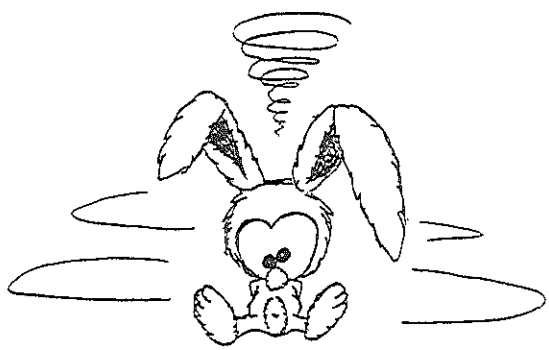
GAZE STABILIZATION IN THE RABBIT: THREE-DIMENSIONAL ORGANIZATION AND CHOLINERGIC FLOCCULAR CONTROL



Publicatie van dit proefschrift werd mede mogelijk gemaakt door financiële steun van Bayer Nederland B.V.

GAZE STABILIZATION IN THE RABBIT: THREE-DIMENSIONAL ORGANIZATION AND CHOLINERGIC FLOCCULAR CONTROL

(BLIK STABILISATIE IN HET KONIJN:
DRIE-DIMENSIONALE ORGANISATIE EN
CHOLINERGE FLOCCULAIRE REGELING)

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof. Dr. C.J. Rijnvos
en volgens besluit van het college van dekanen.
De openbare verdediging zal plaatsvinden op
woensdag 24 juni 1992 om 15.45 uur

door

HENDRA TAN

geboren te Jakarta (Indonesië)

universiteits
Ezafung
DRUKKERIJ
1992

Promotiecommissie:

Promotor:	Prof. Dr. H. Collewijn
Overige leden:	Prof. Dr. M.W. van Hof
	Prof. Dr. P.R. Saxena
	Prof. Dr. J. Voogd

Aan mijn ouders
Aan Marian

Table of contents

Publications on which this thesis is based:

Chapter 2: The three-dimensional organization of optokinetic responses in the rabbit
H.S. Tan, J. Van der Steen, J.I. Simpson and H. Collewijn
Submitted to the Journal of Neurophysiology

Chapter 3: Cholinergic modulation of optokinetic and vestibulo-ocular responses: a study with microinjections in the flocculus of the rabbit.
H.S. Tan and H. Collewijn
Experimental Brain Research (1991) 85: 475-481

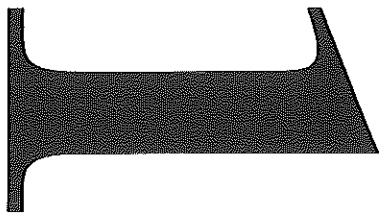
Chapter 4: Cholinergic and noradrenergic stimulation in the rabbit flocculus have synergistic and facilitatory effects on optokinetic responses
H.S. Tan and H. Collewijn
Submitted to Brain Research

Chapter 5: Muscarinic nature of cholinergic receptors in the cerebellar flocculus involved in the enhancement of the rabbit's optokinetic response
H.S. Tan and H. Collewijn
Submitted to Brain Research

Chapter 6: Optokinetic nystagmus in the rabbit and its modulation by bilateral microinjection of carbachol in the cerebellar flocculus
H.S. Tan, H. Collewijn and J. Van der Steen
Accepted for publication in Experimental Brain Research

Chapter 7: Unilateral cholinergic stimulation of the rabbit's cerebellar flocculus: asymmetric effects on optokinetic responses
H.S. Tan, H. Collewijn and J. Van der Steen
Submitted to Experimental Brain Research

Chapter 8: Shortening of per-rotatory nystagmus by micro-injection of carbachol in the rabbit's cerebellar flocculus
H.S. Tan, H. Collewijn and J. Van der Steen
Submitted to Experimental Brain Research



General introduction

Eye movements: man versus rabbit

To categorize the movements we make with our eyes, one can distinguish between movements that are reflexive and those that are voluntary in nature. The reflexive eye movements serve to stabilize the eye relative to the visual surroundings during head movements, a goal which is achieved by moving the eyes in an equivalent, but opposite manner as the head. As a result, the image of the surroundings on the retina remains relatively stable, as is necessary for clear vision during head movements.

Higher mammals, like humans, have developed a specialized area in the retina with a high photo-receptor density: the fovea. With the development of such a fovea, it became necessary to voluntarily control the direction of the line of sight, independently from head movements, to enable fixation of a visually interesting object on the fovea. For that purpose, the oculomotor system of "foveated" animals is equipped with a saccadic system, that serves to aim the fovea towards an object of visual interest, and a pursuit system, that allows the following of a moving object within a stationary visual environment.

Although the pursuit system would theoretically be capable also of gaze stabilization, voluntary movements have not entirely taken over this task. Instead, the brain has chosen to maintain the gaze-stabilizing system and to superpose upon it an array of voluntary movements. Thus, we can consider our eye movement behavior as the output of a control system with two hierarchical levels: a primitive stabilizing system and a higher-level, voluntary gaze-direction system. Indeed, abolition of the cortical, voluntary input to the oculomotor system in the monkey revealed eye movements that had characteristics of a gaze-stabilizing system (Zee et al., 1987).

The rabbit is among the animals that lack a fovea. Accordingly, its eye movements are fully dedicated to gaze stabilization. The oculomotor system of the rabbit, therefore, provides us with the opportunity to study gaze stabilization in a strictly isolated form.

Self-orientation and gaze stabilization

Appropriate, compensatory eye movements for the stabilization of the visual image on the retina can only be generated on the basis of exact information on head displacements. Orientation and displacement of the head could theoretically be monitored through a variety of senses in different modalities. Obviously, evolution has forced the brain to use every single one of them to provide the best possible accuracy in estimating self-orientation at any given moment (see Young, 1977; Collewijn, 1989a, 1989b).

The internal representation of self-motion and self-orientation relative to the surroundings is not only used to generate appropriate compensatory eye movements, but

is crucial for the proper execution of all motor behavior that operates to interact with those surroundings (see Igarashi and Black, 1985; Hwang et al., 1988; Roberts, 1978; Wilson and Melvill Jones, 1979; Peterson and Richmond, 1988). The processing of an internal representation of self-motion and the generation of gaze-stabilizing signals to the eye are, therefore, probably features of one and the same mechanism.

One of the phylogenetically oldest sources of information is the orientation of the gravitational vector relative to the head. The orientation of this vector is sensed by otolith organs, which are part of the vestibular labyrinth in our inner ear (see Kornhuber, 1974; Wilson and Melvill Jones, 1979; Graham and Kemink, 1987; Melvill Jones, 1991). All changes in head position are sensed, except for horizontal rotations of the head, because these movements occur in a plane that is perpendicular to the gravitational vector.

