VISUO-VESTIBULAR INTERACTIONS IN THE RABBIT: THE ROLE OF THE FLOCCULUS AND ITS MONO-AMINERGIC INPUTS

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VISUO-VESTIBULAR INTERACTIONS IN THE RABBIT: THE ROLE OF THE FLOCCULUS AND ITS MONO-AMINERGIC INPUTS

(VISUO-VESTIBULAIRE INTERACTIES BIJ HET KONIJN: DE ROL VAN DE FLOCCULUS EN ZIJN MONO-AMINERGE INPUTS)

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CONTENTS

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Gaze-holding eye movements

To provide adequate vision, retinal images should be kept reasonably stable in all conditions. Although this could theoretically be accomplished by head movements, it is mainly done by several types of eye movements which have one goal in common: the reduction of retinal slip, whatever its cause. These eye movements are called *gaze-holding* eye movements. This thesis will mainly deal with two types of gaze-holding eye movements, which are both the output of a gaze-stabilizing reflex. These reflexes, the vestibulo-ocular reflex (VOR) and the optokinetic reflex (OKR), serve to stabilize vision during movements of the head, or relative movement of the surroundings, respectively. The purpose of these eye movements, the reduction of retinal slip, has led to naming them *compensatory eye movements*. In case of the VOR, the compensatory eye movements oppose the stimulus direction, while in the OKR, stimulus and compensatory eye movements are similarly directed. The smooth compensatory eye movements of the VOR or OKR can be interrupted by very fast *gaze shifting* eye movements in opposite direction, the so-called *saccades*.

Gaze-holding eye movements occur in all animals with mobile eyes, even in those who are unable to generate other, voluntary types of eye movements. If one wants to study gaze-holding reflexes, it is an advantage if the animal can not make other than the tested type of eye movement. For this reason, we used the rabbit in the present experiments. Many animals have a retinal area with highly specialized function, the *fovea*. This is an area with high receptor and neuron density, and therefore high visual acuity. Foveated animals are able to voluntarily fixate or smoothly pursue an object of interest. Such intentional eye movements or fixations can disguise, or suppress the gaze-stabilizing reflex eye movements. The rabbit, a non-foveated animal, does not show such voluntary

oculomotor behavior and, therefore, its compensatory eye movements of the VOR and the OKR can be measured without interference by eye movements of the voluntary type. Because the vestibulo-ocular and the optokinetic reflexes take an important place in this thesis, their function and characteristics will be described in the next sections.

The vestibulo-ocular reflex (VOR)

This reflex serves to stabilize retinal images during movements of the head or the whole body. The sensory information of this reflex is derived from several parts of the vestibular apparatus. The semicircular canals, which are arranged perpendicularly in three dimensions, detect angular accelerations. Two other parts of the vestibular apparatus, the utriculus and the sacculus, are sensitive to linear accelerations. Angular acceleration is detected by hair cells in the cupula-endolympe system, which convert the stimulus into a pulse train in the vestibular nerve. This nerve projects to the vestibular nuclei in the brain stem, which in turn activate -by several connections- the oculomotor nuclei (see Fig. 1.1). Vestibular information is also sent to the cerebellar flocculus, which is part of a parallel side-path to the vestibulo-ocular reflex loop. In short, the vestibular input results in an oculomotor output, which drives the eye muscles. The direction of the resulting eye movement is opposite to the direction of the head movement.

The VOR is a feed-forward system and thus, output can not be checked directly at the input level. Of course, this system should have another oppertunity to control its adequacy, and indeed it has, as will be explained shortly.

Dynamic characteristics of the VOR. Input-output relations of the VOR can be studied by oscillating an animal on a platform around a fixed vertical axis in total darkness, thus providing a vestibular stimulus only. To abolish retinal slip in this situation, the amplitude of the compensatory eye movements should be identical to the stimulus amplitude. However, this is never accomplished in darkness, not even by humans. Compensation is always less than 100%.

In rabbits, the VOR in darkness acts most efficiently at stimulus frequencies above 0.1 Hz, where compensation is maximally about 70%. The VOR is amplitude-dependent, compensating better at higher amplitudes. At lower stimulus frequencies and amplitudes (i.e., at lower stimulus velocities), the compensatory capacity is seriously impaired (Baarsma and Collewijn, 1974). The system responds very fast: eye movements are started within 10 - 14 ms after presentation of the vestibular stimulus (Lisberger, 1984).

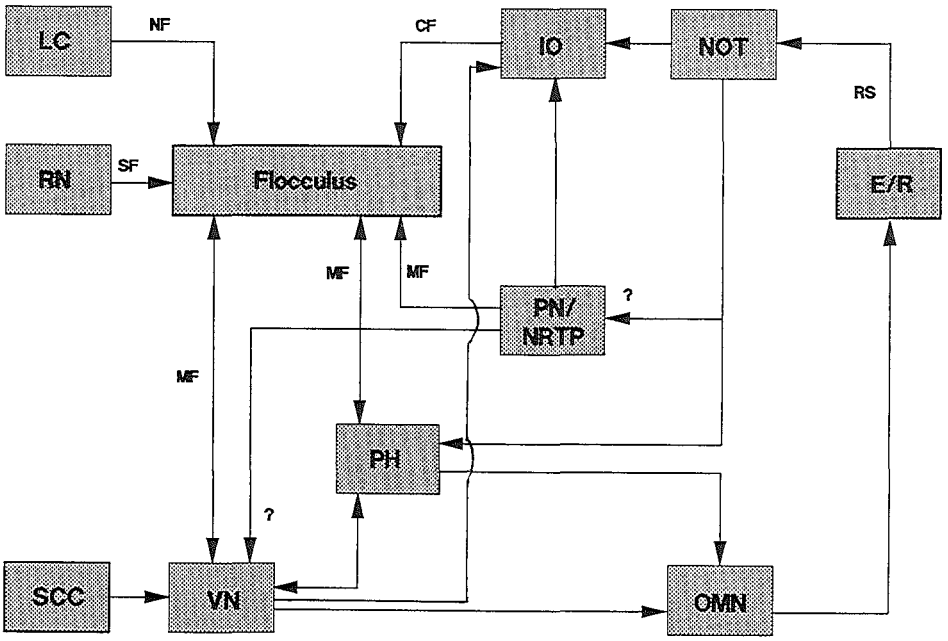

Fig. 1.1. Schematic diagram, showing the neuronal connections of the vestibulo-ocular reflex (VOR), the optokinetic response (OKR), and the interaction between these systems. The cerebellar flocculus with its inputs is indicated as the central structure in this figure. Abbreviations are explained underneath.

SCC = *semi-circular canals*
VN = *vestibular nuclei*
OMN = *oculomotor nuclei*
E/R = *eye, retina*
NOT = *nucleus of the optic tract*
IO = *inferior olive, dorsal cap*
PH = *nucleus prepositus hypoglossis*
PN = *pontine nuclei*
NRTP = *nucleus reticularis tegmenti pontis*
LC = *locus coeruleus*
RN = *raphe nuclei*
rs = *retinal slip signal*
cf = *climbing fibers*
mf = *mossy fibers*
nf = *noradrenergic fibers*
sf = *serotonergic fibers*

The optokinetic response (OKR)

This reflex serves to reduce retinal slip, caused by relative movements of the surroundings with respect to the head. Retinal slip velocity is the input to the optokinetic system. The anatomical pathways involved in the OKR are less well-defined than those of the VOR. The direction selective visual information from the retina is transferred to the pretectal nucleus of the optic tract (NOT) (Collewijn, 1975). The projections from the NOT are in part controversial, because considerable species differences in anatomical connections have been demonstrated. The efferent connections

of the NOT in the rabbit have been studied by an anterograde tracing technique (Holstege and Collewijn, 1982), which revealed both ascending and descending fiber bundles. The ascending fibers, which projected to the lateral geniculate nucleus and other thalamic nuclei, are probably not involved in the OKR, and will therefore not be discussed in detail. The descending fibers from the NOT projected to several eye-movement related brain stem areas, of which the nucleus prepositus hypoglossi (PH), the nucleus reticularis tegmenti pontis (NRTP) and the inferior olive (IO) are important with respect to the OKR.

The nucleus prepositus hypoglossi receives input from several eye-movement-related areas: the NOT, the flocculus and the vestibular neurons (McCrea and Baker, 1985) and projects to the oculomotor neurons, to the vestibular nuclei and to the flocculus (McCrea and Baker, 1985). Its involvement in vestibulo-ocular pathways was physiologically demonstrated by Baker and Berthoz (1975).

The projection from the NOT to the NRTP remains controversial, because it was anatomically demonstrated in the cat (Graybiel, 1974) and in the rat (Teresawa et al., 1979), but these findings could not be confirmed in the rabbit (Holstege and Collewijn, 1982). Physiological experiments by Maekawa and Kimura (1981) provided evidence for a connection between the NOT and the NRTP because NOT neurons were antidromically activated after electrical stimulation of the NRTP. The involvement of the NRTP in the generation of the OKR is, however, without doubt, because the NRTP has been shown to be a source of mossy fibers to the flocculus (Yamamoto, 1979; Maekawa and Takeda, 1981; Gerrits et al., 1984). A projection from the NRTP to the vestibular nuclei was demonstrated in rabbits (Balaban , 1983), but this could not be confirmed in the cat (Gerrits and Voogd, 1986).

The dorso-lateral pontine nuclei, which are situated near to the NRTP, do also convey direction-selective information (Mower et al., 1979) and receive projections from the NOT (Holstege and Collewijn, 1982). Their projection to the flocculus (Hoddevik et al., 1977; Yamamoto et al., 1979) is controversial.

Lastly, the NOT was found to project to the dorsal cap of the inferior olive, which moreover receives input from the vestibular nuclei and from the paramedian pontine reticular formation (Gerrits and Voogd, 1986). The dorsal cap of the inferior olive sends climbing fibers to the cerebellar flocculus (Maekawa and Simpson, 1972; Takeda and Maekawa, 1976; Gerrits and Voogd, 1982).

Figure 1.1 demonstrates these main connections involved in the generation of the OKR. Most of these OKR-related areas project, directly or indirectly, to the vestibular nuclei, which in turn project to the oculomotor nuclei.

A pure optokinetic response can be elicited by rotation of the visual surroundings around a stationary subject, a condition which provides only visual input to the oculomotor system. The output, i.e., a compensatory eye movement, constitutes a direct feedback to the input level, and is therefore called a feedback system. Thus, while the VOR output can not be compared directly to the stimulus at the level of the input, the OKR output can. Any remaining retinal slip will thus immediately re-activate the system, to reach optimal compensation.

Dynamic characteristics of the OKR. In contrast with the VOR, the OKR acts most efficiently in the low frequency / low velocity range. In the rabbit, the OKR compensates for 90% of the retinal slip with velocities up to 2 deg/s. At higher velocities, compensation becomes less adequate (Collewijn, 1969). The system is rather slow, compared to the VOR: compensatory eye movements start no sooner than about 100 ms after presentation of the optokinetic stimulus (Collewijn, 1972).

Visuo-vestibular interaction

Although the VOR and OKR are often investigated separately, they are simultaneously activated in most natural situations. During rotation of an animal in lighted surroundings, both visual and vestibular information are transferred to the brain. As was already suggested by ter Braak (1936), the VOR, being the fastest system, will induce a compensatory eye movement, which is mostly not of optimal amplitude. Then, the deficit will be complemented by the OKR. As can be expected, the amount of compensation resulting from combined visuo-vestibular stimulation is always higher than compensation from either system separately. Moreover, the complementary action of the OKR and VOR offers opportunities for optimal compensation of retinal slip over a wide range of frequencies and amplitudes. In the rabbit, a constant percentage of the stimulus can be compensated for in the range from 0.05 to 1.5 Hz. Thus, the VOR in light is not frequency or amplitude dependent (Baarsma and Collewijn, 1974), due to complementary dynamics of the individual VOR and OKR systems.

With regard to the improvement of the performance of the VOR when visual information is added to the vestibular input, it should be concluded that the two signals are combined in a very efficient way. It is an interesting question how and where the brain handles the integration of visual and vestibular input signals in order to generate an appropriate compensatory eye movement.

Theoretically, visuo-vestibular interaction could occur in all brain regions which receive both visual and vestibular information. As the schema in Figure 1.1 shows, at least four sites are of importance in this respect. Firstly, the vestibular nuclei, where both visual and vestibular responses have been demonstrated in several animal species. Another site is the nucleus prepositus hypoglossi, which receives projections from the vestibular nuclei and from the NOT in the rabbit. Moreover, this nucleus receives an input from the cerebellar flocculus in the rabbit (Yamamoto, 1979; Yingcharoen and Rinvik, 1983), and it projects to the oculomotor nuclei (Graybiel, 1974; Yingcharoen and Rinvik, 1982; McCrea and Baker, 1985). Thirdly, the oculumotor neurons could theoretically be a site of visuo-vestibular interaction, because they serve as an output station for both the vestibular as well as the optokinetic system.

A fourth site of interaction is the cerebellar flocculus, a tiny lobe on the ventrolateral part of the cerebellum, near to the brain stem (Fig. 1.2). Its afferent connections are derived from the vestibular nuclei, from which vestibular input signals are directly received, but also from the inferior olive and the NRTP, two regions which transfer optokinetic information. The efferent connections of the flocculus are directed to the vestibular nuclei, and probably to the nucleus prepositus hypoglossi, which in turn projects to the oculomotor nuclei (Fig. 1.1).

It seems that the flocculus plays an important role in visuo-vestibular interaction, but also in the generation of the basic characteristics of the VOR and OKR, as has been shown by lesion experiments in several animal species (for a review, see Ito, 1982; Precht et al., 1984). It is for this reason that the flocculus, and in particular its relation to the VOR and OKR, is an important topic of this thesis.

The function of the flocculus in the generation of basic output values of the VOR and OKR was investigated by reversible inactivation of this cerebellar lobe. This was done by local micro-injection of GABA-ergic agonists into the flocculus, the results of which are presented in **Chapter 3.**

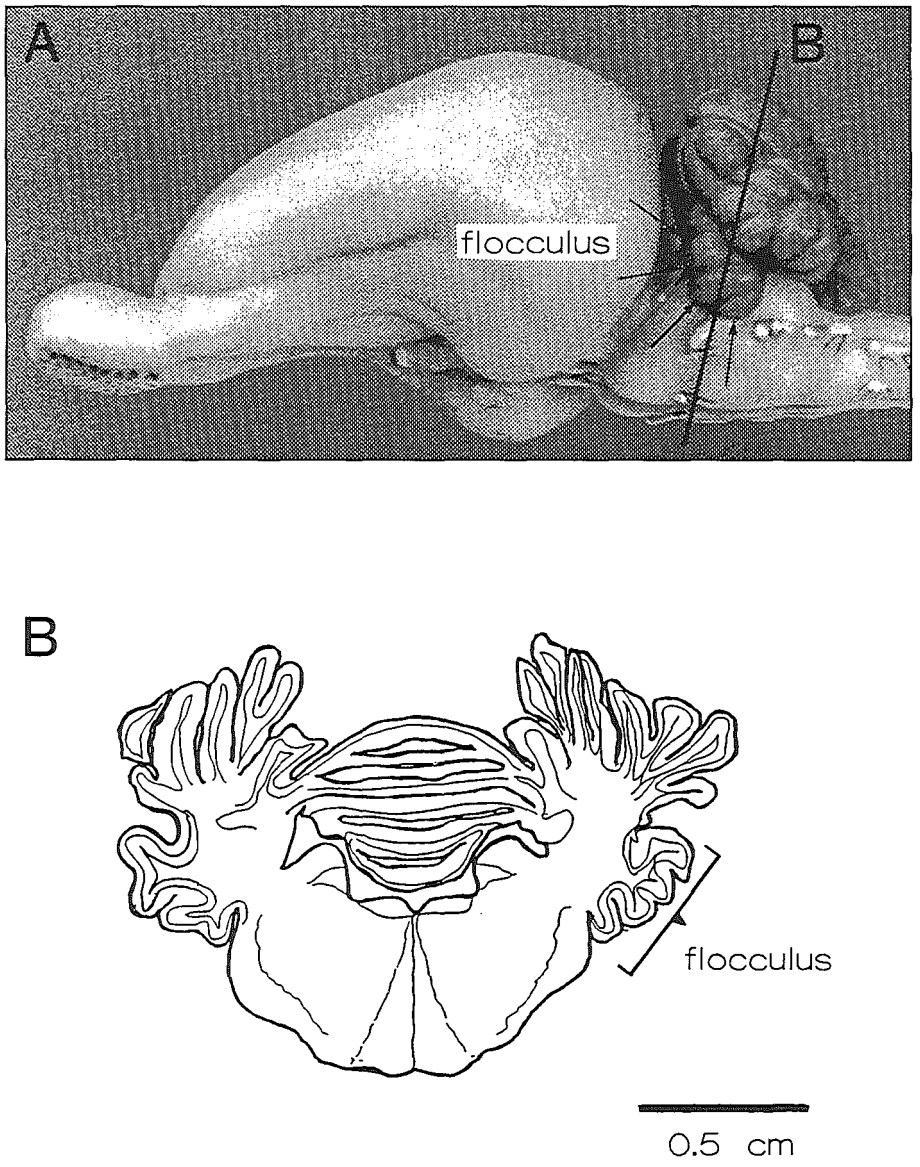

Fig. 1.2. The upper section (A) of this figure shows a schematic drawing of the rabbit's brain, indicating the position of the flocculus. The line indicates where the transverse section, illustrated in the lower part of this figure (B), was taken.

Another main topic of this thesis is visuo-vestibular interaction. It is important to distinguish two aspects in this respect, which have both been investigated:

1) *Direct interaction.* This type of interaction takes place as soon as visual and vestibular information are simultaneously activating the oculomotor system, generating a single output to reduce retinal slip.

2) *Long-term interaction.* In this situation, combined visual and vestibular stimulation occurs during a prolonged period. When this combination is an unnatural one, which is not normally encountered by the animal, the result of such long-term stimulation is an adaptive change in the input-output relations of the VOR, which is shortly called *adaptation.*

Chapter 4 of this thesis deals with direct visuo-vestibular interaction of the VOR and OKR. To understand visuo-vestibular interaction during adaptation, it is important to know whether even the immediate interaction of VOR and OKR may show marked deviation from linearity. Because non-linearity may not show up clearly in the normal, synergistic combination of visual and vestibular stimuli, I designed two experiments in which unusual combinations of visual and vestibular inputs were presented. I concentrated on the question whether these visual and vestibular input signals are processed by simple linear addition, or rather in a much more complex fashion.

Lastly, several aspects of long-term visuo-vestibular interaction were which resulted in adaptation of the VOR were studied. Adaptation can be elicited by unusual visuo-vestibular input combinations, which result in inadequate compensatory eye movements. The vestibulo-ocular system is informed about this deficiency by the visual input, which contains information about the higher amount of retinal slip, resulting from the unusual stimulus combination. As a result, the system is recalibrated in such way that the increase or decrease in retinal slip velocity can be adequately compensated for.

The site where adaptive changes in the input-output relations of the VOR are initiated has not been completely determined yet, but it is generally accepted that the flocculus plays an important role in these adjustments. As was already mentioned, the flocculus is one of the sites where both visual and vestibular afferents arrive. Moreover, the flocculus forms a parallel path to the vestibulo-ocular reflex loop, and would thus be an excellent place for adaptation to be initiated (see Ito, 1984). However, other brain

stem regions could be the site of the actual adjustments, as has been proposed by some investigators (Lisberger, 1988).

Adaptation has been suggested to be affected by mono-aminergic neurotransmitters, namely noradrenaline and serotonin (for a review, see McElligot and Freedman, 1988b). These transmitters would not induce direct excitation or inhibition, but would modulate the efficiency of other excitatory or inhibitory transmitters (see Nicoll, 1982). Such actions might thus occur at Purkinje cell level in the flocculus, where interaction of afferent vestibular mossy fibers and visual climbing fibers might be influenced by these mono-aminergic substances. These neurotransmitter systems might thus affect adaptation of the VOR.

In the second part of this thesis, the possible influence of these modulatory neurotransmitters on VOR adaptation was investigated. The flocculus was chosen as the site to test the influence of these substances on VOR adaptation. The effects of local micro-injection of appropriate agonists and antagonists of these neurotransmitters were investigated during adaptive adjustment of the VOR. The effects of noradrenergic substances on VOR adaptation are described in **Chapter 5**, while the experiments on possible serotonergic influence on VOR adaptation are presented in **Chapter 6.**

This thesis contains the results of experiments on generation and adaptation of visuo-vestibular interaction, with special interest in the role of the cerebellar flocculus in these functions. In **Chapter 7**, an attempt will be made to synthesize these new results and the previous literature on the general function of the flocculus. I will also speculate about the possible mechanisms of the demonstrated effects at the Purkinje cell level.

The methods which have been used in the various experiments are reviewed in **Chapter 2.**

CHAPTER 2

GENERAL METHODS

Young adult (6-12 months) Dutch belted rabbits of either sex with a weight of about 2 kg were used in all investigations. Although the animals took part in various types of experiments, all of these involved the registration of the VOR and OKR. Therefore, all rabbits were provided with equipment for the accurate recording of their eye movements. We used the magnetic induction method, for which the rabbits had scleral search coils permanently implanted on both eyes. When a rabbit was positioned in an alternating magnetic field, an a.c. potential was induced in the coil. The amplitude and phase of this potential were related to the angular position of the eye, as will be explained in one of the following paragraphs. An electromagnetic method for measuring eye movements was first described by Robinson (1963) and later modified by Collewijn (1977). It is by far the most accurate method of eye movement measurement employed so far.

Implantation of the eye coils

The rabbits were anaesthetized using ketamine (Nimatek, 100 mg/ml, AUV, Holland), acepromazine 1%, (Vetranquil, 10 mg/ml, Sanofi, France) and xylazine - HCl 2% (Rompun, 22.3 mg/ml, Bayer, Germany). Initial doses of 0.7 ml/kg of a mixture of ketamine and acepromazine (in 10:1 proportion by volume) and, in a separate injection, 0.25 ml/kg xylazine HCl were given intramuscularly. These initial doses, which maintained a good anaesthesia for about 1 hr, were supplemented with a 1:2 diluted mixture of all three substances as necessary.

Eye coils were implanted on both eyes. Three small openings were made in the conjunctiva, and a multistranded, teflon-coated, stainless steel wire (type AS 632, Cooner,

Chatsworth, CA) was woven underneath the superior and inferior rectus muscles, thus forming a 5 turns coil *in situ*. The conjuctiva was closed with catgut stitches, and a gel containing an anti-inflammatory corticoid and an antibiotic (Sofradex, Roussel) was applied on the eyes. The leading wires of the coils were led underneath the skin, and soldered to a small connector. The skull was exposed in the area surrounding the bregma suture, where 6 small screws were inserted. The connector, together with an assembly of fixation screws was anchored to the skull screws with dental acrylic. This construction was used to fixate the rabbit's head in a headholder to prevent spontaneous movements during the experiments and to connect the eye coil to the detection apparatus. The rabbits recovered very well from this operation, and their eye movements were not impaired by the implanted coils. In general, the coils remained functionally intact for several months.

In a few rabbits, we implanted experimental 80-turn coils. These preformed coils of insulated copper wire of 0.05 mm diameter were embedded in a silicone coating. They were implanted on the eye after circular incision of the conjuctiva. However, the conventional method of implantation of the 5-turn coil proved more satisfactory as indicated by a trend to larger amplitudes of eye movements and a much longer functional life time of the coils. Therefore, the experimental implantation of the 80-turn coil was discontinued after a few animals. It should be noted here that we were interested in the *changes* in amplitude of the eye movements induced by the VOR and OKR in the various experiments. Because these changes were comparable in the conventionally and experimentally implanted rabbits, the data of rabbits with 80-turn coils have not been discarded.

Magnetic angular position detection

When a wire coil is placed in an alternating magnetic field, this field will induce a sinusoidal potential in the coil. The amplitude of this potential depends on several constant parameters: the power of the magnetic field, the number of turns in the coil, and the area of the coil. Moreover, the potential depends on an important variable parameter, namely the number of magnetic field lines that pass the area of the coil, called *the flux*. The value of this parameter depends on the angle between the coil and

the magnetic field lines. Thus, the induced voltage is a measure of the position of the eye. The idea to detect eye movements by using this principle was initially proposed by Robinson (1963).

A somewhat different method, based on phase detection, was introduced by Collewijn (1977) based upon Hartmann and Klinke (1976). This modified method was used in the current experiments.

A rotating magnetic field was generated by two sets of field coils, which were arranged perpendicularly to each other. Both coils were fed with a alternating voltage of identical frequency (f=1300 Hz). The currents through both sets of coils were 90 degrees out of phase. The magnitude of each separate field (B) can thus be described as follows:

$$B_X = c \cos\omega t$$

$$B_Y = c \sin\omega t$$

in which ω is $2\pi f$, t is time and c is a constant which depends on the number of turns and the area of the coils. The combination of these two fields can be described by vectorial addition of B_X and B_Y (Fig. 2.1).

As is clear from this diagram, the vector sum of B_x and B_y is equal to c, and thus constant. In practical terms, the combination of these two magnetic fields results in the generation of a horizontally rotating magnetic vector with a constant amplitude and a constant angular velocity. In this case, the vector rotates 1300 times per second.

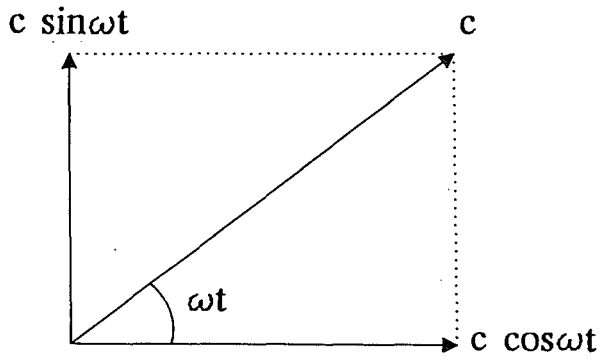

Fig. 2.1 Vector diagram, illustrating the generation of a rotating magnetic vector of constant magnitude and angular velocity from two perpendicularly arranged field coils.

Figure 2.2 illustrates the principle of phase detection. If a stationary coil is positioned in the rotating magnetic field, the amplitude of the induced potential will vary sinusoidally over the period of 1/f (one circular movement of the magnetic vector). This voltage is maximal when the magnetic vector is parallel to the windings of the coil, because the rate of change in flux is maximal at that time. When the magnetic vector reaches the position perpendicular to the windings of the coil, the induced voltage is zero because the rate of change in flux is zero at that moment.

If a rabbit with an implanted eye coil is placed in this magnetic field, the value of the induction voltage in this coil depends not only on the direction of the magnetic vector at each moment in time, but also on the position of the eye. The phase of the induced voltage in the coil will be compared to that of a stationary reference coil. Whenever the eye coil position differs from that of the stationary coil, the zero-crossings of the induced voltage will appear at different moments.

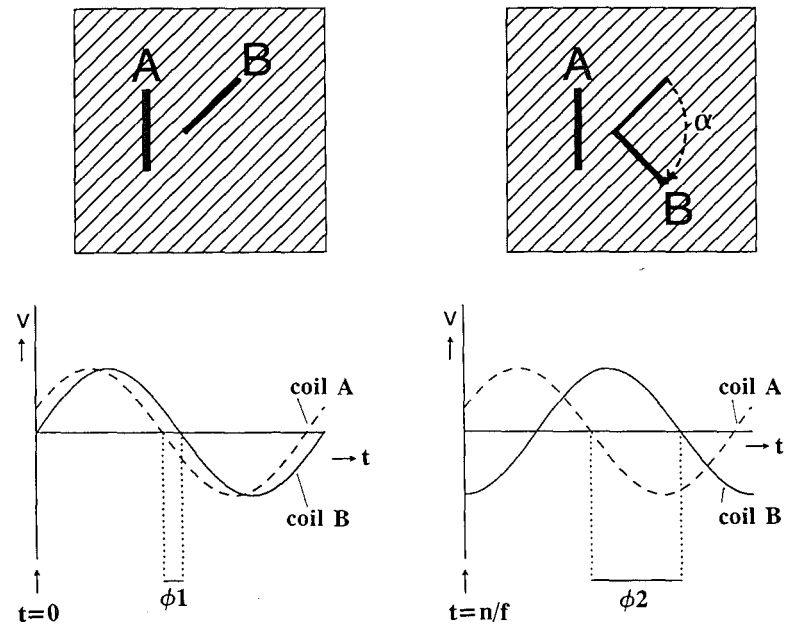

Fig. 2.2. Diagram of the phase detection method. The phase of the inducted voltage (V) in the eye coil (B) is compared to that in a stationary reference coil (A). The different positions of coil B in the magnetic field are represented by different moments of zero-crossings of the inducted voltage in coil B, compared to the zero-crossings in coil A. This phase difference is ϕ_1 at t=0. If the eye rotates over an angle α, the new position of coil B is reflected in a larger phase ϕ_2 of the induction voltage with respect to that of the reference coil A. Thus, ϕ represents at all times the angle of coil B with respect to the reference coil A.

A displacement of the eye will be represented as a time difference in appearance of zero-crossings: a *phase difference*, as is illustrated in Fig. 2.2. This phase difference, which is linearly related to the eye rotation, varies between 0 and 360 degrees. An important property of this phase difference is its independence of the absolute magnitude of the signals. Thus, calibration of the system is unaffected by variations of the effective size of the eye coil.

Phase was detected by a phase- detector, built by the Central Research Workshop (CWR) of the Faculty of Medicine (EUR, Rotterdam), and represented by an analog signal. Induction coils were also attached to the experimental devices, a platform and an optokinetic drum, to record their angular position in a similar way as the eye position.

Signal processing

The displacement of the eye (the gaze-signal) as well as the displacements of the optokinetic drum and of the platform were monitored by phase detection. The eye-in-head signal was obtained by subtraction of the gaze position from the platform position. These four signals were then amplified in such a way that a displacement of 10 deg in the horizontal plane was represented by an output signal of 0.8 V. The signals were connected to a penrecorder for direct observation during the experiment, and also to the computer for data acquisition and analysis.

Data acquisition and analysis

The data acquisition and analysis of all experiments were computer driven (Digital Equipment Corporation, PDP 11/73). The signals of the optokinetic drum, the platform and the eye-in-head were sampled at a frequency of 51.2 or 102.4 Hz by a data acquisition program. Three to twelve periods of sinusoidal oscillation were sampled, depending on the type of experiment and on the stimulus frequency.

In subsequent off-line analysis, the fast phases of the eye movement responses were removed from the record. Saccades were detected by the criteria of a minimal velocity of 6 deg/s, a minimal duration of 10 ms and a maximal duration of 150 ms. After removal of the saccades, the slow phases of the eye movement were quadratically interpolated. The *gain* (the ratio between the amplitudes of the eye movement response and the stimulus), and the *phase* of the eye movement response relative to that of the stimulus were determined after a fast Fourier transformation.

Stimulus generation

An experimental apparatus was designed to generate vestibular and optokinetic responses. It consisted of a circular platform (diameter 0.75 m), surrounded by an optokinetic drum of the same diameter. The drum and the platform could be driven separately or simultaneously by velocity-controlled motors.

The rabbits were fixated in a bag and mounted on a small board, with their heads secured to the headholder. The board was placed on the platform in such way that the midpoint between the eyes was exactly in the centre of both the platform and the optokinetic drum. The drum was decorated with black, randomly distributed dots on a white background. It did not contain obvious borders or sutures, so that the rabbit's whole visual field was occupied by a continuous random dot pattern. The drum was lighted from the inside, and the surroundings were not illuminated (Fig. 2.3).

