by modulation of their complex spike (CS) activity. After localisation of the VA area, the guide cannula surrounding the micro-pipette was fixed in position by securing it to the skull with dental acrylic. The micro-pipette was then withdrawn from the guide cannula after its depth had been marked. For a more detailed description of this method, see van Neerven et al. (1990)

3.2.3 Experimental procedure

The rabbit was restrained in a linen bag and the head was fixed to a head holder with the nasal bone at an angle of about 35° off-vertical. The rabbit was placed on a vertical axis turntable (diameter 70 cm) with the centre of the inter-aural axis in the axis of rotation.

At the start of each experiment, baseline OKR and VOR (in the dark) were measured in response to a single visual pattern or the turntable, oscillating at 0.1 Hz with an amplitude of 2.5°. Next, 1 µl Carbachol (1*mg/ml* saline; pH adjusted to 7.0-7.4; see Tan and Collewijn, 1991) was injected in both flocculi. After the injections the baseline measurements were repeated. Subsequently two transparent visual patterns were presented to the rabbit. Both patterns consisted of light spots on a dark background (see below). One pattern was stationary relative to the head of the rabbit (S-pattern), whereas the other moved sinusoidally about the yaw axis with a frequency of 0.1 Hz and amplitude of 2.5° for 11 cycles (M-pattern). We used 11 luminance steps (76.0, 36.0, 30.0, 16.0, 9.3, 6.0, 4.0, 2.7, 1.7, 1.0, 0.4 and 0 cd/m^2) of the M-pattern, at a fixed luminance level of the S-pattern (4.0 cd/m^2).

The gain of the eye movements in response to the 11 luminance levels was determined under two conditions: 1- optokinetic stimulation (with the head stationary) and 2- vestibulo-optokinetic stimulation (with the rabbit rotating with an amplitude and phase that was identical to the moving visual stimulus). Thus, in the latter condition, the pattern that oscillated relative to the world was stationary relative to the head of the rabbit while the earth-fixed stationary pattern moved relative to the head (see chapter 2 for a more extensive description of the stimulus). In the remainder of this chapter we will use a head-fixed co-ordinate system to describe the visual stimuli, similar to chapter 2. For example, the S-pattern is stationary with respect to the world in the OKR-condition, but moves with respect to the world in the VOKR-condition.

All 22 conditions were presented in random order. At the end of the experiment, baseline OKR and VOR measurements were repeated. In order to ascertain that the effects were not due to pressure artefacts, control data of all conditions

were obtained by injection of saline instead of the Carbachol solution in the flocculi and running the complete experimental protocol. This was done at least 24 hours preceding the experiment with Carbachol.

3.2.4 Histology

After all experiments were performed, the rabbits were perfused. The skull covering the flocculus at both sides of the head was carefully removed and an injection cannula was positioned into the guide tube. In all animals the tip of the cannulas was located in the floccular cortex of the cerebellum, indicating that all observed effects were related to neurochemical influences inside the flocculus.

3.2.5 Data analysis

The gain and phase relative to the stimulus were determined off-line from the eye position data (see chapter 2, for further details). Normalised Luminance (NL) of the visual stimulus was defined as:

$$NL = \frac{Lum(M) - Lum(S)}{Lum(M) + Lum(S)} \qquad (1),$$

where *Lum(M)* and *Lum(S)*, are the luminance of the M-pattern and the S-pattern respectively (see chapter 2). A four-parameter sigmoidal function was fitted to the Gain / Normalised Luminance curves.

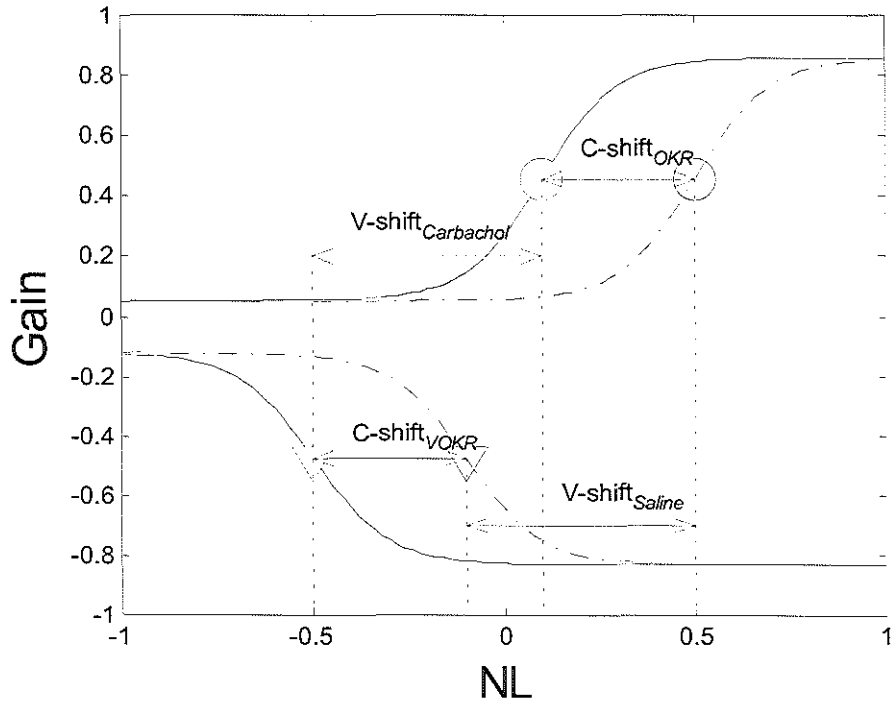

Figure 3.1 *- Definitions of vestibular and neuromodulatory effects This figure schematically shows the relation between the normalised luminance of the optokinetic patterns (NL) and the gain of the eye movements. Dashed lines indicate movements after saline injection, solid lines represent Carbachol-modulated movements. Because the eye-in-head movements are in counterphase with vestibular stimulation, and for presentation purposes, VOKR gains are plotted as negative values. The effect of concurrent vestibular stimulation is indicated for the Carbachol and saline curves (V-shift$_{Carbachol}$ and V-shift$_{Saline}$ respectively). Similarly, the effect of Carbachol is shown as C-shift$_{OKR}$ and C-shift$_{VOKR}$. These shifts are defined as the differences between the relevant tNL values shown as triangles or circles.*

One of the parameters determined by the fit was the 'transitional normalised luminance' (tNL). This parameter was defined as the NL value for which stimulation resulted in half the maximum gain. The most prominent effects were expected in the shift of the tNL points. Fig. 3.1 schematically shows what the data could look like. The curves in this figure are similar to sigmoids used to fit the data. The positions of the curves on the abscissa are fictitious and were chosen to illustrate the shifts clearly.

V-shift$_{Saline}$ quantifies the shift of the curve in NL, due to vestibular stimulation after saline injections. The shift of curves quantified by V-shift$_{Carbachol}$ is the vestibularly induced shift after Carbachol injections. Likewise, C-shift$_{OKR}$ and C-shift$_{VOKR}$ quantify the shift of the optokinetic curve and the vestibulo-optokinetic curve due to the effect of intrafloccular Carbachol injections.

3.3 Results

3.3.1 Effects of Carbachol injections on OKR and VOR

.

We measured the effect of Carbachol injections on the gain of 'pure' OKR and VOR directly after injection as well as after the presentation of the (vestibulo-) transparent stimuli. As expected the changes in OKR gain were more prominent than those in the VOR gain. For the OKR the mean relative gain change (rGC, see Frens et al, 2000) was 0.11 ± 0.07 (SE) directly after injection and 0.14 ± 0.08 at the end of the experiment. Relative VOR gain change failed to reach significance directly after injections, and increased to 0.06 ± 0.04 at the end of the experiment.

3.3.2 Effect on visual normalisation

Figs. 3.2 and 3.3 show data of one rabbit (G). Fig.3.2 shows raw eye position traces during (V)OKR stimulation after saline and Carbachol injections, while keeping the visual stimulation identical. A clear difference between the saline data and the Carbachol data is observed in the OKR-responses. Effects on VOKR are considerably smaller. The gain of the OKR (positive values) and the VOKR (negative values) is plotted as a function of NL in Fig. 3.3. In this animal a C-shift$_{OKR}$ of 0.24 was the result of Carbachol injection. This means that after Carbachol-injection, the rabbit made eye movements at lower intensities of the moving pattern. Concurrent vestibular stimulation showed a smaller C-shift$_{VOKR}$ of 0.07.

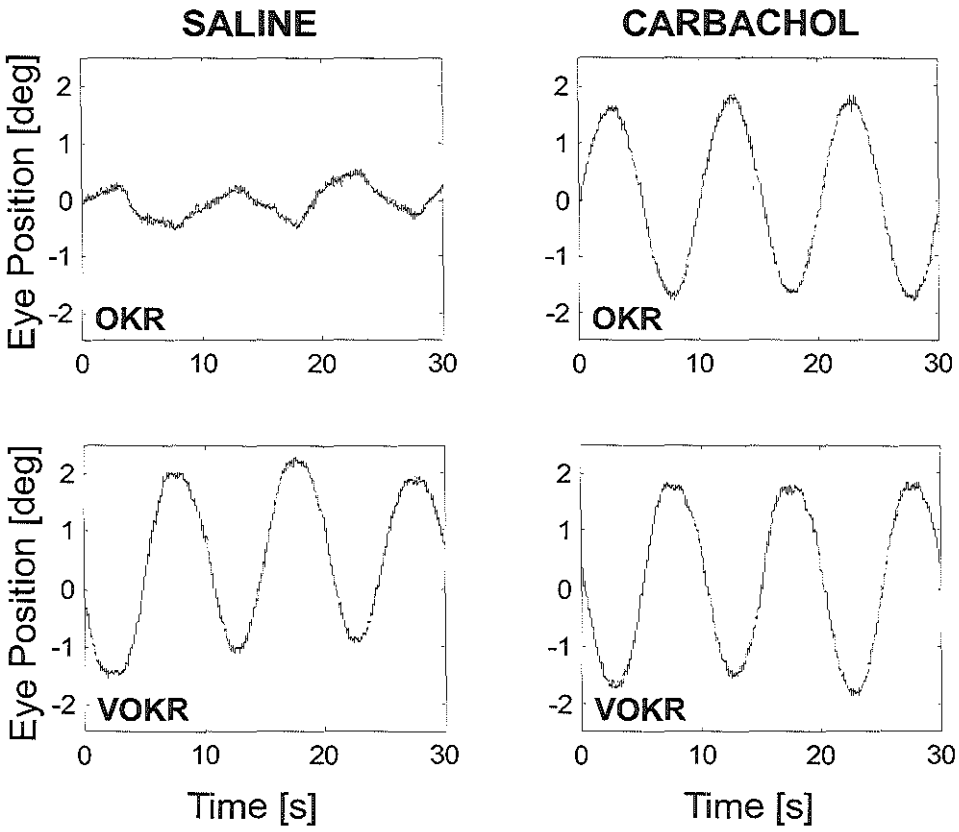

Figure 3.2 *- Eye Position Traces [Rabbit G]*
This figure shows oculomotor responses to transparent visual stimulation (NL=0.2) in isolation (top row), or in combination with vestibular stimulation (bottom row). The effects of floccular Carbachol injections (right) are shown in comparison to saline injections (left).