In addition, angular accelerations of the head in all three dimensions are sensed by the semi-circular canals (see Kornhuber, 1974; Wilson and Melvill Jones, 1979; Schwartz, 1986; Graham and Kemink, 1987; Melvill Jones, 1991). Due to the mechanical properties of the cupula, ampulla and hair cells, described as an "overdamped torsion pendulum" (Steinhausen, 1933), head acceleration is mechanically integrated so that the canals actually provide the brain with a head velocity signal.

A large part of the head movements during everyday life is self-induced. An obvious strategy in the estimation of self-orientation during such voluntary movements would be to use a corollary signal of the intended action. Such signals were actually encountered in tadpoles (Stehouwer, 1987), but their significance in the rabbit has not been established.

Furthermore, proprioception in the neck informs the gaze-stabilizing system about the motion and position of the head, relative to the trunk (Dichgans et al., 1973; Jürgens and Mergner, 1989).

Visual input plays a central role in gaze stabilization. This input is conveyed by image-movement detectors that are velocity-sensitive and directionally specific, so that sensory visual signals encode the velocity and direction of the image motion over the retina (Barlow et al., 1964; Oyster and Barlow, 1967; Oyster, 1968; Oyster et al., 1972). This signal is actually an error signal, providing the gaze stabilizing system with feedback about its performance.

All these different signals originate from different receptors and encode head motion or head-orientation in different modalities. They are transformed into a unitary reference frame before convergence can take place, probably at the level of the vestibular nuclei.

Three dimensional gaze stabilization

The retina of the rabbit contains no fovea, but it does have a horizontal zone of elevated density of ganglion cells and other receptive elements, the "visual streak", that is aligned with the horizon (Chievitz, 1891; Hughes, 1971). Due to this elongated receptive area on the retina, the rabbit enjoys almost panoramic vision. Since there is no distinctly preferred area in the projection of the horizon on the retina, the main concern of gaze stabilization in the horizontal plane is velocity stabilization. During head rotation, the position of the eye is stabilized in space, but when the physical limit of eye deviation in the orbit is approached, velocity stabilization is preferred over position stabilization and the eye undergoes a fast resetting in the opposite direction to be able to continue the tracking movement. The tracking movement with oppositely directed fast resettings is called "nystagmus".

It is obvious, however, that perturbations of the head occur in all directions. Gaze stabilization must therefore compensate angular perturbations in three dimensions. With rotation of the head about the roll axis (naso-caudal axis) gaze stabilization will ensure alignment of the visual streak with the horizon by generating vertical eye movements. While, during horizontal rotation, fast phases are interposed to reset the eyes when they reach the mechanical limits of the oculomotor range, such fast phases would carry the visual streak off the horizon, and are therefore undesirable. In the control of vertical gaze stabilization position control is expected to dominate over velocity control.

Gaze stabilization in the laboratory

In the laboratory, the contribution of each individual sensory component can be studied by manipulation of stimulus conditions. With the animal's body fixated relative to its head, to abolish the influence of neck-proprioception, the isolated *vestibular* component of gaze stabilization can be investigated by rotating the animal in total darkness. Since long, this compensatory eye movement response is called the *vestibulo-ocular reflex* (*VOR*; see Kornhuber, 1974; Wilson and Melvill Jones, 1979; Schwartz, 1986; Graham and Kemink, 1987; Melvill Jones, 1991). Studies of the isolated VOR have revealed that this component is most sensitive for fast head perturbations. For slower head movements the canals provide incorrect information.

During rotation of the visual surroundings around a stationary animal, only visual information is imparted to the gaze-stabilizing system. The response elicited by this stimulus is known as the *optokinetic reflex* (*OKR*; see Collewijn, 1985; Collewijn, 1990). In contrast to the VOR, the OKR is most sensitive to very slow motion (1-10°/s) of the retinal

image.

The output of both reflexes is time-dependent. In response to a step in angular speed, slow phase velocity of the vestibular nystagmus jumps to a value approaching stimulus velocity but subsequently decays to zero within about 10-20 seconds. This response is also called post- or per-rotatory nystagmus (PRN). In response to a step in angular rotation of the visual surroundings, the optokinetic response shows a build-up of slow phase velocity until stimulus velocity is approached. When, after a period of optokinetic stimulation with constant speed the lights are turned off, optokinetic nystagmus continues as optokinetic afternystagmus (OKAN), which is very similar in shape to the PRN. From the first observations of these phenomena, the gradual build-up of OKN and decay of OKAN have been associated with the cancelling of PRN when a subject is rotated in the light (Ter Braak, 1936; Collewijn, 1969).

Gaze stabilization as a model of sensori-motor processing by the brain

The neural control of eye movements is an attractive model for motor control and the use of this model has provided insight into strategies of the brain employed in motor control in general (Robinson, 1986). The favorable position of oculomotor research in comparison to the study of the control of limb and axial musculature stems from the simplifying and unique features of the oculomotor system that make oculomotor data easier to interpret.

A main difference is the simplicity of the eye movement. The movements generated by skeletal and axial muscles are complicated by multi-articulate limbs and the complex pattern of contraction and relaxation of numerous agonist and antagonist muscles that control the movements of each of these joints. Eye movements, in contrast, are movements about a single joint, with three pairs of eye muscles that, conveniently, are orthogonally attached to each eye ball. Moreover, the muscles are wrapped around the eye ball so that the moment-arm for the eye muscle's force remains constant during eye movement, whereas the arm of limb muscles depend heavily on limb position. These features grossly simplify the description of muscle behavior and therefore facilitate the interpretation of neuronal activity in relation to motor behavior.

A second important simplifying point is the lack of a stretch reflex. The oculomotor system has to deal with a constant load, the eyeball, and does not have to cope with unexpected external disturbances.

Furthermore, the entire neuronal network, from sensory neurons to motoneurons, is retained in the skull and therefore easily accessible for electrophysiological analysis. This is a problem when studying limb and axial motor systems, since the last, premotor stages

in their processing are localized in the spinal cord, which is hardly accessible for electrophysiological analysis in a behaving animal. As a result, a large body of knowledge has appeared about the anatomical localization of pathways of the gaze-stabilizing system, and about the properties of these signals at several stages in their processing.

Finally, the development of a convenient, but highly accurate measuring system of eye position has greatly facilitated quantitative analysis of the eye movements (Robinson, 1963).

Cerebellar flocculus and gaze stabilization

Although the cerebellum receives information from a wide variety of different receptors, it is not concerned with conscious sensory perception. Rather, the sensory information transmitted to the cerebellum is used in the automatic coordination of somatic motor activity, the regulation of muscle tone, and mechanisms that influence and maintain equilibrium.