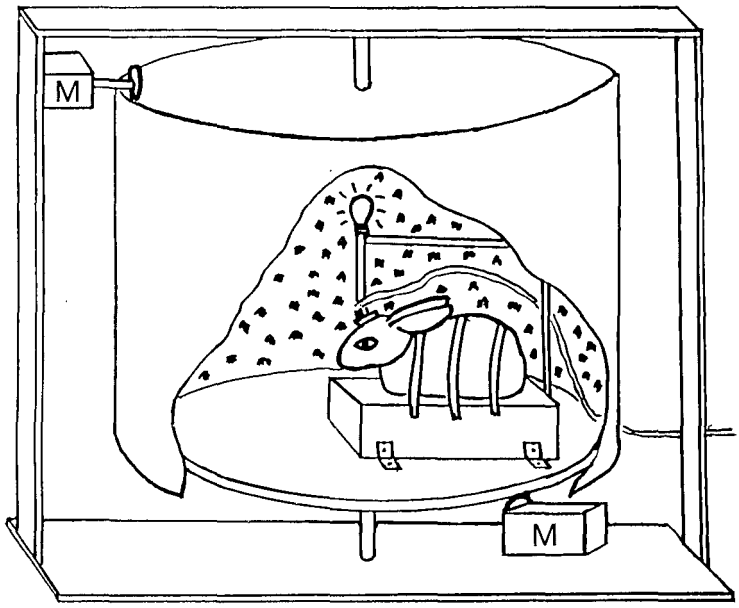

Fig. 2.3. Arrangement of the experimental devices: an optokinetic drum (cut out in the diagram to show the interior of the drum), decorated with random dots, which completely surrounded the platform. The drum was illuminated from the inside. The devices were separately driven with servo-controlled motors (M). The rabbit was fixated on a bench, the head secured to a headholder. The eye coils, which were soldered to a connector on the head, were connected to the detection apparatus by a flexible cable

The vestibulo-ocular reflex could be evoked by sinusoidal oscillation of the platform in darkness. Oscillation of the optokinetic drum alone resulted in an optokinetic response, and by oscillating the platform in the light within the stationary drum, the synergistic interaction between the VOR and OKR was investigated. By driving both devices simultaneously, we could test several non-synergistic interactions between VOR and OKR.

Several input modes were used to drive the drum and platform, depending on the experimental settings. We used either a sine wave generator, for continuous oscillation of either or both devices, or special signals generated by the computer. The exact stimuli used in the various experiments will be described in the respective chapters.

PROCEDURES FOR MICRO-INJECTIONS

In the experiments described in the Chapters 3, 5 and 6, we performed injections of various substances into the cerebellar flocculi of the rabbits via outer cannulae. The procedures for these implantations will be described in the following section.

Implantation of guide cannulas.

About a week after the implantation of the scleral coil, the rabbits were anaesthetized again, using the same anaesthetics as described for the eye coil implantation. The skull was opened bilaterally in the area overlying the paramedian cerebellar lobes. A well of dental acrylic was built to enable fixation of the cannulas to the skull. The dura mater was opened to expose the cerebellar cortex.

Figure 2.4 illustrates the implantation procedure. The rabbit was fixated on the bench with the head stabilized in a headholder. The flocculus was localized on the guidance of electrophysiological recordings. A stereotaxic apparatus and a microdrive were used to manipulate the recording electrode (a glass micropipette with a 4 μ tip, filled with 2.0 M NaCl). As the electrode penetrated the cerebellar tissue, the ipsilateral eye of the animal was stimulated by a slowly moving random-dot pattern.

The flocculus was identified by the recording of modulation of complex spike activity of Purkinje cells synchronous with the movement of the random dot pattern (Simpson et al., 1981; Graf et al., 1988). A site responding to horizontal movement of the stimulus was chosen as the preferred target for the injection of substances. Once such a point was localized, the outer cannula, which surrounded the recording electrode, was lowered until it almost touched the surface of the cortex. The cortex was protected by a gel containing an anti-inflammatory corticoid and an antibiotic (Sofradex, Roussel) and a thin layer of bone wax. Then, the cannula was permanently fixed with dental acrylic. As the acrylic hardened, the electrode was marked just above the upper border of the outer cannula and then retracted. The length of the recording electrode was taken as the appropriate length for the injection cannula, which fitted exactly inside the outer cannula. The whole procedure was repeated on the opposite side, so that the flocculus could be injected on either side.

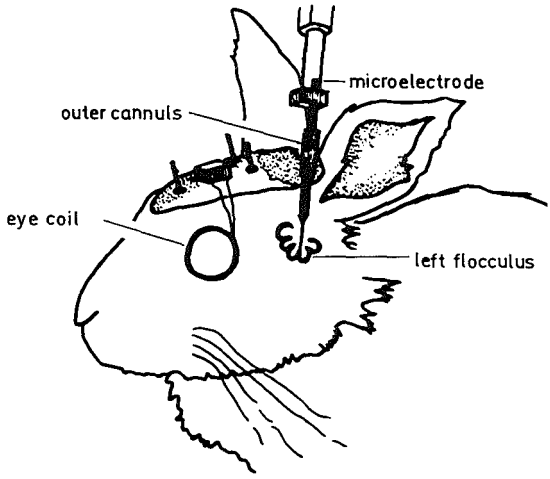

Fig. 2.4 Schematic drawing of the method used to localize the flocculus.

Histology

At the end of the last experiments of a series, each rabbit was injected with a dye (Pontamine sky blue or ink), through the same injection cannula as used in the former injections. The rabbit was then deeply anaesthetized and perfused from the left cardiac ventricle with saline and 10% formaldehyde. The brain stem and cerebellum were dissected and sectioned on a freezing microtome. The sections (30 μm thickness) were mounted and stained with Neutral Red, after which the injection spot could be verified.

CHAPTER 3

FUNCTION OF THE CEREBELLAR FLOCCULUS IN RELATION TO THE VOR AND OKR: EFFECTS OF GABA-ERGIC INJECTIONS

INTRODUCTION

As was reviewed in Chapter 1, the cerebellar flocculus is incorporated in the parallel side path of both the VOR and the OKR. Its function in the generation or regulation of these gaze-stabilizing reflexes has been studied extensively, anatomically as well as physiologically. Several lines of evidence have suggested that the flocculus would influence the basic characteristics of the vestibulo-ocular and optokinetic reflexes.

The first findings that pointed at this role were again derived from experiments on flocculectomized animals. Ito and coworkers (Ito, 1982; Ito et al., 1974a; Ito et al., 1974b) studied the effects of flocculectomy on the characteristcs of VOR and OKR in albino rabbits by whole-body rotation on a platform and sinusoidal oscillation of a striped drum. In these experiments it was demonstrated that chemical unilateral flocculectomy by application of kainic acid, as well as surgical flocculectomy, led to a decrease in the gain and an increase in phase lag of both the horizontal VOR and the horizontal OKR. These deficiencies mainly affected the movements of the ipsilateral eye, while in the gain of the contralateral eye only a transient depression was seen.

The reduction of the horizontal VOR gain as described above is in agreement with results in the chinchilla (Hassul et al., 1976). In pigmented rabbits (Nagao, 1983), only the OKR gain decreased after bilateral flocculectomy, while the gain of the VOR remained almost unchanged. Similar long-term results were obtained after unilateral flocculectomy in the rabbit by Barmack and Pettorossi (Barmack and Pettorossi, 1985), while the short-term effects were complicated by drift and bias of the smooth eye movements in the direction contralateral to the lesions. On the other hand, Robinson

(1976) found that bilateral surgical ablation of the flocculus in the cat produced an increase in the VOR gain. Other experiments in the cat by Keller and Precht (1978) demonstrated that total cerebellectomy or flocculectomy reduced the gain of the VOR, elicited at relatively low frequency rotation of the head, in light and in darkness. The OKR gain was also reduced, but only at high stimulus velocities. Flocculectomy in the monkey resulted in variable effects on the VOR gain: both increases and decreases were shown in individual monkeys (Zee et al., 1981).

Similar effects as after ablation of the flocculus on the gain characteristics of the OKR were obtained after a lesion of the nucleus reticularis tegmenti pontis, which reduced the OKR gain without affecting the dynamic characteristics or adaptability of the VOR (Miyashita et al., 1980). The flocculus receives afferent visual mossy fibers via the nucleus reticularis tegmenti pontis (Maekawa and Takeda, 1978), which may mediate direction-selective modulation of simple spike discharge of the floccular Purkinje cells. Ito (1977) showed that the simple-spike discharge rate of Purkinje cells was increased by backward and depressed by forward movement of a light-slit. This was also demonstrated in more recent and elaborate work by Graf et al. (1988). Because stimulation of the floccular zone related to motion around a vertical axis causes backward movement of the ipsilateral eye (Dufossé et al., 1977; Simpson et al., 1989), these observations suggest that the floccular output facilitates the OKR. The pathway described above sends also visual signals to the relay cells of the VOR in the vestibular nuclei (Balaban, 1983; Hoddevik, 1978; Keller and Precht, 1978).

The comparison of the results obtained in the various lesion experiments is often complicated. For example, some lesions were made unilaterally while in other experiments the lesions were made bilaterally. Furthermore, lesions sometimes exceeded the borders of the flocculus. An important side-effect of surgical lesions is the retrograde degeneration of neurons in the inferior olive (Barmack and Simpson, 1980; Ito et al., 1980), which could also contribute to some of the effects demonstrated after flocculectomy. Although kainic acid lesions produced less retrograde influences in the inferior olive, it cannot be excluded that in these instances changes in contacts of olivary neurons with their cerebellar target cells modify the number and properties of the receptors by which excitatory transmitters act on olivary neurons, as documented in other neuronal networks (Rotter et al., 1984). This would alter the influences which the olivocerebellar axons exert on brainstem and cerebellar neurons in their course to the cerebellar cortex. Moreover, flocculectomy may cause retrograde degeneration of

vestibular neurons, and thus lead to a reduction of the VOR gain (Ito, 1982). Lastly, strain differences in anatomical connections such as those described inbetween albino and pigmented rabbits could blur the comparison of several lesion experiments.

Taking all these complications together, it would be of interest to substitute these irriversible surgical and chemical lesioning methods with a reversible method to inactivate the floccular Purkinje cells. The response of Purkinje cells to excitatory input by mossy and climbing fibers is limited by the activity of three populations of inhibitory interneurons. The basket and stellate cells exert direct inhibition on the Purkinje neurons through axosomatic and axodendritic synapses. The Golgi cells inhibit granule cells, which are in turn relay cells of the excitatory mossy fiber input to the Purkinje cells. These three groups of interneurons are all GABA-ergic (see Ito, 1984). Moreover, the Purkinje cells themselves are GABA-ergic, and their axon collaterals may contribute to intracortical inhibition (Ito, 1984, Palay and Chan-Palay, 1974).

In view of these relations it may be expected that application of a high concentration of GABA in the Purkinje cell layer of the floccular cortex may functionally inactivate these neurons. The function of the flocculus will thus be impaired in a reversible way, which provides an interesting possibility for investigation of the role of floccular Purkinje cells on the dynamic characteristics of the VOR and OKR.

We designed a series of experiments to test the effects of floccular injections of GABA-ergic agonists on the VOR and OKR gain, the results of which are reported in this Chapter. A method of chronic implantation of injection cannulas was developed for these experiments, the description of which was given in Chapter 2.

METHODS

Ten pigmented Dutch belted rabbits were implanted with scleral search coils and bilateral cannulas. They were used to test the effects of floccular micro-injections of the GABA-A-agonist muscimol and the GABA-B-agonist baclofen on the vestibulo-ocular and optokinetic responses.

Experimental conditions

To elicit an optokinetic and vestibulo-ocular response, I used the optokinetic drum and the platform, as described in the method Chapter, Fig. 2.3. The VOR and OKR were tested with two stimuli: 0.25 Hz at an amplitude of 5 deg, and 0.10 Hz at an

amplitude of 2.5 deg. At the start of each experiment, four baseline measurements of the VOR in light and in darkness and of the OKR were recorded in a period of 15 minutes. The mean value of these four baseline measurements was normalized to 100% at time t=0 (see Figs. 1 and 2). After the baseline measurements, a bilateral injection with muscimol or baclofen was made into the flocculus. One minute was taken to inject 1.0 μl. As soon as the injection was completed, a next recording was made. Subsequent recordings were made every 15 minutes, during 3 hrs. Each recording lasted 4 min; during the intervening resting periods of 11 min the rabbit was kept stationary on the platform in lighted surroundings.

Four out of the ten rabbits were used in both the muscimol and the baclofen experiment. One rabbit was used for a dose-dependence study of both substances. Three rabbits were injected only with baclofen and two only with muscimol. Those rabbits which were used in more than one experiment got always at least one day of rest in between two sessions to allow full recovery from the previous injection.The experiments were performed in random order.

Injected solutions

We used the GABA-A-agonist muscimol (Sigma) 16 μg/μl and the GABA-B-agonist baclofen (Ciba-Geigy) 5 μg/μl. Each of these substances was dissolved in saline,with subsequent adjustment of the pH to 7.0 - 7.4. The solutions were injected through stainless steel injection cannulas (outer diameter 0.35 mm) which fitted exactly inside the permanently implanted outer guide cannulas. A Hamilton 1.0 μl syringe was used to deposit precise amounts of the solutions. In a few control experiments, only the solvent (saline) was injected.

RESULTS

Baclofen injections

The absolute baseline gains of the VOR and OKR had rather consistent values, which did not differ markedly in the 7 rabbits at either test frequency. The average baseline gain of the VOR in light was about 0.70 for both frequencies. The average gains of the VOR in darkness and of the OKR were both about 0.45 at 0.10 Hz. At 0.25 Hz, the average baseline gain of the VOR in darkness was 0.60, while the OKR gain was only 0.09. The average baseline values are shown in Table 3.1.

The overall result of bilateral baclofen injections into the flocculus was a strong decrease in gain of the VOR (both in light and darkness) as well as of the OKR, at either test frequency. Table 3.1 lists the average absolute gain reductions of the VOR in light and darkness and of OKR 1.5 hr and 3 hrs after the injection. Fig. 3.1 shows the time course of these decreases in gain at the two test frequencies. This figure shows the gain as a percentage of the baseline values (normalized at 100%).

The effects of baclofen were largely similar for the two frequencies tested. After 3 hrs, the VOR in light and in darkness were reduced by about 50%, and OKR was reduced by about 60-70% at both frequencies (Fig. 3.1). Thus, the decline of the OKR was in general more pronounced than the decline of the VOR. The time course of the VOR-gain was identical in the light and in the dark, and for the two frequencies. Only the time course of the decrease in gain of the OKR showed an effect of stimulus frequency: the decline was steeper and stronger for the stimulus with a frequency of 0.25 Hz and an amplitude of 5 deg (peak velocity 7.9 deg/s), than for the stimulus with a frequency of 0.10 Hz and an amplitude of 2.5 deg (peak velocity 1.6 deg/s).

At both frequencies, the effects reached nearly asymptotic values in the first 2 hrs. Recovery from the depression was slow; the original baseline values were not re-obtained until the next day.

Table 3.1. Decrease in absolute gain after a 1.0 μl injection of baclofen (5 μg/μl). Average values of 7 rabbits. L = light, D = darkness.

condition	baseline gain ± S.D.	decrease in gain after 1.5 hrs ± S.D.	decrease in gain after 3 hrs ± S.D.
VORL 0.25 Hz	0.71 ± 0.22	-0.26 ± 0.13	-0.35 ± 0.11
VORD 0.25 Hz	0.60 ± 0.21	-0.20 ± 0.06	-0.27 ± 0.08
OKR 0.25 Hz	0.09 ± 0.02	-0.05 ± 0.02	-0.06 ± 0.02
VORL 0.10 Hz	0.73 ± 0.20	-0.30 ± 0.13	-0.37 ± 0.10
VORD 0.10 Hz	0.44 ± 0.19	-0.19 ± 0.06	-0.17 ± 0.09
OKR 0.10 Hz	0.47 ± 0.12	-0.22 ± 0.17	-0.31 ± 0.17

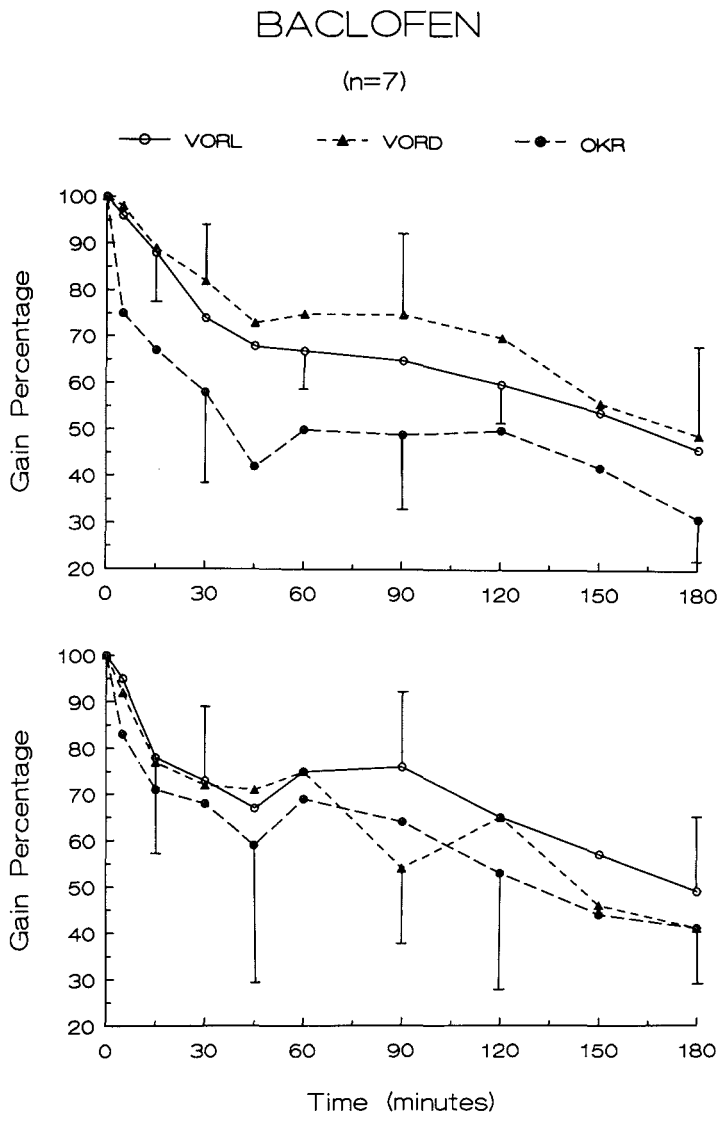

Fig. 3.1. Time course of the effects of bilateral floccular injection of 1.0 μl baclofen (5.0 μg/μl) on the VOR in light, the VOR in darkness and the OKR. Upper graph shows the effects at the 0.25 Hz frequency, lower graph at 0.1 Hz. The average value of 4 baseline measurements was normalized to 100%. Mean values of 7 rabbits.

Muscimol injections

The absolute baseline values of the 6 rabbits used in these experiments were comparable to those obtained in the baclofen experiments. The average values are listed in Table 3.2.

As in the baclofen experiments, the effect of the muscimol injection was a large decrease in gain of the VOR in light and in darkness, and an even stronger decrease in gain of the OKR. Table 3.2 summarizes the average decreases in the gains after 1.5 hr and after 3 hrs of experimentation for both frequencies. Fig. 3.2 shows the time course of these effects in a similar way as Fig. 3.1.

Similarly as in the baclofen experiments, we found little difference between the effects of the injection on gain when comparing the two test frequencies. After 3 hrs, the reduction of the gain was about 50% for the VOR in light, and in darkness, and about 65% for the OKR. These values, and also the time course at which they were reached, were similar to those after the baclofen injections. Once more, the decline of OKR was steeper at 0.25 Hz than at 0.10 Hz (Fig. 3.2). With both substances, the largest part of the decrease occurred during the first 2 hrs of the testing period, and the OKR was affected more severely than the VOR. In fact, the only difference between the substances was that the rabbits injected with baclofen showed less variability in the amount of gain reduction than the muscimol-injected rabbits, as demonstrated by smaller standard deviations (Tables 3.1 and 3.2).

Table 3.2. Decrease in absolute gain after a 1.0 μl injection of muscimol (16 μg/μl). Average values of 6 rabbits. L = light, D = darkness.

condition	baseline gain ± S.D.	decrease in gain after 1.5 hrs ± S.D.	decrease in gain after 3 hrs ± S.D.
VORL 0.25 Hz	0.78 ± 0.12	-0.27 ± 0.21	-0.43 ± 0.18
VORD 0.25 Hz	0.64 ± 0.16	-0.13 ± 0.17	-0.32 ± 0.16
OKR 0.25 Hz	0.12 ± 0.04	-0.07 ± 0.05	-0.08 ± 0.04
VORL 0.10 Hz	0.79 ± 0.14	-0.20 ± 0.24	-0.48 ± 0.30
VORD 0.10 Hz	0.48 ± 0.19	-0.20 ± 0.09	-0.26 ± 0.06
OKR 0.10 Hz	0.59 ± 0.11	-0.21 ± 0.22	-0.35 ± 0.21

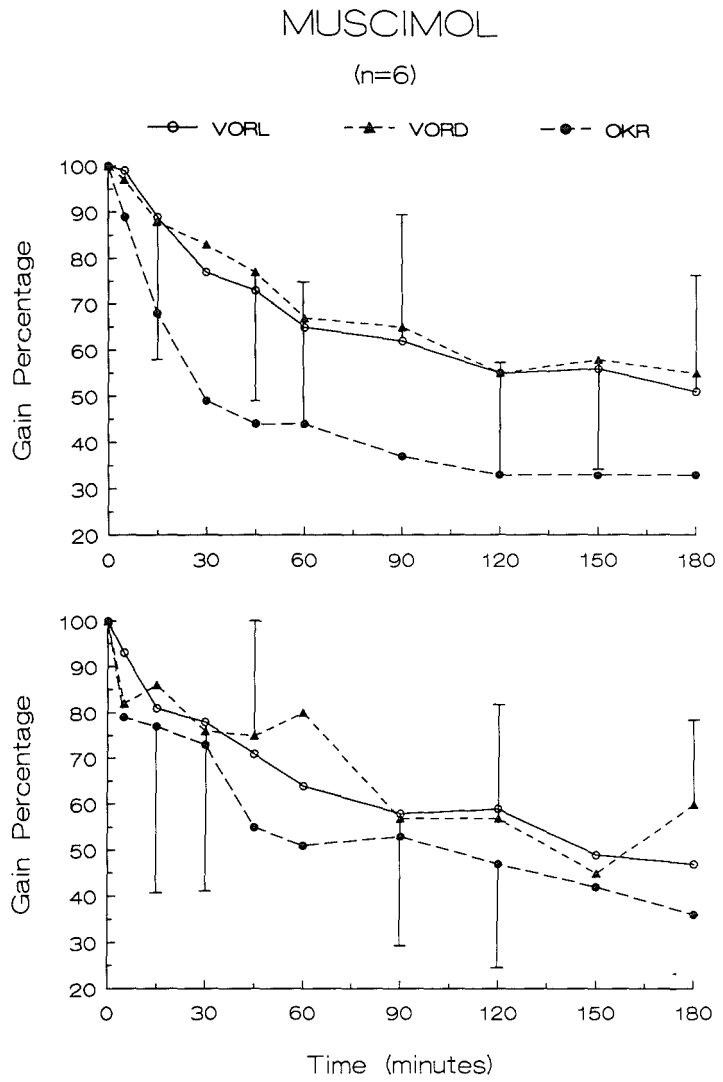

Fig. 3.2. Time course of the effects of bilateral floccular injection of 1.0 μl muscimol (16.0 μg/μl) on the VOR in light, the VOR in darkness and the OKR. Upper graph shows the effects at the 0.25 Hz frequency, lower graph at 0.1 Hz. The average value of 4 baseline measurements was normalized to 100%. Mean values of 6 rabbits.

Muscimol, like baclofen, did have a long-lasting effect. The rabbits did not recover their original gain until the next day. Some rabbits showed minor symptoms of a cerebellar syndrome 3 hrs after the muscimol injection, such as ataxia and a slight

tremor, which recovered spontaneously within the next 12 hours. These behavioural effects were never seen after a baclofen injection.

In neither the baclofen nor the muscimol experiments did we find any significant change in the phase of the eye movement responses with respect to the stimulus, although in all cases the gain decreased dramatically.

Unilateral injection

All the rabbits that received bilateral injections of baclofen or muscimol showed symmetrically reduced compensatory eye movements in each horizontal direction. The number of saccades was equal to or smaller than that during the baseline measurements. There was no predominance in the direction of the saccades.

This was not the case in one rabbit with a unilaterally implanted cannula. After unilateral injection of muscimol, this rabbit showed a decrease in gain which was smaller than that observed in the bilaterally injected animals. Moreover, the sinusoidal compensatory eye movements were asymmetrical, because the amplitude of the eye movement was smaller in the ipsilateral direction. There was no systematic unilateral drift or spontaneous nystagmus, either in light or in darkness. The number of saccades was increased compared to the baseline measurements, and the majority of the saccades were directed towards the injected side. These findings are in agreement with the short-term

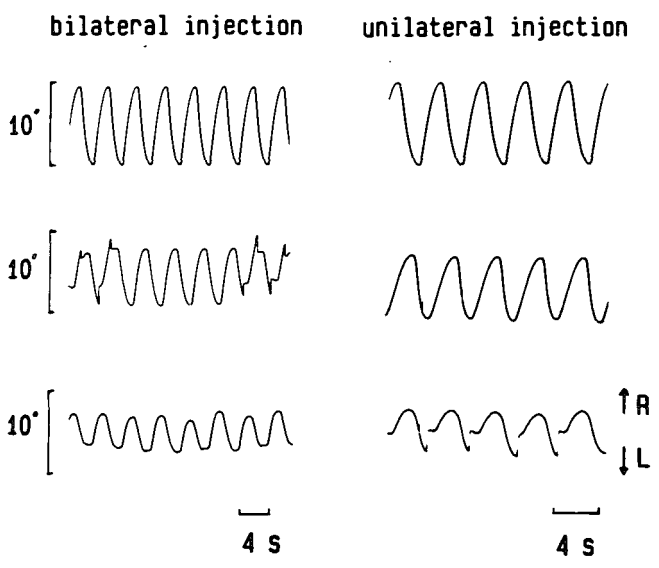

Fig. 3.3. Pen recordings, demonstrating the compensatory eye movements of the VOR in light during the baseline measurement (middle trace) and at 2 hrs after floccular injection of 1.0 μl muscimol (16.0 μg/μl) (lower trace). The left colum shows the effect in a bilaterally injected rabbit, the right colum shows the effects in a unilaterally injected rabbit, which had its cannula on the right side. The upper trace represents the platform oscillation.

effects of unilateral flocculectomy (Barmack and Pettorossi, 1985; Flandrin et al., 1983). Figure 3.3 compares the effects of unilateral and bilateral injection of muscimol.

Visuo-vestibular interaction

In either the baclofen experiments and the muscimol experiments, the gain reduction occurred both in the VOR in light and in darkness, but the visuo-vestibular interaction was still intact. In most of the tested animals, the difference in gain between the VOR in light and the VOR in darkness, (i.e., the improvement of the VOR by visual information), remained roughly similar to the difference in the baseline measurements (see Tables 3.1 and 3.2). However, in three animals the values of the gain of VOR in light approached that of the VOR in darkness, indicating a diminished improvement of the VOR by the visual input. This effect was only present at the 0.25 Hz frequency, where the OKR underwent the strongest reduction in gain. Still, even in these rabbits, the VOR in light remained always larger than the gain of the VOR in darkness. It can thus be concluded that direct visuo-vestibular interaction was not fully abolished by floccular injections of GABA-agonists.

In summary, the effects of injection of baclofen, a GABA-B agonist, and muscimol, a GABA-A agonist into the cerebellar flocculus were highly similar. Either substance caused an impressive decrement in the gain of both VOR in light and dark, and OKR, at both of the two tested frequencies. With the faster stimulus (0.25 Hz, peak velocity 15.7 deg/s), the reduction of the OKR gain was consistenly steeper and stronger than that of the VOR.

Injection of the solvent only

The experiment was repeated in two rabbits, in the same way as described before, but with a bilateral injection of 1.0 μl saline alone. These injections did not have any effect; the gains of the VOR and OKN remained within the normal range of the baseline values. These experiments demonstrate that the effects, observed after injection of GABA-agonists should be attributed to the pharmacological actions of these drugs, and not to mechanical stimulation of the tissue by the injected volume.

Dose-dependence

One rabbit was injected with baclofen and muscimol solutions in various concentrations. Each concentration was injected on different days and in random order. The bilaterally injected volume was always 1.0 μl. There was always one day of rest between successive sessions to allow complete recovery from the previous injection. The baseline gain values in this rabbit were about 1.0 and 0.80 for the VOR in light and darkness, respectively, and about 0.20 for the OKN. These baseline values remained very constant over the period of the entire experiment (18 days), i.e., complete recovery occurred after each injection. As in the other experiments, the averages of the four baseline measurements at the beginning of the experiment were normalized to 100% (Figs. 3.4 and 3.5). In these experiments, we used 0.15 Hz as the test frequency, with an amplitude of 5 deg.

The reductions in gain, as a function of time after injection of baclofen or muscimol, showed a clear dose-dependence. At the lowest baclofen concentration (0.5 μg/μl) there was still a slight decrease (by about 15%) in the gain for the VOR both in light and in darkness, and even more prominently (by about 40%) for the OKR, but these effects were clearly weaker than at the 5.0 μg/μl concentration, where the gain reduction was about 30% for the VOR in light and darkness, and 90% for the OKR (Fig. 3.4).

Injections of various concentrations of muscimol showed an extinction of the effect at the lowest concentration (2 μg/μl), at which the gain reduction of the VOR in light and darkness was only 5%, while the OKR gain was even slightly increased (5%). At a concentration of 8.0 μg/μl however, the effects were about as strong as at the concentration which was used in the main experiments (16.0 μg/μl), indicating a saturation of the effect at those doses (Fig. 3.5).

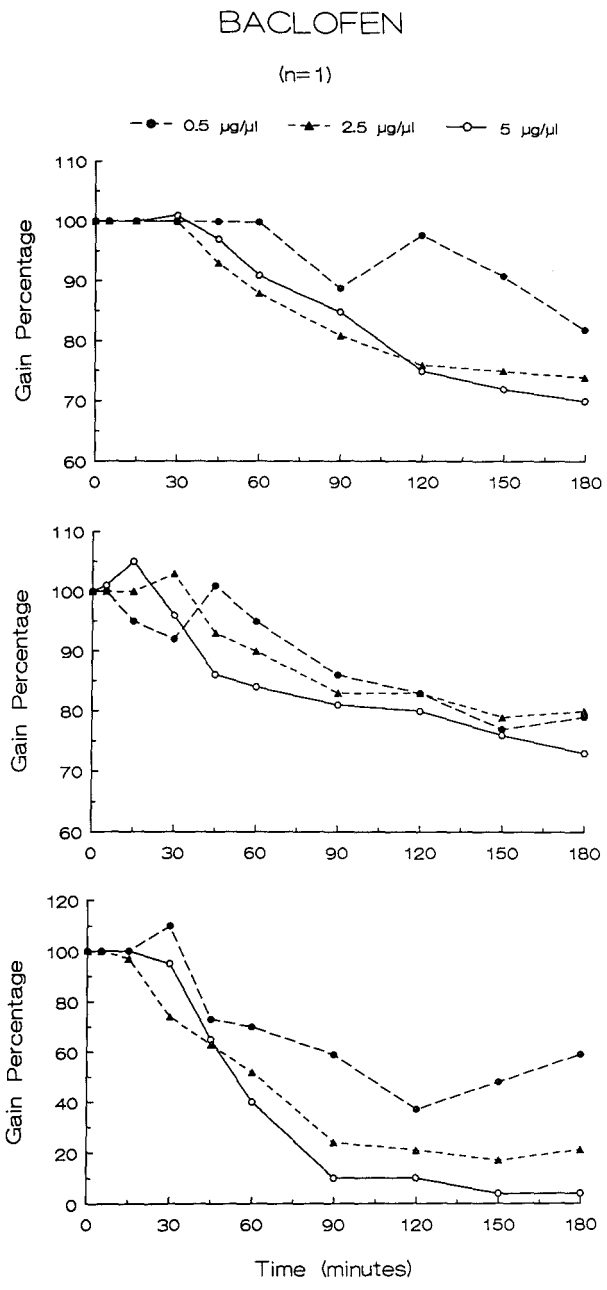

Fig. 3.4. Dose-dependence of the effects of baclofen on the gain of the VOR in light (upper graph), the VOR in darkness (middle graph) and on the OKR (lower graph).