The absolute gain in the lowest and highest parts of Carbachol and saline OKR and VOKR curves were unchanged. This observation held true for all subjects, even though a clear Carbachol effect on pure OKR gain was seen.

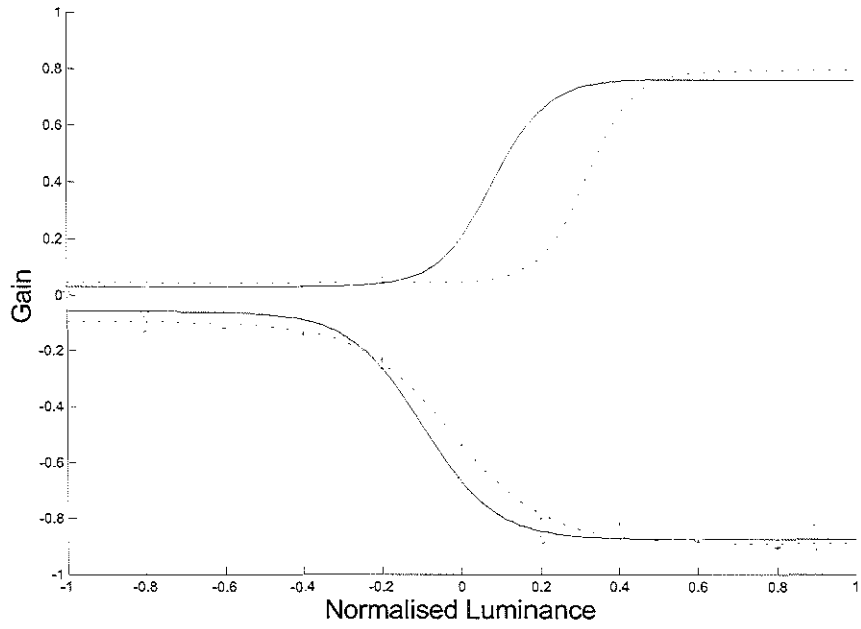

Figure 3.3 *- Gain as a function of Normalised Luminance [Rabbit G]*
In this figure the gain of the eye movement responses are plotted as a function of the normalised luminance of the optokinetic stimulus (see Methods). Dashed lines represent responses after floccular injection of saline, whereas solid lines represent responses after Carbachol injections. Positive gain-values were recorded in the absence of vestibular stimulation. Negative gain-values are VOKR-responses. See also Fig. 3.1.

3.3.3 Effect on visual-vestibular interaction

Fig. 3.4A shows the C-shift $_{VOKR}$ plotted against the C-shift $_{OKR}$ for all rabbits (n = 6) together with the unity line. In all rabbits the C-shift $_{OKR}$ was larger than the C-shift $_{VOKR}$, which means that Carbachol had a larger influence on the OKR than on the VOKR (Contingency test: $p < 0.05$). As a direct consequence, the effect of vestibular stimulation (the V-shift, see Fig 3.1) is smaller after application of Carbachol than after saline injections. There is a positive correlation between C-shift $_{OKR}$ and C-shift $_{VOKR}$ ($r=0.89$; $p < 0.02$), which is probably due to individual differences in the sensitivity to Carbachol.

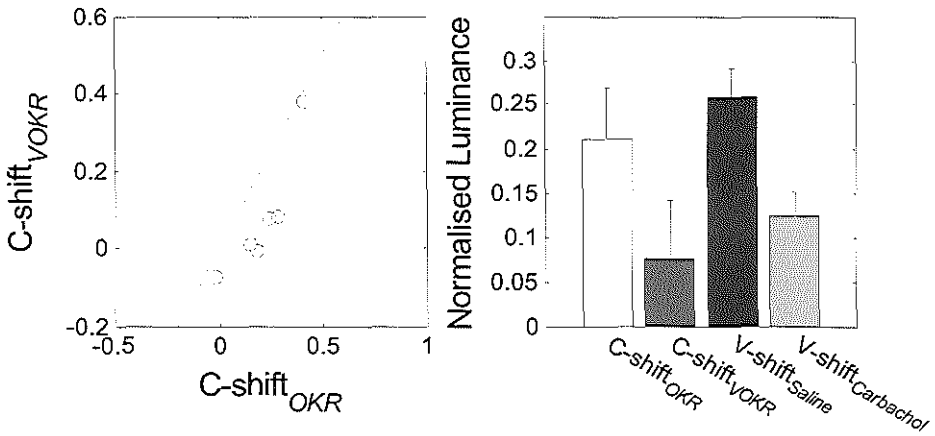

Figure 3.4 *- Overview of shifts.*
A. *C-shift$_{VOKR}$ as a function of C-shift$_{OKR}$. Each point in this figure represents one animal. Note that the C-shift$_{OKR}$ exceeds the C-shift$_{VOKR}$ in all rabbits. Mean shift values. See text for definitions. Note that the effect of Carbachol is larger on the OKR responses than on the VOKR responses (see also panel 4A). Consequently, the vestibularly induced shift of the saline curve is larger than the shift of the Carbachol curve. Thus, vestibular influence is smaller after Carbachol injection.*

In Fig. 3.4B the average curve shifts are shown for all rabbits (N = 6). Here one can see that Carbachol diminishes the vestibular effect by shifting the OKR curve more toward lower intensities of the moving pattern (the average Vshift$_{carbachol}$ is smaller than the Vshift$_{saline}$). Except for the mean C-shift$_{VOKR}$ all mean curve shifts were significantly different from zero ($p<0.05$, Student-t test). One may argue that the effects of Carbachol were substantially larger in one of the animals than in the others (arrow in Fig. 3.4A). However, the significance levels do not change when this animal is left out of the data analysis.

3.4 Discussion

In this chapter we have investigated the role of the flocculus in the processing of (vestibulo-) transparent stimulation for the generation of compensatory eye movements. Based on the data in chapter 2 we have defined two subsequent stages in such processing: a normalisation of the visual motion signals and a visual/vestibular summation. By means of neuro-pharmacological modification of the flocculus we have selectively changed the gain of the OKR. As is outlined in the introduction, specific effects, depending on the role of the flocculus in the process are predicted for the effect on (vestibulo-) transparent motion processing.

3.4.1 The effect of Carbachol injections on OKR and VOR

Injections of Carbachol in the flocculus selectively increased the gain of the OKR. An increase of the VOR gain was only observed at the end of the experiments. This increase was considerably smaller than the OKR gain change. In absolute terms the injections increased the OKR gain somewhat less than in earlier studies (Tan and Collewijn, 1991; Frens et al, 2000). This may partially be due to higher baseline gains in our animals. Despite the smaller effects on pure OKR, the induced changes were sufficient to cause consistent changes in the responses to transparent stimulation.

3.4.2 Effect on visual-visual trade-off and visuo-vestibular interaction

Injections of Carbachol changed the processing of transparent stimuli. The increased OKR-sensitivity caused leftward shifts of the curves that describe the eye movement gain as a function of NL (e.g. Fig 3.2). We quantified this by determining the shift of the tNL values.

On average Carbachol caused a significant leftward shift of the OKR curve with respect to saline baseline data, while the VOKR curve showed no statistically significant shift. Consequently, the effect of vestibular stimulation during transparent optokinetic stimulation was smaller compared to the control experiment (Fig. 3.3B). As was pointed out in the Introduction, this is compatible with the notion that the flocculus has a role in the stage, where visual motion signals have already been normalised. Therefore, Carbachol selectively boosts the normalised optokinetic signal, causing the relative contribution of the VOR in the visuo-vestibular interaction stage to decrease.

3.4.3 Neurophysiological interpretation

It is very well conceivable that the visual normalisation occurs upstream of the flocculus. The assessory optic system (AOS) is considered to be the main source of visual information for the OKR. It receives input from retinal ganglion

cells (Oyster et al. 1980) and projects to the vestibular nuclei, the inferior olive and the nucleus prepositus hyperglossi, all of which in turn project to the flocculus. In the AOS, neurons have been identified that respond to large flow fields (Soodak and Simpson 1988). Responses of neurons in both the AOS and the nucleus of the optic tract (NOT) of anesthetized monkeys were inhibited when a counter-moving pattern was added to a pattern that moved in the preferred direction (Hoffman and Distler 1989). Thus visual patterns already interact at a stage prior to the flocculus.

Possible working mechanism of cholinergic neuromodulation.
Two types of cholinergic innervation of the flocculus exist. One is a ChAT-positive mossy fiber projection that originates from the raphe obscurus and the lateral paragigantical nucleus (Jaarsma et al, 1997). The second projection is a sparse plexus of thin beaded fibers (Ojima et al, 1989; Illing, 1990) that are presumably afferents from the pedunculopontine tegmental cholinergic complex. Cholinergic projections may act directly on the P-cells, but anatomical data suggest that their influence is more likely to be exerted through floccular interneurons (Jaarsma et al, 1997).

Although the exact working mechanism of cholinergic substances in the flocculus is not completely clear, recordings of simple spike Purkinje cell activity during iontophoretic application of acetylcholine indicate that cholinergic substances act as a neuromodulator (Van der Steen and Tan, 1997). Neurotransmission in the flocculus is mainly glutamatergic. A possibility is that ACh presynapticly enhances glutamatergic transmission, in a similar way to what has been demonstrated in other parts of the CNS (McGehee et al, 1995; Gray et al, 1996).

3.5 Conclusion

In this chapter we show that the flocculus is involved in the generation of the oculomotor response to transparent stimuli. Its role is not to determine the relative contribution of the various flow patterns, but rather to combine the visual motion signals with vestibular information. In order to further elucidate the mechanisms underlying these processes, single unit recordings from floccular Purkinje cells in response to transparent stimulation are required. These are described in the next chapter.

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4

Properties of Gain Changes of theOptokinetic Reflex[3]

[3] M.A. Frens, A.L. Mathoera, and J. van der Steen. Prog Brain Res 124: 247-256

4.1 Introduction

Compensatory eye movements serve to minimize retinal slip. By rotating the eyes in a direction opposite to movements of the head, the orientation of the eyes in space remains relatively stable, which prevents blurring of the retinal image by movements of the visual scene relative to the head. In head-restrained afoveate species, such as the rabbit, these reflexive eye movements (most importantly the vestibulo-ocular reflex (VOR) and the optokinetic reflex (OKR)) form the sole oculomotor output. Therefore the rabbit is an ideal animal to study compensatory eye movements in isolation, without interference of pursuit or saccades.