Embryologically and functionally, the cerebellum can be divided into three parts: the archicerebellum, the paleocerebellum and the neocerebellum. The archicerebellum is phylogenetically the oldest part and lesions in this area produce disturbances of locomotion, equilibrium and eye movements. The cerebellar flocculus is a tiny part of the archicerebellum, and is involved specifically in the control of eye movements. Anatomical studies have revealed that a large number of brainstem areas project as mossy fiber afferents to the flocculus. The largest projection arises in the vestibular nuclei, the nucleus prepositus hypoglossi (Langer et al., 1985; Alley et al., 1975; Yamamoto, 1979; Maekawa et al., 1981) and the nucleus reticularis tegmenti pontis (Gould, 1980; Hoddevik, 1978; Gerrits et al., 1984). All of these structures have been related to the control of eye movements.

The study of effects of flocculus lesions has provided insight into the function of that structure (rabbit: Ito et al., 1982; Nagao, 1983; Barmack and Pettorossi, 1985; cat: Carpenter, 1972; monkeys: Zee et al., 1981, Waespe et al., 1983). Despite differences in the extent and laterality of lesions, and the interval between lesioning and eye movement testing, certain patterns of oculomotor dysfunction are evident from these lesion studies. A consistent effect of lesioning is a decreased optokinetic response. The effect of lesions on the VOR is inconsistent across species and studies. Even within the rabbit, results have varied. In one study, unilateral flocculectomy in albino animals produced a lasting reduction in VOR gain for the eye ispilateral to the lesion (Ito et al., 1982). In contrast, other studies have described that unilateral flocculus lesions in pigmented and albinos produced no lasting changes in the gain of the VOR (Barmack and Pettorossi, 1985) or

only slight changes in the gain of the VOR in pigmented rabbits with bilateral flocculectomy. Functional inactivation of floccular signal transmission by local, floccular injection of GABA agonists reduced the gain of the VOR (Van Neerven et al., 1989).

Other evidence for the involvement of the flocculus in the control of gaze stabilization stems from electrophysiological studies. Recordings during optokinetic stimulation revealed that the discharge activity of the floccular Purkinje cell, the sole output of the flocculus, encodes retinal image slip velocity, in addition to eye movement related signals. Differences exist between monkey and rabbit data. During vestibular stimulation, the Purkinje cells encode both sensory vestibular signals and eye movement related signals in monkey, while only the eye movement related activity was encountered in the rabbit (Leonard, 1986).

The cerebellar cholinergic system

A potential role of acetylcholine (ACh) as a cerebellar neurotransmitter has been suggested by the biochemical demonstration of substantial amounts of ACh, acetylcholinesterase (AChE) and choline-acetyltransferase (ChAT; Israel and Whittaker, 1965; Goldberg and McCaman, 1967; Kása and Silver, 1969). More recent histochemical and immunocytochemical techniques have revealed, notwithstanding considerable species differences, the archicerebellum (lobules IX and X) as a preferred site of high concentrations of AChE and ChAT (Kása et al., 1982; Ojima et al., 1989). The mossy fibers in these areas of the cerebellum originate mainly from the vestibular complex (except from the lateral vestibular nucleus) and from the prepositus hypoglossi nucleus (Alley et al., 1975; Yamamoto, 1979; Langer et al., 1985). In an immunohistochemical double-labelling study, Barmack et al. (1986) demonstrated the cholinergic nature of a subset of secondary vestibulo-cerebellar neurons, located in the caudal half of the medial and descending vestibular nuclei and in the prepositus hypoglossi nucleus, and projecting to the cerebellar flocculus and nodulus. In addition to mossy fibers, thin varicose fibers, closely associated with the Purkinje-cell layer, similar fibers in the molecular layer in the rat (Ojima et al., 1989) and cat (Illing, 1990) and a subpopulation of Golgi cells in the cat (Illing, 1990) were found to be ChAT-immunoreactive.

The functionality of the presynaptic cholinergic elements described above is further supported by the identification of muscarinic receptors of the M_2 type (Mash and Potter, 1986; Neustadt et al., 1988) and nicotinic receptors (Hunt and Schmidt, 1978, Swanson et al., 1987). The nicotinic receptors appear to be located in the granular layer in the rat (Swanson et al., 1987). In the rabbit, muscarinic receptors, labelled by [^{3}H]quinuclidinyl benzilate (Neustadt et al., 1988) in lobules IX and X, including the flocculus, were most dense in the Purkinje cell layer; moderate and low densities were present over the granular

and molecular layers, respectively. Furthermore, parasagittal columns of very high density were present over the molecular layer of several cerebellar cortical regions, including the vermis and hemispheres of lobules IX and X.

In comparison to these detailed histochemical studies, the physiological actions of acetylcholine in the cerebellum have remained unclear and no specific role of the cholinergic system in cerebellar signal-processing has been demonstrated. Although application of Ach was found to mildly excite Purkinje cells in the cat (Crawford et al., 1966; McCance and Phillis, 1968) a significant amount of cholinergic transmission at the level of the Purkinje cell was regarded as unlikely, because synaptic responses of Purkinje cells were unaffected by application of ACh antagonists (McCance and Phillis, 1968; Crepel and Dhanjal, 1982). Moreover, a more recent study of De la Garza et al. (1987) reported inhibition of rat Purkinje cells by pressure-injected ACh. For application of ACh to cerebellar granule cells no effect was found by Crawford et al. (1966), whereas excitation was reported by McCance and Phillis (1968).

Short outline of the present thesis

Whereas a large body of knowledge is available on the rabbit's optokinetic responses about a vertical axis, only fragmentary data have been obtained about horizontal-axis optokinetic responses. With emerging knowledge on the spatial organization of the three dimensional visual messages in the flocculus there is a need for a detailed description of three-dimensional optokinetic responses. We conducted a behavioral study of three-dimensional eye movements, elicited by optokinetic stimulation about horizontal axes, which will be presented in *Chapter 2* of this thesis.

Chapter 3 describes the positive modulatory effects of floccular injection of the cholinergic agonist carbachol and the AChE inhibitor eserine on the OKR and the VOR. A possible mechanism for the positive action of carbachol is proposed in *Chapter 4*, in the context of a synergistic action between injections of carbachol and the β-noradrenergic agonist isoproterenol. Specification of the receptor type involved in the action of carbachol is attempted in *Chapter 5*.

The effects of bilateral and unilateral injections of carbachol on optokinetic nystagmus and afternystagmus are presented in *Chapters 6 and 7*, whereas *Chapter 8* describes the effect of bilateral injection of carbachol on vestibular, post-rotatory nystagmus.