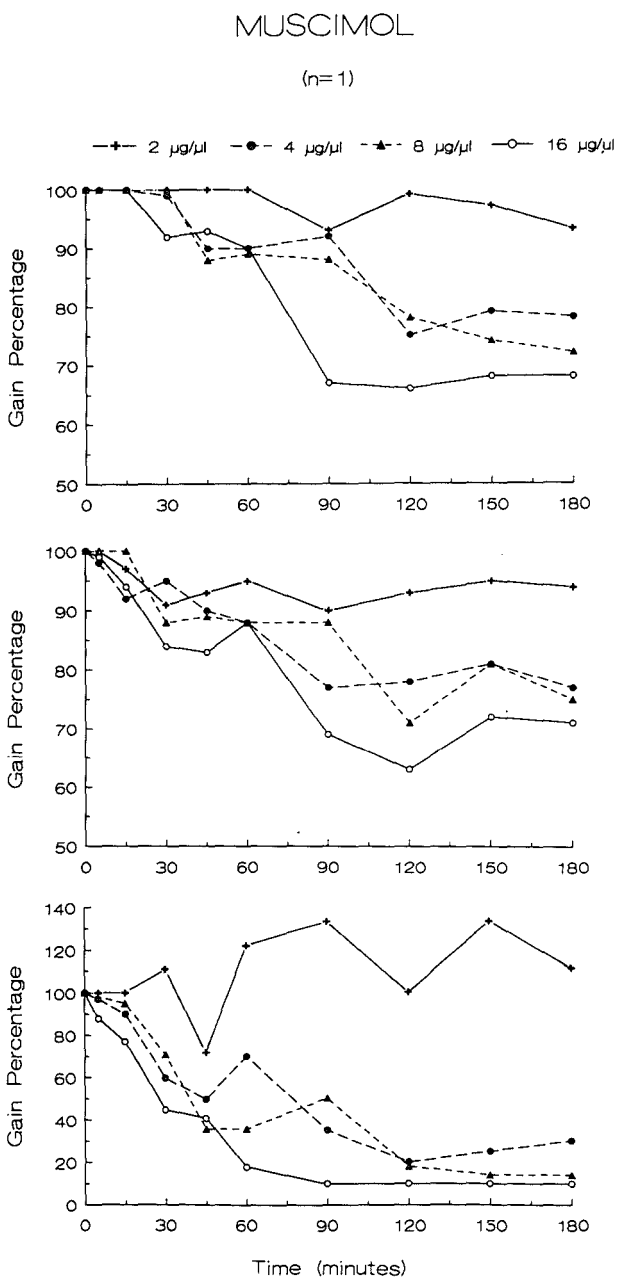

Fig. 3.5. Dose-dependence of the effects of muscimol on the gain of the VOR in light (upper graph), the VOR in darkness (middle graph) and on the OKR (lower graph).

DISCUSSION

From the results presented in this Chapter, it can be concluded that the effects described in the present experiments are specific for the injected drugs, because the effects showed a straightforward dose-dependence. Furthermore, the concentrations chosen in the present experiments were within the optimal range for reaching a clear depression of the VOR and OKN, without causing strong generalized behavioural effects. Lastly, the absence of a trend in the baseline gains over the period of the experiment (a total of 18 days and 7 injections) documents the absence of any permanent change due to the repeated introduction of the cannulas and injection of the GABA-ergic drugs.

General effects of injection of GABA-agonists

The present experiments demonstrate that bilateral injection of GABA-agonists in the flocculus causes a dramatic depression of the gain of the VOR in the light, of the VOR in darkness, and of the OKR. Remarkably, the effects of injection of baclofen (a GABA-B-agonist) and muscimol (a GABA-A-agonist) were virtually identical, at both tested frequencies of oscillation. The effects were specific for the GABA-ergic drugs, because injections of the drugs in various concentrations demonstrated a dose-dependence of the effects, and injection of saline had no effect at all.

The time course of the gain of OKR was different from that of the VOR only to the extent that the decline of the gain of the OKR was steeper at the 0.25 Hz testing frequency. This was the case after the baclofen as well as after the muscimol injections.

Localization of the effects

It can be concluded that the effects found after injection of the GABA-agonists are elicited in the cerebellar flocculus, because the floccular injection sites were electrophysiologically identified and histologically verified at the end of the experiment. However, we can only speculate about the radial distribution and the pharmacological activity of the substances, because the pharmacokinetics of these substances in brain tissue are unknown. From the histological material, the radial spread of the drugs was estimated to be about 2-3 mm diameter, certainly not exceeding the borders of the flocculus, and thus not resulting in GABA-ergic inhibition of brain stem structures such as the vestibular nuclei (see Chapter 7).

Receptor types

These results suggest that the depression of the gain of the VOR and OKR is mediated through GABA-receptors.

Pharmacological, biochemical and electrophysiological data (Bormann, 1988; Bowery, 1983; Bowery et al., 1984a; Bowery et al., 1984b; Bowery et al., 1984c; Hill and Bowery, 1981) have demonstrated that GABA receptors can be subdivided in two different subtypes: GABA-A and GABA-B. GABA-A receptors seem to be associated with Cl^- channels, while GABA-B regulates Ca^{2+} and K^+ channels (Bormann, 1988). Moreover, GABA-B receptors act by regulation of adenyl- cyclase activity (Hill, 1985; Karbon and Enna, 1985, Woczik and Neff, 1984); GABA-A receptors, however, have no apparent linkage with this enzyme system.

The existence of both GABA-A and GABA-B receptors has been demonstrated in the mammalian brain by radioligand binding techniques (Bowery et al., 1984c; Hill and Bowery, 1981). Moreover, these techniques have been extended to obtain receptor autoradiograms of brain sections, which allowed to detect the qualitative and quantitative distribution of both GABA subtypes in the central nervous system in various species. In particular, it was demonstrated in the rat (Bowery et al., 1987) and in the mouse (Rotter et al., 1988) that GABA-A receptors are predominantly and in large numbers present in the granular layer of the cerebellar cortex, but with some component in the Purkinje cell and molecular layers. The sites detected in these studies were bicuculline-sensitive and exhibited a high affinity for muscimol, which is characteristic for GABA-A sites (Palacios et al., 1981; Wilkin et al., 1981). Moreover, the distribution of these sites was very similar to the localization profile shown by GABA-A receptor complex monoclonal antibodies (Schoch et al., 1985). In contrast, GABA-B sites, which are insensitive to bicuculline and have a high affinity for baclofen (Bowery et al., 1985; Gehlert et al., 1985), were found almost exclusively and in very large numbers in the molecular layer of the cerebellum (Bowery et al., 1980; Bowery et al., 1987; Hill and Bowery, 1981; Wilkin et al., 1981).

The cell types on which the two receptor subtypes are located have also been identified. It appears from light microscopical (Chan-Palay et al., 1978; Wilkin et al., 1981) and electron microscopical autoradiographic studies (Chan-Palay et al., 1978; Chan-Palay and Palay, 1978) that GABA-A receptors are found in particular on Purkinje cell somata, in the basket axon formation surrounding the cell body and the initial axonal segment of Purkinje cells and somewhat less on basket and stellate cells in the

molecular layer, as well as on dendrites of granule and Golgi cells in the granular layer. It has also been suggested that presynaptic GABA-A receptors regulate the transmitter release from parallel fibers (Aloisi et al., 1983). The GABA-B binding sites in the molecular layer are probably located pre- and postsynaptically. Presynaptic receptors seem to exist on the terminals of parallel fibers (Woczik and Neff, 1984) as well as on noradrenergic fibers (Bowery et al., 1980). The postsynaptic receptor sites would be on Purkinje cell dendrites (Bowery et al., 1984a).

The evidence in the literature demonstrates a clear difference in the localization and in the mechanisms of activation between GABA-A and GABA-B receptors in cerebellum. However, our results show that floccular injections of muscimol, the GABA-A agonist, and baclofen, the GABA-B agonist, induce identical effects on the VOR and OKR. This would suggest that these drugs are equally potent in inhibiting the floccular Purkinje cells, although they act in very different ways. Presumably, baclofen abolishes the discharge of Purkinje cells by inhibiting them directly at the dendritic level, as well as by suppressing the dendritic excitatory input represented by the parallel fibers. On the other hand, muscimol abolishes the activity of Purkinje cells by direct inhibition of their cell somata as well as by suppressing the discharge of excitatory granule cells and inhibitory interneurons.

The fact that the absolute value of the gain reduction after baclofen injection is similar to that after muscimol injection could indicate that a maximal gain reduction is produced by both drugs.

Cerebellar mechanisms

The results of the present experiments can be understood if we consider that all the inhibitory interneurons located in the cerebellar cortex are GABA-ergic. It is known that the mossy fiber input excites, through the granular cells, not only the Purkinje cells, but also the Golgi, basket and stellate cells. The Golgi cells exert an inhibitory influence on the dendrites of granular cells, thus depressing the transmission of the mossy fiber input to the Purkinje cells. The basket and stellate cells exert direct inhibitory influence on the Purkinje cell somata and their dendrites, respectively. Activation of the GABA-ergic synapses made by inhibitory interneurons may not only directly or indirectly depress the spontaneous activity of the Purkinje cells, but also reduce the efficacy of the conventional excitatory and inhibitory synaptic inputs, thus decreasing the signal-to-noise ratio of the evoked versus spontaneous activity (see Ito, 1984).

It is known that the activity of the vestibular neurons, which drive the oculomotor neurons during horizontal VOR in the dark, is not only determined by the direct response to the primary vestibular afferents but in addition by the output of the floccular side path, which also sends efferents to these vestibular neurons. The floccular Purkinje cells receive visual as well as vestibular afferent information, which enables these cells to contribute not only to changes in VOR gain during horizontal rotation in the light, but also to the generation of OKR (see Ito, 1984). It has been demonstrated that, for a given direction of animal rotation, the peak of the simple spike discharge of the Purkinje cells is out-of-phase with the stimulus velocity. On the other hand, the primary vestibular afferents are modulated in-phase with the head velocity. The interaction of the primary vestibular afferents and the floccular Purkinje cells at the level of the floccular target neurons would result in disinhibition of these neurons by the floccular output, in-phase with the excitation of the floccular target neurons by the primary vestibular afferents. This interaction will result in a positive influence on the VOR and OKR gain.

If the GABA-A and GABA-B agonists suppress the modulation of the firing rate of the Purkinje cells by mossy and climbing fibers, this would lead to a reduction of the disinhibition of the floccular target neurons by the floccular Purkinje cells, and thus to a decrease in the gain of the VOR and OKR. It is important to stress this point, because simplistic interpretations of the inhibitory nature of the Purkinje cell output have sometimes led to the misunderstanding that the cerebellar side loop as a whole would have a negative effect on the VOR, thus leading to the expectation that cerebellar lesions should result in an enhanced gain of the VOR (Robinson, 1976), although this is contradicted by the vast majority of the literature.

The gain of the OKR was reduced to a larger extent than that of the VOR in our reversible inactivation experiments, especially at relatively high stimulus velocities. However, OKR was never totally abolished and some immediate visuo-vestibular interaction was preserved. Except for this last aspect, our observations agree with those of Ito et al. (1982) after unilateral flocculectomy. The impairment of rapid visuo-vestibular interaction observed by Ito et al, (Ito, 1982; Ito et al., 1974a) is probably an artefact of their use of a weak optokinetic stimulus (a single light-slit) and of the albino rabbit, a strain with anomalous OKR (Collewijn et al., 1978). Our observations agree also with those of Keller and Precht (1978), who found that cerebellectomy in the cat reduced the gain of the VOR at 0.10 Hz to about half, while the OKR gain was

was still improved by vision. Moreover, Keller and Precht (1978) found that after cerebellectomy visual modulation of neuronal activity in the vestibular nuclei was still qualitatively present, although quantitatively reduced in comparison to intact animals.

These results can be explained from the knowledge that part of the visual input used for OKR and immediate visuo-vestibular interaction reach the vestibular nuclei independently of the cerebellum. Holstege and Collewijn (1982), on the basis of a tracing study of the projections of the pretectal NOT, suggested a pathway through the prepositus hypoglossi nucleus and/or the dorso-medial medullary reticular formation, whereas Precht (1982) suggested the nucleus reticularis tegmenti pontis as the main intermediate station for visual signals to reach the vestibular complex. This part could therefore not be abolished by floccular GABA-injections.

In this Chapter it was demonstrated that floccular injection of muscimol or baclofen results in a strong depression of the gain of both the VOR and the OKR. Both substances, although activating different GABA-receptors, generated identical effects. It is suggested that the injections enhance GABA-ergic inhibition of floccular Purkinje cells either directly, or indirectly through granular cells, thus leading to the functional inactivition of Purkinje cells. Since these Purkinje cells act out-of-phase with respect to the vestibular neurons which are driven by the same afferent vestibular signal, their reduced activity would remove one of the main components which contribute to the gain of the VOR and OKR. These experiments demonstrate that reversible inactivation of floccular Purkinje cells results in a reversible depression of the gain of the VOR and OKR. This evidence indicates that one of the functions of the flocculus is to exert a positive influence on the VOR and OKR.

CHAPTER 4

VISUO-VESTIBULAR INTERACTION

INTRODUCTION

Compensatory eye movements elicited by the vestibulo-ocular reflex (VOR) are greatly improved by vision (Baarsma and Collewijn, 1974; Ito et al., 1974a, 1974b). It is presumed that the output of the VOR in light is created from a combination of separate vestibular-ocular and optokinetic reflexes. The input-output relations of these reflexes have been studied in the monkey (Skavenski and Robinson, 1973) and in the rabbit (Collewijn, 1969; Baarsma and Collewijn, 1974). Both the VOR and the OKR system have been demonstrated to be frequency and amplitude dependent. The VOR is most effective in the higher frequency and amplitude range, while the OKR has an exactly complementary action, acting most adequately at lower frequencies and amplitudes. Combined visuo-vestibular stimulation activates both systems, and results in perfect compensatory eye movements over a wide range of frequencies and amplitudes.

Visual and vestibular information can also interact in a non-synergistic situation, for example during suppression of the VOR. In foveate animals, this condition can be tested during rotation of the animal in the presence of a fixation target which is stationary with respect to the head. Suppression of the VOR can also be induced in non-foveate animals by rotating them on a platform with platform-fixed visual surroundings. In this situation, the compensatory eye movements of the VOR and OKR are opposite in action, which results in a decrease in the gain of the VOR in light. Suppression of the VOR has been shown to be more effective at lower stimulus velocities, and both gain and phase are markedly frequency and amplitude dependent in this situation (Baarsma and Collewijn, 1974; Godaux et al., 1983; Barnes and Edge, 1984).

As was mentioned in Chapter 1, the cerebellar flocculus has been proposed as a main site where visuo-vestibular interaction may take place. The flocculus receives vestibular signals as a mossy fiber input (Brodal and Høivik, 1964; Precht and Llinas, 1969), but also visual signals, which arrive either through climbing fibers derived from the dorsal cap of the inferior olive (Maekawa and Simpson, 1972, 1973; Simpson and Alley, 1974; Gerrits and Voogd, 1982), or through mossy fibers, derived from the nucleus reticularis tegmenti pontis (Maekawa et al., 1981; Gerrits et al., 1984). It has been shown that both the mossy and climbing fiber input can modulate the firing activity of floccular Purkinje cells, and that in case of simultaneous visual and vestibular stimulation in the natural situation, the mossy and climbing fibers interact synergistically (Lisberger and Fuchs, 1974; Ghelarducci et al., 1975; Waespe and Henn, 1979; Waespe and Henn, 1981).

The way in which the flocculus affects visuo-vestibular interaction has been studied by lesion experiments in various animal species, and pathology in humans. The results of such experiments are not always unequivocal. Lesions of the vestibulo-cerebellum in humans resulted in an inability to modulate the VOR by vision (Baloh et al., 1981; Zee et al., 1976). In these patients, the compensatory eye movements in light had approximately the same amplitude as those in darkness. Takemori and Cohen (1974) demonstrated severe impairment of visual suppression of caloric nystagmus in the monkey. They emphasize the fact that this disturbance was only found in animals with complete or nearly-complete flocculectomy. In general agreement with these results, Zee and coworkers (1981) found that bilateral flocculectomy in monkeys resulted in impairment, but not total abolition of visual suppression of vestibular nystagmus.

In the chinchilla, an animal in which complete removal of the flocculus is relatively easy, visuo-vestibular interaction was totally abolished after such lesions (Hassul et al., 1976; Daniels et al., 1978).

On the other hand, Waespe et al. (1983) described conflicting results in monkeys. They found no impairment of visuo-vestibular interaction in flocculectomized monkeys. Moreover, these animals were perfectly able to suppress vestibular nystagmus.

The effects of flocculectomy on visuo-vestibular interaction have also been studied in the rabbit. A bilateral lesion of the flocculus resulted in an impairment of visuo-vestibular interaction (Ito et al., 1982). However, Koehn and coworkers (1981) and Honrubia and coworkers (1982) still found visuo-vestibular interaction as well as possibilities to suppress the VOR gain after lesions of the vestibulo-cerebellum. These

responses were, however, reduced with respect to normal animals. Honrubia and colleagues noticed that a very small remnant of intact floccular tissue could greatly improve the interaction between visual and vestibular inputs.

Visuo-vestibular interaction occurs not only in the flocculus, but also in the vestibular nuclei. This has been shown in rabbits (Dichgans and Brandt, 1972), cats (Bauer et al., 1976), fish (Allum et al., 1976) and monkeys (Waespe and Henn, 1977). It has been suggested that the combined of visuo-vestibular signals in the vestibular nuclei may be derived from the vestibulo-cerebellum, and in particular from the flocculus. In this view, it is of interest to investigate the effects of flocculectomy on visuo-vestibular interaction in the vestibular nuclei.

Waespe and Cohen (1983) did this experiment in monkeys, and they found no disturbance of interaction in the vestibular nuclei after flocculectomy. They did, however, demonstrate an increase in the time constant of the build-up of steady state firing levels of the vestibular neurons during combined visuo-vestibular stimulation. The authors conclude from these results that the site of interaction of the floccular output with the VOR appears to be external to the vestibular nuclei.

These results from Waespe and Cohen (1983) in the monkey are in line with earlier experiments by Keller and Precht (1978, 1979) and by Precht and Strata (1980) in the cat. Precht and his coworkers found visuo-vestibular responses in the vestibular nuclei, although the whole cerebellum, including both flocculi, had been extirpated. Moreover, the gain of the VOR was still improved by vision in these flocculectomized animals. Therefore, these authors reach the same conclusion as later arrived at by Waespe and Cohen (1983) that the flocculus is not directly responsible for visuo-vestibular interaction in the vestibular nuclei.

This review of the function of the flocculus in visuo-vestibular interaction indicates that final conclusions can not be drawn yet. It would take further research to find out if the contradictions in the literature are due to species differences, or to other variables in the employed methods. It seems of special interest that testing the effects of flocculectomy at the output level (i.e., on the gain and phase of the VOR in light) or, on the other hand, at the level of signal interaction in the vestibular nuclei, leads to different results. When tested at the output level, flocculectomy results mostly in an impairment of visuo-vestibular interaction, while testing interaction at the level of the

vestibular nuclei reveals less impairment. Because the connections by which the flocculus affects the VOR are not completely clear, it seems best to study visuo-vestibular interaction at the output level.

Although the question *where* visuo-vestibular interaction takes place offers quite a number of possibilities for further investigation, the experiments which will be presented in this Chapter are mainly concentrating on *the nature* of the interaction. An important question in this respect is whether visuo-vestibular interaction is attained by simple, linear addition of the separate visual and vestibular components, or, whether a much more complex strategy is used to combine these two input signals.

Allum and coworkers (1976) investigated the nature of the interaction at the level of the vestibular nuclear neurons in the cat. They suggested a linear combination of weighted vestibular and optokinetic responses. Waespe and Henn (1977), on the basis of similar experiments in monkeys, suggest that the interaction is affected by a switching mechanism, which allows either the optokinetic or the vestibular input to pass into the vestibular nuclei while the other input is turned off.

Interaction by linear addition of weighted VOR and OKR components has also been suggested by Baarsma and Collewijn (1974) and Batini and coworkers (1979). Both groups did experiments in rabbits, and judged visuo-vestibular interaction from VOR output-characteristics. Baarsma and Collewijn (1974) succesfully predicted visuo-vestibular interaction by linear addition of the VOR component and the OKR response to the retinal slip which remained after compensation by the VOR. Optokinetic input-output relations had been determined beforehand. A weak point in their method was that phase relations were disregarded.

The experiment by Batini and coworkers (1979) was more complete in this respect. They tested the dynamic characteristics of the VOR and OKR and their interactions in albino rabbits. Eye movements were measured by means of a closed circuit television system, which recorded the position of a fluorescent spot on the cornea. Beside the natural synergistic interaction of VOR and OKR, suppression as well as enhancement of the VOR gain by visual input was investigated. Interaction of the VOR and OKR was predicted from the individual dynamic characteristics, taking the phase differences as well as the velocity sensitivity of the OKR into account. Batini et al. conclude that visual modification of the VOR is due to linear interaction of the two systems, in agreement with the earlier conclusions by Baarsma and Collewijn (1974).

Batini et al.'s experiment could be improved at a few points. In the first place, they used albino rabbits, which are known to have abnormal anatomical connections in the visual system (Collewijn et al., 1978). Furthermore, the authors used a single light slit to elicit an optokinetic response, and they employed an eye movement detection method which is not very accurate.

The experiments described in this Chapter were developed to re-investigate the hypothesis of linear interaction in pigmented rabbits. As in the experiments by Batini et al., we started from the assumption that during vestibular stimulation in light in natural conditions, the vestibular system is the first to be activated and to generate a compensatory eye movement. The remaining retinal slip will thereafter serve as an input to the optokinetic system. In a parallel way, one might predict the gain of the VOR in light from the separate values of the gain of the VOR in darkness and the gain of the optokinetic response to an appropriate optokinetic stimulus. This stimulus would be a copy of the residual retinal slip, remaining after the compensatory eye movement produced by the VOR in darkness. If the hypothetical linear addition of visual and vestibular components would be correct, this strategy would predict a gain value of the VOR in light (VOR_p) which would be identical to the actual value of the gain of the VOR in light (VOR_a)

To test the linearity of interaction, visuo-vestibular interaction was studied in two non-synergistic situations: 1) during a conflict situation which causes suppression of the vestibulo-ocular reflex and 2) during a conflict situation which causes enhancement of the vestibulo-ocular reflex.

METHODS

The experiments were done on 6 Dutch belted rabbits, using the eye measurement method described in *General methods* (Chapter 2). The two situations of conflicting visuo-vestibular interaction will now be described in detail.

1) Suppression of the VOR

During these experiments, the animal was sinusoidally rotated with platform-fixed surroundings. This has proved to be a suitable stimulus to induce partial suppression of the VOR in rabbits. The platform and the optokinetic drum moved together in phase

with identical amplitudes. The stimulus frequency, which was 0.15 Hz, was presented at three different amplitudes: 2.5 deg, 5 deg and 10 deg. Thus, three different maximum stimulus velocities (2.4 deg/s, 4.7 deg/s and 9.4 deg/s) were investigated. In this conflict situation, the compensatory eye movement evoked by the vestibulo-ocular reflex should be totally suppressed in order to eliminate retinal slip. Theoretically, in case of linear visuo-vestibular interaction, this would require an optokinetic eye movement which should be equally large as, but opposite in direction to the compensatory eye movement elicited during the VOR in darkness. Obviously, such a result is unlikely to occur in a linear interaction between VOR and OKR, unless OKR would have unity gain. Indeed, a total suppression of the VOR has never been demonstrated in the rabbit (Baarsma and Collewijn, 1974; Batini et al., 1979).

In the present experiments, the VOR and OKR components were separately generated and vectorially added (i.e. with appropriate phase relations) to a predicted response of the VOR in light. This predicted response was compared to the effect of simultaneous visual and vestibular stimulation.

The VOR component was obtained by oscillation of the rabbit in the dark. The velocity signal of the compensatory eye movement, obtained in this condition, was introduced into an electronic circuit where the fast phases of the eye movement were eliminated. The basic principle of this saccade detection method is shown in Fig. 4.1. A delay technique was used to circumvent the usual flaw of hardware, real-time saccade

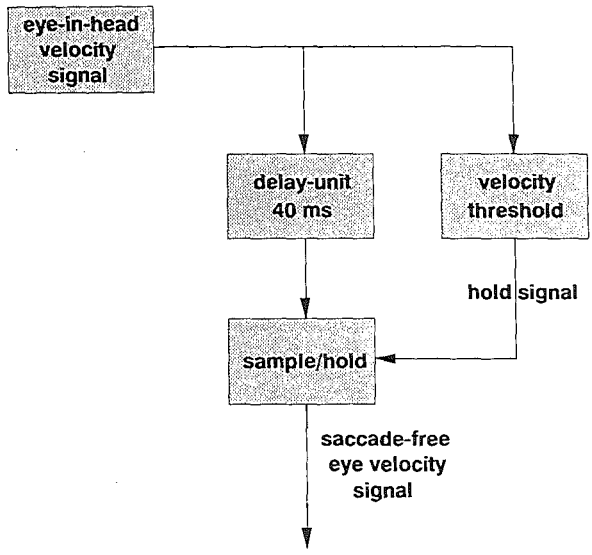

Fig. 4.1. Schematic representation of the circuit which was used to eliminate fast phases of the compensatory eye movement from the trace. The output of the circuit was a smooth eye velocity signal with a 40 ms delay, which could be used to drive the optokinetic drum.

detectors, that rejection of a saccade only starts after its reliable detection, i.e., long after its real onset. The velocity signal of the eye-in-head in darkness, obtained by analogue, active differentiation of the eye position signal, was the input to the circuit. This signal was processed in two parallel paths, one of which contained the pure input signal, while the other introduced a 40 ms delay to the input signal. The delay was produced by a digital device (YEW, type 3382) employing a shift-register. The undelayed signal was scanned for fast phases of the eye movement by an adjustable threshold velocity criterion. Once the onset of a saccade was detected, a "hold-signal" was generated and transferred to the other parallel path which carried the delayed signal. The imminent saccade was suppressed by clamping the voltage to the sample value prior to the saccade onset for the next 200 ms. In this way, the circuit generated a smooth, saccade free eye velocity signal, delayed by 40 ms with respect to the input. This signal could be stored and then used in subsequent trial as the input to the velocity control of the optokinetic drum.

By generating a drum movement equal to the smooth component of the prior VOR in the dark, the corresponding optokinetic response was determined and used as the OKR component for calculating the predicted VOR in light. This OKR was vectorially subtracted from the compensatory eye movements during the VOR in darkness, taking the phase-angles and amplitudes of both components into account (see Fig. 4.2). The VOR and OKR components could not be directly subtracted, because the optokinetic signal had a 40 ms delay with respect to the compensatory eye movements of the VOR in darkness, and was therefore not in time-register with that signal.

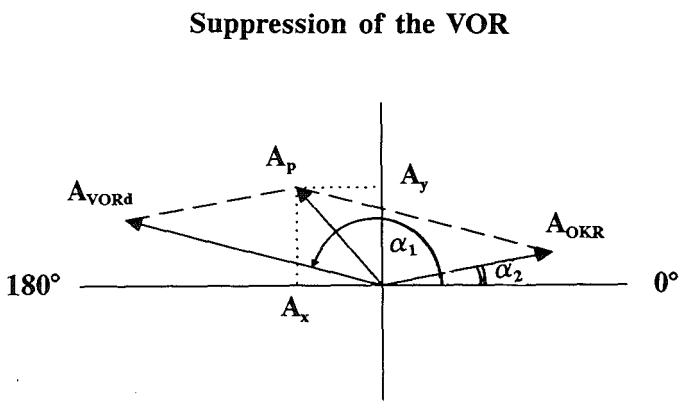

Fig. 4.2. Vector diagram illustating the calculation of the predicted amplitude of the VOR in light (A_p) from separate VOR and OKR components (A_{VORD}, A_{OKR}) during a suppressive conflict condition.

The predicted amplitude of the VOR in the light could be calculated as follows:

$$A_x = A_{VORd} \cos\alpha_1 - A_{OKR} \cos\alpha_2$$

$$A_y = A_{VORd} \sin\alpha_1 - A_{OKR} \sin\alpha_2$$

$$A_p = \sqrt{A_x^2 + A_y^2}$$

In which A is the amplitude and α is the phase angle of the compensatory response. The amplitudes were calculated from the gain values:

$$A_{OKR} = G_{OKR} \times A_{VORd}$$

$$A_{VORd} = G_{VORd} \times A_{platf}$$

The phase of the VOR component in darkness was very close to -180 deg, and the phase of the OKR was maximally 15 deg at all three stimulus amplitudes. It follows from the equations that the sine or cosine of these angles is close to zero or 1, and therefore the phase angles did not have a great impact on the calculation of the predicted amplitude.

It is important to stress the point that, in the suppression as well as in the enhancement experiments, each measurement of the amplitude of the VOR in darkness was immediately followed by the measurement of the amplitude of the OKR, using the compensatory eye movement of the previous VOR_d measurement as an input for the optokinetic drum. In this way, any disturbing effect of natural variability of the VOR and OKR over periods of more than a few minutes was significantly reduced. The predicted amplitude of the VOR in the light was calculated for each separate measurement. The combination of VOR_d, OKR, and VOR_l was repeated 5 times at each stimulus velocity, and the mean values of the A_p and A_a of these 5 measurements were computed for each rabbit.

In case of a linear interaction of VOR and OKR, the predicted value of the VOR in light (A_p) should be identical to the actually measured value (A_a).

2) Enhancement of the VOR

In this situation, the VOR in the light was tested while the platform and the optokinetic drum were moving simultaneously but in counter-phase. The drum oscillated at half the amplitude of the platform. We used the same three stimulus velocities as in the suppression experiments.

This conflict situation causes an increase of the retinal slip, which in turn results in an increase of the required amplitude of the compensatory eye movement of the VOR. Theoretically, retinal slip would be completely abolished if the OKR not only compensated for the remaining slip of the VOR in darkness, but also for the additional slip induced by the drum movement. In case of linear visuo-vestibular interaction, the eye movement response of the VOR in light would be predicted by summation of the compensatory eye movements elicited during the VOR in darkness and the optokinetic response to the actual retinal slip, which occurs during the simultaneous movement of platform and drum.

The actual retinal slip can be simulated by vectorial addition of the non-compensated part of the platform amplitude during the VOR in darkness, the *gaze,* to the amplitude of the drum. This signal was constructed in a similar way as was described in the former section. The gaze velocity signal, which was recorded during oscillation of the rabbit in darkness, was used as the input to the saccade-detection circuit, where fast phases of the eye movement were eliminated in an identical way as was described in the former section. The smooth gaze velocity output signal of the circuit was added to the velocity signal of the drum-position, thus creating the input signal for the optokinetic drum. The compensatory eye movement of the OKR to this constructed amplitude was recorded (OKR*).