Both the VOR and the OKR are highly plastic, and changing conditions can modify the performance of the reflexes within short periods of time. Nonetheless, somewhat surprisingly the gain (the ratio of eye movement amplitude and stimulus amplitude) of the reflexes of the rabbit is usually less than 1. In other words, the response is not perfect but rather shows a consistent undershoot (Collewijn, 1969). Only during combined vestibular and visual stimulation the gain reaches a value that is close to unity.

Plasticity of compensatory eye movements has been most extensively studied in the VOR (e.g. Collewijn and Grootendorst, 1979; Demer et al., 1989; De Zeeuw et al., 1998). For instance, a *gain-increase* paradigm in which the animal is sinusoidally rotated, while the visual environment is rotated in the opposite direction, leads to an increased amount of retinal slip with respect to a rotation in a stable environment. The VOR adapts to this new condition within hours by increasing the amplitude of the oculomotor response (Collewijn and Grootendorst, 1979). This increase persists even when the animal is subsequently rotated in the dark. Likewise, a *gain-decrease* paradigm in which the visual environment moves in phase with the vestibular stimulation leads to a decrease in the amplitude of the eye movements. In the rabbit VOR-adaptation is somewhat specific for the frequency of the adapting stimulus. At other stimulus frequencies the response changes less (Collewijn and Grootendorst, 1979).

Also the OKR has plastic properties. This has been shown when the animal is subjected to prolonged optokinetic stimulation. The gain of the OKR increased from 0.65 to 0.8 after 4 hours of stimulation at a frequency of 1/6 Hz (Collewijn and Kleinschmidt, 1975). This type of plasticity is, similarly to the VOR, frequency specific. Furthermore it not only increases the performance of the OKR but also of the VOR.

Although there is controversy about the exact neural substrate of VOR-plasticity, it is generally accepted that the flocculus of the cerebellum plays an important role. The flocculus receives two major inputs. One source is de *parallel fiber* system that consists of afferents from the vestibular nuclei. This input consists of mixed visual and vestibular information, as well as eye velocity signals. Furthermore, the flocculus receives input from the inferior olive through *climbing fibers* that carry an –almost purely visual- retinal slip

signal (e.g. Simpson and Alley, 1974; Graf et al., 1988). Since retinal slip is the parameter that should be minimized by compensatory eye movements, the climbing fiber input has been considered an 'error signal' (Ito, 1970). Both the parallel and climbing fibers connect, directly and indirectly through floccular interneurons, with the Purkinje-cells. These neurons form the sole output of the flocculus. The inputs result in different activity in the Purkinje-cells. The parallel fiber input induces 'conventional' Na^{+}-spikes in the Purkinje-cells ('simple spikes'), whereas climbing fiber activity results in large Ca^{++}-spikes with a long duration ('complex spikes').

In vitro, a form of plasticity has been demonstrated at the level of the Purkinje-cells (Ito et al, 1982; Linden, 1994). When climbing fiber input arrives at the Purkinje cell simultaneously with parallel fiber input, the strength of the synapse between that parallel fiber and the Purkinje cell is reduced. Thus the error signal changes the effectivity of synapses, and therefore the strength of specific inputs. This phenomenon is known as long term depression (LTD).

Cerebellar LTD could very well be the mechanism that is responsible inducing the plasticity in the VOR in paradigms such as described above. Here, the vestibular input is accompanied by an abnormal amount of retinal slip. As a consequence, there is a different amount of climbing fiber input, which causes the parallel fiber activity to change accordingly. Recent data support this view. Knockout mice that do not express cerebellar LTD in vitro are also not capable of VOR adaptation within hours of stimulation (De Zeeuw et al., 1998).

It is much less understood if and how LTD could be responsible for OKR-plasticity. During the prolonged presentation of an optokinetic stimulus no mismatch is created between the stimulus, the oculomotor response, and the resulting retinal slip. The climbing fiber input at the beginning of an adaptation paradigm is identical to the activity that is encountered by the animal during the normal movements it makes. Rather it seems that during the adaptation phase the stimulus becomes more salient, and the *setpoint* for the proper response to be made to a certain stimulus changes. In other words, the errors that are created by the natural undershoot of the response are apparently tolerated in normal life, but are not tolerated anymore by the system after prolonged optokinetic stimulation.
Not only prolonged optokinetic stimulation can change the response of the optokinetic system. Microinjections of cholinergic agonists and antagonists in the flocculus affect the gain of the OKR (Tan and Collewijn, 1991). For instance the non-specific cholinergic agonist Carbachol has been shown to increase the gain within minutes after injection. This increase, which never causes the gain to rise above unity, persists for at least several hours. Similar to the stimulus induced gain changes, the effect of cholinergic substances is to a lesser extent, present in the VOR.

There is anatomic evidence for cholinergic mossy fibers that stem from the vestibular complex and the nucleus prepositus hypoglossi (Barmack et al, 1986; Barmack et al 1992b). Furthermore there is evidence for a thin-beaded network of afferents, terminating in in granular and molecular layers (Ojima et

al, 1989; Illing, 1990; Barmack et al, 1992a). Nicotinic receptors have been demonstrated on several types of floccular interneurons (Jaarsma et al, 1997). Electrophysiological studies have shown that iontophoretic application of Carbachol increases the amplitude of the simple spike modulation of Purkinje cells (Van der Steen and Tan, 1997). However, at present it is not clear what the exact working mechanism of these cholinergic agents in the flocculus is.

In this chapter we investigate the functional effect of floccular micro-injections of Carbachol. Therefore we injected Carbachol into the flocculus, and applied subsequent optokinetic stimulation. If Carbachol and prolonged optokinetic stimulation act on the same mechanism, the setpoint for the response should be changed. Therefore optokinetic stimulation that is presented *after* the Carbachol-injection should either not change the oculomotor response or increase the gain even more.

Alternatively, if the setpoint is unaffected by Carbachol, a mismatch is created between subsequent stimulation and the oculomotor response. Thus, a situation arises that is similar to the VOR-adaptation paradigm, and it is predicted that a plastic mechanism (e.g. LTD) changes the gain back to its original value. Note that in the original experiments of Tan and Collewijn (1991), the rabbits were kept in a stable visual environment after the injection. Therefore the optokinetic system in these experiments had no means to evaluate its gain.

4.2 Materials and Methods

4.2.1 Animal Preparation

Six female Dutch belted rabbits were used in this study. All experimental and surgical procedures were in accordance with the *Principles of laboratory animal care* (NIH publication no.86-23, revised 1985), and were approved by the ethical committee of the Erasmus University.

The rabbits were equipped with a headholder that provided stable head fixation in the experimental setup, as well as induction coils in both eyes for recording eye position (for surgical details see chapter 2). In addition the skull and dura mater were removed above both paramedial lobules of the cerebellum. Stainless steel recording chambers were placed above both openings.

A few days after surgery we started localizing the VA-zone of both flocculi. The VA-zone in the region in the flocculus where units respond optimally to stimulation about the vertical axis (Graf et al, 1988). To that means, we recorded single unit activity with glass micropipettes filled with 4 M NaCl. The VA-zone was identified on the basis of activity of Purkinje-cells that modulated their activity in response to vertical axis optokinetic stimulation.

When the VA-zone was thus localized, the position of the tip of the electrode was reconstructed. A guide tube was subsequently cemented in the recording

chamber, in such a way that, during the experiments, it allowed an injection canula of the proper length to enter the VA-zone (for procedural details see Tan and Collewijn, 1991).

4.2.2 Experimental Protocol

When a rabbit was provided with two guide tubes we applied the following protocol.

4.2.2.1 Pre-injection test

We started by testing the oculomotor response of the rabbit to optokinetic and vestibular stimulation. The animal was first vestibularly stimulated in the dark with a sinusoidal stimulus at a frequency of 0.1 Hz (amplitude 5° p.p.). Then optokinetic stimulation was given at frequencies of 0.05, 0.1 and 0.2 Hz (amplitude 10°, 5°, and 2.5° p.p. respectively). The amplitudes of stimulation were chosen in such a way that the maximum stimulus velocity was always 1.7 °/s. All stimuli were about the vertical axis, and presented over 11 cycles. A detailed description of the stimulus apparatus is given in chapter 2

4.2.2.2 Injections

Subsequently we injected 1.0 µl of Carbachol (1 g/l; Carbamylcholine Chloride, Sigma Chemical Co., St. Louis, USA) bilaterally in the VA-zones of the flocculi. To that means a canula was filled with the Carbachol solution and attached by a micro-tube to a water-filled 1.0 µl syringe (Hamilton, Reno, USA). After the canula was placed in the guide tube the Carbachol was applied by pressure injection. The volume was injected in roughly 10 to 15 seconds in order to avoid pressure artifacts on the neural tissue. Both injections were made within 3 minutes.

4.2.2.3 Post-injection test

In order to assess the effect of these floccular injections of Carbachol on the compensatory eye movements, we performed the post-injection test immediately after the injections. The stimuli that were applied were identical to those in the pre-injection test.

4.2.2.4 Prolonged optokinetic stimulation

Subsequently, the animal was subjected to constant sinusoidal optokinetic stimulation about the vertical axis. The stimulus that was given had a frequency of 0.1 Hz at an amplitude of 5 ° p.p. Stimulus duration was 120 minutes.

4.2.2.5 *Post-stimulation test*

Immediately after stimulation, the same stimuli as in the pre- and post-injection tests were given once more. This was done in order to investigate the frequency-specificity of the effects of optokinetic stimulation, as well as a possible transfer to the VOR. In order to rule out that (part of) the effects were due to fatigue or other such factors, the animal was then kept stationary in the light in a stable visual environment for ten minutes, and a second post-stimulation test was given. However, since no significant differences were observed between both post-stimulation tests, we only present the data of the first post-stimulation test in this chapter.

4.2.3 Controls

Two types of control experiments were performed.
1. In all rabbits we performed an experiment that was identical to the protocol described above, except that we injected saline instead of Carbachol.
2. In two rabbits (M and N) we replicated the experimental protocol of Tan and Collewijn (1991). To that means we performed the normal protocol, but kept the rabbit in a stable environment for 2 hours, instead of presenting prolonged optokinetic stimulation. Every 20 minutes the animal was briefly stimulated (11 periods), in order to measure its OKR gain.

4.2.4 Data recording and analysis

Eye position was measured with the induction coil technique (Collewijn et al, 1975), in a three-field system (Remmellabs, Ashland, USA).
Position signals of the optokinetic and vestibular apparatus, as well as both eyes were sampled at a frequency of 250 Hz (CED1401, Cambridge, UK) and stored on hard disc for offline analysis. During the prolonged optokinetic stimulation 11 periods were recorded every 20 min.