References

Alley K, Barker R, Simpson JI (1975) Afferents to the vestibulo-cerebellum and the origin of the visual climbing fibers in the rabbit. Brain Res 98: 582-589

Barlow HB, Hill RM, Levick WR (1964) Retinal ganglion cells responding selectively to direction and speed of image motion in the rabbit. J. Physiol 173: 377-407

Barmack NH, Baughman RW, Eckenstein FP, Westcott-Hodson M. (1986) Cholinergic projections to the cerebellum. Soc Neurosci Abstr 12: 577

Barmack NH, Pettorossi VE (1985) Effects of unilateral lesions of the flocculus on optokinetic and vestibuloocular reflexes of the rabbit. J Neurophysiol 53: 481-496

Carpenter RHS (1972) Cerebellectomy and the transfer function of the vestibulo-ocular reflex in the decerebrate cat. Proc R Soc Lond B 181: 353-374

Chievitz JH (1891) Ueber das Vorkommen der Area centralis retinae in de vier höheren Wirbeltierklassen. Arch Anat Entwicklungsgesch Suppl 139: 311-334

Collewijn H (1969) Optokinetic eye movements in the rabbit: input-output relations. Vision Res 9: 117-132

Collewijn H (1985) Integration of adaptive changes of the optokinetic reflex, pursuit and the vestibulo-ocular reflex. In: Berthoz A, Melvill Jones G (eds), Adaptive Mechanisms in Gaze Control. Amsterdam, Elsevier, pp 51-69

Collewijn H (1989a) The vestibulo-ocular reflex: is it an independent subsystem? Revue Neurol (Paris) 145: 502-512

Collewijn H (1989b) The vestibulo-ocular reflex: an outdated concept? In: Allum JHJ, Hulliger M (eds) Progr Brain Res 80: 197-209

Collewijn H (1991) The optokinetic contribution. In: Carpenter RHS (ed), Eye Movements. Vol. 8 in "Vision and Visual Dysfunction", London, Macmillan, pp 45-70

Crawford JM, Curtis DR, Voorhoeve PE, Wilson VJ (1966) Acetylcholine sensitivity of cerebellar neurones in the cat. J Physiol (Lond) 186: 139-165

Crepel F, Dhanjal SS (1982) Cholinergic mechanisms and neurotransmission in the cerebellum of the rat. An in vitro study. Brain Res 244: 59-68

De la Garza R, Bickford-Wimer PC, Hoffer BJ, Freedman R (1987) Heterogeneity of nicotine actions in the rat cerebellum: an in vivo electrophysiologic study. J Pharmacol Exp Ther 240: 689-695

Dichgans J, Bizzi E, Morasso P, Tagliasco V (1973) Mechanisms underlying recovery of head-eye coordination following bilateral labyrinthectomy in monkeys. Exp Brain Res 18: 548-562

Gerrits NM, Epema AH, Voogd J (1984) The mossy fiber projection of the nucleus reticularis tegmenti pontis to the flocculus and adjacent ventral paraflocculus in the cat. Neuroscience 11: 627-644

Goldberg AM, McCaman RE (1967) A quantitative microchemical study of choline acetyltransferase and acetylcholinesterase in the cerebellum of several species. Life Sci 6: 1493-1500

Gould MM (1980) Organization of afferents from the brain stem nuclei to the cerebellar cortex in the cat. Adv Anat Embryol Cell Biol 62: 1-90

Graham MD, Kemink JL (eds) (1987) The Vestibular System, Neurophysiologic and Clinical Research. NY, Raven Press

Hoddevik GH (1978) The projection from the nucleus reticularis tegmenti pontis onto the cerebellum of the cat. Anat Embryol 153: 227-242

Hughes A (1971) Topographical relationships between the anatomy and physiology of the rabbit visual system. Doc Ophthalmol 30: 33-159

Hunt S, Schmidt J (1978) Some observations on the binding patterns of α bungarotoxin in the central nervous system of the rat. Brain Res 157: 213-232

Hwang JC, Daunton NG, Wilson VJ (eds) (1988) Basic and Applied Aspects of Vestibular Function. Hong Kong: Hong Kong University Presss

Igarashi M, Black FO (eds) (1985) Vestibular and Visual Control on Posture and Locomotion Equilibrium. Basel, Karger

Illing RB (1990) A subtype of cerebellar Golgi cells may be cholinergic. Brain Res 522: 267-274

Israël M, Whittaker VP (1965) The isolation of mossy fibre endings from the granular layer of the cerebellar cortex. Experientia 21: 325-326

Ito M, Jastreboff PJ, Miyashita Y (1982) Specific effects of unilateral lesions in the flocculus upon eye movements in albino rabbits. Exp Brain Res 45: 233-242

Jürgens R, Mergner T (1989) Interaction between cervico-ocular and vestibulo-ocular reflexes in normal adults. Exp Brain Res 77: 381-390

Kása P, Silver A (1969) The correlation between choline acetyltransferase and acetylcholinesterase activity in different areas of the cerebellum of rat and guinea pig. J Neurochem 16: 389-396

Kása P, Bánsághy K, Rakonczay Z, Gulya K (1982) Postnatal development of the acetylcholine system in different parts of the rat cerebellum. J Neurochem 39: 1726-1732

Kornhuber HH (ed) (1974) Handbook of Sensory Physiology, Vestibular System, Part I, Basic Mechanisms, Vol VI/1. Berlin, Springer

Langer T, Fuchs AF, Scudder CA, Chubb MC (1985) Afferents to the flocculus of the cerebellum in the rhesus macaque as revealed by retrograde transport of horseradish peroxidase. J Comp Neurol 235: 1-25

Leonard CS (1986) Signal Characteristics of Cerebellar Purkinje Cells in the Rabbit Flocculus During Compensatory Eye Movements (PhD Dissertation). New York, New York University

Maekawa K, Takeda T, Kimura M (1981) Neural activity of nucleus reticularis tegmenti pontis - The origin of visual mossy fiber afferents to the cerebellar flocculus in the rabbit. Brain Res 210: 17-30

Mash DC, Potter LT (1986) Autoradiographic localization of M1 and M2 muscarine receptors in the rat brain. Neuroscience 19: 551-564

McCance I, Phillis JW (1968) Cholinergic mechanisms in the cerebellar cortex. Int J Neuropharmacol 7: 447-462

Melvill Jones G (1991) The vestibular contribution. In: Carpenter RHS (ed), Eye Movements. Vol. 8 in "Vision and Visual Dysfunction", London, Macmillan, pp 13-44

Nagao S (1983) Effects of vestibulocerebellar lesions upon dynamic characteristics and adaptation of vestibulo-ocular and optokinetic responses in pigmented rabbits. Exp Brain Res 53: 36-46

Neustadt A, Frostholm A, Rotter A (1988) Topographical distribution of muscarinic cholinergic receptors in the cerebellar cortex of the mouse, rat, guinea pig, and rabbit: a species comparison. J Comp Neurol 272: 317-330

Ojima H, Kawajiri SI, Yamasaki T (1989) Cholinergic innervation of the rat cerebellum: qualitative and quantitative analyses of elements immunoreactive to a monoclonal antibody against choline acetyltransferase. J Comp Neurol 290: 41-52

Oyster CW (1968) The analysis of image motion by the rabbit retina. J Physiol 199: 613-635

Oyster CW, Barlow HB (1967) Direction-selective units in rabbit retina: Distribution of preferred directions. Science 155: 841-842