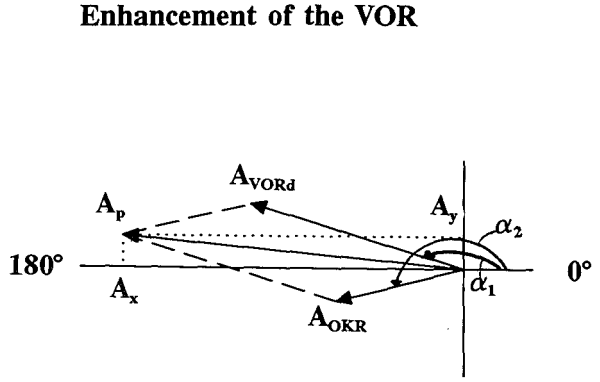

Fig. 4.3. Vector diagram illustating the calculation of the predicted amplitude of the VOR in light (A_p) from separate VOR and OKR components (A_{VORd}, A_{OKR}) during a enhancing conflict condition.

The predicted value of the amplitude of the VOR in light during this enhancing situation could be calculated by vectorial addition of the eye movement amplitude during the VOR in darkness and the amplitude of OKR*, as is demonstrated in Fig. 4.3.

$$A_x = A_{VORd} \cos\alpha_1 + A_{OKR*} \cos\alpha_2$$

$$A_y = A_{VORd} \sin\alpha_1 + A_{OKR*} \sin\alpha_2$$

$$A_p = \sqrt{A_x^2 + A_y^2}$$

In which A is the amplitude while α is the phase-angle of the response. The amplitudes were calculated from the gains:

$$A_{OKR*} = G_{OKR*}[\ (1-A_{VORd}) + (0.5 \times A_{platf})\ ]$$
$$A_{VORd} = A_{platf}\ (\ 1 - G_{VORd}\ )$$

Again, as in the suppressive conflict situation, the predicted and actually measured values of the VOR in light are expected to be similar in case of linear visuo-vestibular interaction.

Because the phase angles of the compensatory eye movements in darkness and of the optokinetic responses in both conflict situations were close to -180 deg and 10 deg respectively, these phases had only little influence on the predicted amplitudes, as was the case in the suppression experiments.

RESULTS

1) Suppression of the VOR

In this group of 6 rabbits, the gain of the VOR in darkness ranged from 0.70 - 0.90, depending on the stimulus velocity. The normal VOR in light had a gain above 0.90 in most animals.

The amount of suppression of the VOR was lowest at the stimulus with the largest amplitude. At the stimulus 0.15 Hz, 10 degrees amplitude, the average gain of the VOR in light during normal synergistic interaction was 0.99 ± 0.08 (S.D), while the gain of the VOR in darkness was 0.89 ± 0.06 (S.D.). During suppression of the VOR at this stimulus velocity, the gain dropped to 0.84 ± 0.11 (S.D.). At the middle amplitude, (5 deg), the average normal VOR gain was 0.95 ± 0.09 (S.D.) in the light, 0.87 ±0.08 (S.D.) in darkness, and 0.73 ± 0.18 (S.D.) during suppression of the VOR. At the lowest amplitude (2.5 deg) and thus the lowest stimulus velocity, the VOR suppression was most pronounced. The gain of the VOR in light was 0.94 ± 0.10 (S.D.), while it was 0.83 ±0.09 (S.D.) in darkness. During suppression of the VOR, the gain decreased to 0.71 ± 0.17 (S.D.). The relation between stimulus amplitude and VOR suppression is represented in Fig. 4.4.

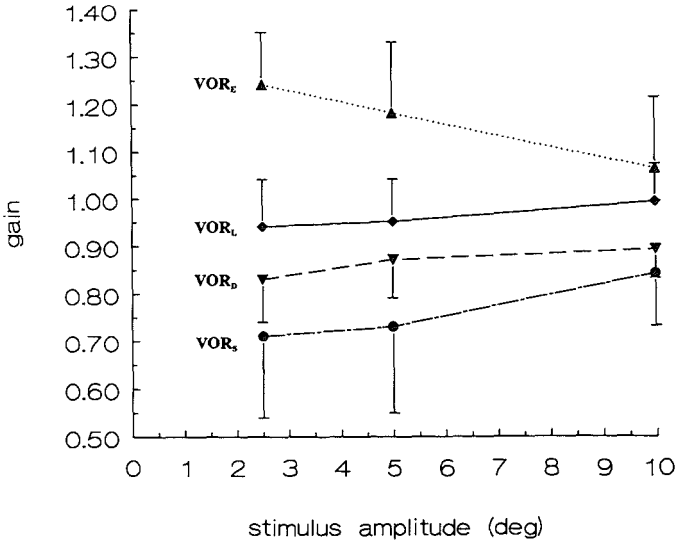

Fig. 4.4. This graph demonstrates the amplitude dependence of the gain of the VOR in light in normal, synergistic visuo-vestibular interaction (VOR_L) and in darkness (VOR_D), compared to the gain during suppression of the VOR (VOR_S) and during enhancement of the VOR (VOR_E). Average values and standard deviations of 6 rabbits. In all four conditions, the same stimulus frequency (0.15 Hz) was used.

For each rabbit, I tested whether the actual amplitude of the VOR during suppression could be predicted by linear addition of the separate VOR and OKR components, in which phase angles as well as amplitudes were considered. The values of A_a and A_p were calculated for every measurement. I did 5 measurements at each of the 3 stimulus velocities. The average values of A_a and A_p for each rabbit are listed in Table 4.1.

Table 4.1. Suppression of the VOR: comparison of actual (A_a) and predicted amplitude (A_p) of the VOR in light at the three tested stimuli.

rabbit	0.15 Hz, 10°		0.15 Hz, 5°		0.15 Hz, 2.5°	
	A_a	A_p	A_a	A_p	A_a	A_p
77	9.2	9.3	4.7	4.3	2.4	1.8
78	8.8	8.0	3.4	3.6	1.5	1.2
76	7.6	7.1	3.3	2.7	1.6	1.0
94	9.8	8.1	4.6	4.0	2.1	1.7
54	6.9	6.5	2.3	1.6	1.2	0.6
46	8.0	8.2	3.7	3.2	1.8	1.4
mean	8.4	7.9	3.7	3.2	1.8	1.3
± S.D.	±1.1	±1.0	±0.9	±1.0	±0.4	±0.4

The table shows that the actual amplitude of the VOR in light was slightly higher than the predicted value in 4 of the 6 rabbits at the highest stimulus velocity. At the middle stimulus velocity, the actual amplitude was higher than the predicted amplitude in 5 out of the 6 rabbits, while at the slowest stimulus, A_a was in all cases higher than A_p. Nevertheless, the differences between the average predicted and the average actual amplitudes of the VOR in light were in none of these cases statistically different from zero.

The data are plotted in a correlation diagram, which represents the relation between A_a and A_p for the three stimulus velocities in 6 rabbits (Fig. 4.5.). All data points are located approximately on the line $A_a = A_p$. Overall, the results show that the actual amplitude of the VOR can be very well predicted by summation of separate VOR and OKR components.

It is important to emphasize the reason why I did compare the *absolute amplitudes* of the eye movement responses instead of the *gains*. As Fig. 4.5 demonstrates, the absolute difference between predicted and actual amplitudes is very small for all three stimuli (at average 0.5 degree), and this difference is not amplitude dependent. If these small amplitudes were reported as a percentage of the stimulus amplitude (the gain), a slight difference in amplitude would be represented as a large percentage. Comparing actual and predicted gains would result in rather large differences, especially at the stimulus with the smallest amplitude. Because of this distortion, comparison of predicted and actual *gain* values suggested that the difference between G_a and G_p was amplitude dependent, and most pronounced at the lowest stimulus velocity. To prevent these misleading results, I decided to assess the differences between actual and predicted *absolute amplitudes*.

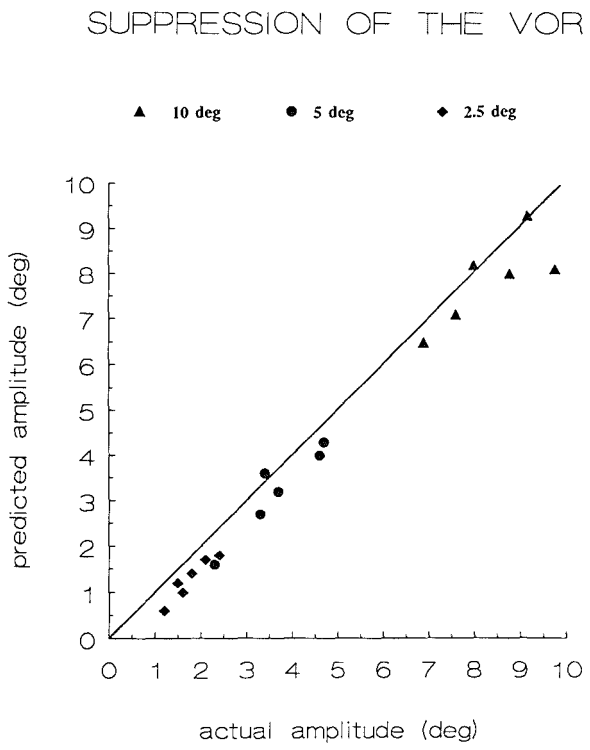

Fig. 4.5. Correlation diagram, demonstrating the linear relation between actual and predicted amplitude of the VOR in light during visual suppression. Average values of 5 measurements are indicated for each rabbit. Markers indicate the three different stimulus amplitudes.

In conclusion, these experiments have demonstrated that actual combined visuo-vestibular output during visual suppression of the VOR can be quite accurately predicted from the responses to separate visual and vestibular stimuli. This was the case

at all three stimulus amplitudes. Moreover, the amount of suppression of the VOR was amplitude dependent; it was larger at the lower stimulus amplitude.

2) Enhancement of the VOR

Similarly as in the suppression experiments, the 6 rabbits were tested at three stimuli with different amplitudes, at each of which 5 measurements were recorded. The basic values of the VOR in light and in darkness were comparable to those obtained in the suppression experiments. The actual values of the gain of the VOR in light in the enhancing situation were mostly higher than unity, and they depended on the stimulus velocity.

The amount of increase in gain of the VOR was largest at the lowest stimulus velocity. The gain of the VOR was 0.94 ± 0.10 (S.D.) in the light and 0.83 ±0.09 (S.D.) in darkness. It increased to 1.24 ± 0.11 (S.D.) in the enhancing situation. At the middle stimulus amplitude, the normal gain of the VOR was 0.95 ± 0.09 (S.D.)in the light, and 0.87 ± 0.08 (S.D.) in the dark, while during enhancement of the VOR, it became 1.18 ± 0.15 (S.D.). At the highest stimulus velocity, the average gain was enhanced from 0.99 ± 0.08 (S.D.) in the light and 0.89 ±0.06 (S.D.) in darkness to 1.06 ± 0.15 (S.D.) in the enhancing situation. The amplitude dependence of VOR enhancement is demonstrated in Fig. 4.4.

Table 4.2. Enhancement of the VOR: comparison of actual (A_a) and predicted amplitude (A_p) of the VOR in light at the three tested stimuli.

rabbit	0.15 Hz, 10°		0.15 Hz, 5°		0.15 Hz, 2.5°	
	A_a	A_p	A_a	A_p	A_a	A_p
77	11.5	11.0	5.7	5.5	2.7	2.8
78	12.2	11.8	6.4	6.6	3.3	3.1
76	9.7	9.5	5.9	5.5	3.0	2.8
94	11.1	10.8	5.9	5.5	3.1	2.8
54	11.1	11.4	7.0	6.3	3.5	3.2
46	8.1	8.8	4.8	5.4	3.0	2.8
mean	10.6	10.6	6.0	5.8	3.1	2.9
±S.D.	±1.5	±1.2	±0.7	±0.5	±0.3	±0.2

To test the hypothesis of linear visuo-vestibular interaction, the values of A_a were measured and compared to the values of A_p as was explained in the method section. The data from the six rabbits are listed in Table 4.2, and Fig. 4.6 shows a correlation diagram of the average actual and calculated amplitude values from 5 measurements in each rabbit. Note that the phase angles have been taken into account in the amplitude calculations.

Comparing the values of A_a and A_p in this situation, it appears from the table and the figure that the actual amplitude exceeds the predicted value in 13 of the 18 cases. These differences are, however, not significantly different from zero at any of the tested amplitudes. The correlation diagram shows that all data are very near to the hypothetical line which represents identical actual and predicted amplitudes.

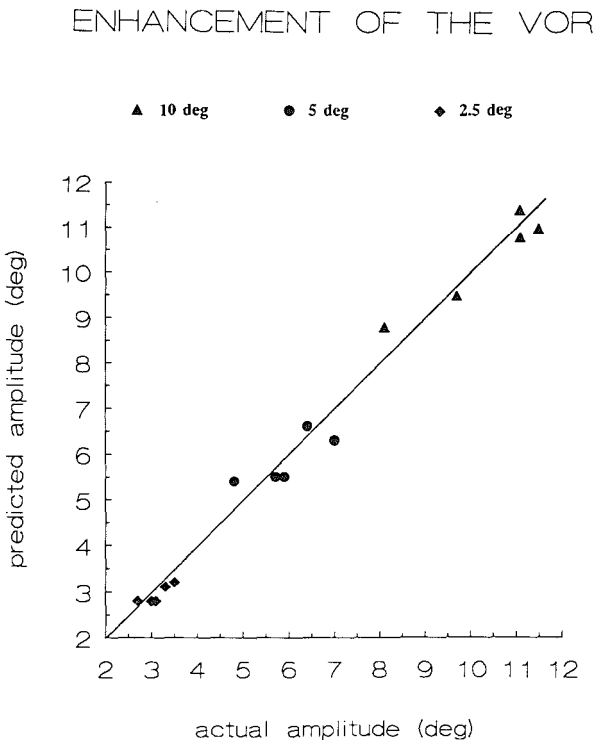

Fig. 4.6. Correlation diagram, demonstrating the linear relation between actual and predicted amplitude of the VOR in light during visual enhancement. Average values of 5 measurements are indicated for each rabbit. Markers indicate the three different stimulus amplitudes.

The conclusion should therefore be that this unusual combination of visual and vestibular stimuli, which requires an increase in gain of the VOR in light, results in identical enhancement of the VOR by the visual input as was predicted by linear addition of the amplitude of the separate components. Moreover, the amount of enhancement of the compensatory eye movement of the VOR is largest at the lower stimulus velocities. In other words, like suppression of the VOR, enhancement of the VOR is amplitude-dependent.

Normal, synergistic visuo-vestibular interaction (rotation of the animal with earth-fixed surroundings) was also investigated, but only in a limited number of pilot studies. In these experiments, visuo-vestibular interaction was tested with small amplitudes at the frequency of 0.1 Hz. The gain of the VOR was rather low in these conditions, and therefore, the optokinetic drum could be driven with the residual retinal slip signal to obtain valid data for the OKR component. Although only few data were obtained in this condition, the predicted and actual amplitudes of the VOR in light were in close agreement, as in the non-synergistic experiments.

DISCUSSION

The results of the experiments on conflicting visuo-vestibular interaction suggest that this interaction is approximately linear at all tested stimulus velocities. In both the suppression and the enhancement experiments, the actual and predicted amplitudes were virtually identical at the tested stimuli (Figs. 4.5 and 4.6).

These results fit well to those obtained by Baarsma and Collewijn (1974) and Batini et al. (1979), who also investigated visuo-vestibular interaction in the rabbit. They suggested the combination of visual and vestibular signals to be mainly linear. In line with Batini and his collegues, these experiments were designed in such a way that the separate components of VOR and OKR were indeed of an appropriate magnitude and phase to allow a meaningful comparison to the actual amplitude of the VOR in light in the non-synergistic interaction. The fact that the optokinetic signal was directly constructed from the eye-in-head or gaze signal during registration of the VOR in darkness makes the experimental setting optimally comparable to the natural situation.

The linearity of combination of vestibular and optokinetic components seemed to be universally present, independent of the stimulus amplitude, and thus independent of

the magnitude of the response of either VOR or OKR. It should be noticed that only three stimuli with identical frequency were tested. The amplitudes were chosen in such range that the resulting stimulus would in any case activate the VOR, as well as the OKR. Therefore it is not known what type of interaction would occur in the high and low stimulus velocity range, which would activate mainly the VOR or mainly the OKR. This experimental method can not be used to compute a drum-driving signal from a very small eye-in-head response.

For the same reason, we mostly avoided to study the normal, synergistic visuo-vestibular interaction, in which oscillations would be tested in lighted, but earth fixed surroundings. This type of interaction would result in fairly adequate compensatory eye movements in darkness at the tested frequency and amplitudes, and therefore, the residual retinal slip, which would serve as the input to the optokinetic drum in this situation, would be very small. The mechanical properties of the drum made it impossible to reproduce such small amplitudes accurately, and, as a consequence, the results would be very insensitive to small discrepancies. However, the results obtained in a number of pilot experiments suggested that the synergic visuo-vestibular interaction is also approximately linear.

In the past, several authors have suggested mechanisms by which visual and vestibular input signals would be combined (see Koenig et al., 1978). One of the possibilities, the so-called "switching hypothesis", was initially proposed by Young (1970) and later adopted by Waespe and Henn (1977). It was based on the idea that during visuo-vestibular interaction either the visual or the vestibular input was transferred to the vestibular nuclei, while the other input was turned off. The vestibular system would dominate in the high velocity range, while the optokinetic system would generate the output at the lower stimulus velocity range. This hypothesis, which was based on experiments in primates, was abandoned after a while because it could not explain the improvement of the VOR in light with respect to darkness in those velocity ranges where the optokinetic system was supposed to be inactivated. Neither could the switching hypothesis account for conflicting interaction of vestibular and visual components, as occurs for example during suppression of the VOR.

Other hypotheses were based on the assumption that both the VOR and the OKR inputs were used in order to generate a single output. If the individual non-linear characteristics of the VOR and the OKR were taken into account, the linear addition of the separate components could be predicted by mathematical simulation-models. For

example the model by Schmid et al. (1980) could predict both synergistic and conflicting combinations of the VOR and OKR. The model uses linear addition of vestibular and optokinetic components, and it also represents the non-linearities of either system. Schmid and coworkers could reproduce the results on visuo-vestibular interaction which had been obtained in man by Koenig et al. (1978). The latter authors as well as others (Baarsma and Collewijn, 1974; Allum et al., 1976; Batini et al., 1979; Keller and Precht, 1979) all agreed in the conclusion that visuo-vestibular interaction was very close to linear, if the non-linear characteristics of the subsystems were taken into account.

Both suppression and enhancement of the VOR were amplitude dependent (Fig. 4.4). This finding had already been reported by Gilson et al (1974), Baarsma and Collewijn (1974), Godaux et al. (1983) and Barnes and Edge (1984). It is obviously due to the fact that enhancement or suppression of the VOR is induced by the incoming visual information. Therefore, the required changes will be most adequately brought about at the lower stimulus velocities, at which the OKR is most efficient.

The absolute amount of suppression obtained in the rabbit was rather low (maximal 25%), compared to results obtained in cats. Godaux and colleagues (1983) reported a total suppression of the VOR in the cat at frequencies lower than 0.05 Hz, but the gain of the VOR is very low at this frequency; even rabbits show total suppression of the VOR under these conditions (see Baarsma and Collewijn, 1974). Nevertheless, the overall amount of suppression in the cat is higher than in the rabbit, which is probably due to the fact that rabbits are non-foveated animals. They are therefore unable to fixate, and this seems to be an important capability to perform suppression of the VOR. However, the untrained cat, which has an area centralis and not a true fovea, will not fixate a spot voluntarily, and will therefore not suppress the VOR a great deal better than the rabbit.

On the other hand, more pronounced differences were found on comparing the present data in rabbits to those in primates. Takemori and Cohen (1974a) demonstrated that vestibular nystagmus in the cat could be suppressed to 50% by visual input. In humans, almost complete suppression of the VOR can be obtained when a fixation spot is presented, which is stationary with respect to the head. Obviously, suppression of the VOR in non-foveated animals by oscillation on a platform with platform-fixed visual surroundings is less effective. It seems that the presence of a fovea, in combination with the intention to fixate a spot are needed to produce good suppression. These conditions are not satisfied to in the rabbit.

In conclusion, it was demonstrated in this Chapter that visuo-vestibular interaction is accomplished by linear addition of the separate components, whatever their magnitude. Enhancement and suppression per se are amplitude dependent, but the combination of VOR and OKR takes place in identical ways, whether suppressive or enhancing combinations of VOR and OKR are presented. It seems safe to conclude that the normal, synergistic interaction between visual and vestibular responses will be linear as well, because this situation is intermediate inbetween the tested, unusual combinations of visual and vestibular stimuli. Pilot experiments suggested that this is indeed the case.

The experiments which were presented in this Chapter were designed to investigate the nature of visuo-vestibular interaction. As was mentioned in the introduction, the cerebellar flocculus seems to be an important structure in the generation and combination of VOR and OKR signals. The following Chapter will provide more evidence with respect to the function of the flocculus in these reflexes.

CHAPTER 5

EFFECTS OF FLOCCULAR INJECTIONS OF NORADRENERGIC SUBSTANCES ON ADAPTIVE CHANGES IN THE VOR GAIN

INTRODUCTION

In the previous Chapters, two aspects concerning the generation of adequate compensatory eye movements during the VOR were discussed. Firstly, we concentrated on the role of the flocculus in the accomplishment of the basic characteristics of the VOR and OKR and secondly, the nature of direct visuo-vestibular interaction was investigated. In the present and the following Chapter, evidence will be presented on a second important function of the flocculus: the regulation of *adaptation* of the VOR.

Adaptation can be defined as an adjustment of the gain of the VOR, due to long-lasting unusual combinations of visual and vestibular input. The goal of these adaptive gain changes is to maintain compensatory eye movements of adequate magnitude during head movement in all occasions, even when part of the system gets damaged for some reason.

It has been demonstrated that remarkable changes in gain, in an appropriate direction, occur when the relations between head movements and the associated relative motions of the visual surroundings are modified by such devices as reversing prisms, magnifying or minifying lenses, or by uncommon combinations of visual and vestibular stimuli (for a general review see Berthoz and Melvill Jones, 1985). These changes in gain will be referred to as *adaptive changes* from now on. The visual effect of magnifying or minifying lenses can be simulated by oscillating an animal on a platform which is surrounded by an optokinetic drum, which rotates in-phase or out-of-phase with the platform. This set-up has proved to be an efficient and practical laboratory condition for the study of adaptive changes. It leads to substantial adaptation of the VOR in a matter

of hours in rabbits (Ito et al., 1974a, 1979; Collewijn and Grootendorst 1978, 1979; Collewijn and Van der Steen, 1987), cats (Keller and Smith, 1983; McElligott and Freedman, 1988a, b) and goldfish (Schairer and Bennett, 1986a, 1986b).

From the beginning, the cerebellar flocculus has been implicated in the adaptation of the VOR. Although experimental evidence has been presented which either supports (Ito et al., 1974b, 1982; cf. Ito, 1984) or contests (see Miles and Lisberger, 1981) the hypothesis that the floccular cerebellar cortex is the actual site of the neuronal changes instrumental in adaptation, it is generally agreed that damage or removal of the flocculus prevents adaptation of the gain of the VOR (Ito et al., 1982; Nagao, 1983).

Apart from the question where adaptation is established, it is not known what mechanisms are involved. Several major theories of "cerebellar learning" have been proposed to explain the occurrence of adaptive changes. Some of these suggested that heterosynaptic interactions between climbing and parallel fiber systems would be responsible for "cerebellar learning" (Marr, 1969; Albus, 1971). In addition to these two cerebellar afferent systems, the noradrenergic (NA) system has been proposed to affect consolidation of cerebellar learning (Gilbert, 1975).

A key role for NA has been postulated also for other processes involving neuronal plasticity, notably the development of the visual cortex in early life. Electrophysiological (Kasamatsu and Pettigrew, 1976) and behavioral (Gordon et al., 1986) studies have shown that general depletion of brain-NA by 6-hydroxydopamine (6-OHDA) prevents the occurrence of ocular dominance shifts after monocular deprivation in kittens. On the other hand, local perfusion with NA restored visual cortical plasticity (Kasamatsu et al., 1979). For a recent evaluation of these experiments, see Kasamatsu, 1987.

In addition to these effects on sensory processing, NA-depletion has been shown to impair the acquisition of a locomotor task (Watson and McElligott, 1983, 1984). Another example of motor-related effects of the NA-system is the recent demonstration that electrolytic lesions of the LC in the decerebrate cat may either increase (d'Ascanio et al., 1985) or decrease (d'Ascanio et al., 1989) the gain of vestibulospinal reflexes, recorded from forelimb extensors during sinusoidal roll of the animal. Similar results were also obtained after functional inactivation of the noradrenergic and NA-sensitive LC neurons produced by local injections into this structure of either the α_2-adrenoceptor agonist clonidine (Pompeiano et al., 1987) or of the β-adrenoceptor agonist isoproterenol (Stampacchia et al., 1988), which act by inhibiting these neurons.

The finding of a noradrenergic modulation of the vestibulo-spinal reflex raises the question in how far NA may be also directly involved in changes in the gain of the vestibulo-ocular reflex. The projection of the LC to the flocculus, originating mainly from the locus coeruleus (LC; Hokfelt and Fuxe, 1969; Olson and Fuxe, 1971), offers one obvious pathway for such an interaction (Fuxe, 1965; Bloom et al., 1971). It appears that this projection is bilateral but with an ipsilateral preponderance, and reaches the whole area of the cerebellar cortex (Dietrichs, 1988) including the flocculus (Kimoto et al., 1978; Somana and Walberg, 1978; Langer et al., 1985). It has been reported that the noradrenergic afferents terminate within the granular and the molecular layers of the cerebellar cortex (Bloom et al., 1971, Olson and Fuxe, 1971, Segal et al., 1973, Yamamoto et al., 1977, Kimoto et al., 1981).

The noradrenergic afferents to the flocculus could potentially affect the VOR in two ways: (1) direct modulation of the amplitude of the VOR, similarly as was described for vestibulo-spinal reflexes; or (2) control of the degree of adaptability of the VOR to modified visual input.

The latter possibility is supported by some studies on the effect of general depletion of brain-NA on adaptation of the VOR. Intracisternal injections of 6-OHDA, which severely depleted the central stores of NA, reduced or abolished VOR-adaptation in cats (Keller and Smith, 1983; McElligott and Freedman, 1988a, b). These findings contrast with an earlier report by Miyashita and Watanabe (1984) that no loss in VOR-adaptation occurred after a similar 6-OHDA injection in pigmented rabbits. Such a loss was, however, obtained in rabbits after depletion of both NA and serotonine (5-HT) by intraventricular injection of 5,7 dihydroxytryptamine (5,7-DHT). Miyashita and Watanabe concluded from these experiments that serotonin, and not noradrenaline, is crucial in maintaining adaptive modifiability of the VOR, at least in the rabbit. This discrepancy will be further discussed in Chapter 6.

All of these general depletion techniques have an important drawback: they do not identify a specific site where noradrenaline or serotonin may affect adaptability. More seriously, it is not certain whether the effects described are due to a specific impairment of long-term visuo-vestibular interactions or to more general impairments related to the overall depression of the noradrenergic system.

If we summarize the main facts about VOR adaptation that can be gained from the literature, we can conclude that:

1) an intact flocculus is needed to generate VOR adaptation,

2) the noradrenergic and/or serotonergic systems seem to affect VOR adaptation.

This raised the question whether the noradrenergic or serotonergic systems act through the cerebellar flocculus to modify adaptation of the VOR gain.

The experiments described in this and in the following Chapter were designed to answer this question. The presumed actions of these two neurotransmittor systems will be investigated by floccular injection of their agonists and antagonists, thus imitating or blocking their natural function.

In this Chapter I shall concentrate on the role of the noradrenergic system, while effects of the serotonergic system on VOR adaptation will be presented in the next Chapter. The injection technique we used in these experiments has been described in Chapter 2, with the general methods.

The Results-section of this Chapter will concern the effects of injections of adrenoceptor agonists and antagonists on VOR adaptation. Because both β_1- and β_2 (Minneman et al., 1979, 1981; M. Pompeiano et al., 1989) as well as α_1 and α_2-noradrenaline receptors (Bylund and U'Prichard, 1983) have been demonstrated in the cerebellum, we should test agonists and blockers to either receptor subtype. Because all these experiments were performed with identical procedures, the method will be explained at the beginning of this Chapter.

METHODS

Procedure for adaptation experiments

The rabbits were fixated in a hammock and mounted on a small bench, which was centered on the platform (Chapter 2, Fig. 2.3). We made sure that none of the fixtures intruded into the visual field. Adaptive enhancement of the VOR gain was elicited by sinusoidally oscillating the platform at the frequency of 0.15 Hz with an amplitude of 5 deg, while the optokinetic drum moved in counter-phase at the same frequency but with an amplitude of 2.5 deg (see Ito et al., 1974a, 1979). This stimulus, which was continuously presented for 3 hrs, induced an adaptive increase in the VOR gain in light as well as in darkness. Ideally, this situation should lead to a gain of the compensatory eye movements of about 1.5, as contrasted to a normal ideal value of about unity (an increase by 50%), but such complete adaptation was never seen in rabbits.

Each experiment was started with 4 baseline measurements of the VOR during passive oscillation in light, as well as in darkness. During these baseline measurements, which took about 15 minutes, the platform was oscillated at 0.15 Hz with an amplitude of 5 deg, while the drum remained stationary. The platform did not move in between the measurements. In the appropriate cases, a bilateral injection with the agonist or antagonist of choice was made after the baseline measurements. One minute was taken to inject 1.0 μl of the solution at a chosen concentration. As soon as the injection was completed, motion of both the platform and the drum in couterphase was started.

A first measurement was taken as soon as possible after the injection. During the rest of the experiment, recordings of the VOR in the light as well as in darkness were taken every 15 minutes. Each recording took 90 seconds. In this way, the adaptive process was continuous over successive periods of 13.5 minutes, and interrupted only for 1.5 minutes to record the VOR in darkness. This short interruption did not noticeably affect the adaptive process.

In each rabbit, the experiment described above was performed in three conditions: a) a control adaptation without any injection, b) an adaptation after injection of the appropriate agonist, and c) an adaptation after injection of the appropriate antagonist. The three experiments were performed in a random order. There was always at least one day of rest in between two sessions to allow the rabbit to recover fully from the previous experiment.

Procedures for non-adaptation experiments

Control experiments were performed to find out whether the injected substances would affect the basic characteristics of the VOR or OKR. These control experiments were especially important in those cases where the injections did affect VOR adaptation. In such case it was crucial to establish whether the effect of the agonist or antagonist was specific for the adaptive process itself, or whether the basic VOR gain characteristics were affected independently of adaptation.

In order to investigate this, the rabbits were oscillated on the platform at the same frequency as used during the adaptation experiments (0.15 Hz, 5 deg amplitude). This time, however, the drum was kept stationary and the platform did move only during the control measurements and not continuously, as during the adaptive process. In each control, the VOR in the light and in darkness, as well as the optokinetic response (OKR) were registered. To evoke an optokinetic response, only the optokinetic drum

surrounding the rabbit was oscillated at the same frequency and amplitude as used in the combined stimulus (0.15 Hz, 5 deg). Also in this condition, 3 experiments were performed in each rabbit and in random order to determine the time course of: a) changes in the VOR and OKR gain without injection; b) changes in the VOR and OKR gain after injection of the appropriate agonist; and c) changes in the VOR and OKR gain after injection of the appropriate antagonist. There was always a recovery period of at least one day between successive experiments. As in the adaptation experiments, 1.0 μl of the solutions was injected bilaterally.