From the 11 periods that were obtained in each condition the gain of the response was determined. To that means the first period was discarded, in order to avoid responses that were due to the onset of the stimulus. From the remaining ten periods, the velocity signal of the stimulus and the eye was calculated by differentiation of the position signals. Through the stimulus signal a sinus was fitted. Fitted parameters were amplitude, frequency and phase. The amplitudes and frequencies that were thus obtained differed less than 1% from the parameters that were specified in the stimulation program.

Fast phases in the eye velocity signal were removed on the basis of a velocity/duration criterion. Through the remaining slow phase signal a sine was fitted with a frequency that was identical to the one obtained from the stimulus, leaving two free parameters: amplitude and phase. Analysis was always performed on the signals from the right eye. Only incidentally, when the induction coil in this eye was broken, we used the signal from the left eye.

The gain of the response was now defined as the ratio of eye velocity amplitude and stimulus velocity amplitude. Note that this is mathematically identical to the –more commonly used- ratio between eye and stimulus *position* amplitude, because differentiation is a linear operation. The advantage of defining gain in the velocity domain is that no signal needs to be substituted for the removed fast phases. Confidence intervals of the fitted gain-values were obtained by calculating the gain of each individual period separately and determining the variation (expressed as standard deviation) in these values.

4.2.5 Histological Verification

After all experiments were performed, the rabbits were perfused. The skull covering the flocculus at both sides of the head was carefully removed and an injection canula was positioned into the guide tube. In all animals the tip of the canulas was located in the floccular cortex of the cerebellum, indicating that all observed effects were related to neurochemical influences inside the flocculus.

4.3 Results

4.3.1 Effects of Carbachol injection

In all rabbits (N=6) except one (rabbit G) the injections of Carbachol caused a significant increase of the gain of the OKR at the frequency that was used for prolonged optokinetic stimulation (0.1 Hz). The absolute values of this increase varied considerably between rabbits (Fig. 4.1). This was probably due to the fact that Carbachol injections never increased the gain to values above unity (Tan and Collewijn, 1991). Therefore rabbits with a relative high gain can only increase their performance slightly, whereas the converse is true for animals with a low gain. Figure 4.1 shows that there is a negative correlation between the gain before injection, and the size of the Carbachol induced gain shift. To overcome this problem we will express gain change in this chapter as a fraction of the pre-adaptation undershoot. Thus,

$$rGC = \frac{G_i - G_p}{1 - G_p}$$

where rGC is the relative gain change, and G_p and G_i are the gains of the OKR before and after injection, respectively. Note that if the Carbachol injection has no effect, rGC equals 0, whereas rGC is 1 when the injection causes the gain to become equal to unity.

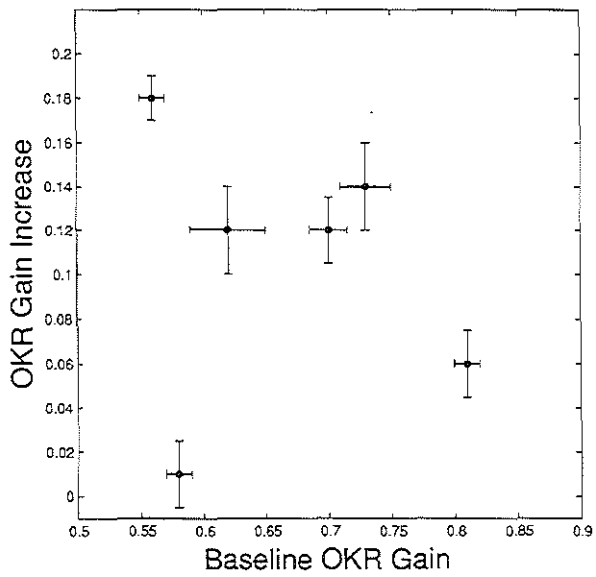

Figure 4.1 *This figure shows for each rabbit the increase of gain due to Carbachol injection, as a function of the baseline optokinetic gain at 0.1 Hz. Note that one rabbit (G) hardly responded to the application of Carbachol. For the other rabbits a negative correlation can be observed between baseline gain and gain increase. Errorbars represent standard deviations (see Methods).*

The closed bars in the top panel of Figure 4.2 summarize the effects of Carbachol for the different frequencies. In the means of this figure the data of rabbit G have been excluded. Since a Carbachol induced gain change is a prerequisite for this study, its data were not relevant for the rest of the chapter.

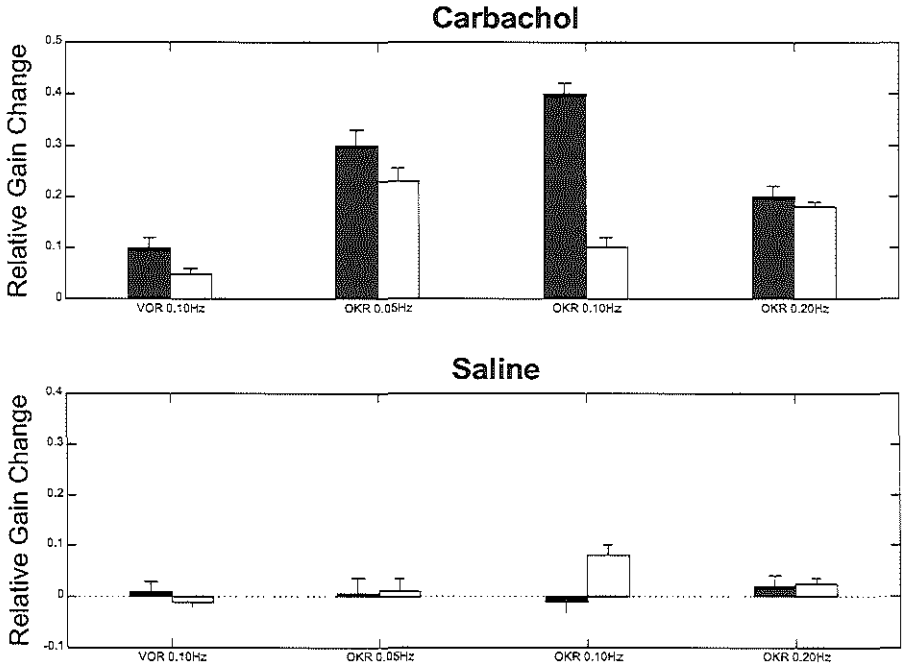

Figure 4.2 *Top panel: Mean relative gain changes (±SD) for each stimulus condition, directly after floccular application of Carbachol (closed bars), as well as after subsequent prolonged optokinetic stimulation (open bars). Note that a reduction is present in all gains. The largest reduction is observed at the frequency applied during the optokinetic stimulation (0.1 Hz).*
Bottom panel: Same graph, but with saline injections. Note that the gain at 0.1 Hz is slightly increased due to the prolonged optokinetic stimulation.

As is shown in this figure, Carbachol caused an increase of the gain in all conditions tested. The increase of the gain in response to optokinetic stimulation exceeded the gain increase of the VOR. This is consistent with the literature (Tan and Collewijn, 1991). As expected, injections of saline had no effect on the oculomotor response of the animals (Fig.4.2, bottom panel).

4.3.1 Effects of optokinetic stimulation

When the animals were subjected to optokinetic stimulation after the floccular application of Carbachol, the gain of the OKR decreased systematically over time (Fig. 4.3, solid line). This was not due to a limited time range in which Carbachol was effective. When Carbachol was given, but no subsequent optokinetic stimulation was applied, the gain increase was more or less constant over the whole recording period of 120 minutes (Fig. 4.3, dashed line). When saline was injected into the flocculus, the gain of the optokinetic response of the rabbit shown in Fig. 4.3 did not change as a result of prolonged stimulation (dotted line). Averaged over all rabbits however, a slight

increase of gain was observed (Fig.4.2), consistent with earlier findings (Collewijn and Kleinschmidt, 1975).

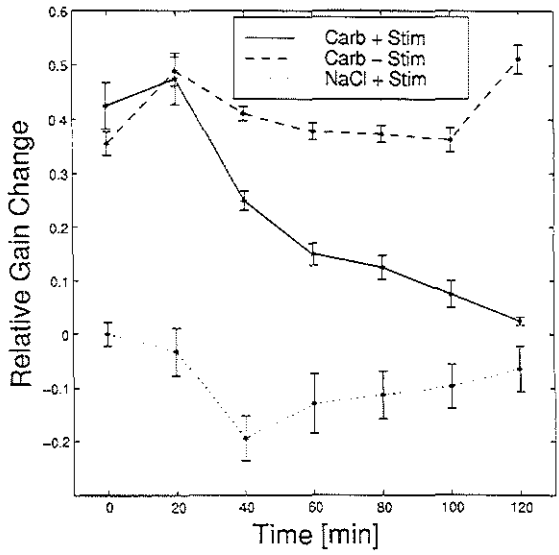

Figure 4.3 *Time course of adaptation (data from rabbit N). This graph shows the data of three different experiments with rabbit N. The solid line represents optokinetic stimulation after Carbachol injection. The dashed line shows the gain values after Carbachol injection, but without subsequent stimulation. The dotted line shows the effect of optokinetic stimulation after injection of saline. Time is given relative to the onset of optokinetic stimulation.*

4.3.2 Frequency specificity and transfer to VOR

The mean values in the post-stimulation test are shown in Fig. 4.2 (open bars). The gain increase of the OKR due to the injection of Carbachol is substantially reduced at 0.1 Hz as a result of prolonged optokinetic stimulation at this frequency. However smaller reductions can be observed at the other optokinetic frequencies, as well as at in the vestibular response. Therefore the adaptation is not strictly frequency or modality specific, but it shows a limited transfer to other stimulus conditions.

4.4 Discussion

4.4.1 Effects of Carbachol injection

In line with earlier findings by Tan and Collewijn (1991) we found that Carbachol has a stimulating effect on the gain of the OKR. However, the absolute effect that we found is considerably smaller than what is reported in

their paper (a mean absolute gain increase of 0.12 in our data, compared to a gain increase of 0.4 in their paper). Several factors may contribute to this. Firstly our rabbits on average had a somewhat larger gain of their optokinetic response before injection (means 0.69 vs. 0.63), and therefore this gain could increase less. Another factor may be that the used frequencies and amplitudes in both studies were slightly different.

4.4.2 Effects of optokinetic stimulation

When an effect of Carbachol was present in the eye position data, this effect remained more or less constant over a period of at least two hours (Fig.4.3, dashed line), provided that the rabbit was kept in a visually stable environment. This condition is in fact a replication of the experiment of Tan and Collewijn (1991), and the results are comparable. However, when the optokinetic system of the rabbit was allowed to evaluate its gain by giving optokinetic stimulation, the gain increase that was created by Carbachol quickly reduced (Fig.4.2 & Fig.4.3).