Oyster CW, Takahashi E, Collewijn H (1972) Direction selective retinal ganglion cells and control of optokinetic nystagmus in the rabbit. Vision Res 12: 183-193

Peterson BW, Richmond FJ (eds) (1988) Control of Head Movement. New York, Oxford University Press

Roberts TDM (1978) Neurophysiology of Postural Mechanisms. 2nd Edn, London, Butterworths

Robinson DA (1963) A method of measuring eye movement using a scleral search coil in a magnetic field. IEEE Trans Biomed Electron. BME-10: 137-145

Robinson DA (1986) Is the oculomotor system a cartoon of motor control? Prog Brain Res 64: 411-417

Steinhausen W (1933) Über die Beobachtung der Cupula in den Bogengangsampullen des Labyrinths der lebenden Hecht. Pflügers Arch Ges Physiol 232: 500-512

Schwartz DW (1986) Physiology of the vestibular system. In Otolaryngology-Head and Neck Surgery. Cummings CW (ed), St Louis, Mosby, pp 2679-2721

Stehouwer DJ (1987) Compensatory eye movements produced during fictive swimming of a deafferented, reduced preparation in vitro. Brain Res 410: 264-268

Swanson LW, Simmons DM, Whiting PJ, Lindstrom J (1987) Immunohistochemical localization of neuronal nicotinic receptors in the rodent central nervous system. J Neurosci 7: 3334-3342

Ter Braak JWG (1936) Untersuchungen über optokinetischen Nystagmus. Arch Néerl Physiol 21: 309-376

Van Neerven J, Pompeiano O, Collewijn H (1989) Depression of the vestibulo-ocular and optokinetic responses by intraflocculer microinjection of GABA-A and GABA-B agonists in the rabbit. Arch Ital Biol 127: 243-263

Waespe W, Cohen B, Raphan T (1983) Role of the flocculus and paraflocculus in optokinetic nystagmus and visual-vestibular interactions: effects of lesions. Exp Brain Res 50: 9-33

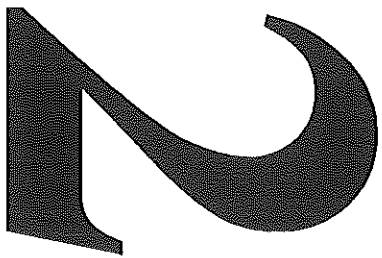
Wilson VJ, Melvill Jones G (1979) Mammalian Vestibular Physiology. New York, Plenum

Yamamoto M (1979) Topographical representation in rabbit cerebellar flocculus for various afferent inputs from the brainstem investigated by means of retrograde axonal transport of horseradish peroxidase. Neurosci Lett 12: 29-34

Young LR (1977) Pursuit eye movement - What is being pursued? In: Baker R, Berthoz A (eds). Control of Gaze by Brain Stem Neurons. Amsterdam, Elsevier, pp 29-36

Zee DS, Tusa RJ, Herdman SJ, Butler PH and Güçer G (1987) Effects of occipital lobectomy upon eye movements in primate. J Neurophysiol 58: 883-907

Zee DS, Yamazaki A, Butler PH, Güçer G (1981) Effects of ablation of flocculus and paraflocculus on eye movements in primate. J Neurophysiol 46: 878-899



Three-dimensional organization of optokinetic responses

Introduction

During head rotation, the eyes are stabilized in space by compensatory eye movements which ensure fixation of the image of the surroundings on the retina. This is achieved primarily by the vestibulo-ocular reflex (VOR) and the optokinetic reflex (OKR). These reflexes have been studied extensively in the rabbit. In the horizontal plane, the VOR responds to angular head-accelerations and functions optimally at the higher frequency range (Baarsma and Collewijn, 1974) whereas the OKR is maximally sensitive to low angular velocities of the whole visual surround (Collewijn, 1969). The combined, synergistic action of the VOR and the OKR, elicited by head rotation in the light, results in a fairly good ocular stability over the entire natural frequency range of head rotation (Baarsma and Collewijn, 1974). A similar synergy between visual and vestibular control has been found for head-rotation in the frontal and sagittal planes (Van der Steen and Collewijn, 1984). To accomplish this close synergy between the VOR and the OKR it seems necessary for the brain to transform the (different) sensory input modalities of the two reflexes into a common coding in three-dimensional (3-D) space. The present study aimed at elucidating the intrinsic 3-D reference frame of the rabbit's OKR.

The coding of the vestibular input is related to the architecture of the vestibular canal system. The best-response axes of the three pairs of canals are organised in a three-dimensional reference frame with a vertical axis and two axes in the horizontal plane, at angles of approximately 45° and 135° relative to the sagittal plane. The input to the OKR, retinal image-slip, is conveyed by direction-selective ganglion cells in the retina (Oyster, 1968), in particular the ON-type ganglion cells (Oyster et al., 1972; Soodak and Simpson, 1988). By studying subsequent levels of processing of retinal image-slip information in the accessory optic system (AOS; Soodak and Simpson, 1988; Simpson et al., 1988), inferior olive (IO; Leonard et al., 1988), floccular climbing fibers and floccular Purkinje cells (Graf et al., 1988), it was recognized that flow-detection by ganglion cells and subsequent neuronal stages is organized in three pairs of channels, each pair conveying signals about flow of the visual surround about one of three rotation axes, which were approximately collinear with the best-response axes of the semicircular canals as well as with the rotation axes of the extraocular muscles. In addition, these studies showed that the three pairs of channels of optokinetic information are anatomically segregated in the AOS and the IO. More recently, it was shown that also in the flocculus the different channels processing optic flow have an anatomical substrate, consisting of a series of zones distinguishable with acetylcholinesterase staining. Electrical micro-stimulation in these different anatomically distinguishable floccular zones elicited eye movements organized in a canal-like

reference frame (Van der Steen et al., 1991; Simpson et al., 1989).

In representing 3-D space and controlling 3-D eye movements, the brain can be postulated to utilize an intrinsic sensory and motor reference frame, based on axes intrinsic to the anatomy of the semicircular canals and extraocular muscles (Pellionisz and Llinas, 1979, 1980; Simpson et al., 1981). This view differs from that using the extrinsic reference frame based on vertical, sagittal and transverse axes, mediating yaw, roll and pitch, which is conventionally used in most VOR and OKR experiments. However, the strong neurophysiological evidence for a canal-like intrinsic organisation of the OKR has not yet been complemented by an adequate study of the 3-D organization of the OKR at the behavioral level. Behavioral data on the OKR are largely confined to its horizontal component. Stimulation at a constant speed about the vertical axis leads to a regular sequence of tracking ("slow") and resetting ("fast") phases, forming an optokinetic nystagmus (OKN). In the rabbit, the response about the vertical axis (VA) is characterized by a steady-state gain slightly below unity for speeds up to 20°/s during binocular stimulation; the gain declines progressively for higher stimulus speeds. Furthermore, a strong directional preference for temporal-to-nasal as opposed to nasal-to-temporal motion is present during monocular stimulation. The VA response is further characterized by a velocity build-up during continuous rotation at higher speeds, followed by optokinetic afternystagmus (OKAN) in darkness. During monocular stimulation the build-up is asymmetric, occurring only in the temporal-to-nasal direction. (For reviews see Collewijn, 1981, 1991).