It should be mentioned here that the rabbits were not always used in both the adaptation and the non-adaptation experiment; sometimes the non-adaptation experiments were done on a separate group of rabbits. This will be specified in the respective paragraphs.

I. EFFECTS OF INJECTION OF AN α_1-ADRENOCEPTOR AGONIST AND ANTAGONIST ON VOR ADAPTATION

Six rabbits were prepared with eye coils and guiding cannulas as explained in Chapter 2. All of them went through both the adaptation as well as the non-adaptation experiments. The procedures for the experiments were standardized as described in the method section of this Chapter.

Injected solutions

Phenylephrine HCl (Sigma, USA) 16 μg/μl was used as an α_1-agonist, prazosin (Pfizer, Belgium) 4 μg/μl as the α_1-antagonist. Both substances were dissolved in saline and adjusted at a neutral pH. In each experiment, 1.0 μl of the solution was injected bilaterally into the flocculi, using a stainless steel injection cannula which was connected to a Hamilton 1.0 μl syringe.

RESULTS

Adaptation experiments

Before adaptation of the VOR was started, 4 baseline measurements of the VOR in light and in darkness were recorded. As an average, the gain of the VOR in light in these 6 rabbits was 0.80 ± 0.04 (S.D.), while in darkness the mean value was 0.62 ± 0.08 (S.D.). Adaptive gain increase was measured for 3 hrs. All rabbits adapted the gain of their compensatory eye movements upward to some extent in this condition. The *difference* (△G) *adapted gain* minus *baseline gain* was used as an index of adaptation. A positive number indicates an increase in gain, whereas a negative number indicates a decrease in gain during the adaptation period.

Table 5.1 shows a synopsis of the data on the adaptation experiments, as described in the following paragraph. Figure 5.1 gives the time course of the average increase in gain (△G) after injection of the α_1-agonist and the α_1-antagonist, compared to the control adaptation curve. The *zero* values represent the average initial values, obtained in the 4 baseline measurements of the VOR in the light and in darkness, before the adaptation was started.

Table 5.1. Average gain increase during the adaptation experiments (n=6)

Condition	Time (hrs)	△G VOR light (mean ± S.D.)	△G VOR dark (mean ± S.D.)
control	1.5	0.37 ± 0.09	0.15 ± 0.09
	3.0	0.39 ± 0.05	0.17 ± 0.08
phenylephrine (16 μg/μl)	1.5	0.36 ± 0.10	0.13 ± 0.11
	3.0	0.36 ± 0.07	0.16 ± 0.12
prazosin (4 μg/μl)	1.5	0.34 ± 0.07	0.12 ± 0.15
	3.0	0.33 ± 0.12	0.15 ± 0.14

The upper graph of Fig. 5.1 shows the effect of the injections on the VOR in light. In these 6 rabbits, the average ΔG after 1.5 hrs of adaptation was 0.37 ± 0.09 (S.D.) in the control experiment, 0.36 ± 0.10 (S.D.) in the adaptation after injection of phenylephrine, and 0.34 ± 0.07 (S.D.) in the prazosin experiments. These values do not differ significantly.

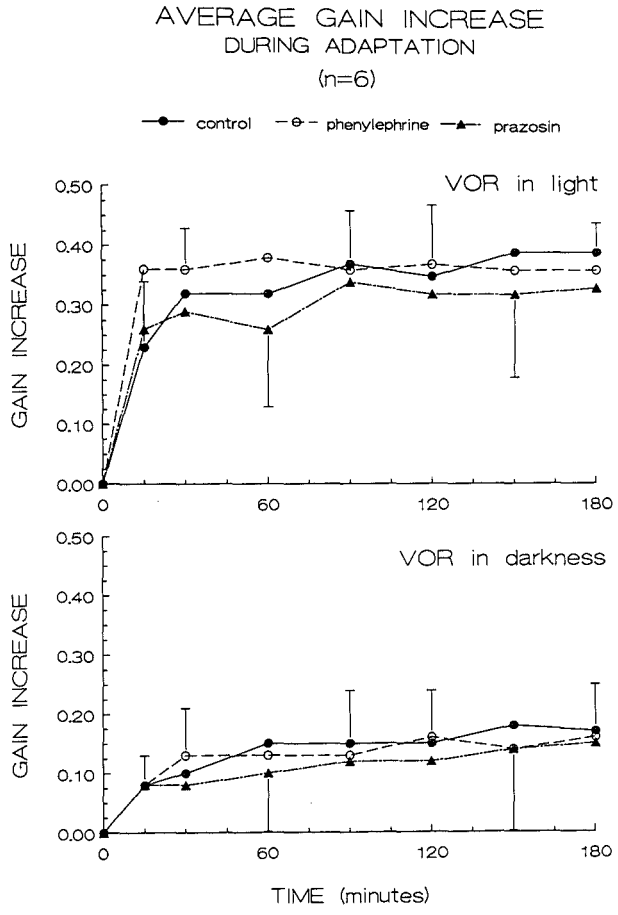

Fig. 5.1. Graphs of the mean time course of the increase in gain during the adaptation experiments, comparing injections of phenylephrine and prazosin to the control adaptation experiments. The upper graph represents the effects in the light, the lower graph in darkness. Mean values of 6 rabbits, with bars representing 1 S.D.. The zero values represent the initial values, obtained in 4 baseline measurements which were taken in the first interval of 15 minutes.

The upper part of Fig. 5.1 suggests that the injections do seem to affect adaptation during the first hour. However, statistical testing of these data in a Multiple Analysis of Variance (MANOVA) has shown that they are not significantly different from the control values. Moreover, if the injections really affected VOR adaptation, this should especially be reflected in the gain increase of the VOR in darkness. In order to conclude that an adaptive change has taken place, this change should also be manifest in a situation without the stimulus that induced it. This implies that a change in adaptability should be found in the VOR in darkness, without the presence of the visual information that induced the change. This was not the case in these experiments. The mean ΔG after 1.5 hrs of adaptation was 0.15 ± 0.09 (S.D.) in the control experiment, 0.13 ± 0.11 (S.D.) after phenylephrine injections, and 0.12 ± 0.15 (S.D.) after injection of prazosin. These data do not show a significant difference between the control experiments and the injection experiments in darkness, therefore we conclude that the α_1-agonist and antagonist did not affect VOR adaptation.

Non-adaptation experiments

In the same 6 rabbits, the time course of the VOR in light and in darkness, as well as the OKR was tested without pressure for adaptation. Stimuli with the same frequency and amplitude as in the adaptation experiments were employed. Four baseline measurements were taken in the first interval of 15 minutes, as in the adaptation experiments.

Table 5.2. Average change in gain during the non-adaptation experiments (n=6)

Condition	Time (hrs)	ΔG VOR light (mean ± S.D.)	ΔG VOR dark (mean ± S.D.)	ΔG OKR (mean ± S.D.)
control	1.5	-0.02 ± 0.02	-0.02 ± 0.06	0.04 ± 0.03
	2.5	-0.01 ± 0.03	-0.02 ± 0.04	0.04 ± 0.05
phenylephrine	1.5	0.02 ± 0.03	0.06 ± 0.04	0.02 ± 0.09
(16 μg/μl)	2.5	0.01 ± 0.01	0.07 ± 0.06	0.03 ± 0.06
idazoxan	1.5	0.01 ± 0.04	0.06 ± 0.08	0.03 ± 0.05
(4 μg/μl)	2.5	0.02 ± 0.02	0.03 ± 0.08	0.02 ± 0.05

The average value of these 4 baseline measurements is represented as *zero* in Fig. 5.2. The average baseline gain of the VOR in light was 0.79 ± 0.05 (S.D.), the mean gain of the VOR in darkness was 0.57 ± 0.06 (S.D.), and the mean OKR gain was 0.57 ± 0.22 (S.D.).

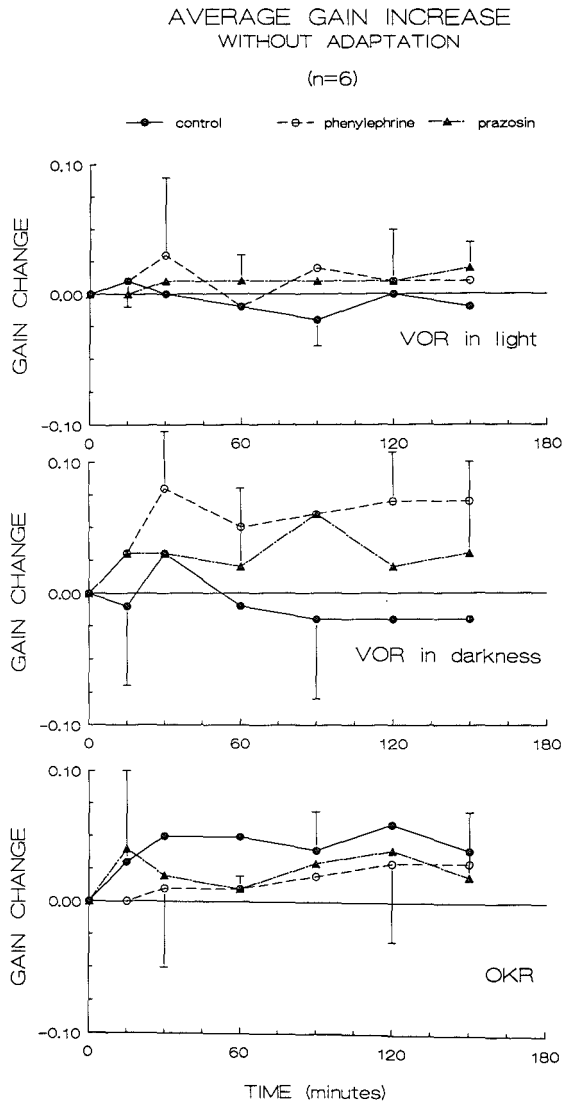

Fig. 5.2. Graphs of the mean time course of the changes in gain in the non-adaptation experiments, comparing injections of phenylephrine and prazosin to the control non-adaptation experiments. The upper graph represents the effects on the VOR in light, the middle graph shows the VOR in darkness, and the lower graph represents the OKR. Mean values of 6 rabbits; bars represent 1 S.D..

The results obtained in the non-adaptation experiments are summarized in Table 5.2 and Fig. 5.2. The representation of the data in the figure is comparable to that used in Fig. 5.1. The time course of the injections was followed for 2.5 hrs. During this period, the VOR in light did hardly show changes in gain, either in the control experiments, or in the phenylephrine or prazosin injected cases. The gain of the VOR in darkness was somewhat less constant. The average gain change in these 6 rabbits changed upwards by +0.08 in the phenylephrine injected experiments, but this increase did not differ significantly from the control experiment. The results obtained after injection of prazosin differed even less from the control. The OKR gain was very constant during the 2.5 hrs testing period and it was comparable in the experiments after α_1-noradrenergic injections and the control experiment.

Summarizing these results, we can conclude that the adaptive stimulus resulted in a good enhancement of the gain of the VOR in light and in darkness. However, injection of an α_1-noradrenergic agonist or blocker did not affect this gain adjustment positively, or negatively.

II. EFFECTS OF INJECTION OF AN α_2-ADRENOCEPTOR AGONIST AND ANTAGONIST ON VOR ADAPTATION

For these experiments, nine rabbits were implanted with eye coils and bilateral cannulas. Six of them were used in non-adaptation experiments as well as in the adaptation experiments, while the other 3 were only tested in the adaptation experiments. Each rabbit was tested in a control adaptation, an adaptation after injection of an α_2-agonist and an adaptation after injection of the α_2-antagonist in random order, as was described before. The non-adaptation experiments were similarly randomized.

Injected solutions

We injected the α_2-agonist clonidine (Sigma, USA) 4 μg/μl and the α_2-antagonist idazoxan, (Reckitt and Colman, England) 8 μg/μl. The substances were dissolved, neutralized and injected as described in the first paragraph. We used identical procedures for adaptation and non-adaptation experiments as described for the α_1 experiments, presented in the former paragraph.

RESULTS

Adaptation experiments

Four baseline measurements of the VOR in light and in darkness were recorded before adaptation of the VOR was started. The average gain of the VOR in light in these 9 rabbits was 0.82 ± 0.03 (S.D.) while in darkness the mean value was 0.64 ± 0.05 (S.D.). These baseline values are similar to those obtained in the α_1-noradrenergic experiments. Adaptive gain change was measured during 3 hrs. The increase in gain, relatively to the baseline value is indicated again as ΔG in Table 5.3. Figure 5.3 shows the effects of injection of clonidine and idazoxan compared to the control adaptation experiment in a similar way as Fig 5.1.

Table 5.3. Average gain increase during the adaptation experiments (n=9)

Condition	Time (hrs)	ΔG VOR light (mean ± S.D.)	ΔG VOR dark (mean ± S.D.)
control	1.5	0.34 ± 0.06	0.06 ± 0.12
	3.0	0.35 ± 0.07	0.11 ± 0.13
clonidine (4 μg/μl)	1.5	0.31 ± 0.12	0.06 ± 0.12
	3.0	0.32 ± 0.13	0.12 ± 0.12
idazoxan (8 μg/μl)	1.5	0.33 ± 0.06	0.04 ± 0.08
	3.0	0.37 ± 0.07	0.12 ± 0.08

The figure clearly shows that the adaptive capacity of the VOR gain remained unaffected by the α_2-adrenergic injections, either in light or in darkness. The average ΔG of the VOR in light after 1.5 hrs of adaptation was 0.34 ± 0.06 (S.D.) in the control experiment, 0.31 ± 0.12 (S.D.) after clonidine injection and 0.33 ± 0.06 (S.D.) after idazoxan injection. These values are even less variable than those obtained after the α_1-

injections. The same is true for the VOR in darkness: the mean ΔG after 1.5 hrs of adaptation was 0.06 ± 0.12 (S.D.) in the control experiment, 0.06 ± 0.12 (S.D.) after clonidine injections, and 0.04 ± 0.08 (S.D.) after injection of idazoxan. Again, the values obtained after the injections did not differ significantly from the values in the control experiments.

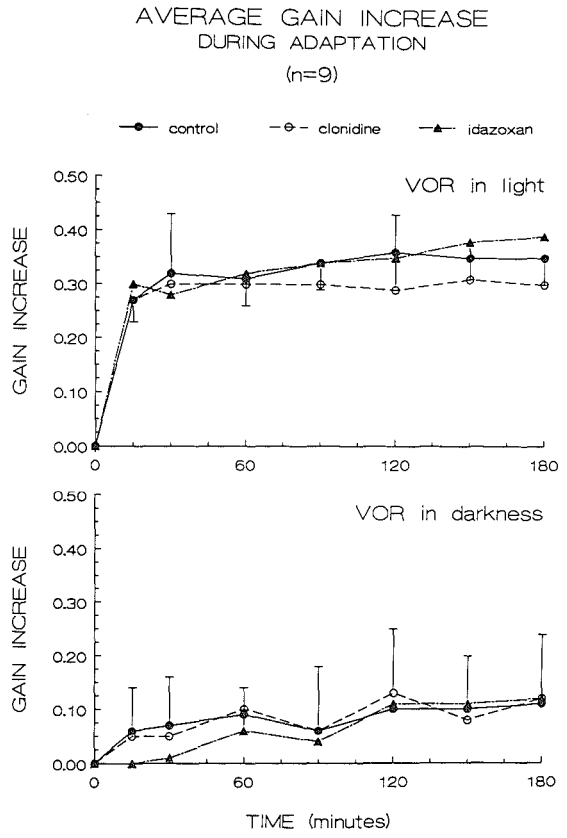

Fig. 5.3. Graphs of the mean time course of the increase in gain during the adaptation experiments, comparing injections of clonidine and idazoxan to the control adaptation experiments. The upper graph represents the effects in the light, the lower graph in darkness. Mean values of 9 rabbits, with bars representing 1 S.D.. The zero values represent the initial values, obtained in 4 baseline measurements which were taken in the first interval of 15 minutes.

Non-adaptation experiments

In 6 rabbits, the time course of the VOR in light and in darkness, as well as the OKR were tested without the pressure for adaptation, as was described in the former paragraph. The average baseline gain of the VOR in light was 0.81 ± 0.04 (S.D.), the mean gain of the VOR in darkness was 0.67 ± 0.06 (S.D.), and the mean OKR gain was 0.39 ± 0.12 (S.D.).

The average results obtained in the non-adaptation experiments of the 6 rabbits are summarized in Table 5.4 and Fig. 5.4. During the testing period, the VOR in light did not show significant variations in gain in the control experiments, nor in the clonidine or idazoxan injected cases. The variations in gain never exeeded 0.04. The average gain of the VOR in darkness always showed more variations, which was due to a rather large variability of this gain among individual rabbits, resulting in large standard deviations. In the control experiment, the gain fluctuated around the baseline value, with a maximum increase of 0.08. After injection of either clonidine or idazoxan, the gain decreased at most by 0.10 with respect to the baseline value. However, the standard deviations were considerably larger than in the control, and thus this decrease in gain was not significant (MANOVA). Moreover, it would not be expected that clonidine and idazoxan produced similar effects, because these substances are pharmacologically opposite in action.

Table 5.4. Average change in gain during the non-adaptation experiments (n=6)

Condition	Time (hrs)	ΔG VOR light (mean ± S.D.)	ΔG VOR dark (mean ± S.D.)	ΔG OKR (mean ± S.D.)
control	1.5	0.02 ± 0.03	0.00 ± 0.10	0.06 ± 0.10
	2.5	0.02 ± 0.02	0.00 ± 0.08	0.05 ± 0.08
clonidine	1.5	0.00 ± 0.01	-0.01 ± 0.06	0.10 ± 0.15
(4 μg/μl)	2.5	0.02 ± 0.03	-0.03 ± 0.06	0.13 ± 0.15
idazoxan	1.5	0.01 ± 0.09	-0.10 ± 0.11	0.07 ± 0.14
(8 μg/μl)	2.5	0.02 ± 0.08	-0.06 ± 0.07	0.09 ± 0.13

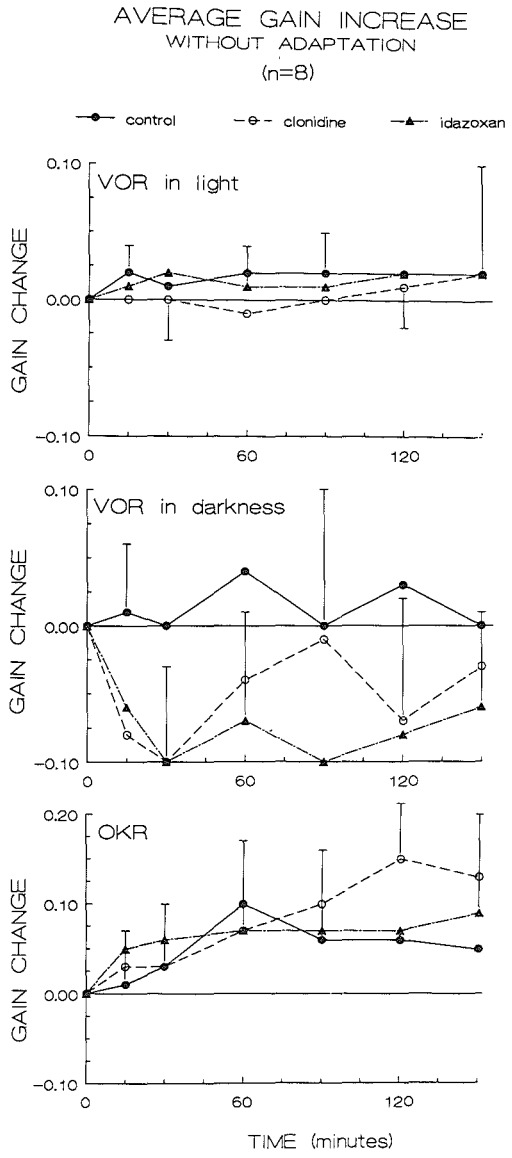

Fig. 5.4. Graphs of the mean time course of the changes in gain in the non-adaptation experiments, comparing injections of clonidine and idazoxan to the control non-adaptation experiments. The upper graph represents the effects on the VOR in light, the middle graph shows the VOR in darkness, and the lower graph represents the OKR. Mean values of 6 rabbits; bars represent 1 S.D..

The average gain of the OKR showed a slight increase over the period of 2.5 hrs in the control as well as in the injected experiments, as was often seen in non-adaptation experiments. Remarkably, injection of clonidine resulted in an increase in gain by a maximum of +0.15, whereas injection of idazoxan produced identical results as the non-injection experiments (about + 0.05). However, this increase in gain occurred in only 3 out of the 6 rabbits, and this effect of clonidine proved not to be statistically significant (student-T $p<0.15$).

The absence of effects of the clonidine injections in the flocculus on the basic characteristics of the VOR gain is of special interest in one aspect. During a series of pilot-experiments where we tested the effects of *intravenous* injection of all selected substances on the basic VOR, we *did* find an effect of clonidine. The gain of the VOR decreased to about 50% of the original value after a 2.0 ml injection at a concentration of 1.5 mg/ml. The gain of the OKR was not tested, so we do not know whether the increase in OKR gain we found in 3 of the 6 rabbits did also appear after systemic injection of clonidine. Although the effect on the VOR was impressive, it could not be reproduced after *floccular* injection of the same substance. Whatever may have caused the decrease in VOR gain after systemic injection, we know now that it has not been generated in the flocculus. As in the former paragraph, we can thus conclude that floccular injection of α_2-substances do not affect adaptation, nor the basic characteristics of the VOR gain.

III. EFFECTS OF INJECTION OF A β-ADRENOCEPTOR AGONIST AND ANTAGONIST ON VOR ADAPTATION

Sixteen rabbits were prepared with eye coils and permanently implanted cannulas. Ten of them were used in experiments where adaptive changes of the VOR gain were tested, and the other six served as controls in the non-adaptation experiments.
The procedures for both types of experiment have been described before.

Injected solutions

We used bilateral 1.0 μl injections of isoproterenol HCl, an aselective β-agonist (Sigma, USA) 16 μg/μl and sotalol, an aselective β-antagonist (Bristol Myers, Holland) 4 μg/μl. Both substances were dissolved in saline and adjusted to pH 7.0 - 7.4.

RESULTS

Adaptation experiments

The average baseline values of the VOR gain were similar to those obtained in all other experiments. The average gain of the VOR in the light was 0.86 ± 0.16 (S.D.), whereas in the dark the average value was 0.68 ± 0.21 (S.D.). Each individual rabbit maintained approximately its own typical baseline values on the successive testing days.

Table 5.5. Average gain increase during the adaptation experiments (n=10)

Condition	Time (hrs)	ΔG VOR light (mean ± S.D.)	ΔG VOR dark (mean ± S.D.)
control	1.5	0.26 ± 0.09	0.10 ± 0.04
	3.0	0.26 ± 0.11	0.14 ± 0.07
isoproterenol	1.5	0.26 ± 0.09	0.17 ± 0.08
	3.0	0.18 ± 0.14	0.14 ± 0.12
sotalol	1.5	0.17 ± 0.09	0.04 ± 0.04
	3.0	0.23 ± 0.12	0.08 ± 0.05

In the control adaptation experiments, the average ΔG after 1.5 hr was 0.26 ± 0.09 (S.D.) in the light and 0.10 ± 0.04 (S.D.) in darkness. The final gain increase after 3 hrs of adaptation was 0.26 ± 0.11 (S.D.) in the light and 0.14 ± 0.07 (S.D.) in darkness. These data are represented in Table 5.5 and Fig. 5.5. As these data indicate, most of the gain increase was acquired in the first 1.5 hr, after which a plateau value was

maintained. This was the case both in the light and in darkness, although the final ΔG in darkness was always lower than in the light (Fig. 5.5).

Administration of the β-agonist did not result in a very clear effect on the VOR measured in the light in most rabbits. Only 2 rabbits showed a distinct enhancement of their VOR adapation (+0.10), as demonstrated in Fig. 5.6 for an individual rabbit.

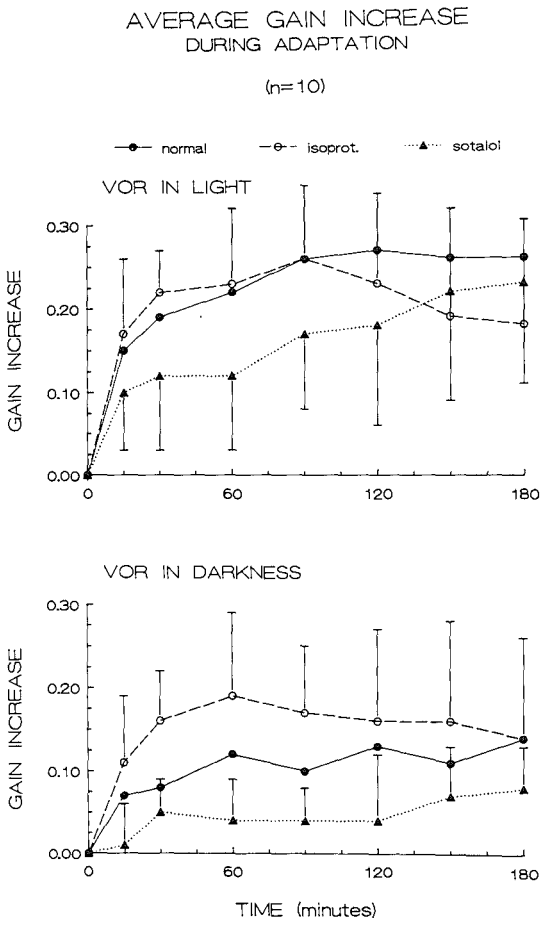

Fig. 5.5. Graphs of the mean time course of the changes in gain in the adaptation experiments, comparing injections of isoproterenol and sotalol to the control adaptation experiments. The upper graph represents the effects in the light, the lower graph in darkness. Mean values of 10 rabbits, with bars representing 1 S.D.. The zero values represent the initial values, obtained in 4 baseline measurements which were taken in the first interval of 15 minutes.

The other rabbits showed no or only marginal gain changes compared to the normal adaptation experiment. However, the values of the gain of the VOR measured in darkness were systematically higher after floccular injection of isoproterenol than during normal adaptation. This enhancement, clearly visible in Fig. 5.5, was present in 9 out of the 10 rabbits. Remarkably, in some rabbits the prominent gain increase in the first 1.5 hr was followed by a decrease of the gain in the last hour. Such an effect was found only once in a normal adaptation experiment.

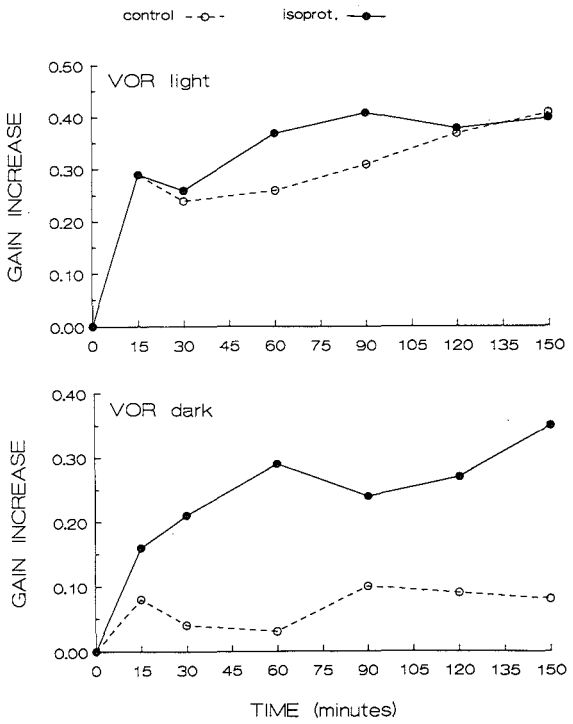

Fig. 5.6. Example of the increase in gain of the VOR in light and darkness after injection of isoproterenol, compared to that in the control experiment in one rabbit.

This effect, together with the absence of an enhancement of the VOR adaptation in the light, resulted in the absence of a significant overall-effect of isoproterenol in a multiple analysis of variance (MANOVA).

Injection of the β-blocker affected adaptation negatively in the light as well as in darkness. In either light and darkness, 7 of the 10 rabbits showed a clear decrement in adaptive gain change after sotalol injection. The remaining animals showed only a slight

or no reduction of adaptation. In no case was adaptation increased by sotalol. Figure 5.7 demonstrates the increase in gain of the VOR in light and darkness after injection of sotalol compared to that in the control adaptation in one rabbit.

Adaptation of the VOR in darkness was greatly affected by sotalol in those rabbits which adapted well, i.e., which had a large ΔG in the control adaptation experiment. On the other hand, rabbits with a marginal ΔG of the VOR in darkness in the control adaptation were less susceptible to the effects of sotalol (Fig. 5.8, dots). This correlation ($r = -0.73$) was only found in darkness for sotalol; for isoproterenol there was no correlation in darkness (Fig. 5.8, circles), or in light between the control level of adaptation and the effects of the drug.

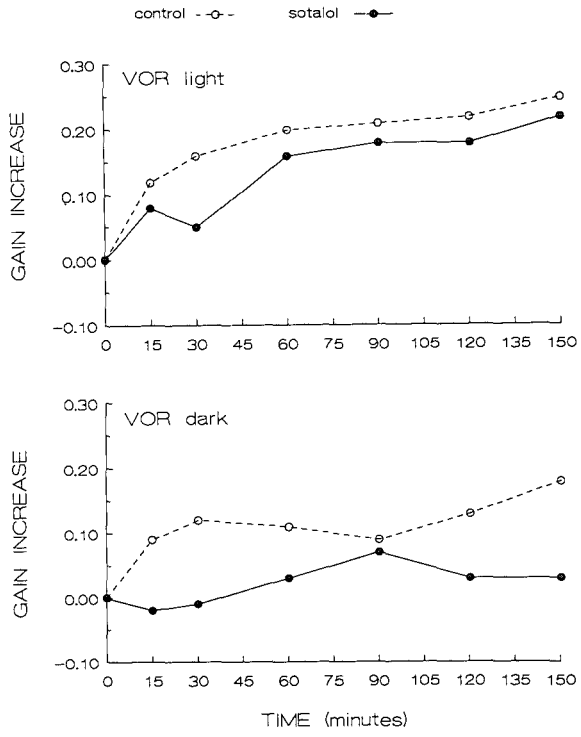

Fig. 5.7. Example of the increase in gain of the VOR in light and darkness after injection of sotalol, compared to that in the control experiment in one rabbit.

The overall effects of sotalol were statistically significant. The mean ΔG of the compensatory eye movements was lower than that obtained in the control adaptation experiments, in the light ($p < 0.009$, MANOVA) as well as in darkness ($p < 0.003$, MANOVA). Contrary to the normal adaptation, the rabbits showed little increase in gain during the first hour after the injection of sotalol, and a considerable part of the gain change was acquired in the last hour. At this time, the effect of the β-blocker may have been sufficiently reduced to permit the resumption of the adaptive process.

In conclusion, it can be said that after injection of sotalol, the adaptive change in gain was mostly smaller than and sometimes equal to the baseline values, in the light as well as in darkness; a relatively large part of any gain increase was delayed until the third hour of adaptation. Isoproterenol injections had hardly any effect in the light, although a decrease in gain occurred during the last hour, whereas in darkness a consistent enhancement of the gain change was found, especially in the first 1.5 hr.