The OKR can be considered to be a closed loop system. The output of the system is directly compared with its input through sensory feedback. As was discussed in the introduction, the only way to induce lasting changes in a closed loop system is to change its setpoint, which is in the case of the OKR the 'desired' amount of retinal slip. Apparently, floccular application of Carbachol does not change this setpoint value, since the reduction of retinal slip that is induced by such injections, is compensated for by a plastic response that changes the gain to its original value.

Therefore the gain change of the OKR that is neurochemically induced in this study is fundamentally different from the effects that have been found for gain changes that are due to prolonged optokinetic stimulation (Collewijn and Kleinschmidt, 1975). In the latter study a change in the response was found, despite a normal amount of slip at the onset of stimulation. This is indicative for a change of setpoint. Conversely, at the onset of stimulation in our study an unusual amount of slip was created by a gain increase, and this was not accepted by the optokinetic system, suggesting that the setpoint had not altered. This finding is somewhat surprising, since the retinal slip at the onset of stimulation was *less* than the normal slip and the gain of the OKR was closer to unity.

We therefore conclude that the undershoot that is normal for the OKR of the rabbit (expressed as a response gain < 1), is not due to the incapability of the rabbit to perform better, since plastic increase of the OKR gain can be induced (Collewijn and Kleinschmidt, 1975; Tan and Collewijn, 1991; this study). The gain is also not determined by 'the maximum error that is acceptable', which could cause plastic mechanisms to cease their work. If this were the case, the better gain that was induced by Carbachol in our study would not have been actively reduced. Therefore the normal gain undershoot must represent a true optimum for the rabbit. The value of this optimum gain may change under different conditions, for example when a certain optokinetic

stimulation is given for a long period (i.e. the setpoint change in the study of Collewijn and Kleinschmidt, 1975). We can only speculate why a gain that is less than unity is optimal. It seems reasonable to assume that this is due to the fact that under physiological conditions the OKR often works in conjunction with the VOR, and their combined action should be optimal.

4.4.3 Is plasticity of the OKR due to the same mechanism as plasticity of the VOR?

The gain reduction that we observe for the OKR in this study may very well be due to the same mechanism that causes gain changes in the VOR (see Introduction). Both the time course of adaptation and the frequency specificity are comparable (e.g. Collewijn and Grootendorst, 1979). Also the finding that the gain of the VOR is somewhat altered by the adaptation of the OKR is in line with this hypothesis.

However, it must be stressed that the driving force for the adaptation that we observe is 'restoration of the original gain', which, in our case, *increases* the amount of retinal slip. Therefore a mechanism that is activated purely on the basis of the amount of slip, such as LTD is thought to work, cannot account for these findings. One possibility is that other mechanisms, in- or outside the flocculus, must play a role as well. A likely candidate may be presynaptic long-term potentiation (LTP) that has been found at the parallel fiber/Purkinje cell synapse (Salin et al, 1996; Storm et al, 1998). Alternatively, LTD may not strive for minimum slip, but rather for 'optimal slip', similar to what we find in this study. The fact that during normal OKR there is a considerable amount of climbing fiber activity present in the floccular Purkinje cells (Graf et al, 1988), without apparent induction of plastic changes is in agreement with this notion.

4.5 References

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5

Floccular Complex Spikes Encode Motor Error, rather than Retinal Slip[4]

[4] Submitted to Neuron

5.1 Introduction

The flocculus of the rabbit is critically involved in the generation and the plasticity of compensatory eye movements, such as the optokinetic (OKR) and the vestibulo-ocular reflex (VOR). Physical and chemical lesions of the flocculus have shown to cause a decrease in response gain (defined as the ratio between response velocity and stimulus velocity) of both VOR and OKR (Collewijn, 1976; Barmack et al., 1980; Barmack and Pettorossi, 1985; van Neerven et al., 1989) and a blockade of adaptation of the VOR and OKR to new sensorimotor conditions (Ito, 1993).

Like all other regions in the cerebellar cortex, the flocculus receives two major sources of input. The climbing fibers (CF) are projections from the inferior olive (IO). They project directly to the Purkinje cells (P-cells), which form the sole output of the Flocculus. Climbing fiber activation results in complex spiking in the P-cells. The mossy fibers originate in a variety of nuclei, including the medial vestibular nucleus (MVN), and the Nucleus Prepositus Hypoglossi (NPH). They project through the parallel fibers (PF) and floccular interneurons on the P-cells, causing simple spikes.

The role of the climbing fiber signals in cerebellar processing is a topic of vivid debate (for review: Simpson et al., 1996). Some propagate the viewpoint that CF-inputs are related to the timing of cerebellar output (the 'timing hypothesis'; e.g. Llinás and Welsh, 1993; Lang et al., 1999). Others assign a role as teacher-signal to the CF-inputs (the 'learning hypothesis'; e.g. Raymond and Lisberger, 1998). In the latter view the CFs carry a signal that encodes the error between actual and optimal motor output. The fact that CF-inputs can modify the strength of PF/P-cell synapses *in vitro* supports this view (Ito et al., 1982; Linden, 1994).

Complex spikes of floccular Purkinje cells have been recorded in anaesthetized rabbits during rotatory visual stimulation. The modulation of the complex firing rate in these experiments correlated well with the velocity of the retinal slip, i.e. movement of the environment with respect to the retina (Graf et al., 1988). This is thought to be in line with the learning hypothesis, since under these conditions retinal slip is directly related to the motor error of compensatory eye movements. For instance, a response with a gain of 1.0 (perfect compensation) results in no slip.

However, this relation is only true if the visual system is confronted with one flow velocity at a time. eye movements can not compensate for all visual flow on the retina, if multiple flow velocities are simultaneously present. Such circumstances occur as a result of parallax, or of transparent motion. In other words, retinal slip that is due to head translation or rotation equals motor error, only if all objects in a visual scene are at the same distance to an observer, and do not move with respect to each other. If CF-modulation is driven by retinal slip, the full set of flow patterns should determine the response. If CFs encode motor error, the modulation should be determined by the pattern that drives the eye movements.

5.2 Methods

In order to determine whether CFs encode 'retinal slip' or 'motor error', we have recorded from P-cells in the flocculus of the awake and behaving rabbit, while the animal made eye movements in response to transparently moving visual stimuli.

5.2.1 Animal preparation

Eight female dutch belted rabbits were used for this study. All animals had implanted search coils in both eyes for eye position recording. A head-holder made of acrylic cement was placed on the skull, and recording chambers were placed directly above the paramedian lobule of the cerebellum. The details of these procedures are described published in chapters 2 and 5. All surgical procedures as well as all experimental protocols described below are in accordance with the guidelines set by the Animal Welfare Committee of our university, as well as with the principles of laboratory animal care (NIH publication No.~86-23, revised 1985).

5.2.2 Single unit recording

We recorded extracellular potentials of single Purkinje cells in alert rabbits. We used glass micropipettes without filament (outer diameter 1.0 mm, tip 3-4 μm, impedance ±1 MΩ) that were filled with a 4M NaCl solution. The electrodes were advanced into the flocculus by means of a motorized micro drive (Fine Science Tools) that was mounted on a small custom-made XY-table (Fine Science Tools). The XY-table was rigidly attached to the recording chamber. The signal from the electrode was amplified, low pass-filtered (10 kHz, CyberAmp 380, Axon Instruments) and digitized at a frequency of 20 kHz (CED 1401, Cambridge Electronics). The data were stored on hard disk for off-line analysis.

Units in the vertical axis–zone (VA-zone) of the flocculus were selected for recording. This is the area where the P-cells respond to rotations about the vertical axis. A cell was identified as a VA-zone unit by moving a hand-held random dot pattern (40 * 40 cm) in front of the eye of the rabbit in various directions, while monitoring the CS-activity online (Van der Steen et al., 1994).

Only units were selected that provided stable recordings of both the simple and the climbing fiber activity. The pause in simple spike activity that follows a complex spike (CS-SS-pause) is a good parameter to determine whether one is recording from a single Purkinje cell. During the experiment this pause was checked by eye, and verified off-line in the digitized data (see below).

5.2.3 Visual stimulation and eye position recording

For optokinetic stimulation we used a custom-made panoramic apparatus, identical to the setup described in chapter 2. In short, the apparatus consists of an opaque dome (diameter 90 cm) that is white inside and has a black coating on the outside. In the black coating spots are left blank at random locations (Fig 2.1). When illuminated from the outside, these spots light up when viewed from the inside. The dome can be rotated about the vertical axis by means of a servomotor with position feedback. Inside the dome a planetarium is fixed that projects light spots on the interior of the dome, thus creating a second pattern of light spots. In the experiments described in this chapter the planetarium did not move. The light intensity of each pattern could be varied independently.

Eye position was recorded by means of chronically implanted search coils (e.g. van der Steen et al., 1984). The rabbit and the stimulus apparatus were placed in a high frequency oscillating magnetic field system (3*3*3m). These fields were driven and the coil signals were demodulated by a Remmel system (Remmellabs, USA). Eye position signals of both eyes were digitized at 500 Hz and stored on hard disk for off-line analysis.

5.2.4 Experimental protocol

When a P-cell in the VA-zone was isolated, optokinetic stimuli were presented. We always started with a single moving pattern (*SP*, 4.0 cd/m^2), moving at a frequency of 0.1 Hz with an amplitude of 2.5°. The pattern was presented for 11 full cycles.

Subsequently transparent stimuli were presented that consisted of a stationary (S) and a moving pattern (M). The movement parameters of the M-pattern were identical to those of the single pattern described above. The luminance was varied from trial to trial between 0 to 40.0 cd/m^2. The luminance of the S-pattern was fixed (4.0 cd/m^2). In combination, this gave rise to normalized luminances between –1 and 0.9. Normalized luminance (NL) is the contrast between the luminances of the two patterns [NL = (M-S)/(M+S)], and has proven to be the value that determines the ocular response gain of rabbits during transparent stimulation, irrespective of the absolute values of the luminances (chapter 2). An NL of –1 indicates a single stationary pattern, an NL of 0 indicates two patterns with identical luminances, and an NL of 1 is a single moving pattern.

The transparent motion conditions were presented in random order. The total presentation time of all stimuli was roughly 30 minutes.

5.2.5 Histological verification

After the experiments the animals were sacrificed and the electrode tracks were reconstructed based on histology. All recordings were positively identified as being from the VA-zone of the flocculus.

5.2.6 Data analysis

50 Hz hum and its harmonics were removed from the raw electrode signal. Subsequently, possible simple and complex spikes were detected by level discrimination. The height of the level was dynamic. It was set at 3 times the average noise level over the previous second. The set of waveforms of the spikes that were thus detected was decomposed into 4 principal components.