Relatively few studies have examined OKN elicited by stimulation about horizontal axes and nearly all of these used the traditional convention of stimulating and measuring only movements in the sagittal and frontal planes. Collewijn and Noorduin (1972a) looked at vertical (roll-axis) as well as torsional (pitch-axis) OKN in the rabbit. Vertical OKN during binocular stimulation showed a low-velocity preference similar to that of horizontal OKN with a slow phase gain of 0.7 to 0.9 for stimulus speeds below 1 °/s. Some build-up of slow phase velocity, as present in horizontal OKN, could also be observed in vertical OKN, but this did not cause a major improvement in gain. With monocular stimulation, vertical and torsional OKN were similar in that directional (sense of rotation) asymmetries were not noticed.

Later, Erickson and Barmack (1980) compared horizontal and vertical OKN in the rabbit. They showed that with monocular stimulation, vertical OKN had a gain of 0.2 to 0.4 for stimulus speeds between 1 and 5°/s, and a progressively declining gain for higher speeds. This response is similar to the one found for horizontal, monocular OKN in the non-preferred nasal-to-temporal direction. In contrast with the study of Collewijn and Noorduin (1972a) a slight, but statistically significant, preference was found for vertical stimulation of the seeing eye in the downward direction, compared to

the upward direction.

So far, a more complete study of OKN in rabbit, taking into account the 3-D organisation of visual input and motor output, has been missing. A study of this kind was performed in chicken by Wallman and Velez (1985), who examined OKN about a range of horizontal axes, although only monocular recordings were made. In addition to a general preference for upward movement, they found that a maximum in OKN gain occurred for visual world rotation that would correspond to head rotation exciting the anterior canal contralateral to the seeing eye.

The major aim of the present study was to describe the three-dimensional optokinetic responses of both eyes during monocular and binocular optokinetic stimulation about the full range of horizontal axes. The study revealed anisotropies and directional asymmetries in the optokinetic responses that reflect an intrinsic canal-oriented organization of the optokinetic system.

Methods

Surgical procedures

Six young adult Dutch-belted rabbits of either sex were used. General anaesthesia was induced and maintained by a mixture of ketamine, acepromazine and xylazine-HCl (for details see Van Neerven et al., 1990). About a week prior to the experiments, the rabbits were permanently implanted with a vertically and a horizontally oriented coil on each eye and with head-bolts for fixation of the head. The vertical coil consisted of 5 turns of teflon-coated, stainless-steel wire (Miniature bioflex wire, type AS 632, Cooner Sales Company, Chatsworth, CA) wound under the superior, inferior and medial rectus and inferior oblique muscles, parallel to the limbus. The horizontal coil was implanted on top of the eyeball above the superior rectus muscle. This preformed coil had a diameter of 8 mm and contained 40 turns of insulated copper wire (diameter 0.05 mm).

Eye-position recording

We measured eye movements in three dimensions with scleral search-coil techniques. The a.c. magnetic field (1300 Hz) had a large homogeneous area (a cube of about 1 m along each edge) and rotated in the horizontal plane (see Collewijn, 1977). The signals of the vertical eye coils were processed by a phase-detection technique (Collewijn, 1977) for measurement of the horizontal component (VA rotation) of the eye movements. The signals of the horizontal eye coils were processed by an amplitude-detection technique (Robinson, 1963) for measurement of the vertical components

reflecting eye movements about horizontal axes (HA rotations). The horizontal movements, measured by the vertical coils, had absolute calibration because the phase-detection technique was used. The horizontal coils were calibrated prior to implantation. Responses of both eyes were measured simultaneously (for a more detailed description of this combined technique see Van der Steen and Collewijn, 1984).

The orthogonal coordinate system used to measure the eye movements (Fig. 1) consisted of a vertical axis and two horizontal axes, oriented at 45° and 135° azimuth relative to the rabbit's sagittal plane. The latter two components were derived as the orthogonal components, induced in the horizontal coil, by a dual-phase lock-in amplifier (PAR model 129A), with suitable setting of the phase-reference in relation to the field. The three components of the rotations of each eye were named after their respective axis of rotation. We defined the sense of eye rotation around the 45° and 135° axes as clockwise (CW) or counterclockwise (CCW), according to how it was seen when looking along the axis of rotation towards the rabbit's eye. The sense of rotation about the VA was defined as CW and CCW when looking down on the rabbit along the VA. The coordinate system for the eye movements remained locked to the rabbit's head, irrespective of horizontal rotation of the rabbit, because the phase-reference for the detection systems was derived from a head-fixed reference coil.

Data acquisition and analysis of all experiments were done by computer (DEC, PDP 11/73). The eye position signals were sampled at a frequency of 62.5 Hz and stored on disk. In subsequent off-line analysis, the saccades and fast phases were removed and the gain of the slow phase response of each axial component was determined separately. The gain and orientation of the total response was subsequently calculated as the vectorial sum of the three components.

Experimental conditions

The animal was restrained in a bag that was secured to a small board and the head bolts were fastened to a head holder. The horizontal canals of the rabbit were aligned close to the horizontal plane by holding the head with the nasal bone at a pitch-angle of 57° to the horizontal. Optokinetic stimulation was provided by rotation of a cylinder with a length of 100 cm and a diameter of 100 cm, open at one end, and covered on the inside with a random-dot pattern. This drum could be tilted from a vertical position, used for vertical axis optokinetic stimulation, to a horizontal position, to provide stimulation about selectable horizontal axes. The drum was driven by a velocity-controlled motor which received either a steady d.c. current to produce constant speed drum rotation or a d.c. current which was alternated in polarity to achieve triangular motion of the drum.

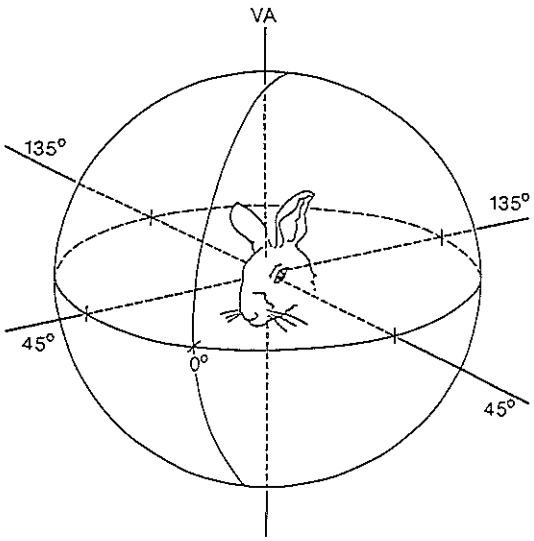


Fig. 1 The orthogonal coordinate system used to measure the angular displacements of the eyes, consisting of an earth-vertical axis (VA) and two horizontal axes (HA) at 45° and 135° azimuth. The three components of rotation of the eyes were named after their respective axis of rotation, i.e. VA, 45° HA and 135° HA. The direction of rotation of each component was defined as clockwise (CW) or counterclockwise (CCW) looking along each axis.