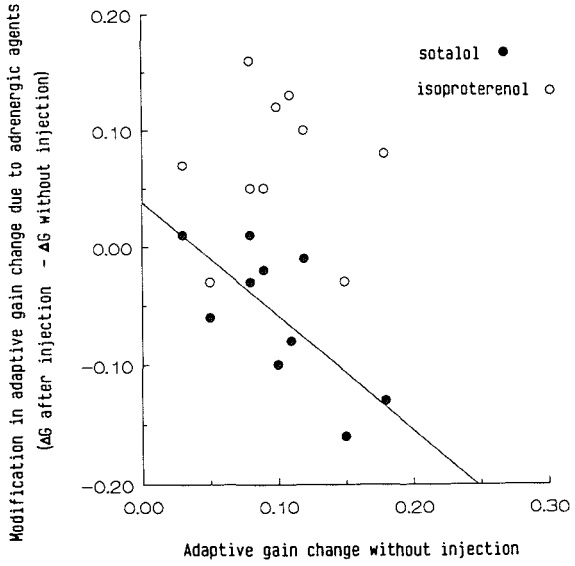

Fig. 5.8. Diagram to demonstrate the correlation between the magnitude of the adaptation of the VOR in darkness in control conditions (without injection), and the change in the magnitude of the adaptation due to the injected drugs. For sotalol, there was a correlation (see linear regression line, $r = -0.73$). For isoproterenol, no correlation between the level of the control adaptation and the modification caused by the drug was apparent.

Non-adaptation experiments

After the finding of a noradrenergic influence on adaptive changes in the VOR, it was especially important to make sure that these injections did really affect the adaptive process rather than the basic gain characteristics of the VOR. Therefore, the results of the non-adaptation experiments were needed before conclusions could be drawn.

In 6 rabbits, the OKR as well as the VOR in the light and in darkness were recorded every 15 minutes during 2.5 hrs. In this group of rabbits, the absolute baseline gain of the VOR was 0.92 ± 0.08 (S.D.)in the light, and 0.63 ± 0.14 (S.D.) in darkness. The baseline of OKR was 0.30 ± 0.08 (S.D.). The data gathered in these non-adaptation experiments are summarized in Table 5.6, and Fig. 5.9.

In the experiments without injection, there was hardly any change in the gain of either the VOR or the OKR during the testing period. At the end of the experimental period (after 2.5 hrs) the average ΔG of the VOR was 0.03 ± 0.05 (S.D.) in the light and 0.04 ± 0.15 (S.D.) in darkness, while the average ΔG of the OKR was 0.04 ± 0.04 (S.D.). None of the ΔG values measured over 2.5 hrs were significantly different from *zero* (MANOVA). It is, however, of interest that the average ΔG values at the end of 2.5 hrs were always positive, indicating an increase of the gain of the VOR and OKR compared to the baseline values. This suggests a very small adaptation of the initial sub-unity levels of the gain towards unity. This phenomenon was also seen in the non-adaptation experiments where α_1 or α_2 noradrenergic substances were injected.

Table 5.6. Average change in gain during the non-adaptation experiments (n=6)

Condition	Time (hrs)	ΔG VOR light (mean ± S.D.)	ΔG VOR dark (mean ± S.D.)	ΔG OKR (mean ± S.D.)
control	1.5	0.05 ± 0.06	0.04 ± 0.07	0.01 ± 0.05
	2.5	0.03 ± 0.05	0.04 ± 0.15	0.04 ± 0.04
isoproterenol	1.5	0.04 ± 0.10	0.09 ± 0.15	0.07 ± 0.08
	2.5	0.09 ± 0.10	0.07 ± 0.14	0.04 ± 0.05
sotalol	1.5	0.03 ± 0.05	0.00 ± 0.13	0.04 ± 0.06
	2.5	0.06 ± 0.07	-0.02 ± 0.05	0.02 ± 0.07

Similar experiments were done after injection of the β-agonist isoproterenol. Compared to the values in the non-injection experiments, the VOR in the light showed no difference in the average gain change; neither did the OKR. The VOR in the dark showed a slightly larger increase in gain compared to the experiments without injection, but this difference was not systematic and not statistically significant in the pooled data (MANOVA). Overall, the gain changes demonstrated in this group of isoproterenol experiments were almost the same as those in the non-injected group, and thus it can be concluded that the isoproterenol injections did not directly affect the VOR or the OKR gain characteristics.

The variations in gain were also monitored after injection of the β-antagonist sotalol. In this case, the change in gain of the VOR in the light was comparable to the gain increase in the experiments without injection. However, the VOR in darkness and the OKR showed a slightly lower change in gain. The ΔG of the VOR in darkness was 0.00 $\pm$ 0.13 (S.D.) after 1.5 hr, and after 2.5 hrs there was even a decrease (-0.02 $\pm$ 0.05, S.D.). The OKR gain change seemed to be slightly depressed only during the first hour. Although neither of these effects was statistically significant (MANOVA), the overall results suggest a slight depressive effect of sotalol. This effect was very small compared to that obtained in the adaptation experiments.

The results of this group of non-adaptation experiments show that injection of isoproterenol or sotalol did not affect the VOR in light or in darkness, nor the OKR. It can thus be concluded that the effects of the β-adrenergic substances on adaptation of the VOR gain are established by affecting the adaptive process itself, and not by modification of the basic VOR gain. As was demonstrated in the former two paragraphs, α_1 and α_2 agonists and antagonists had no influence on the adaptive proces.

Injections of the solvent

In two rabbits, a bilateral injection of the solvent (saline) was made, using an equal amount as administered during the adaptation experiments (1.0 μl bilaterally). These experiments were done to exclude a possible mechanical effect of the injected volume on the VOR gain. One rabbit was tested in an adaptation experiment, the other in a control experiment without adaptation. These injections caused neither a significant change in ΔG, compared to the normal adaptation experiment, nor changes in the gain

of the VOR and OKR as compared to the baseline values. It can thus be concluded that the effects observed during the adaptation experiments were due to the pharmacological properties of the injected substances.

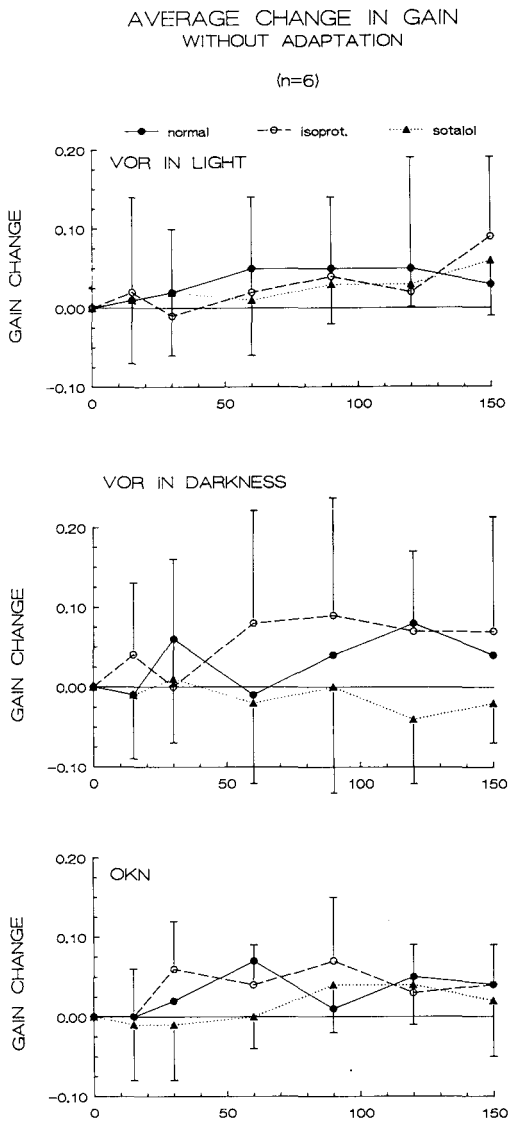

Fig. 5.9. Graphs of the mean time course of the changes in gain in the non-adaptation experiments, comparing injections of isoproterenol and sotalol to the control non-adaptation experiments. The upper graph represents the effects on the VOR in light, the middle graph shows the VOR in darkness, and the lower graph represents the OKR. Mean values of 6 rabbits; bars represent 1 S.D..

DISCUSSION

Main effects of α - and β-noradrenergic substances

The main result of this study is the demonstration that activation or inactivation of the β-noradrenergic system in the flocculus of rabbits affects adaptation of the VOR gain. The noradrenergic influence could only be demonstrated when using a β-noradrenergic agonist or antagonist, while injection of α_1 and α_2-agonist and blocker had no effect. Thus, it appears that the noradrenergic effects on eye movements are mediated through β-receptors. We can not specify for β_1 or β_2 subtypes because we used an aselective β-agonist and antagonist.

It appears from the results that it is mainly the adaptive process which is affected by the β-adrenergic substances. In the absence of pressure for adaptation, injection of the β-agonist isoproterenol or the β-antagonist sotalol caused on average only small changes in the basic gain of the VOR in darkness, which were not statistically significant. The gain of both the OKR and the VOR in the light remained almost unaffected in this condition. Moreover, injections of only the solvent had no effect.

On the other hand, injection of the β-adrenergic substances induced significant changes in the adaptive capacity of the VOR gain: isoproterenol enhanced adaptation of the VOR gain, while sotalol induced depression of this process. The finding that injections of α_1- or α_2-agonists or antagonist did not affect adaptation of the VOR gain does corroborate the robustness of the effects of the β-noradrenergic injections (see figs. 5.10 and 5.11).

The time course of the gain of the compensatory eye movements in the non-adaptation experiments shows a slight trend of the gains to increase during the 2.5 hrs, but this increase was not found in the first hour and can thus not be attributed to the pharmacological injections. It was probably due to a very small adaptation of the initial sub-unity levels of the gain towards unity, caused by the cumulative effects of the short, but repeated measurement periods in which some retinal slip was perceived.

The time course of the gain during the adaptation experiments was very different. The major part of the increase in gain occurred during the first 1.5 hr. In the experiments where we injected β-noradrenergic substances, the 3 curves representing the different pharmacological treatments approached each other (Fig. 5.5), which might be partly due to a decline of the pharmacological effects. However, too little is known about the pharmacokinetic properties of these substances when injected in the central

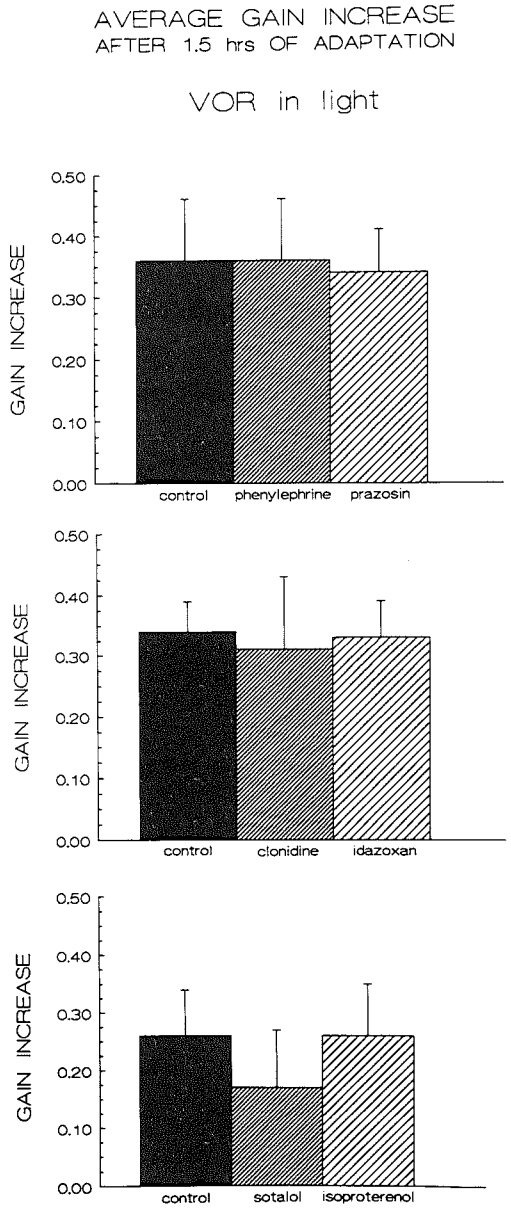

Fig. 5.10. These block diagrams demonstrate the effect of injection of the β-agonist and antagonist on the gain of the VOR in the light during adaptation (lower graph), compared to identical block diagrams of adaptation after injection of $\alpha 1$ (upper graph) or $\alpha 2$ (middle graph) substances. Only the effect of the β-injections were statistically significant (see text).

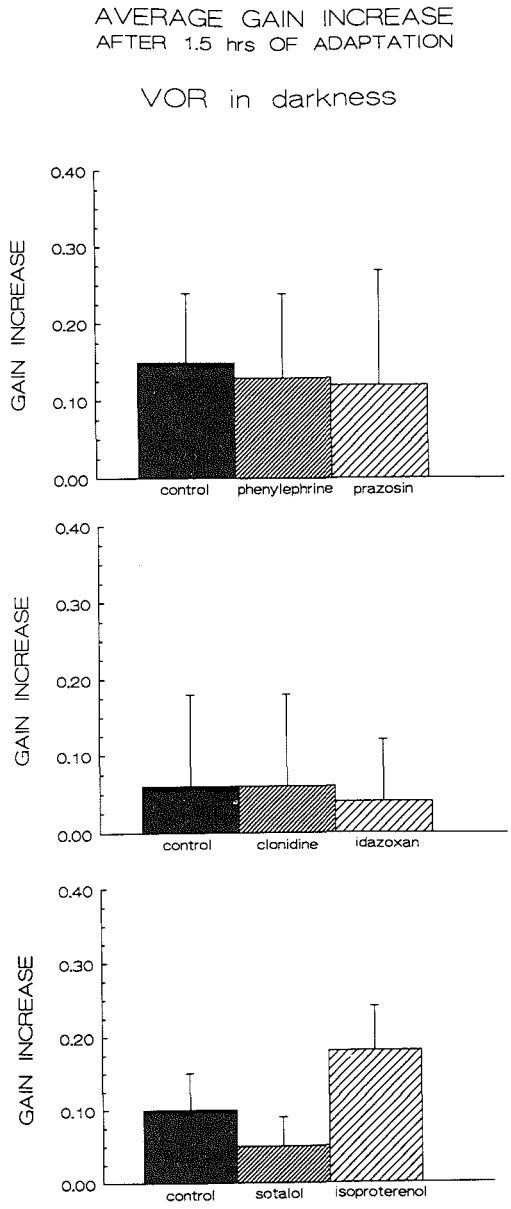

Fig. 5.11. Block diagrams to demonstrate the effect of injection of the β-agonist and antagonist on the gain of the VOR in darkness during adaptation (lower graph), compared to identical block diagrams of adaptation after injection of $\alpha 1$ (upper graph) or $\alpha 2$ (middle graph) substances. Only the effect of the β-injections were statistically significant (see text).

nervous system to ascertain during what time the substances were pharmacologically active. Moreover, the isoproterenol curve in the light (Fig. 5.5) shows even a decrease in gain in the third hour, which can hardly be explained by inactivation of the substance, since no distinct enhancement of the VOR adaptation was observed during the first 1.5 hrs. This effect could be attributed to *desensitization* of the β-receptors by isoproterenol, due to plastic changes of these receptors. This hypothesis is supported by experiments on the pineal gland, where it has been shown that injection of isoproterenol caused a rapid decrease in the number of specific binding sites for 1-[^{3}H] alprenolol, a competitive β-antagonist of high specificity. In these experiments, the maximal loss of specific binding sites occurred after 2 hrs (Kebabian et al., 1975). In a similar way, the adaptive increase in the VOR gain in the third hour after the sotalol injection could be attributed to *sensitization* of the β-receptors, due to an increase in the number of specific binding sites.

In the non-adaptation experiments related to the β-substances, only the VOR in darkness showed a trend to being slightly affected by the injections. In darkness, the vestibular signals are the only input for the generation of compensatory eye movements, which occurs under feed-forward conditions. Therefore, this condition is the most sensitive indicator of subtle changes in the parameters of oculomotor control. The gain produced in darkness is never fully compensatory for the head movements. During rotation of the animal in the light, additional visual input will provide feedback and will initiate compensation for the residual retinal slip. This feedback operation will tend to conceal the effects of the β-adrenergic substances on the basic VOR gain.

In the adaptation experiments, injection of the β-adrenergic substances affected both adaptation of the VOR in the light and in darkness, but the increase in gain in the light was always larger than the increase in gain in darkness. This difference could be due to a stronger expression of the adaptive changes during simultaneous visual and vestibular input, but it could also be caused by adaptation of the OKR gain. Although the gain of the OKR remained almost unaffected by the injections of a β-agonist or a β-antagonist during the non-adaptation experiments, it is known that the OKR undergoes adaptive changes under conditions of long-term retinal image slip (Collewijn and Grootendorst, 1979). We do not know whether an increase in OKR gain occurred during our adaptation experiments because we omitted measurements of OKR alone in the interest

of continuity in the adaptive process, but the earlier evidence makes such an increase very likely. Moreover, the adaptation of OKR gain could be under noradrenergic influence, just as the adaptation of the VOR gain is.

The increases in gain after 3 hrs of adaptation without injection are in agreement with those found by others, with regard to the absolute values, as well as to the time course (Ito et al., 1982; Nagao 1983). As to the effects of β-agonists and antagonists on adaptation of the VOR gain, our study harmonizes well with the earlier work by Keller and Smith (1983) and McElligot and Freedman (1988a). These authors showed that adaptive change in the VOR gain could be reduced by generalized depletion of NA in the cat. In a recent review, McElligot and Freedman (1988b) have reported that depletion of cerebellar NA by injection of 6-OHDA in the coeruleo-cerebellar pathways had the same effect as generalized depletion. Therefore, they concluded that the noradrenergic influence on VOR adaptation takes place in the cerebellum. However, they could not determine the exact localization of the effects, because the coeruleo-cerebellar afferents terminate in the whole cerebellar cortex and in all of the nuclei, as shown in anatomical (Dietrichs, 1988) and binding studies (M. Pompeiano et al., 1989). Furthermore, they did not exclude the possibility that the effects were due to changes in the basic VOR characteristics, rather than to changes in adaptive capacity. The present results deal with both these problems, and also provide evidence on the responsible receptor type.

On the other hand, the results contradict those described by Miyashita and Watanabe (1983). These authors could not demonstrate a decreased adaptability after depletion of noradrenaline, but they did demonstrate a serotonergic effect. The conclusion of their work was that the serotonergic instead of the noradrenergic system is involved in VOR adaptation. We did investigate their hypothesis as well, and the results of those experiments will be presented in the following chapter.

It could be supposed that the effects on VOR adaptation were caused by changes in the animal's state. However, we did not observe postural or motor changes, nor changes in the oculomotor activity after bilateral injection of either the α_1 and α_2-, or the β-adrenergic substances. Moreover, the animals appeared to be normally attentive, and were not noticeably drowsy or agitated after the injections.

We found quite some variability in adaptation of the VOR gain among the rabbits, and also in the increase or decrease of the adaptation after injection of isoproterenol or sotalol. For example, some rabbits showed a prominent increase in gain in the experiments without injections in the light as well as in darkness, whereas others displayed smaller increases. It may well be that the VOR gain change during adaptation depends partly on the resting discharge of the central noradrenergic neurons projecting to the cerebellum (Kimoto et al., 1978; Somana et al., 1978; Langer et al., 1985). This background discharge may increase to a various extent in different animals according to the different amounts of alertness (cf. Foote et al., 1983) or stress (Abercrombie and Jacobs, 1987) occurring in the restrained condition. It should also be mentioned in this respect that increases in turnover of NA and decreases in the concentration of NA occur following various types of stress (Minneman et al., 1981).

Localization of the effects

Because the floccular injection sites were electrophysiologically identified and histologically verified after the last experiment we can conclude that the effects found after injection of the β-adrenergic substances are elicited in the cerebellar flocculus. Spread of the injected substances to nearby areas was minimized by injection of 1 μl in 1 minute, because low infusion volumes (Myers, 1966; Silberman et al., 1960) and infusion rates (Edwards and Hendrickson, 1981) are known to be essential to ensure anatomical specificity (see also Greenshaw, 1985). Our histological material showed that a solution of pontamine sky blue 5% in 1 μl saline spread to a maximum diameter of 3 mm as estimated during the cutting of the frozen sections. The pattern of diffusion was always uniform and remained within the limits of the flocculus. Moreover,the eye movement responses were never asymmetrical and no spontaneous nystagmus was observed, indicating a homogeneous spread of the substance in both flocculi. As already mentioned, it is unknown over which distance and time the pharmaca remain active, and therefore it is impossible to say if injections in different parts of the flocculus would induce different effects.

The possibility remains that apart from the flocculus other brain regions that affect adaptive changes of the VOR gain may be under noradrenergic influence.

Receptor type

It was demonstrated that neither α_1, nor α_2 agonists or blockers affect VOR adaptation, while β-noradrenergic substances do. Therefore, it should follow that the described actions on VOR adaptation were mediated through β-receptors. Moreover, the effects elicited by isoproterenol and sotalol were opposite.

The existence of β-receptors in the cerebellum is supported by several lines of evidence: binding studies (Minneman et al., 1981; M. Pompeiano et al., 1989), histofluorescence and autoradiographic methods (Palacios and Kuhar, 1980; 1982; Rainbow et al., 1984 ; Sutin and Minneman, 1985; Lorton and Davis, 1987). In the rat, the majority of β-receptors in the cerebellar cortex is of the β_2 subtype (Minneman et al., 1979, 1981; M. Pompeiano et al., 1989). The β_2 subtype is prominently present in the molecular layer, where the density β_1-subtype has low levels (Rainbow et al., 1984). Both β_1 and β_2 receptors surround the Purkinje cell somata; however, there seems to be a threefold relative density of β_2 with respect to β_1 receptors (Sutin and Minneman, 1985). As isoproterenol and sotalol bind aselectively to both β_1- and β_2-receptors, more experiments are needed to find out which β-receptor subtype is responsible for the changes in adaptation of the VOR gain.

The general question is how the noradrenergic effect on adaptive change of the VOR gain is brought about. To understand this, we should know precisely how the mossy fiber and climbing fiber input interact on the Purkinje cells which control the activity of the vestibulo-oculomotor neurons, and how this interaction is influenced by NA. The hypotheses on this topic will be discussed in Chapter 7.

This Chapter concentrated on the noradrenergic influence on VOR adaptation. Whether or not this adaptive process is also affected by serotonergic afferents to the flocculus will be investigated in the following Chapter.

CHAPTER 6

MICRO-INJECTIONS OF SEROTONIN IN THE FLOCCULUS

INTRODUCTION

After finding an enhancing effect of ß-adrenoceptor agonists on VOR adaptation, we were left with one more important question: can VOR adaptation be affected by serotonergic injections as well? Suggestive evidence in this respect was presented by Miyashita and Watanabe (1984), who used intraventricular injection (5,7)-dihydroxy tryptamine (5,7-DHT) to deplete the brain stores of 5-hydroxytryptamine (5-HT; serotonin). This study on pigmented rabbits demonstrated that general depletion of serotonin abolished adaptive gain change in rabbits, while the dynamic characteristics of the VOR and OKR remained unchanged. Since (5,7)-DHT depletes both the serotonergic and the noradrenergic stores, Miyashita and Watanabe (1984) could not exclude the participation of the noradrenergic system, either exclusively or in addition to the serotonergic system, in regulation of adaptation of the VOR. Therefore, these authors repeated the experiments in rabbits that were depleted of noradrenaline only, using intra-cerebroventricular administration of 6-hydroxydopamine (6-OHDA). In these experiments, no changes in adaptability were found, and thus the authors concluded that it was the serotonergic rather than the noradrenergic system that affected adapation of the VOR. Their results on depletion of noradrenaline are, however, somewhat debatable, because they did not succeed in inducing complete depletion (only -61%, whereas McElligot and Freedman (1988a) achieved a depletion of -87% in cats). Despite this lack of proof that the effects were solely induced by depletion of serotonin, the results of Miyashita and Watanabe (1984) make it imperative to test both the serotonergic and the noradrenergic influence on VOR adaptation.

There is additional evidence that serotonin may affect the VOR. Ternaux and Gambarelli (1987) tested the effects of intraventricular injection of serotonin on the amplitude of the VOR in the rat. They demonstrated that such injection resulted in an increase of the amplitude of the VOR, an effect which could be blocked by intraventricular injection of methiothepine, a serotonergic antagonist which binds to both 5-HT1-like and to 5-HT_2 receptors (Saxena et al., 1986). The authors suggest that these effects on the VOR dynamics are exerted through a direct effect on the oculomotor neurons, by which the tone of the oculomotor muscles would be changed. A modulatory effect of serotonin on the activity of motoneurons of other muscles has been previously demonstrated (McCall and Aghajanian, 1979; White and Neuman, 1980) for spinal motoneurons. These experiments produced evidence that serotonin mediates overall facilitation of spinal motoneurons, and thus acts as a gain-setting system (for a review, see Holstege and Kuypers, 1987).

Serotonin has also been suggested to modulate central learning processes, either directly or indirectly via interactions with other neurotransmitters (Ögren, 1985; Hunter, 1988). For example, avoidance tasks seemed to be inhibited by administration of serotonin before training (Ögren, 1982; 1986). The use of several distinct methods to either deplete or increase central serotonergic stores, as well as the use of different learning tasks in different animal species might have led to the discrepancies which were observed in the various studies. Moreover, the employed techniques exert generalized effects, which can not exclude indirect serotonergic effects.

In general, the actions of the serotonergic system appear to be rather similar to those of the noradrenergic system. Both systems seem to act as level-setting mechanisms, and they seem to be involved in the modulation of learning mechanisms as well. Because a noradrenergic effect on VOR adaptation has already been demonstrated, these general concepts are a further reason to investigate whether the adaptive adjustment of the VOR is under serotonergic influence as well.

In an attempt to identify a region in the central nervous system that could be the site of action of the noradrenergic and serotonergic systems in influencing VOR adaptation, we have once more focussed on the cerebellar flocculus. As a parallel to the testing of the effects of noradrenergic floccular injections, experiments will be described in which the effects of serotonin injections on VOR adaptation were investigated.

METHODS

The experiments were performed in a similar way as those described in the Chapter 5. Eight Dutch belted rabbits were implanted with eye coils and bilateral guide cannulas (see Chapter 2). All of them were tested in adaptation as well as in non-adaptation experiments, the procedures of which were described in Chapter 5. As was explained in the introduction, I only tested serotonin (5-hydroxytryptamine creatine sulphate complex, 16 μg/μl, Sigma, USA), as an agonist in comparison to the control adaptation experiment.

RESULTS

Adaptation experiments

At the start of each experiment, baseline values of the VOR in light and in darkness were measured. In all rabbits used in these series, these values remained approximately constant during the successive experiments.

The mean baseline value of the gain of the VOR in 8 rabbits was 0.81 ± 0.04 (S.D.) in the light, and 0.64 ± 0.04 (S.D.) in the dark. The time course of the control (non-injection) adaptation experiment was compared to that after injection of serotonin. The average values of the increase in gain in the control experiment, compared to those after injection of serotonin are listed in Table 6.1, which shows these data after 1.5 and after 3 hrs of adaptation.

Table 6.1. Average gain increase during the adaptation experiments (n=8)

Condition	Time (hrs)	ΔG VOR light (mean ± S.D.)	ΔG VOR dark (mean ± S.D.)
control	1.5	0.35 ± 0.10	0.10 ± 0.12
	3.0	0.37 ± 0.09	0.12 ± 0.13
serotonin (16 μg/μl)	1.5	0.36 ± 0.10	0.16 ± 0.08
	3.0	0.38 ± 0.08	0.21 ± 0.06

Figure 6.1 shows the time course of adaptation with and without injection in light and in darkness, and Fig. 6.2 demonstrates the effects after 1.5 hrs of adaptation in a block diagram. The table and the figures give similar representations of the data as those which were presented in the Chapters on noradrenergic influence on VOR adaptation.

During the 3 hrs testing period, the gain of the VOR in light increased by about 0.37 ± 0.09 (S.D.) with respect to the baseline value in the control experiments.

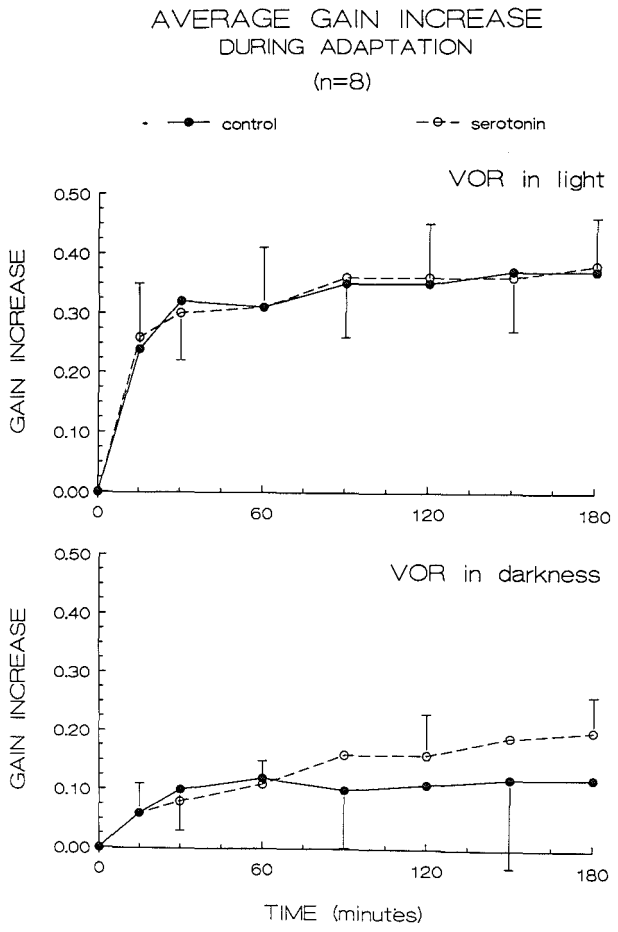

Fig. 6.1. Mean time course of the changes in gain in the adaptation experiments, comparing the effects of injection of serotonin to the control adaptation curve. The upper graph represents the effects in the light, the lower graph shows the effects in darkness. Mean values of 6 rabbits.

Floccular injection of serotonin did not affect this result at all: the increase in gain was 0.38 ± 0.08 (S.D.) after 3 hrs of adaptation. The major part of the increase in gain was achieved well within the first 15 minutes, as was the case in the former experiments where noradrenergic substances were injected.

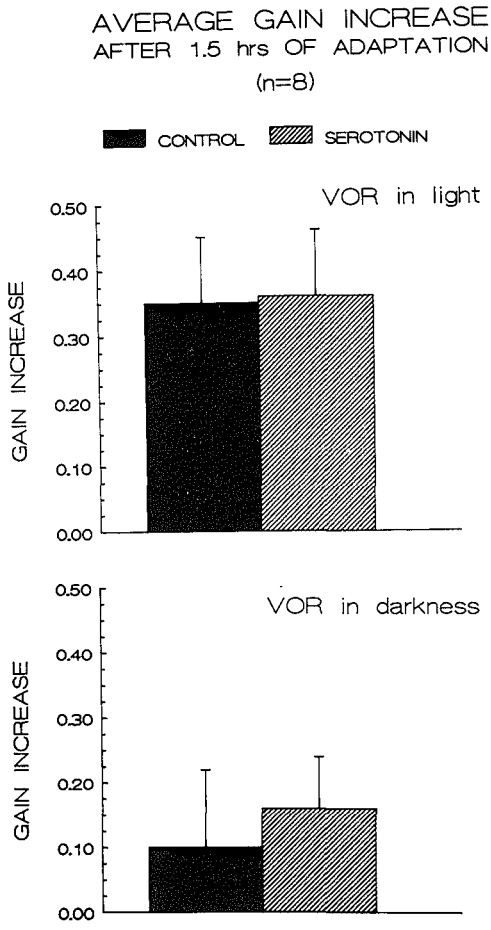

Fig. 6.2. Block diagrams, showing the increase in gain after 1.5 hrs of adaptation in light (upper graph) and darkness (lower graph), comparing the serotonergic injections to the control adaptation experiments. Means and standard deviations of 6 rabbits.