Comparing these principal components allowed filtering out artifacts and possible spikes from neighboring neurons (Epping and Eggermont, 1987). The timing of the remaining simple and complex spikes was stored for further analysis. On the basis of these timing moments the CS-SS-pause was determined for each trial. Trials in which this pause was absent were discarded from further analysis. Under our stimulus conditions rabbits hardly make fast phases. However, when an occasional fast phase occurred, the period of the sine during which it occurred was discarded in order to eliminate possible saccade related changes of activity.

Subsequently, the CS-modulation was related to the stimulus parameters and to the movement parameters of the ipsilateral eye. Sine functions were fitted through the spike histograms and the average stimulus and eye slow phase velocity traces. The sine function had a fixed frequency of 0.1 Hz. Fits were made using the Nelder-Mead simplex method. The response gain was defined as the fitted amplitude of the eye velocity trace divided by the amplitude of the stimulus velocity trace. All off-line analysis was done in Matlab (the Mathworks, USA).

5.3 Results

We successfully recorded from 138 Purkinje cells in the VA-zones of 11 flocculi while monitoring eye position. 28 of these units could be recorded long enough to reliably determine the complex spike modulation over a sufficient number of transparent conditions (24 from the left flocculus, 4 from the right).

5.3.1 Behavior

The eye movement behavior of the animals in response to the transparent stimulation was similar to what we reported before (chapter 2). Depending on the luminances of both patterns, the eyes followed with a response gain that was virtually constant throughout a trial. Fig.4.1A shows that the oculomotor behavior was comparable to the response to a single optokinetic pattern (NL=1) at higher values of NL, whereas the eyes were not moving at lower values of NL. The transition between these two states happened over a short trajectory of luminances, and was often complete within one measured step of NL.

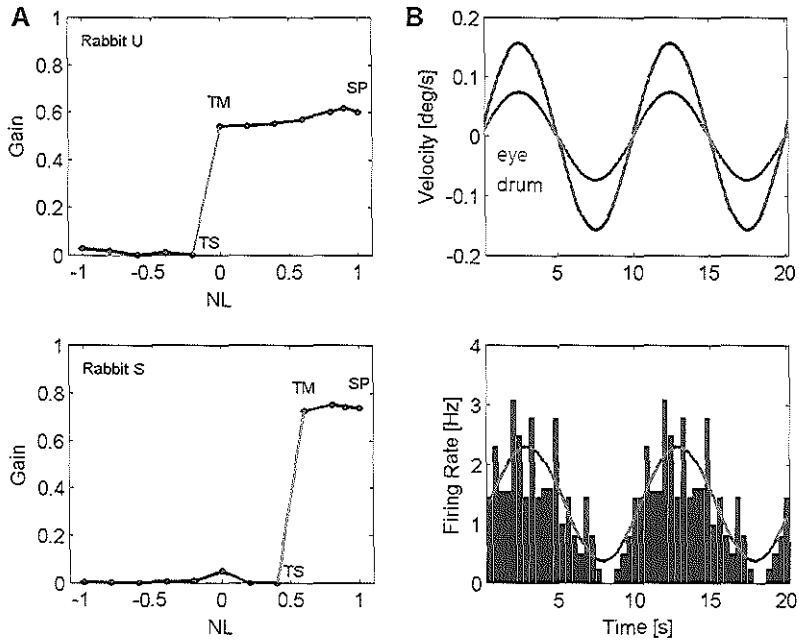

Figure 5.1 *A: Two examples of eye movement behavior in response to transparent stimulation. The gain of the response is plotted as a function of normalized luminance (see text). Note that the responses under transparency are equal to either a single moving pattern (NL=1) or to a stationary pattern (NL=0). TS is the highest NL-value where no movement occurs, TM is the lowest NL-value where the eye moves, and SP is the response to a single pattern.*
B: *Complex spike modulation. This graph shows the response of the CS spikes to a single optokinetic pattern (lower panel). The histogram represents values averaged over 10 cycles. The sine has been fitted through the data. The upper panel shows that the peak of the CS-modulation is roughly in phase with the maximum velocity of the stimulus and the eye.*

This means that in the majority of transparent conditions the eye movement behavior was either identical to the behavior in response to the S-pattern alone, or to the M-pattern alone. However, the NL-values where the shift from one type of behavior to the other occurred varied between animals and from day to day (compare the two panels of Fig 4.1A). For each recording we marked these values *TM* (transparent moving) and *TS* (transparent stationary) respectively. TM varied between -0.2 and 0.6 and TS between -0.4 and 0.4.

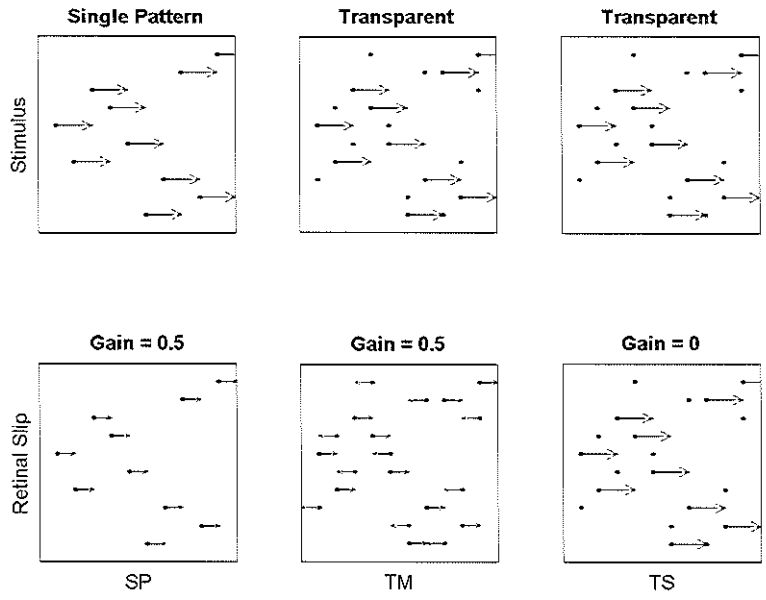

Fig 5.2 A: *Retinal slip due to a single moving pattern. Here we schematically show the relation between a stimulus and the ensuing slip signal. The upper graph shows the movement of the stimulus in head centered coordinates. When the eye compensates for this movement with a gain smaller than 1 (0.5 in this example), the slip in retinal coordinates is reduced (see lower panels) and is in the same direction as the stimulus. For sinusoidal stimulation this results in a slip signal that is in phase with the stimulus.*
B: *Retinal slip at TM. Due to the movement of the eyes the dots of the stationary pattern now move in retinal coordinates, creating retinal slip. This slip is exactly in counterphase to the slip that is due to the moving dots.*
C: *Retinal slip at TS. Since the eyes do not move, no compensation for slip occurs. Therefore the slip is in phase with SP, but at a higher velocity. TS, TM and SP correspond to the values depicted in Fig 1A.*

Though the oculomotor behavior at TM is identical to the response to a single pattern (SP; see Fig 5.1), the retinal slip in these conditions is considerably different. This is shown schematically in Fig. 5.2. When a single optokinetic pattern moves, all retinal slip is approximately in phase with the stimulus (Fig

5.2A). This is because the response gain is always smaller than 1. With the chosen parameters in this study it is in the order of 0.5. When the rabbit makes an identical movement in response to a transparent stimulus such as those used in our paradigm, the stationary dots of the S-pattern create slip that is exactly in counterphase to the slip that is generated by the M-pattern (Fig 5.2B), with roughly similar speeds. At TS, when the eyes do not move, a slip pattern is present on the retina which moves in phase with the M-pattern and has a higher velocity than in the SP condition (Fig 5.2C).

5.3.2 Complex spike modulation

Fig 5.1B shows an example of the modulation of the complex spikes over time in the SP condition. As can readily be seen, the modulation was mild enough to prevent 'clipping', i.e. completely silencing the climbing fiber input while rotating to the non-preferred direction. Accordingly saturation affects in CS firing are not assumed. We could therefore reliably fit a sine function through the spike histogram . The phase and amplitude of this sine were taken as the phase and amplitude of the CS-modulation. Sine fits with an $r^2>0.5$ were considered to represent a significant spike modulation.

The critical test to compare motor error coding with retinal slip coding is to compare the CS-modulation at SP and at TM. If the CS-modulation encodes motor error, the response at TM should be equal to the response at SP, since the motor error is the same in the two conditions. If retinal slip is encoded, the response at TM should be determined by the two slip patterns. Then, two possible outcomes are possible (see Fig 5.3). (a) If the cell responds stronger to the M-pattern than to the S-pattern, the modulation amplitude should decrease, but the phase of the response should remain the same. (b) However, if the S-pattern dominates the phase of the response changes over 180°. The amplitude can in principle increase and decrease, depending on how strong the response to the S-pattern is.

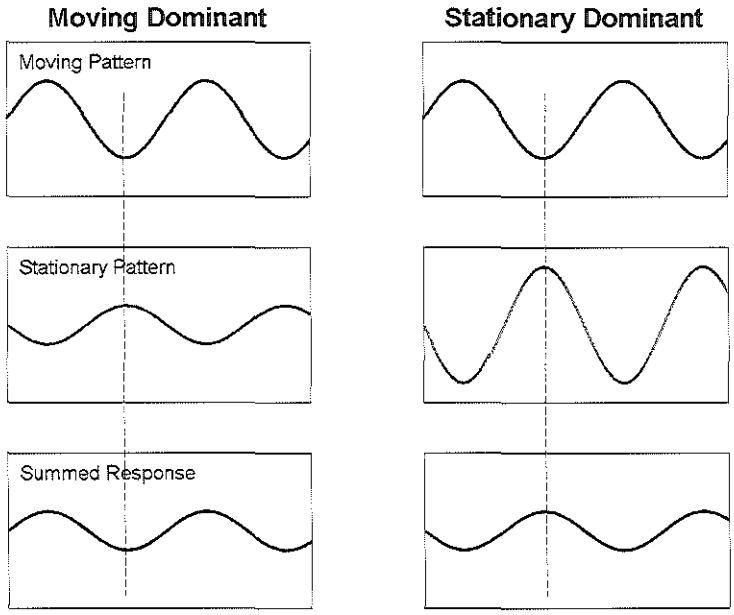

Fig. 5.3 *Hypothetical CS-responses to transparent stimulation. Sine waves in this figure represent modulation of CS-activity. The summed response can vary in amplitude, based on the relative weights of the inputs of the two slip signals. However, the phase of the summed response is either in phase with the slip of the M-pattern or with the S-pattern slip and will not adopt an intermediate value.*

Therefore we compare both phase and amplitude of the CS-responses in both conditions. An example of the modulation of a representative P-cell at SP, TM and TS is shown in Fig. 5.4A. The phase differences of the CS modulations with respect to the SP condition are shown in Fig. 5.4B. None of the neurons changed its phase over 180° (see Fig 5.3, right panel). In 5 neurons a small but significant modulation was also present at TS. As expected this modulation was also in phase with the responses in the other conditions.