The rabbit was carefully positioned so that the drum axis (which was oriented either vertically or horizontally) ran through the midpoint between the eyes. In order to change the drum axis orientation in the horizontal plane, the rabbit itself was repositioned within the stationary drum and field coils by rotation about an earth-vertical axis that intersected the midpoint between the eyes. We defined the 0° horizontal axis (HA) orientation as that orientation for which the rotation axis of the drum was in the sagittal plane of the rabbit. Note that the rabbit retained its normal orientation towards gravity in all stimulus conditions.

Constant-speed drum rotation

Vertical-axis, constant speed (2°/s) stimulation was presented to 6 rabbits, during both monocular and binocular viewing with the drum moving CW and CCW. With the drum axis tilted into the horizontal plane, the rabbit was positioned in such a way that its sagittal plane included the rotation axis of the drum (0° azimuth position) and measurements were made during binocular viewing for both rotation directions (CW and CCW). With constant speed rotation it was not possible to test the response to binocular stimulation about axes other than the roll axis, as this would permit the rabbit to view the open end of the drum. This limitation was not present during monocular stimulation, which was provided by covering each of the eyes in turn with an opaque cap. Measurements were made with the drum axis oriented at 0°, 45°, 90° and 135° relative to the seeing eye. By pooling the sets of measurements derived from 2

eyes in 6 rabbits, we obtained 12 sets of measurements for the monocular condition. Each measurement had a duration of 82 s, and included both the initial build-up and the steady-state phase of OKN. Gain was determined as the ratio between slow-phase velocity (averaged over the entire measurement) and stimulus velocity.

Triangular drum rotation

Triangular stimulation consisted of drum rotations with a speed of 2°/s, alternating every 10 sec (0.05 Hz) in the CW and CCW directions, with an amplitude of 20° peak-to-peak. Measurements during binocular, left-eye-only and right-eye-only viewing were made for a range of 180° at increments of 15°. This procedure covered the entire range of possible stimulus axis orientations. With triangular stimulation, full field stimulation was technically possible because the small excursions of drum rotation permitted the open end of the drum to be occluded with a random-dot pattern. The measurements had a length of 64 s. After removal of the saccades, gain was calculated as the ratio between the response amplitude and the stimulus amplitude. The gains of the 45°- and 135°-components were computed separately and then combined vectorially to obtain the vectorial magnitude of the response (expressed as gain) and the orientation of the response axis (expressed as deviation from the stimulus axis). Once again, the data sets of the monocular experiments with the left or right eye covered were pooled for the 6 rabbits tested, giving a total of 12 monocular experiments. A full set of binocular responses was measured in 3 of the 6 rabbits.

Results

Responses to constant-speed drum rotation

Continuous drum rotation at a speed of 2°/s with the drum axis oriented vertically and both eyes viewing elicited a well-developed VA-OKN with regularly alternating slow and fast phases in both CW and CCW directions (Fig. 2A). After a moderate build-up of eye velocity at the start of the response, a steady state was reached with a gain of about 0.9.

VA drum rotation during monocular viewing elicited OKN with a strong nasal-to-temporal asymmetry. Optokinetic stimulation in the temporal-to-nasal direction elicited an optokinetic response which was similar to OKN during binocular vision. Fig. 2B (lower records) shows the response during monocular optokinetic stimulation in the preferred (temporal-to-nasal) direction. The speed of the slow phases was constant and resetting saccades were frequently and regularly interposed. A slight build-up of eye velocity was also present, after which a steady-state gain of about 0.9 was reached.

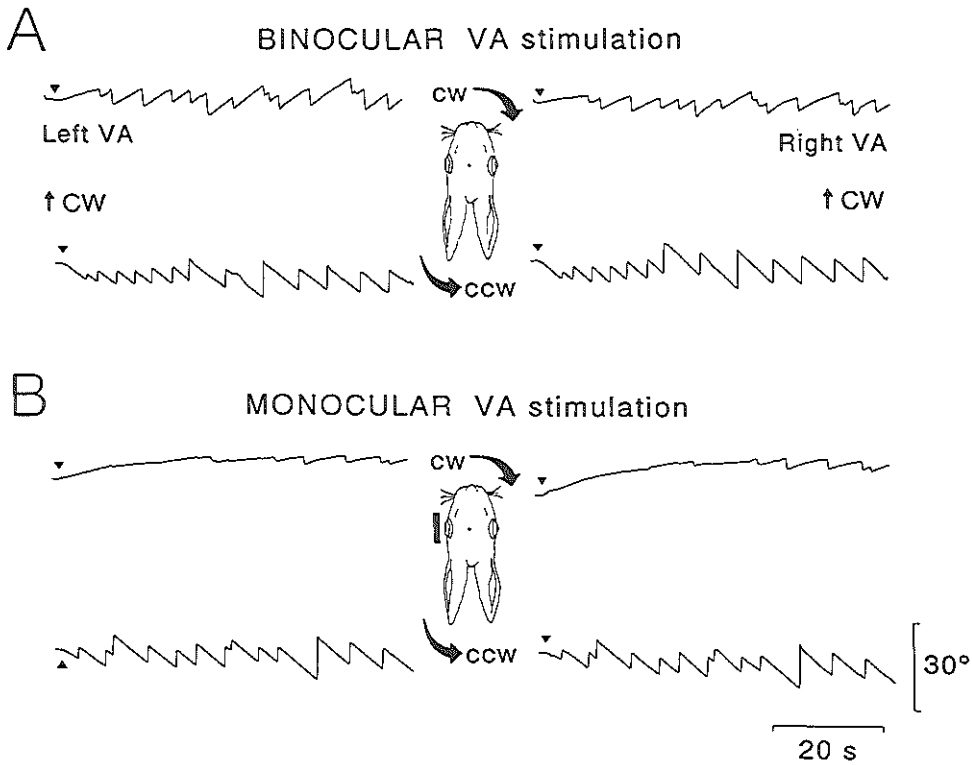


Fig. 2 Eye position recordings during optokinetic VA stimulation with a constant speed of 2°/s. The VA eye rotations of both the left and right eye in response to stimulation in CW and CCW directions under binocular (A) and monocular (B) viewing conditions are shown.