During the first 1.5 hrs of the control experiments, the average increase in gain in darkness was 0.10 ± 0.12 (S.D.). After injection of serotonin, the gain change was slightly higher (0.16 ± 0.08, S.D.). After completing the 3 hrs of adaptation, the average increase in gain was 0.12 ± 0.09 (S.D.) in the controls, whereas the serotonin-injected animals produced an average increase in gain of 0.21 ± 0.06 (S.D.). Although these data suggest an enhancing effect of 5-HT on adaptation of the VOR in darkness, the difference with respect to the control experiments was not statistically significant (t-test, $p < 0.15$ after 1.5 hrs of adaptation). The enhancement of adaptability was not consistently found in all of the 8 rabbits. In fact, 4 of them performed better after the serotonin injection, while the other 4 performed similarly or even worse than during the control experiment. Moreover, during the first hour of adaptation, the control curve and the curve of the experiment after injection of serotonin show great overlap, which indicates that serotonin did not affect VOR adaptation in the first hour of the experiment.

Non-adaptation experiments

The possibility of serotonergic effects on the basic dynamic characteristics of the VOR and OKR was also investigated in experiments without the use of an adaptive stimulus. In these experiments, done in the same 8 rabbits, the average baseline gain of the VOR was 0.82 ± 0.03 (S.D.) in the light, and 0.63 ± 0.06 (S.D.) in the dark, while the baseline gain of the OKR was 0.53 ± 0.20 (S.D.). These values were very similar to those obtained in the adaptation experiments.

Table 6.2. Average change in gain during the non-adaptation experiments (n=8)

Condition	Time (hrs)	ΔG VOR light (mean ± S.D.)	ΔG VOR dark (mean ± S.D.)	ΔG OKR (mean ± S.D.)
control	1.5	-0.02 ± 0.03	-0.02 ± 0.05	0.03 ± 0.05
	3.0	-0.02 ± 0.03	-0.01 ± 0.05	0.04 ± 0.05
serotonin	1.5	0.01 ± 0.03	-0.03 ± 0.08	0.02 ± 0.05
	3.0	0.02 ± 0.03	0.02 ± 0.08	0.01 ± 0.07

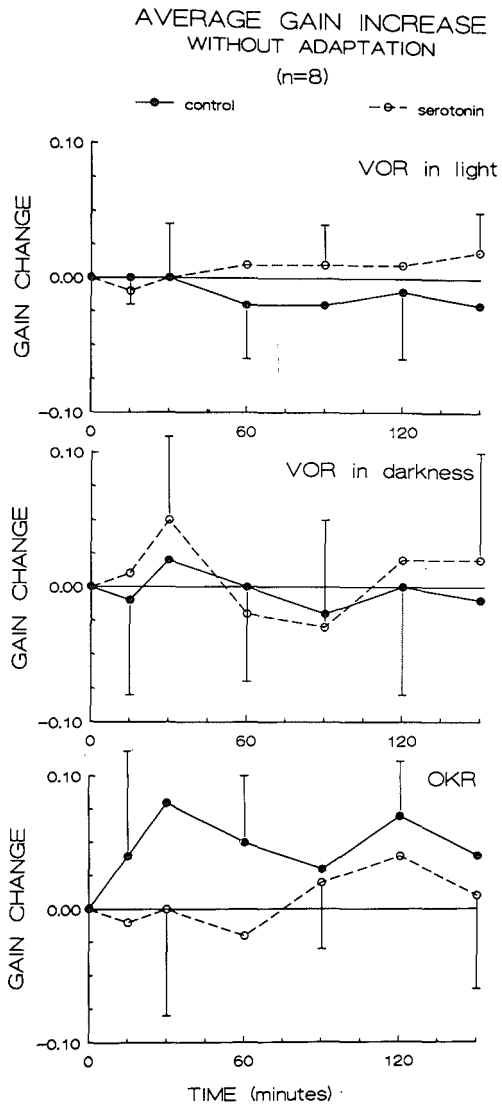

Fig. 6.3. Graphs of the average time course of the changes in gain in the non-adaptation experiments, comparing the effects of serotonergic injections to the control experiments. Upper graph represents the VOR in the light, the middle in darkness, and the lower shows the OKR. Means and standard deviations of 6 rabbits.

As can be seen from Table 6.2 and Fig. 6.3, which give comparable representations of the data as Fig 6.1, but at a much more sensitive scale, injections of serotonin did not change the gain of the VOR in light. The variability in gain did never exceed ± 0.02,

which was similar as in the control experiments. The gain of the VOR in darkness fluctuated a little more, and had larger standard deviations. This was also the case in previous non-adaptation experiments (Chapter 5). However, injection of serotonin did not change the gain of the VOR in darkness or its variability significantly. The OKR gain showed a slight increase during the 2.5 hrs testing period (+0.06), as was also the case in other non-adaptation experiments. Serotonin did not affect these fluctuations in the gain.

The serotonin injections did not affect the behaviour of the rabbits. Their attention level remained unchanged, as far as this could be could be judged from the gain of their eye movements and the constant frequency of saccades in the course of the experiment.

DISCUSSION

The results from these adaptation and non-adaptation experiments lead to the conclusion that micro-injection of serotonin in the cerebellar flocculus does not enhance or impair VOR dynamics, nor does it affect adaptation of the VOR gain.

Although general depletion of serotonin has been shown to affect adaptation in rabbits (Miyashita and Wanatabe, 1984), while intraventricular injection of serotonin affected VOR dynamics in rat (Ternaux and Gambarelli, 1987), no trends confirming these findings could be recorded after intra-floccular injection of serotonin. This does not disprove the above mentioned results; it does only indicate that those effects were probably not elicited in the flocculus, but at another site in the brain. However, definitive exclusion of the flocculus as a possible site where the effects might be generated will have to await the results of floccular injection of an appropriate serotonergic antagonist.

The lack of effect of serotonergic injections in the flocculus on the amplitude of the VOR, or on its adaptability could be due to a low concentration of the injected solution. From the results of Ternaux and Gambarelli this seems not to be the case, because they used a concentration of 10^{-5} M serotonin, which got even further diluted after intraventricular injection. Our solution was more concentrated, and was used for local injections. On the other hand, the dosis used in my experiments was not too high either, because the rabbits showed no behavioral changes after the serotonin injections.

The validity of our assumption that the presumed serotonergic influence on VOR adaptation might be exerted through the flocculus depended on two important facts. First of all, the flocculus should receive serotonergic afferents and secondly, the flocculus should contain serotonergic receptors.

The existence of mono-aminergic nerve terminals in the cerebellum, in addition to mossy and climbing fibers, was already demonstrated by Hökfelt and Fuxe in 1969. These authors reported that these fibers ran in the transverse plane of the folium, parallel to the surface. It has been suggested that the main serotonergic projection to the cerebellum would be derived from the raphe nuclei in the brain stem. These projections have been demonstrated by anatomical retrograde tracing techniques (Lavail et al., 1973; Shinnar et al., 1973; Batini et al., 1977; Frankfurter et al., 1977; as well as by anterograde techniques (Bobiller et al., 1976; Chan-Palay et al., 1977). However, it has recently been demonstrated that not all raphe neurons contain serotonin (Bishop and Ho, 1985), and therefore, these projections are not invariably serotonergic.

The physiological effects of serotonin on cerebellar neurons seem to be variable. Electrical stimulation of raphe neurons resulted in modulation of cerebellar neuronal activity (Strahlendorf et al., 1979, 1987). Both excitation and inhibition have been demonstrated on various cerebellar neurons after iontophoretical application of serotonin (Hoffer et al., 1969; Bloom et al., 1972). From several sources in the literature it has become clear that serotonin exerts a modulatory action on Purkinje cells, in a similar way as was described for noradrenaline (For a review, see Strahlendorf et al., 1987).

Bishop and Ho (1985) investigated the origin and distribution of serotonergic afferents to the cerebellum in the rat. They used a double labeling technique, which combined immuno-cytochemical identification of serotonin-containing fibers with retrograde staining of the neurons of origin. These authors concluded that the most important origins of serotonergic fibers are the n. reticularis gigantocellularis, the n. reticularis paragigantocellularis and the n. reticularis pontis oralis. Cerebellar projections from the raphe nuclei were only derived from the raphe magnus. The serotonergic fibers were diffusely distributed all over the cerebellar cortex, but with different laminar appearance in different lobules. In the flocculus, labeling was primarily found in the molecular layer, with few scattered fibers in the granular and Purkinje cell layers (Takeuchi et al., 1982; Bishop and Ho, 1985).

The distribution of serotonergic fibers in the cerebellum has also been studied in the cat (Takeuchi et al., 1982), and in the opossum (King et al, 1983; Bishop et al., 1985). In the cat, the fibers were distributed in a diffuse way, similarly as in the rat. However, the cerebellum of the cat received less fibers, which appeared to be predominantly present in the granular layer. The opossum showed a distribution pattern which differed from that in the rat and the cat. In this species, serotonergic fibers projected mainly to the caudal vermal lobes, while other parts of the cerebellum were left unlabeled. As in the rat, the fibers were predominantly present in the granular layer, but in this case only at the border with the Purkinje cell layer.

There are no detailed studies about the origin and distribution of serotonergic fibers in the rabbit's cerebellum. The studies on the rat, the cat and the opossum have shown a considerable species difference and, moreover, a difference in laminar distribution of serotonergic fibers. This indicates that the effects of serotonin may differ in the various cerebellar regions, and also in the various animal species.

As to the serotonergic receptor subtypes, no specific information is available about their occurence in the cerebellar flocculus. Until now, three main functional subtypes of serotonergic receptors have been classified, on the basis of their specific sensitivity to the various agonists and antagonists. The first category, the 5-HT_1-like receptors, is heterogeneous (Bradley et al., 1986), as four different binding sites (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D}) have been demonstrated (see Marsden et al., 1989; Peroutka, 1988). All 5-HT1-like receptors and their putative subtypes are highly sensitive to serotonin and some specific agonists such as methysergide and methiothepin, which also block 5-HT2 receptors (Bradley et al., 1986). The 5-HT_1-like receptors have been demonstrated in several parts of the central nervous system of various animal species. In the rat, 5-HT_1 receptors have been demonstrated in the cerebellar molecular and granular layer, albeit in very low concentrations (Pazos and Palacios, 1985).

The second subtype of serotonergic receptors, the 5-HT_2 receptors, are highly sensitive to the antagonistic actions of several drugs, including ketanserine and cyproheptadine (Bradley et al., 1986). The 5-HT_2 receptors have been demonstrated in the frontal cortex and the striatum in the rat, the cat, and in man (Leysen et al., 1983; Schotte et al., 1983; see also Leysen et al., 1984). They seem to be absent in the cerebellum of the rat (Pazos et al., 1985).

Thirdly, a subclass of serotonergic receptors has been classified which are called 5-HT_3 receptors. This receptor type is predominantly present in the peripheral nervous system, but it has also been demonstrated in the central nervous system of the rat (Kilpatrick et al., 1987; Peroutka and Hamik, 1988).

Before jumping to the conclusion that serotonergic injections in the flocculus do not affect VOR adaptation, the present experiments should be complemented by testing an appropriate antagonist. The fact that injection of serotonin did not change the adaptability of the VOR might be due to a high intrinsic level of serotonin, so that an additional dose would not result in detectable effects. In that case, injection of an antagonist should lead to an effect. The presumed serotonergic effect on VOR adaptation could theoretically be exerted through all three receptor-subtypes, because they have all been demonstrated in the central nervous system. However, specific data about the presence of the various subtypes in the cerebellum are only sparcely available, although 5-HT_1-like receptors have been demonstrated (Pazos and Palacios, 1985; Pazos et al., 1985). To complete these experiments, we could therefore test the effects of either methiotepine or methysergide, which bind mainly to the 5-HT_2 receptors, but also slightly to 5-HT_1 receptors.

The serotonergic effect on VOR amplitude and adaptation could also be exerted through several other brain regions which are related to oculomotor functions, and which are known to contain serotonergic receptors. Ternaux and Gambarelli (1987) already suggested that serotonin might affect oculomotor neurons. Indeed, the oculomotor nuclei receive serotonergic afferents and they do contain a high level of endogenous amines (Palkovits et al., 1974) as well as serotonergic receptors (Steinbusch, 1981; Soghomoniam et al., 1988). Nevertheless, this hypothesis is in contradiction with the results of Miyashita and Watanabe (1984), who found no change in VOR dynamics after generalized serotonin depletion, which, of course, also affects oculomotor neurons. Other possibilities are the olivary pretectal nucleus, the superficial grey layer of the colliculus superior and the lateral geniculate nucleus (Pazos and Palacios, 1985).

Moreover, it should be further investigated whether the results obtained in the experiments on general depletion or intraventricular injection are due to general effects of serotonin, resulting in changes in the animal's state (Hole et al, 1976).

Lastly, adaptation of the VOR could well be affected in the flocculus or in another brain site by interaction of the noradrenergic and serotonergic systems, and perhaps with other neurotransmitters. Such interactions have been demonstrated at the level of target neurons in the cortex (Ferron et al., 1982, Schwarts et al., 1984; Bröcher et al., 1989), and offer an interesting topic for further investigations.

The experiments described in this Chapter were designed to test the possible effect of floccular injections of serotonin on adaptation of the VOR. Together with identical experiments on the effects of noradrenergic substances on VOR adaptation, which were described in Chapter 5, we can now integrate these results with the preceding literature on this subject. This general integration will constitute the final Chapter of this thesis.

CHAPTER 7

GENERAL DISCUSSION

This thesis has focussed on several aspects of visuo-vestibular interaction in the rabbit. Direct as well as long- term interactions have been studied, and special attention has been paid to the role of the cerebellar flocculus and its noradrenergic and serotonergic inputs in these functions. This final chapter attempts to integrate the present results with the literature on previous investigations in this field. The current views about the function of the flocculus will be discussed, as well as some general topics which had their impact on all experiments.

GENERAL ASPECTS

Attention level of the animal

An important question in all animal behavioral studies is whether the animal remains alert, especially during long-lasting experiments. A number of alerting measures could be considered to prevent the diminishing of attention, for example injections of amphetamines or other alerting drugs. It is obvious that such methods can not be used in experiments where drug effects are investigated, because of the possibility of drug-interactions. The rabbits in the present experiments were not alerted in any way; neither by drugs, nor by generation of loud noises during the experiment, nor by somatosensory stimuli such as application of vibration to the abdomen, as was suggested by Magnusson (1986). The gradual enhancement of the gain in an adaptation experiment could be interrupted by a loud noise, which tended to enhance the gain temporarily, after which it would decrease again. For this reason, we did not apply alerting stimuli, because this

should have been done in exactly the same way, with reproducible results, in each experiment. Moreover, *changes in gain* were tested with respect to the baseline, so the absolute level of the gain had no great impact on the results.

Although no alerting stimuli were used, we found very high baseline gains, compared to identical experiments by others (see Baarsma and Collewijn, 1974, 1975; Ito et al., 1974a, 1982; Nagao, 1983, 1988, 1989a, 1989b). The baseline gain values remained at this high level throughout the experimental series which lasted about three weeks for each rabbit. Moreover, we demonstrated good enhancements of the gains during adaptation. The VOR gain was only adapted upwards, which is an advantage with respect to attentional problems. The possibility of a low attention level is much more dangerous when investigating adaptive suppression of the VOR gain, because loss of attention would, by itself, also tend to reduce the gain of the VOR.

Gaba-ergic, noradrenergic or serotonergic injections did never affect the animal's general behavior, except for a few rabbits which showed minor symptoms of a cerebellar syndrome after muscimol injections (see Chapter 3).

Spread of the injected substances

This problem has been shortly mentioned in the respective chapters. It is definitively true that no precise definition can be given of the spread and, more importantly, the action radius of the injected substances. We can only judge the spread from the dye-injections and their appearance in the histology. Although the dye remained always within the borders of the flocculus, it is possible that the pharmacological substances diffused further, because they did not contain such large particles as pontamine sky blue or ink. However, diffusion will cause dilution of the injected substance, proportional to the third power of the distance from the injection spot. Moreover, this diffusion takes time, during which the pharmacological activity subsides, due to metabolic elimination and removal in the circulation. Although the pharmacokinetics of the injected substances in brain tissue are unknown, it seems safe to consider only those effects which started within 15 minutes after the injection, assuming that at least those effect are elicited in the flocculus. The most plausible second target where visuo-vestibular processing could be affected would be the vestibular nuclei, the nearest of which (group Y) is probably about 2 mm away from the flocculus, while the other vestibular nuclei which have floccular connections are located at a distance of about 4 mm from the nearest floccular area.

Myers (1966) injected several dye-solutions with variable molecular weights in thalamic and hypothalamic regions in the central nervous system of the rat. Injections of 1.0 μl of the solution with the smallest particles resulted a spread of 2.2 mm from the injection spot, when measured 10-25 minutes after the injection. The dye solution with the largest particles diffused upto 1.6 mm from the injection spot. These data are comparable to our injections. It seems therefore not very likely that the substances could diffuse to the vestibular nuclei, at sufficiently high concentrations and in less than 15 minutes.

Damage of floccular tissue by the injections

Each rabbit was injected at the average four times during the course of the experiment. The histological material revealed no tissue damage or necrosis in the vast majority of the rabbits. A few animals, which were used in a pilot experiment where they received more than 10 injections, showed edema of the paramedian lobe and the flocculus. In these rabbits, the tissue lesions were correlated with decreased performance of the VOR and OKR compensatory eye movements. It seemed therefore that the tissue-state could be reliably estimated from the values of the baseline gains of VOR and OKR during the respective experiments. In general, these gains tended to remain constant. For example one rabbit, which was used in the dose-dependence experiment on muscimol and baclofen (Chapter 4), received 7 injections in the experimental series without showing any deterioration of the baseline values of the reflexes. No detectable lesions of floccular tissue were found in the histological material of this animal. Thus, traumatic side effects of the injection technique appear to be virtually absent.

SPECIFIC ASPECTS

Basic characteristics of the VOR and the OKR

As was explained in the introduction of Chapter 3, investigation of the function of the flocculus in generation of the VOR and OKR has revealed species differences. However, in the rabbit, such experiments have produced quite consistent findings. Total flocculectomy impaired the basic dynamics of the VOR and the OKR in albino rabbits (Ito et al., 1974b, 1982).

Identical experiments on pigmented rabbits could initially only confirm the effect on the gain of the OKR, while the gain of the VOR remained unchanged (Nagao, 1983), but in a more recent study both the VOR and the OKR were affected (Nagao 1989).

In the experiments which were described in Chapter 3 of this thesis, I injected the GABA-ergic agonists muscimol and baclofen, which caused a reversible inactivation of the floccular Purkinje cells. The gain of both the VOR and the OKR diminished at least by 50%, but a complete abolition was never encountered. This is comparable to the results of flocculus lesions by kainic acid (Nagao, 1989b), in which similar gain reductions were obtained: -35 to -58% for the OKR and -30% for the VOR. This means that the flocculus exerts a substantial positive influence on the basic dynamics of the vestibular and optokinetic reflexes However, the flocculus is not the only structure supporting the basic dynamics of the VOR and OKR; other brain stem areas do also contribute in this respect. This can be expected, because the floccular circuit is a side path to both the vestibular and the optokinetic reflex loop (see Fig. 1.1 in Chapter 1).

It should be noticed that the actual effects of the GABA-ergic injections on Purkinje cell firing activity have not been physiologically investigated, and therefore it is not certain whether the Purkinje cells are completely silenced by the injections. However, the fact that the amount of suppression of the VOR and OKR gain is comparable to the lesions obtained by kainic acid, which is known to be cytotoxic to Purkinje cells (Nagao, 1983, 1989b), makes this very likely.

These results indicate that the flocculus contributes positively to both the VOR and the OKR in the rabbit, in contrast to the suggestion by Robinson (1976), who demonstrated an increased gain of the VOR after flocculectomy in the cat, which he simply ascribed to the inhibitory output of the flocculus to the vestibular nuclei. On the contrary, the inhibitory output of the floccular Purkinje cells has been demonstrated to affect the vestibulo-ocular output in a much more complex fashion. Physiological studies, in which Purkinje cell reponses to vestibular stimulation were investigated, have demonstrated that floccular Purkinje cell simple spike activity is modulated during oscillation of the animal (Dufossé et al, 1978). Modulation is said to be *in-phase* when the maximum spike frequency occurs when the platform reaches its maximal velocity while rotating towards the recorded side. Correspondingly, the modulation is said to be *out-of-phase* when the maximum spike frequency occurs simultaneously with the maximal velocity of platform rotation to the contralateral side of the recording. Both in-phase and out-of-phase modulated Purkinje cells have been identified, but the vast majority of H-

zone floccular Purkinje cells (these induce horizontal eye movements, when electrically stimulated) are modulated out-of-phase with the stimulus velocity (Dufossé et al., 1978; Nagao, 1983; Nagao, 1989a, b).

Out-of-phase firing of Purkinje cells enhances the amplitude of the VOR. Primary vestibular afferents have been shown to be modulated in-phase with head velocity. These afferents form synapses in the vestibular nuclei, from where efferents project to the flocculus. The floccular output signal is conveyed to the so-called floccular target neurons (Lisberger, 1988), where secundary vestibular afferents arrive as well (see Fig. 7.1). The modulation of the vestibular afferent signal will thus be opposed by floccular output during in-phase modulation of the floccular Purkinje cells, resulting in a decrease of the VOR gain.

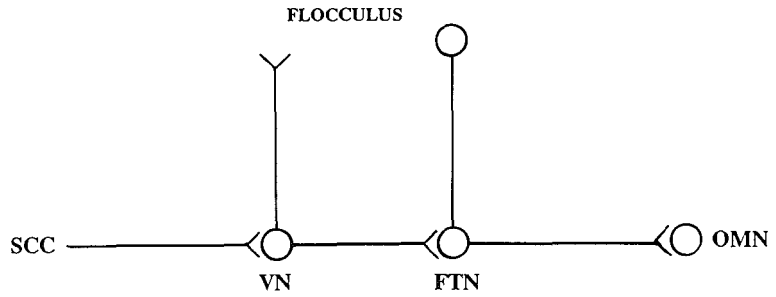

Fig. 7.1. Diagram of the vestibular input-output circuit and its floccular connections, according to Lisberger (1988). VN = vestibular neuron; FTN = floccular target neuron; OMN = oculomotor neurons; FL = flocculus.

On the other hand, out-of-phase modulation of Purkinje cell simple spike activity would result in disinhibition of vestibular neurons coincident with the arrival of the vestibular stimulus in the floccular target neurons, which results in an enhancement of the VOR gain.

Purkinje cell responses have also been investigated during pure optokinetic stimulation (Nagao, 1988). It was shown that simple spike modulation was enhanced by backward rotation of the drum with respect to the recorded side, which is called modulation in-phase with maximal drum velocity.

This situation is comparable to out-of-phase modulation of simple spikes with respect to the platform during vestibular stimulation, because the retinal slip, induced in either situation, is similarly directed (see Fig. 7.2). Simple spike modulation was independent of retinal slip velocity, in agreement with the studies by Miyashita and Nagao (1984).

Complex spike modulation was also demonstrated during optokinetic stimulation; this modulation was out-of-phase with the drum velocity, and thus reciprocal to the simple spike modulation during OKR. Complex spike modulation was correlated with retinal slip velocity; it increased at higher slip velocities. This has also been demonstrated by Simpson and Alley (1974) and Maekawa et al. (1988).

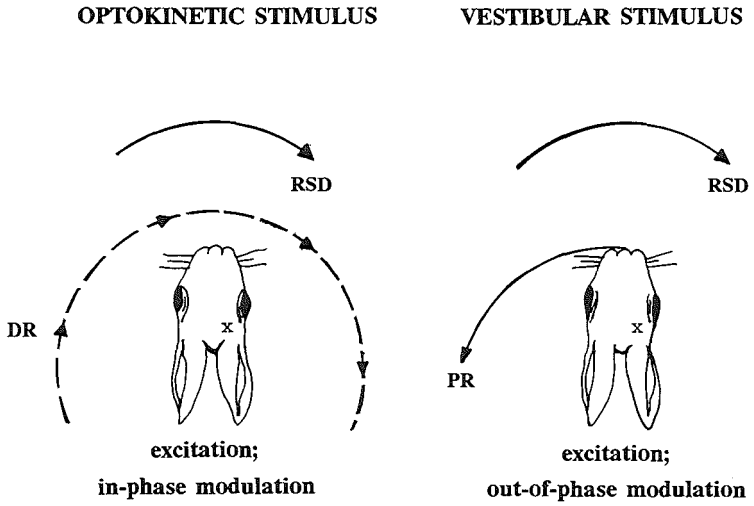

Fig. 7.2. Scematic drawing, explaining simple spike modulation of floccular Purkinje cells during optokinetic (left) and vestibular stimulation (right). Note that the Purkinje cells are excited by ipsilaterally directed retinal slip in both situations.

If these findings about Purkinje cell modulation during optokinetic stimulation are combined with the finding that electrical stimulation of Purkinje cells causes backward movement of the ipsilateral eye (Dufossé et al., 1977; Nagao, 1985) it can be concluded that the flocculus facilitates the OKR, just as it facilitates the VOR.

The electrophysiological findings are very well in line with the results obtained by flocculectomy-experiments as well as with the present results on GABA-injections. Therefore, the present results strongly support the idea that floccular Purkinje cells facilitate both the VOR and the OKR.

Direct visuo-vestibular interaction

In previous studies where flocculectomies were employed to investigate the effects on visuo-vestibular interactions, the results which were obtained in various animal species mostly showed impairment of the interaction. In the rabbit, impairment of visuo-vestibular interaction by flocculectomy was demonstrated by Ito et al., (1982), but some improvement of the VOR by vision remained possible.

In Chapter 4 of this thesis, it was demonstrated that the responses to unusual combinations of visual and vestibular inputs are synthetized by linear addition of the separate outputs. It seemed from those results that linear addition occurs independently of the magnitude of the separate components, and that the normal, synergic visuo-vestibular interaction is most likely also accomplished by linear interactions of the VOR and OKR components.

The effects of GABA-injections on visuo-vestibular interaction, described in Chapter 3, can be compared to similar experiments in flocculectomized animals. Injection of GABA-ergic agonists into the flocculus seemed not to interfere with interaction, although the two separate components were seriously impaired. The gain of the VOR in darkness was still somewhat improved by vision in all rabbits. Whether the combination of the visual and vestibular component was still accomplished by linear addition has not been formally tested, but a rough evaluation of the data suggests so. Two stimuli were used in these experiments, one of which (0.25 Hz) resulted in almost complete compensation by the VOR in darkness. In this case, the resulting retinal slip which should be used as an input to the optokinetic drum would be so small, that, especially at the very low gain of the OKR after GABA-injections, there would hardly be an OKR amplitude to add to the VOR in darkness. Indeed, the average gain of the VOR in light and in darkness are almost similar at this frequency (see Fig. 3.1 and Fig. 3.2). At the other tested frequency (0.10 Hz), the residual retinal slip after compensation by the VOR in darkness was somewhat larger, but the OKR, at its very low gain after GABA injections, would still not result in a large OKR component to add to the VOR in darkness. Indeed, the average gain of the VOR was 0.36 in the light and 0.26 in darkness at the 0.10 Hz stimulus, while the VOR gain was 0.36 in the light and 0.33 in darkness at 0.25 Hz. These data, obtained after baclofen injections, were largely similar after the muscimol injections.

The present results on GABA-ergic injections, as well as previous results from flocculectomized rabbits by others, indicate that direct visuo-vestibular interaction is not solely dependent on the flocculus, because floccular lesions did not completely impair improvement of the VOR by vision. Other brain stem areas are probably contributing to visuo-vestibular interaction, in particular the vestibular nuclei. It has been demonstrated by several authors that visuo-vestibular interaction still occurs in the vestibular nuclei after total cerebellectomy (Allum et al., 1978, Keller and Precht, 1978; 1979). Theoretically, the nucleus prepositus hypoglossi could also be a possible site of interaction outside the flocculus, because physiological studies have demonstrated that the prepositus hypoglossi neurons in the cat respond to vestibular (Baker and Berthoz, 1975; Lopez-Barneo et al., 1982), as well as to visual stimuli (Lopez-Barneo et al., 1982).

Adaptation of the VOR

The effects of flocculectomy on adaptation of the VOR agree very well across the various animal species, but the actual site where adaptation is accomplished remains uncertain. Miles and Lisberger (1981) proposed an undefined brain stem region to be the actual site where plastic synaptic changes, mediating adaptive changes in the VOR gain, would be established. In their opinion, the flocculus would only provide the necessary visual information, which would be projected to the responsible brain stem neurons. According to Ito and Nagao (see Ito, 1982 and 1984, Nagao, 1989a, b), the adaptive changes of both VOR and OKR are accomplished by synaptic changes within the cerebellar flocculus. In any case, the general opinion agrees to the idea that the flocculus is a necessary link in the accomplishment of adaptive changes in the VOR gain.

The way in which adaptation of the VOR is generated can be understood by considering the way in which the floccular output positively affects the VOR, as was explained in the paragraph on basic VOR and OKR characteristics. The evidence on the adaptive mechanism has mainly been collected in the rabbit (Ito et al., 1982; Nagao, 1983; Nagao,1989a, b), although additional work has been done in the monkey (Watanabe, 1984). These studies have led to "the flocculus-hypothesis" on VOR control (Ito, 1982, 1984). This hypothesis explains most results which were obtained in experiments on the function of the flocculus, and the main topics of this theory will therefore be reviewed.

As was explained earlier, out-of-phase simple spike modulation of floccular Purkinje cells enhances the VOR, while in-phase modulation depresses this reflex. The basic idea in Ito's flocculus hypothesis is that adaptive changes in VOR gain are accomplished by a shift towards more in-phase or more out-of-phase modulation of the Purkinje cells, depending on what type of adjustment is required in a particular situation. This assumption has been justified physiologically. Nagao (1983, 1989a,b) studied the Purkinje cell responses in the flocculus during sustained rotation of rabbits in various adaptative conditions. During normal, synergistic visuo-vestibular interaction, the simple spike modulation was mainly out-of-phase with respect to the stimulus, although some Purkinje cells fired in-phase with the stimulus. Enhancement of the VOR gain by simultaneous out-of-phase oscillation of the platform and the drum resulted in an increased out-of-phase modulation of the Purkinje cell simple spike activity. On the other hand, suppression of the VOR was reflected in an increased in-phase modulation of the simple spike activity of the floccular Purkinje cells.

Climbing fiber input, which represents information about retinal slip velocity, is also modulated during combined visuo-vestibular stimulation (Ghelarducci et al., 1975, Watanabe, 1984; Nagao, 1989a,b). It has been demonstrated that complex spike activity is modulated out-of-phase with the head velocity when the screen and the platform are oscillating in phase (i.e., during suppression of the VOR). On the other hand, complex spike activity is modulated in-phase with head velocity when the platform and drum are oscillating out-of-phase (i.e., enhancement of the VOR). Thus, complex spike modulation is exactly opposite to the simple spike modulation.

The shift to predominant in-phase or out-of-phase simple-spike modulation which takes place during adaptation of the VOR is, according to the hypothesis, accomplished by a modification of simple spike responsiveness of the floccular Purkinje cells to vestibular input, under influence of retinal slip information, conveyed by the climbing fibers.