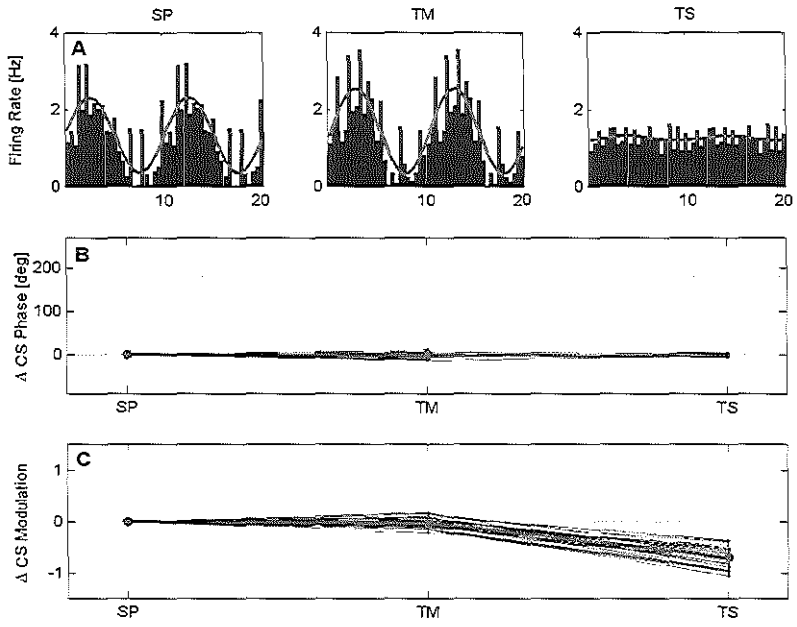

Fig 5.4. *CS-modulation as a result of transparent stimulation.* ***A:*** *Spike histogram of unit U8L at SP, TM and TS.* ***B:*** *Phase changes. Only data are shown of significant modulations (SP,TM: N=28; TS: N=5)* ***C:*** *Amplitude changes. Individual (thin line; N=28) and average (thick line; ±SD) changes with respect to the response to a single optokinetic pattern (SP). The predictions of retinal slip coding are not fulfilled, since in none of the recorded neurons the phase changed over 180° at TM (dashed line in panel B), and no systematic decrease of modulation amplitude can be seen. Therefore these data are in line with the prediction of motor error coding.*

Therefore, if there is an influence of the stationary pattern, it should be reflected in a systematic *decrease* of modulation amplitude. However, the fitted changes of amplitudes at TM scatter around zero and the mean change is insignificant (Fig. 5.4C, $p > 0.05$). In conclusion, no systematic effect of the stationary pattern on the response at TM could be observed. The same is true for all measured transparent conditions at NL-values larger than TM (not shown). At TS the response is small and often insignificant (see above). This is in agreement with the high slip velocity in this condition, for which CS hardly respond (Graf et al, 1988).

5.4 Discussion

This chapter is the first that studies climbing fiber input to the cerebellar flocculus, while 'motor error' and 'retinal slip' are dissociated. By presenting two optokinetic patterns simultaneously ('transparent motion') not all retinal slip could be compensated for by an eye movement. We have shown that the modulation of complex spikes in P-cells of the flocculus does not change when an additional flow pattern is added to the optokinetic stimulation. The complex spike modulation does not change under identical motor conditions (i.e. response gain), despite a considerably different pattern of retinal slip. This means that the climbing fiber input to the flocculus does not simply represents slip signals on the retina. Rather it seems to encode the difference between the velocity of the eye and the stimulus that triggers the movement. This is the *motor error* of the eye.

It must be stressed that a motor error signal is not a motor signal. In order to drive CS-modulation, the presence of a visual stimulus is required. For example during VOR in the dark, when the eye undershoots the head movement no CS-modulation occurs (see however Simpson et al., 1999). Under these circumstances the compensatory eye movements function in an open loop fashion due to the lack of sensory feedback. This means that no error can be obtained. In this way 'motor error' is different from the motor error signals that are found in the deep layers of the superior colliculus (DLSC; e.g. Wurtz and Goldberg, 1971). In the DLSC the error is not between stimulus and eye, but rather between actual eye position and desired eye position. As a consequence motor error bursts in the DLSC precede every saccadic eye movement, even in absence of a stimulus.

If the CF-input to the flocculus is indeed a teacher signal, it functions to shape the P-cell responses to mossy fiber input. Therefore it makes sense from a functional point of view that the nature of this signal is motor related rather than being purely sensory. It is commonly assumed that the role of the floccular output is to optimize compensatory eye movements. The signal that teaches this output should therefore not be 'contaminated' by sensory variation, but should rather represent the motor task that is to be performed as accurately as possible.

When analyzing complex spikes of P-cells, one is actually directly analyzing the output of the IO, since the efficacy of the CF synapse is so high that each action potential on a CF results in a complex spike. Thus, complex spikes directly reflect IO output. If the IO encodes motor error rather than slip, a form of pattern selection must take place at some stage in or upstream of the IO, since it has to be decided with respect to which pattern motor error has to be determined. For the stimuli that were used in this study, such a mechanism could be reasonably straightforward. The slip velocity vector that is caused by the stationary pattern is identical to the velocity of the eye in the head. Therefore, combining a mixed slip signal with an eye velocity signal could filter out the contribution of the S-pattern slip.

Such a mechanism might take place in the IO itself. The nucleus of the optic tract (NOT) and the assessory optic system (AOS) provide the IO with slip signals (Soodak and Simpson, 1988). Furthermore the nucleus prepositus hypoglossi (NPH) of the rabbit, that carries both eye position and eye velocity information has inhibitory projections directly to the IO (De Zeeuw et al., 1993). Thus all signals that are required for filtering out the stationary background are present at the level of the IO. Nevertheless, as stated above, such a mechanism can only be functional to filter out slip that is due to a head-stationary stimulus but is useless for segregation between two or more moving stimuli. Responses to such stimulation are currently under investigation in our laboratory. They are necessary to investigate whether the present findings can be generalized to all stimulus conditions.

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6

Summary and Conclusions

In this thesis the compensatory eye movement system of the rabbit as a general model for VOR, OKR and VOKR was investigated on a behavioral and neuronal level using sinusoidal transparent motion.

OKR and VOKR responses to transparent motion (Chapter 2)

Transparent motion is a visual stimulus condition that generates multiple motion vectors on the retina that can differ in speed, direction, and/or luminance. Transparent motion creates a conflict for retinal stabilization. In chapter 2 we investigated the effect of transparent visual motion on the oculomotor reflexes that provide retinal stabilization in the rabbit. In the first experimental condition, the animals were stationary. We presented one stationary and one oscillating visual pattern to the animals while varying the luminance of the patterns. We found that the optokinetic eye movement gain was fully determined by the luminance of the individual visual inputs, weighted for the total luminance. Thus no effect of absolute stimulus intensity was found. In the second experimental condition we oscillated the animals, while using an identical visual stimulation paradigm. The contribution of the vestibulo-ocular reflex enhanced the response to the visual pattern, that was in agreement with the vestibular stimulus. This effect of vestibular stimulation was independent of the absolute intensity of the visual stimuli. Vestibular stimulation results in an approximately constant shift of the NL-gain relationship. From this result we conclude that the weighting process of the transparent visual patterns occurs upstream from the site of the visual-vestibular interaction. If the vestibular input would be added for example before visual normalisation, the shape of the NL-gain relationship would have been altered, which was not the case. Both the visual weighting and the visual-vestibular interaction were dependent on stimulus frequency. In line with the properties of the visual and vestibular stabilization reflexes in isolation, the contribution of the vestibular system increased, whereas the influence of the optokinetic system decreased with increasing stimulus frequency.

Neuromodulation of the flocculus and transparent motion responses of OKR and VOKR (Chapter 3)

In chapter 3 we investigated the role which the flocculus plays in the OKR and VOKR response to transparent motion by injecting the non-selective acetylcholine agonist Carbachol into the flocculus. These injections are known to increase the gain of the optokinetic reflex, but have a smaller effect on the vestibulo-ocular reflex (Tan and Collewijn, 1991). We investigated the effect on the oculomotor response to (vestibulo-) transparent stimuli, where one pattern oscillated sinusoidally and the other pattern was stable with respect to the head. We found that the injections caused a higher response gain at a

lower luminance of the oscillating pattern. Furthermore the influence of concurrent vestibular stimulation decreased. These findings agree with a role of the flocculus that is downstream of visual normalisation, presumably the AOS and NOT but upstream of the visual-vestibular interaction, very likly the vestibular nuclei.

Properties of gain changes of the optokinetic reflex (Chapter 4).

In the previous chapter Carbachol was used to investigate floccular involvement during transparent stimulation. This chapter uses non-transparent optokinetic stimulation to investigate the functional effect of floccular micro-injections with Carbachol. The optokinetic stimulus consisted of a dome shaped surface on which one pattern is projected moving sinusoidally about the vertical axis. The OKR gain was measured after Carbachol injections at 20 minutes interval for 2 hours. In that 20 minute interval the rabbits were kept in a stable illuminated visual environment or were subjected to continues sinusoidal optokinetic stimulation.
Prolonged optokinetic stimulation after carbachol injections demonstrated that the initial neurochemical gain increase returned to the OKR gain level before injection. While, in the case where the rabbit was kept in a stationary environment, the neurochemical gain enhancement remained unchanged. The OKR is considered to be a closed loop system. The output of the system is directly compared with its input through sensory feedback. The only way to induce lasting changes in a closed loop system is to change its setpoint. We therefore conclude that floccular application of carbachol does not change this setpoint value, since the reduction of retinal slip that is induced by such injections, is compensated for by an adaptive response that changes the gain to its original value. Consequently carbachol and prolonged optokinetic stimulation act on different mechanisms. As P-cell CS are thought to play a key role in natural adaption as was used in this chapter, this study was followed by an investigation on the effect of transparent stimulation on CS firing.

Complex spike responses of floccular Purkinje cells to transparent motion (Chapter 5).

In this paper we investigate the nature of the signal that is encoded by the climbing fiber input to the flocculus of the cerebellum. In the literature it is stated that this input encodes 'retinal slip', which in turn would be a measure for the motor error of compensatory eye movements (for review: Simpson et al., 1996). However, this relation is only true under specific conditions, where all visible objects are at the same distance to the eyes, and objects do not move with respect to each other. We therefore presented transparent motion to rabbits, while recording the ensuing oculomotor behavior and complex spike activity of floccular Purkinje cells. By presenting transparent motion we created an ambiguous retinal slip signal. Nonetheless, in many transparent conditions the animals displayed oculomotor behavior that was identical to the

response to a single optokinetic pattern, in line with the data of chapter 1 of this thesis.
In none of the recorded neurons, the presentation of an additional retinal slip signal during transparent motion affected the phase or amplitude of the complex spike modulation. We therefore conclude that the climbing fiber input to the flocculus does not respond to retinal slip, but rather encodes the motor error of the movement. In order to determine motor error with respect to a stimulus, some form of pattern selection has to take place in the complex spike code when more stimulating patterns are present.