Ito's flocculus hypothesis is very attractive, because it explains most of the findings on basic VOR and OKR dynamics, as well as on VOR adaptation. However, the evidence in favour of this theory is debatable on one important point. The flocculus hypothesis assumes that simple spike modulation is mainly related to head velocity, while others have demonstrated that simple spikes also carry eye velocity signals (Miles and Lisberger, 1981). According to these authors, it could be the case that the modulation of simple spike responsiveness was merely *reflecting* changes in eye velocity, rather than

that it would be the *cause* of a change in eye velocity. In recent papers by Nagao (1989a, 1989b) this possibility was discounted by a calculation of the change in the frequency of simple spikes, induced by a certain change in eye velocity. On the basis of this calculation, Nagao (1989b) concludes that simple spike modulation in the rabbit is largely related to head velocity. Nevertheless, it should be kept in mind that Ito's theory, although compatible with most experimental results on the VOR and its adaptability, is still hypothetical.

Mono-aminergic effects at Purkinje cell level

In Chapter 5 and 6, the results of experiments on the effects of mono-aminergic injections in the flocculus on adaptation of the VOR were presented. Although two neurotransmitter systems had been suggested to affect VOR adaptation, only one has been confirmed to act in the cerebellar flocculus: the noradrenergic system. It was demonstrated that this sytem acts on β-noradrenergic receptors, and not on α_1 or α_2 receptors. Injection of a β-agonist or antagonist did not affect VOR adaptation in an all-or-nothing way, but rather in a gradual, modulatory fashion. This is not surprising, because mono-aminergic transmitters (NA as well as 5-HT) have been shown to act as neuromodulators in several other neuronal systems, as was extensively discussed in the introductions of the respective chapters (Chapter 5 and 6). The next paragraph will concentrate on the presumed actions of these mono-aminergic transmitters at the Purkinje cell level.

There is abundant evidence on the action of NA on Purkinje cells. It has been shown that local application of NA, as well as LC stimulation, depresses the spontaneous discharge of cerebellar Purkinje cells. This effect could be mimicked by the injection of β-agonists, and was blocked by β-antagonists (for a review see Waterhouse et al., 1988). In contrast with these *in vivo* results, *in vitro* experiments on cerebellar slices (Basile and Dunwiddie, 1984) as well as *in oculo* (intra-ocular cerebellar grafts) preparations have revealed that NA can evoke excitation as well as inhibition of the spontaneous discharge of Purkinje cells (Granholm and Palmer, 1988). A consistent finding in the *in vivo* preparations was that local application of NA (Freedman et al., 1976, 1977), as well as LC stimulation (Moises et al., 1981), enhanced the responses of Purkinje cells to both excitatory and inhibitory inputs. It has been demonstrated that the enhanced Purkinje cell responsiveness to GABA after application of a β-agonist is accomplished via the

receptor-coupled cyclic-AMP-system (Sessler et al., 1989) Furthermore, reduction of the level of NA diminished the climbing fiber enhancement of mossy fiber signals to the Purkinje cells (McElligot et. al., 1986).

The hypothesis which emerged from this evidence was that the noradrenergic afferents to the cerebellar cortex, while depressing spontaneous firing activity of the Purkinje cells, actually enhanced the efficacy of excitatory and inhibitory inputs, and thus increased the signal-to-noise ratio of the evoked versus spontaneous activity. For this reason, NA is often implicated as a neuromodulator in the cerebellar cortex (Woodward et al., 1979; van Dongen, 1981, Waterhouse et al., 1988).

Integration of the hypotheses on the modulatory action of NA in the cerebellar cortex and on adaptation of the VOR gain may explain the effects on VOR adaptation obtained after β-noradrenergic injections. The β-agonist would increase the responsiveness of the Purkinje cells to the corresponding mossy and climbing fiber inputs, driven by the vestibular and visual information, thus increasing the signal-to-noise ratio of the evoked versus spontaneous activity. If the mossy fiber - climbing fiber interaction would undergo adaptive changes, the suggested action of noradrenaline would enhance this adaptive change, as has indeed been demonstrated in the present experiments. The results of noradrenergic agonists on VOR adaptation are therefore very well in line with the generally accepted ideas on actions of noradrenergic systems in the central nervous system.

The question remains why the serotonergic injections did not affect VOR adaptation, although previous evidence suggested similar actions as for the noradrenergic system. Besides general modulatory effects of serotonin on spinal motoneurons (see Holstege, 1986) specific actions of serotonin on Purkinje cells have also been investigated. Initially, Hoffer et al. (1969) demonstrated equal numbers of excitatory as inhibitory responses of Purkinje cell simple spike activity after iontophoretic application of serotonin. Electrical stimulation of raphe cells resulted predominantly in depressive effects on the spontaneous activity of Purkinje cells (Strahlendorf et al., 1979), a result which was largely reproduced by iontophoretic application of serotonin on Purkinje cells (Strahlendorf et al., 1984; Strahlendorf and Hubbard, 1983). These authors suggested that Purkinje cells would contain different serotonergic receptors, capable to induce

either excitatory or inhibitory responses. It was also demonstrated that cells firing at higher frequencies are more likely to be inhibited by serotonin, while slowly firing cells are mainly excited by serotonin. In this way, Purkinje cells would be set at a preferable firing rate by the serotonergic input.

Serotonin has also been demonstrated to affect complex spike activity, which was increased after iontophoretic application of 5-HT. Fast firing Purkinje cells with few complex spike discharges were very susceptible to serotonin application, which readily increased complex spike discharges (Strahlendorf et al., 1986).

Besides its action at the postsynaptic level, serotonin might also be active at the presynaptic level. Glutamate-induced excitation of Purkinje cells has been shown to be blocked by serotonin (Lee, 1984; Lee et al., 1985). This presynaptic action of serotonin is opposite to that of noradrenaline, which mainly enhanced the efficacy of other neurotransmitter actions at Purkinje cell synapses. Nevertheless, the present injections of serotonin did not affect VOR adaptability, although it should be kept in mind that antagonists were not tested.

It might as well be that the serotonergic system, when solely tested on adaptive VOR adjustments, does not affect this process, while effects on adaptation might be established by combined actions of the serotonergic system with other transmitters. In this respect, evidence may be mentioned which indicates modulations of the noradrenergic and serotonergic systems by cholinergic transmitters, which act through presynaptic receptors on the mono-aminergic nerve terminals (Imamura and Kasamatsu, 1989; Schwartz et al., 1984; Bröcher, 1989). This possibility of drug interaction should be a topic of further investigations in this field.

Synaptic changes during adaptation

The physiological mechanisms by which adaptive changes in the VOR and OKR gains are accomplished are largely revealed by now. The next step would be to study the synaptic events during these adaptations. Already in the nineteenseventies, Albus (1971) and Marr (1969) proposed a hypothesis in which the dual excitatory input to the cerebellum, the mossy and climbing fibers, was suggested to be responsible for "cerebellar learning" in motor systems. According to these hypotheses, motor learning should be effected by modification of synapses in the neuronal network. The initial learning of a certain movement was assumed to take place in the cerebral cortex, from where it would be send to the cerebellum to be stored. In this way, the cerebellum

would relieve the cerebral cortex from the task to retrieve the learned movement. Storage in the cerebellum was thought to be accomplished by facilitatory changes in parallel fiber synapses to Purkinje cells, due to the concomitant firing of climbing and parallel fibers. Albus (1971) supported this hypothesis, but he also suggested a second site for modification of synapses to occur, namely at the parallel fiber-basket and stellate cell synapses, which also receive climbing fiber input. Gilbert (1975) extended these ideas to the point that also the initial learning could take place in the cerebellum. He proposed an important role of noradrenaline in consolidation of memory functions. Simultaneous activation of climbing and parallel fibers would only modify synaptic strength if the noradrenergic input to the cerebellum was concomitantly increased.

Although these theories on cerebellar learning and Ito's flocculus hypothesis are very much in agreement, further investigations, specifically on the precise events at Purkinje cell level, are required. The present evidence on floccular involvement in VOR dynamics and VOR adaptation fits beautifully into the current views on floccular functioning and cerebellar learning in general.

SUMMARY

This thesis deals with two gaze-stabilizing oculomotor reflexes, the vestibulo-ocular reflex (VOR) and the optokinetic reflex (OKR), which serve to reduce retinal slip during movements of the head, or relative movements of the surroundings respectively.

The anatomical connections related to these two reflexes have been extensively studied. Both the VOR and the OKR pathways are mainly located in brain stem circuits, indicating their early phylogenetical origins. The VOR, which is activated by vestibular stimuli, is a feedforward system, and sends its signals to the oculomotor neurons after synapting in the vestibular nuclei. The OKR, on the other hand, is activated by retinal slip, and induces a compensatory eye movement by a feedback circuit which contains a number of brain stem areas including the vestibular nuclei (see Fig. 1.1). Both systems have been demonstrated to supply collaterals to a parallel cerebellar loop, which passes through the flocculus. These connections strongly suggest involvement of the cerebellar flocculus in the generation of the VOR and the OKR, and probably also in visuo-vestibular interaction and adaptive changes in the VOR gain.

On the basis of anatomical and physiological experiments, it has indeed been demonstrated that the flocculus is involved in three aspects of the VOR and OKR:

1) the flocculus affects the basic dynamic characteristics of the VOR and OKR;
2) direct visuo-vestibular interaction, i.e., the immediate improvement of the VOR by vision, is under floccular control;
3) long-term adaptive changes of the VOR, which can be induced by persistent unusual combinations of visual and vestibular inputs, require an intact flocculus.

This thesis presents new evidence on all of these three functions of the flocculus in VOR and OKR control. The following topics were investigated:

1) The involvement of the flocculus in basic VOR and OKR dynamics was tested by local injections of GABA-ergic agonists in the flocculus, which reversibly inactivate floccular Purkinje cells.
2) Concerning direct visuo-vestibular interaction, we concentrated on the nature of the combination of visual and vestibular components. It was investigated whether the combination of these signals was accomplished by linear addition, or rather in a much more complex fashion. Moreover, the effects of reversible Purkinje cell inactivation on direct visuo-vestibular interaction were tested.
3) The effects of mono-aminergic transmitters on long-term adaptive VOR changes, which are presumed to be accomplished in the flocculus, were studied. This study was based on investigations by others (for a review, see McElligot, 1988b), by whom the role of mono-aminergic substances on general learning mechanisms was revealed.

To investigate vestibulo-ocular and optokinetic reflexes, we assessed the amplitude of the compensatory eye movement in comparison to the amplitude of the stimulus which evoked the reflex. The VOR can be evoked by oscillating the animal horizontally on a platform, either in light or in darkness. The compensatory eye movements which result from this stimulation are opposite in direction to the stimulus. Complete compensation is achieved when the eye movement is 180 degrees out-of-phase with, but of equal amplitude as the platform oscillation. The OKR can be evoked by an oscillating optokinetic drum, which rotates around the stationary animal. The compensatory eye movements of the OKR are optimal when they are equally large as, and in-phase with the stimulus. Adaptation of the VOR can be evoked by simultaneous oscillation of the platform and the optokinetic drum in unusual combinations. The increase in retinal slip, which results from these unnatural stimuli, will eventually be reduced by a long-term adaptive change in the output of the VOR.

The amplitude of the compensatory eye movements was measured with the magnetic induction method (see Chapter 2 for a detailed description). The rabbits were permanently implanted with search coils, in which a voltage was induced by placing them in a rotating a.c. magnetic field. Changes in the induced voltage in the coil are a measure for the displacement of the eye.

Those rabbits, in which pharmacological injections were made, were permanently implanted with outer cannulas, which were used as a guide to insert inner injection cannulas into the flocculus. The outer cannulas were implanted at the guidance of electrophysiological identification of floccular Purkinje cells.

To investigate VOR and OKR dynamic characteristics, experiments were designed in which the VOR and OKR were tested for 2.5 hrs, recording the gain of the VOR and the OKR every 15 minutes, in between which the rabbit remained stationary. The results were presented in Chapter 3. In control situations, this resulted in constant values of the gain of the VOR and the OKR for 2.5 hrs. However, injections of the GABA-A agonist muscimol or the GABA-B agonist baclofen, which inhibit floccular Purkinje cells either directly or indirectly, induced a 50% depression of the gain of the VOR as well as the OKR. The specificity of these results was confirmed by a dose-dependence study of both substances and also by the results of a unilateral injection, which caused asymmetrical depression of the VOR gain. This evidence indicates a strong positive influence of the flocculus on VOR and OKR dynamics.

The nature of visuo-vestibular interaction was also investigated. In order to find out whether the combination of visual and vestibular inputs occurred by linear addition, we designed a special method by which the gain of the VOR in light could be predicted by addition of the VOR in darkness and the optokinetic response to an appropriately sized stimulus. This OKR stimulus was derived from the eye-in-head or gaze signal of the VOR in darkness, depending on the experimental conditions (For details, see Chapter 4). With this method, visuo-vestibular interaction was tested in two rather unusual situations. Firstly suppression of the VOR was tested, in which the platform and the optokinetic drum oscillated together in-phase and with the same amplitude. Secondly, enhancement of the VOR was tested, in which the platform and drum oscillated simultaneously out-of-phase, which results in an increase of the gain of the VOR. In both situations, the predicted and actually measured values of the gain of the VOR in light were statistically identical, indicating linear addition of the VOR and OKR components. The results of these two unusual combinations of visual and vestibular inputs make it very likely that the normal synergistic interaction, in which the platform moves in light with earth-fixed surroundings, is also accomplished by linear addition. This is supported by the outcome of a number of pilot experiments, in which the nature of synergistic interaction was investigated.

Lastly, adaptative changes in the gain of the VOR were studied. Mono-aminergic neuro-transmitters, in particular noradrenaline (NA) and serotonin (5-HT), have been shown to be involved in general modulatory functions, and also in learning mechanisms. Their impact on adaptation of the VOR has also been demonstrated by generalized depletion techniques. This evidence, together with the numerous demonstrations in the literature that flocculectomy abolishes VOR adaptation (for a review see Ito, 1982), led to the idea to investigate whether these presumed mono-aminergic effects on adaptation could be demonstrated in the flocculus. Several adrenoceptor agonists and antagonists, which were specific for the respective noradrenaline receptors, were injected into the flocculus: phenylephrine (α_1 agonist), clonidine (α_2 agonist) and isoproterenol (β-agonist); prazosin (α_1 antagonist), idazoxan (α_2 antagonist) and sotalol (β antagonist). Apart from testing adaptive changes in the gain of the VOR, the basic VOR dynamics were also tested, because a possible effect on VOR dynamics could be misinterpreted as an effect on adaptability of the VOR. The outcome of these experiments, presented in Chapter 5, showed that neither α_1, nor α_2 substances affected VOR adaptation or dynamics. On the other hand, the aselective β-substances did. Adaptation was enhanced by floccular injection of the β-agonist isoproterenol, while it was depressed by sotalol, the β-antagonist. These substances did not affect the basic dynamics of either the VOR or the OKR, so the demonstrated effects only involved the adaptability of the VOR.

The same set of experiments was done with serotonin, testing only the agonist (see Chapter 6). Serotonin did not affect the basic dynamics or the adaptability of the VOR gain, in contrast to the effects of general 5-HT depletion described in the literature (Miyashita and Watanabe, 1984). However, it should be taken into account that the lack of results in the present experiments may be due to high intrinsic levels of serotonin, which prevent further enhancement of adaptation after additional injection of 5-HT. Definitive conclusions should therefore await investigation of the effects of a suitable antagonist.

The last Chapter deals with a few general topics which had their impact on all of the experiments, such as the level of alertness of the animal, the spread of the injected substances and the possible lesions of the floccular tissue by the injections. Moreover, it was shown that the present results fit beautifully into Ito's hypothesis (1982; 1984) on the function of the flocculus, and also into earlier general theories on cerebellar learning (Marr, 1969; Albus, 1971; Gilbert, 1975).

In conclusion, evidence was presented which supports involvement of the cerebellar flocculus in several aspects of the vestibulo-ocular and optokinetic reflexes. Firstly, we demonstrated a positive influence of the flocculus on the basic characteristics of the VOR and OKR. These results underline the importance of the cerebellar parallel loop in the generation of these reflexes. Secondly, it was shown that visuo-vestibular interaction is accomplished by linear addition of the separate components. Apart from unusual situations like suppression or enhancement of the VOR, this is very likely also the case in normal, synergistic interaction. In addition, it was demonstrated that these interactions are only slightly effected by the flocculus, because the GABA-agonists never completely abolished visuo-vestibular interaction. Lastly, we confirmed previous evidence by lesion experiments that the flocculus affects VOR adaptation. We demonstrated an enhancing effect of a β-agonist on adaptive changes in the VOR gain, while a β-noradrenergic antagonist impaired adaptation. In contrast, α_1, α_2 or 5-HT substances had no effect in similar experiments. These results indicate a modulatory action of the noradrenergic system on adaptive changes in the VOR gain, which is exerted through β-receptors in the flocculus.

SAMENVATTING

Dit proefschrift beschrijft twee typen reflectoire oogbewegingen bij het konijn, de vestibulo-oculaire reflex (VOR) en de optokinetische reflex (OKR). Deze reflexen zorgen voor stabilisatie van beelden op het netvlies, waardoor perceptie steeds geoptimaliseerd wordt. De VOR is gevoelig voor bewegingen van het hoofd of het hele lichaam, terwijl de OKR geactiveerd wordt door relatieve bewegingen van de omgeving. In beide gevallen treedt er verschuiving van de netvliesbeelden op, wat ook wel *retinale slip* wordt genoemd. De resulterende oogbewegingen elimineren deze retinale slip en worden daarom compensatoire oogbewegingen genoemd.

Er is veel onderzoek gedaan naar de anatomische verbindingen die ten grondslag liggen aan deze twee reflexen. Het grootste deel van de desbetreffende verbindingen bevindt zich in de hersenstam. De VOR, welke wordt opgewekt door hoofdbewegingen, krijgt een vestibulair signaal als input. Het signaal komt binnen in de vestibulaire kernen, vanwaaruit projecties verlopen naar de oculomotorische motoneuronen die de oogspieren aansturen. De input van de OKR, gerelateerd aan retinale slip, wordt aangevoerd via de nervus opticus, welke verbindingen vormt met een groot aantal hersenstam-kernen. De uiteindelijke reactie komt ook tot stand door projecties naar de oogspier-motoneuronen. Beide reflexbanen hebben behalve hun directe input-output verbindingen ook een zijdelingse verbinding met het cerebellum, met name met de flocculus. Deze anatomische gemene deler van de VOR en de OKR impliceert een interactie tussen de beide reflexen, die inderdaad is aangetoond door middel van fysiologisch onderzoek.

In dit proefschrift wordt vooral ingegaan op de rol van de flocculus in diverse aspecten van de VOR, de OKR en hun interacties. Eerder onderzoek naar de functie van de flocculus toonde de volgende feiten aan:

1) de dynamische eigenschappen van de VOR en de OKR staan onder invloed van de flocculus;
2) directe visuo-vestibulaire interactie, welke optreedt bij de onmiddellijke verbetering van de VOR in het licht ten opzichte van de VOR in het donker, wordt tenminste gedeeltelijk beïnvloed door de flocculus;
3) plastische aanpassingen van de VOR, welke ontstaan ten gevolge van langdurige ongewone combinaties van visuele en vestibulaire signalen, zijn alleen mogelijk indien de flocculi intact zijn.

Het onderzoek beschreven in dit proefschrift, betrof alle hierboven genoemde facetten van de functie van de flocculus. De experimenten zijn in drie groepen te verdelen:
1) een groep experimenten, waarin de invloed van de flocculus op de dynamische karakteristieken van de VOR en de OKR werd bestudeerd na injectie van GABA-agonisten in de flocculus. Deze injecties veroorzaakten tijdelijke inactivatie van de Purkinje-cellen, welke in feite vergelijkbaar is met een reversibele flocculectomie;
2) een groep experimenten waarin een aspect van directe visuo-vestibulaire interactie werd onderzocht, namelijk de vraag of deze interactie tot stand komt door lineaire additie van de visuele en vestibulaire componenten, dan wel via een niet-lineair proces;
3) een laatste groep experimenten, waarin de functie van de flocculus in adaptatie van de VOR werd beïnvloed door injecties van noradrenerge en serotonerge farmaca. De aanleiding hiertoe lag in eerder onderzoek waarin de invloed van de mono-aminerge transmittersystemen op algemene leereffecten werd aangetoond (zie voor een overzicht van de desbetreffende literatuur McElligot, 1988b).

Om de vestibulo-oculaire en optokinetische reflexen te kwantificeren, werd de compensatoire oogbeweging geregistreerd. De amplitude van deze oogbeweging, als fractie van die van de stimulus, werd uitgedrukt als de *gain* van de reflex. De OKR werd opgewekt door oscillatie van een optokinetische trommel om het stilstaande konijn. Het konijn ondervond op deze manier alleen een visuele stimulus en genereerde een pure optokinetische respons waarvan de compensatoire oogbeweging dezelfde richting had als de trommel. Compensatie werd optimaal genoemd als de oogbewegingsamplitude even groot was als die van de stimulus. De VOR werd opgewekt door het dier op een

platform te plaatsen dat in het horizontale vlak oscilleerde in het licht of in het donker. In het laatste geval spreekt men van een pure vestibulo-oculaire reflex, omdat er dan slechts vestibulaire stimuli worden aangeboden. In geval van oscillatie in het licht, waarbij zowel visuele als vestibulaire input aanwezig is, treedt er interactie op van de VOR en de OKR. De richting van de compensatoire oogbeweging van de VOR was tegengesteld aan die van het platform en werd optimaal genoemd als de amplitude gelijk was aan die van het platform. In het donker werd echter nooit volledige compensatie bereikt. De amplitude van de compensatoire oogbeweging in het licht was altijd groter dan die van de VOR of van de OKR afzonderlijk.

Met dezelfde experimentele opstelling kon adaptatie van de VOR worden onderzocht. Adaptatie werd opgewekt door het platform en de optokinetische trommel tegelijkertijd in tegengestelde richting aan te drijven. In zo'n geval ontstond een ongebruikelijke combinatie van vestibulaire en visuele input, die leidde tot een veel grotere retinale slip dan gewoonlijk. De VOR paste zich onder deze omstandigheden geleidelijk aan; en wel zodanig, dat de amplitude van de compensatoire oogbewegingen toenam waardoor uiteindelijk de retinale slip gereduceerd werd. Om dit te bereiken was wel een langere periode van continue stimulatie noodzakelijk, in tegenstelling tot de situatie die beschreven werd onder directe visuo-vestibulaire interactie.

De amplitude van de compensatoire oogbeweging werd bepaald met behulp van de magnetische inductie methode. Bij alle konijnen werden sclerale inductiespoeltjes geïmplanteerd; de optokinetische trommel en het platform waren ook voorzien van dergelijke spoeltjes. De gehele experimentele opstelling bevond zich in een magnetisch draaiveld, waardoor in al deze spoeltjes een sinusiodaal verlopende inductiespanning werd opgewekt. De fase van deze spanning was gerelateerd aan de hoekpositie van het oog, de trommel en het platform. Deze methode, welke uitgebreider is beschreven in Hoofdstuk 2, wordt veel gebruikt in oogbewegingsexperimenten en is zeer accuraat.

De konijnen die gebruikt werden in experimenten waarin farmacologische injecties in de flocculus werden gemaakt werden alle uitgerust met permanent geïmplanteerde canules. Door de buitenste canule kon een injectiecanule tot in de flocculus naar beneden worden geschoven. De buitenste canule werd onder de juiste hoek geïmplanteerd op geleide van een electrofysiologische afleiding, waarmee Purkinje-cellen in de flocculus geïdentificeerd werden. Tijdens deze afleiding werd ook de juiste lengte

voor de injectiecanule bepaald, zodat deze exact in de flocculus terecht zou komen. Ook deze procedure is in Hoofdstuk 2 verder toegelicht.

Het eerste experiment, beschreven in Hoofdstuk 3, was ontworpen om de dynamische karakteristieken van de OKR en de VOR te testen. Iedere 15 minuten werden de optokinetische en vestibulaire stimuli afzonderlijk toegediend, waarna de compensatoire oogbeweging werd beoordeeld. Tussentijds stond het konijn stil in een verlichte omgeving. In controlesituaties, waarbij geen injecties werden gemaakt, bleef de gain van de VOR en van de OKR vrij constant gedurende het 2.5 uur durende experiment. Injectie van de GABA-A-agonist muscimol, zowel als van de GABA-B-agonist baclofen, leidde tot een daling van de gain van de OKR en de VOR tot ongeveer 50% van het controleniveau. Deze effecten waren specifiek voor de geïnjiceerde drugs, want ze waren dosis-afhankelijk en niet aantoonbaar wanneer alleen het oplosmiddel werd ingespoten. Bovendien leidde een enkelzijdige injectie van deze drugs tot een asymmetrische afname van de amplitude van de compensatoire oogbeweging. Deze resultaten tonen duidelijk aan dat de flocculus een positief effect heeft op de dynamica van de VOR en van de OKR.

Naast deze experimenten werd de aard van de directe interactie tussen VOR en OKR onderzocht tijdens oscillatie van het konijn in het licht; met andere woorden: tijdens directe visuo-vestibulaire interactie. Wij richtten ons in het bijzonder op de vraag, of de combinatie van visuele en vestibulaire signalen geschiedde door middel van lineaire optelling van de afzonderlijke componenten of door middel van een niet-lineair proces. Om dit te onderzoeken werd een speciale techniek ontworpen waarmee de gain van de VOR in het licht voorspeld kon worden uit de gain van de VOR in het donker, en de gain van de OKR, gemeten met een passende stimulus. Deze optokinetische stimulus was afgeleid van de oog-in-hoofd beweging of de blikbeweging tijdens de VOR in het donker, afhankelijk van het desbetreffende experiment (zie Hoofdstuk 4).

Visuo-vestibulaire interactie werd getest in twee situaties. In de eerste plaats gedurende onderdrukking van de VOR ten gevolge van gelijktijdige oscillatie van het platform en de optokinetische trommel met gelijke fase en amplitude. De gain van de VOR was in dit geval lager dan gewoonlijk. In de tweede geteste situatie werd de gain van de VOR opgedreven door het gelijktijdig oscilleren van het platform en de optokinetische trommel met tegengestelde fase. De toename van retinale slip leidde in dit geval tot een verhoging van de gain van de VOR. In beide situaties werd de

voorspelde waarde van de gain van de VOR in het licht vergeleken met de werkelijk gemeten waarde en in beide gevallen werd geen statistisch significant verschil gevonden. Dit resultaat impliceert dat visuo-vestibulaire interactie tot stand komt door lineaire sommering van de afzonderlijke componenten.

Indien wordt uitgegaan van het resultaat in deze onnatuurlijke testsituaties, is het zeer waarschijnlijk dat de gewone, synergistische interactie van de VOR en de OKR, zoals optreedt tijdens oscillatie van het dier in het licht met stilstaande omgeving, ook tot stand komt door lineaire additie. Deze veronderstelling werd bevestigd door de resultaten van enige pilot-experimenten, waarin de normale interactiesituatie werd bestudeerd.

Het laatste deel van dit proefschrift betreft experimenten aangaande de adaptieve capaciteit van de VOR. In de literatuur wordt melding gemaakt van het feit dat mono-aminerge neurotransmitters, met name noradrenaline (NA) en serotonine (5-HT), betrokken zijn bij modulatoire acties en algemene leerfuncties in het centraal zenuwstelsel. Gegeneraliseerde depletie van deze transmittersystemen leidde bovendien tot een verminderd adaptief vermogen van de VOR (zie voor een literatuuroverzicht McElligot et al., 1988b). De combinatie van deze gegevens met het feit dat flocculectomie adaptatie van de VOR onmogelijk maakt (zie voor literatuur Ito, 1982), leidde tot de vraagstelling of NA en 5-HT adaptatie kunnen beïnvloeden in de flocculus.

Om dit te onderzoeken, werden konijnen met geïmplanteerde canules gebruikt, waarbij diverse noradrenerge farmaca werden ingespoten tijdens opwaartse adaptatie van de VOR (zie Hoofdstuk 5). Voor deze experimenten werden de agonisten phenylephrine (α_1), clonidine (α_2) en isoproterenol (β) en de antagonisten prazosine (α_1), idazoxan (α_2) en sotalol (β) gebruikt. De invloed van deze drugs op het tijdsverloop van de adaptatie werd gedurende 3 uur vervolgd. Tevens werd het effect van dergelijke injecties op de basale karakteristieken van de VOR en de OKR getest, op vergelijkbare wijze als beschreven voor de GABA-experimenten in Hoofdstuk 3. Het zou immers mogelijk zijn dat een schijnbaar effect op VOR-adaptatie in werkelijkheid werd veroorzaakt door een verandering in de basale waarden van deze reflex.

De resultaten wezen uit dat de α_1- en α_2-agonisten en antagonisten het adaptatievermogen van de VOR niet beïnvloedden. Ook de basale waarden van deze reflex bleven onveranderd. In tegenstelling tot dit negatieve resultaat vonden we een fraai effect na injectie van de β-agonist en antagonist: adaptatie van de VOR werd bevorderd door de agonist en onderdrukt door de antagonist. Omdat de basale VOR-

karakteristieken onaangetast bleven, was hier duidelijk sprake van een beïnvloeding van het adaptatievermogen van de VOR.

Ter aanvulling van deze resultaten werd een identiek experiment verricht met serotonine injecties (zie Hoofdstuk 6). Hoewel voor gegeneraliseerde depletie van serotonine een effect is beschreven op adaptatie van de VOR (Miyashita and Watanabe, 1984), hadden serotonerge injecties in de flocculus noch effect op VOR adaptatie, noch op de basale waarden van de VOR. Het uitblijven van een verandering zou verklaard kunnen worden door hoge intrinsieke serotonine concentraties, maar een definitieve conclusie kan niet worden getrokken zolang het effect van injectie van een adequate serotonerge antagonist niet is getest.

In het laatste Hoofdstuk worden een aantal algemene factoren toegelicht die mogelijk effect zouden kunnen hebben op de beschreven resultaten. Hierbij valt onder meer te denken aan de alertheid van de dieren, de diffusie-afstand van de geïnjiceerde drugs en de mogelijke beschadiging van het weefsel van de flocculus door herhaaldelijke injecties. Verder geeft dit Hoofdstuk een integratie van de gepresenteerde resultaten met de eerdere literatuur, met name toegespitst op algemene hypothesen over de functie van de flocculus (Ito, 1982) en van het cerebellum (Marr, 1969; Albus, 1971; Gilbert, 1975).

Dit onderzoek heeft een aantal resultaten opgeleverd die de diverse functies van de cerebellaire flocculus in de regulatie van oogbewegingsreflexen verduidelijken. In de eerste plaats ondersteunen deze gegevens eerdere vondsten waaruit bleek dat de flocculus een positieve invloed heeft op de gain van de VOR en de OKR. Ondanks het feit dat de flocculus slechts zijdelingse verbindingen onderhoudt met de directe reflexbanen, hebben deze verbindingen een belangrijke functionele invloed. In de tweede plaats werd aangetoond dat visuo-vestibulaire interactie tot stand komt door lineaire sommering van afzonderlijke VOR- en OKR- componenten, zowel in normale als in onnatuurlijke situaties. De flocculus lijkt niet essentieel te zijn voor het lineaire karakter van de directe visuo-vestibulaire interacties, gelet op het feit dat de GABA-injecties wel de dynamica van de afzonderlijke reflexen, maar niet hun interactie aantastten. Tenslotte vonden we een duidelijk effect van een noradrenerge β-agonist en antagonist op VOR-adaptatie. Injecties van α_1- en α_2-noradrenerge farmaca en van 5-HT hadden daarentegen geen effect. Het adaptatievermogen van de VOR wordt dus beïnvloed door het noradrenerge systeem, via β-receptoren in de flocculus.

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