Samenvatting

In dit proefschrift is het compensatoire oogbewegingssysteem van het konijn, in het bijzonder de vestibulo-oculair reflex (VOR), de optokinetische reflex (OKR) en de vestibulo-optokinetisch reflex (VOKR) onderzocht. Van deze reflexen is de verwerking van oscillerende transparante bewegingen op gedragsniveau en op het niveau van het zenuwstelsel bestudeerd.

OKR en VOKR reacties op transparante bewegingen

Transparante beweging is een visuele prikkel waarbij meerdere bewegende patronen op het netvlies ontstaan die over elkaar heen kunnen liggen en kunnen verschillen in snelheid en/of richting. Een voorbeeld waarin dit optreedt is wanneer we ons voortbewegen in de ruimte met meerdere objecten en naar 1 object in die ruimte kijken (zie omslag van dit proefschrift). Hierbij kan een conflict situatie ontstaan voor het stabiliseren van het totale beeld op het netvlies. (voorbeeld: een passagier in een trein die gaat rijden terwijl die naar buiten kijkt).

In hoofdstuk 2 wordt het effect van transparante visuele bewegingen onderzocht op de reflexmatige oogbewegingen van het konijn die het beeld op het netvlies stabiliseren. Om dit te bestuderen werden twee soorten experimentele condities gerandomiseerd aangeboden: in de eerste experimentele conditie bewoog het konijn niet. Er werden vervolgens een om de verticale as oscillerend doorzichtig patroon en een stilstaand patroon gelijktijdig aangeboden waarbij de lichtsterkte van de afzonderlijke patronen gevarieerd werden. De optokinetische oogbewegingen werden volledig door het verschil in de lichtsterkte van de individuele patronen als fractie van de totale lichtsterkte van beide patronen bepaald. De absolute lichtsterkte van de afzonderlijke patronen had geen effect op de oogrotaties. In de tweede experimentele conditie werden de konijnen sinusoidaal om de verticale as geroteerd terwijl dezelfde transparante visuele stimuli werden aangeboden. Dit resulteerde in een verbeterde compensatoire oogbeweging ten opzichte van het bewegend patroon. De bijdrage van de VOR versterkte de reactie op het patroon wat in overeenstemming was met de vestibulaire stimulatie. Dit vestibulair effect was niet afhankelijk van de absolute lichtsterkte van de patronen. Derhalve werd geconcludeerd dat de transparante patronen neuraal verwerkt worden voordat vestibulaire interactie met deze informatie plaats vindt.

Een model wat deze interactie verklaart wordt in dit hoofstuk gepostuleerd. Het effect van verandering in stimulatie frequentie van de transparante stimulus op de VOKR en OKR werd eveneens bestudeerd door het aanbieden van 3 verschillende frequenties. Zowel de OKR als de VOKR onder transparante condities vertoonde een frequentie afhankelijkheid. De *gain* (de amplitude van de oogrotatie als fractie van de stimulus) van de VOKR nam

toe bij lagere genormaliseerde lichtsterktes terwijl de OKR gain afnam bij hogere waarden met het toenemen van de frequentie. Dit is in overeenstemming is met de individuele eigenschappen van de VOR en de OKR.

Neuromodulatie van de flocculus en OKR / VOKR respons op transparante bewegingen.

In hoofdstuk 3 werd de rol die de flocculus speelt in de VOKR en OKR reactie op transparante beweging onderzocht door de aspecifieke cholinerge agonist carbachol in de beide flocculi te injecteren. Van deze injecties is bekend dat ze de gain van de OKR verhogen en tevens in mindere mate de gain van de VOR (Tan en Collewijn 1991). Er werden experimenten uitgevoerd waarbij er of carbachol of fysiologisch zout in de flocculus werd geïnjecteerd waarna dezelfde transparant bewegende patronen in dezelfde condities als hiervoor beschreven (hoofdstuk 2) werden aangeboden wel of niet gecombineerd met vestibulaire stimulatie. Uit de metingen van de oogbeweging bleek dat de er een toename van de optokinetische gain optrad bij lagere lichtsterktes van het bewegend patroon na carbachol in vergelijking met de controle proeven. Tevens verminderde de toename in de gain als gevolg van gelijktijdige vestibulaire stimulatie. Deze bevindingen zijn in overeenstemming met de rol van de flocculus als station na neurale verweking van de visuele normalisatie van transparantie (zoals aangegeven in het model van hoofdstuk 2) en voor vestibulaire interactie met het visueel signaal.

Eigenschappen van gain veranderingen van de OKR als gevolg van carbachol.

In het vorige hoofdstuk werd Carbachol gebruikt om betrokkenheid van de flocculus in neurale verwerking van signalen tijdens transparante bewegingen te onderzoeken. Hoofdstuk 4 maakt als enige in het proefschrift gebruik van niet transparante optokinetische stimulatie om het effect van carbachol injecties in de flocculus nader te bestuderen. De visuele stimulus bestond uit een koepelvormig oppervlak met lichtgevende vlekken erop die om de verticale oscilleerde rondom het stilstaande konijn. Na de injecties met Carbachol werd om de 20 minuten de OKR gain bepaald gedurende 2 uur. Twee experimentele condities werden onafhankelijk van elkaar uitgevoerd en met elkaar vergeleken. In de tussenliggende periode van 20 minuten werd het konijn in de ene conditie in een stilstaand verlichte omgeving gehouden en in de andere conditie het werd het konijn doorlopend visueel gestimuleerd zoals beschreven. Van langdurige optokinetische stimulatie is bekend dat het de OKR verbetert.

Aanhoudende optokinetische stimulatie na injecties met carbachol liet zien dat de gain toename als gevolg van carbachol injecties daalde naar het niveau voor de injecties. Dit is in tegenstelling tot de conditie waarbij de visuele omgeving stil stond, de neurochemische gain toename bleef hierbij

gehandhaafd. De OKR wordt beschouwd als een gesloten systeem met een interne terugkoppeling. De output van dit systeem (de compensatoire oogbeweging) wordt direct vergeleken met de sensorische input (retinale slip). De enige wijze waarop een blijvende verandering in dit systeem kan worden aangebracht is door het omslagpunt voor de optimale OKR gain te wijzigen. Uit de experimenten wordt geconcludeerd dat door het injecteren van carbachol in de flocculi dit omslagpunt niet wijzigt aangezien de vermindering van de retinale slip door deze injecties te niet worden gedaan door een adaptief proces. Derhalve werkt carbachol op een ander mechanisme dan langdurige optokinetische stimulatie. Omdat van Complex Spikes wordt aangenomen dat zij een sleutel rol spelen in adaptatie zoals in dit hoofdstuk is gebruikt, werd dit onderzoek gevolgd door experimenten waar bij het effect van transparante stimulatie op het voorkomen van Complex Spikes werd bestudeerd

Complex spike respons van flocculus Purkinje cellen op transparante beweging.

In dit hoofdstuk wordt onderzocht wat het signaal, wat door de klimvezels naar de flocculus wordt geleid, eigenlijk betekent. Dit signaal is herkenbaar als het repeterend optreden van kenmerkende relatief langdurige actie potentialen van de Purkinje cel, genaamd Complex Spikes. In de literatuur wordt beweerd dat dit signaal *retinale slip* (verschuiving van netvliesbeelden) codeert waarmee het een maat is voor de imperfectie (de motorische fout) van de compenserende oogbeweging (Simpson 1996). Dit geld echter alleen in specifieke omstandigheden waarbij alle bewegende visuele objecten op dezelfde afstand ten opzichte van de ogen staan en deze objecten niet ten opzichte van elkaar bewegen. Transparante visuele stimulatie werd aangeboden op gelijksoortige wijze zoals eerder beschreven terwijl de resulterende oogbewegingen en Complex Spike activiteit van Purkinje cellen in de flocculus werden opgenomen. Door het aanbieden van een transparante stimulus creëerden wij een ambigu retinale slip signaal, er was altijd sprake van retinale slip ongeacht de oogbeweging. In deze experimentele opzet is de imperfectie van de compensatoire oogbeweging dus niet meer gelijk aan de retinale slip. Deze studie is de eerste in de literatuur waarbij deze 2 entiteiten op deze wijze van elkaar gescheiden zijn.

Desondanks reageerde de konijnen in vele transparante condities met compensatoire oogbewegingen alsof een niet transparant bewegend patroon werd gebruikt wat ook in overeenstemming is met de data van hoofdstuk 1 van dit proefschrift. In geen van de neuronen leidde deze additionele retinale slip veroorzaakt door het transparante patroon tot veranderingen in de fase of de amplitude van de Complex Spike modulatie. Derhalve concluderen wij dat de klimvezels naar de flocculus niet reageren op retinale slip maar op de motorische fout van de oogbeweging. Om deze te kunnen vast stellen moet er een patroon selectie hebben plaats gevonden in de complex spike code bij aanwezig van meerdere transparante patronen.

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Curriculum Vitae

De auteur van dit proefschrift werd geboren op 18 maart 1968 te Rotterdam. Na de lagere school jaren deels op Aruba en in Nederland te hebben doorlopen, ging hij, dankzij de inspanningen van zijn ouders, naar het Atheneum-β aan het Rotterdams Lyceum waar hij het diploma in 1986 behaalde. Vervolgens studeerde hij geneeskunde aan de Erasmus Universiteit Rotterdam. In mei 1994 behaalde hij zijn arts-examen.

Om in de kliniek wetenschappelijk beter toegerust te zijn begon hij in het Oogziekenhuis als arts-onderzoeker. Al gauw verdiepte hij zich in fundamentele niet klinische onderwerpen waarop de stap naar het echte wetenschappelijk werk werd gezet in 1995 toen hij AIO/OIO werd op de Afdeling Fysiologie, (afdelingshoofd Prof. H. Collewijn), Faculteit der Geneeskunde en Gezondheidswetenschappen van de Erasmus Universiteit Rotterdam. In juni 1999 waren al de experimenten verricht en verliet hij de afdeling en was voor een korte periode werkzaam als AGIO op de afdeling KNO. Na een zeer leerzame klinische periode werd de curatieve sector verlaten en begon hij als verzekeringsarts bij GAK Nederland. Per januari 2001 is hij aangevangen als adviserend verzekeringsarts bij het USZO/ABP te Rotterdam.

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