Monocular stimulation in the nasal-to-temporal direction evoked a much weaker nystagmus with other characteristics different from temporal-to-nasal OKN (Fig. 2B, upper records). The difference was not simply a general reduction in the gain of the OKR, but also included a different shape of the slow phase of the optokinetic response. The speed of the initial part of the slow phase during nasal-to-temporal optokinetic stimulation was relatively high, with a gain of about 0.7, but the speed decreased as the eyes reached a more eccentric position. The slow phase resumed a high gain right after each fast phase, independent of eye velocity immediately before the resetting saccade, but during the later part of the slow phase, the gain gradually diminished.

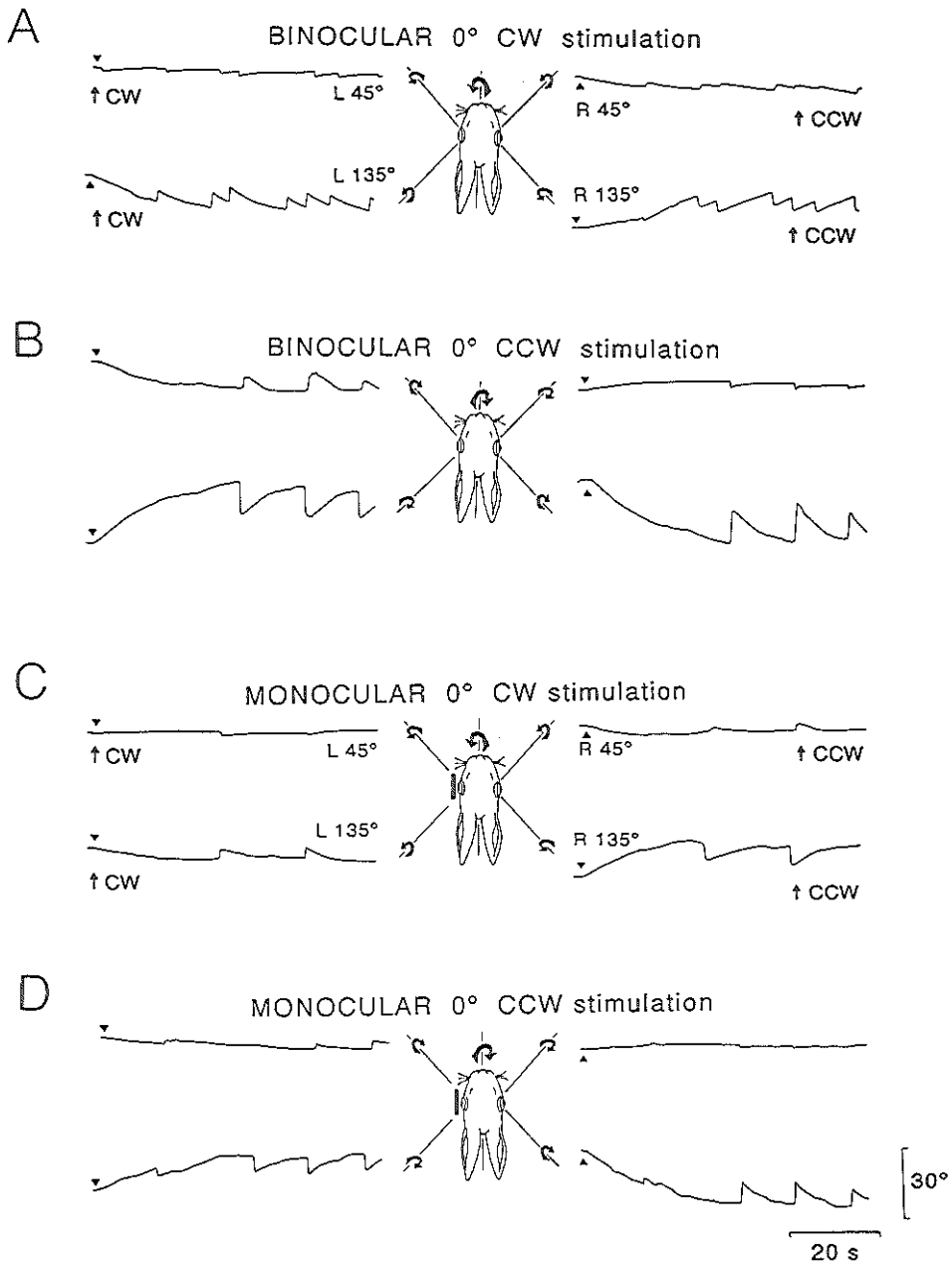
Although the response to binocular VA optokinetic stimulation was essentially a

conjugate movement of the two eyes, a slight convergence occurred during the slow phase, due to the fact that the eye that was stimulated in the temporal-to-nasal direction moved faster than the nasally-to-temporally stimulated eye, as described earlier (Collewijn and Noorduin, 1972b). This vergence-component amounted to about 5-10% of the version-movement. During monocular viewing, the response of the seeing eye was typically about 5-10% larger than the response of the covered eye for both directions of stimulation, in agreement with previous descriptions (Collewijn and Noorduin, 1972b).

Typical optokinetic responses to constant speed drum rotation of 2°/s about axes in the horizontal plane are shown in Fig. 3. Optokinetic stimulation about the 0° HA (roll axis) during binocular viewing elicited a nystagmus with an average gain of 0.44 for upward and 0.38 for downward stimulation. Fig. 3A-B shows a typical response to 0° HA binocular stimulation and illustrates that the net response of each eye was not a pure vertical movement about the 0° axis. For both eyes, the 135°-component of the eye movement was stronger than the 45°-component so that the net axis of rotation of each eye deviated in the horizontal plane from the 0° stimulus axis towards -45° (or 135°) azimuth. Deviations were 36° for the upward and 25° for the downward moving eye, resulting in an average angle between the response axes of the two eyes of 51°.

Monocular stimulation about any of the horizontal axes elicited responses with characteristics similar to those of monocularly elicited horizontal OKN in the non-preferred (nasal-to-temporal) direction. Fig. 3C-D shows typical responses to monocular stimulation about the 0° HA. Compared to binocular OKN about the 0° HA, the average slow-phase gain of the monocular HA optokinetic response was poor. This difference was mainly due to the irregular and saturating nature of the optokinetic response just as with monocular, horizontal OKN in the non-preferred direction. While the slow phases started off with a relatively good initial velocity, the velocity decreased as the eyes reached more eccentric positions. Often the eye velocity even dropped to zero with the eye maintained in a steady eccentric position for a variable time. Therefore, the average slow-phase gain, computed over all of the slow phase intervals, was very low, with values of 0.2-0.3. As was found for binocular viewing, the 135°-component dominated the net response, resulting in a deviation of the response axis of each eye by about 30° from the 0° stimulus axis towards -45° (or 135°) azimuth.

Typical recordings of the optokinetic response to monocular stimulation about the 45° HA and 135° HA, shown in Fig. 4, revealed an even greater lack of yoking between the eyes. A pronounced dissociation between the directions and magnitudes of the movements of the two eyes was present when CCW stimulation was delivered to the seeing (right) eye about its 135° HA, as shown in Fig. 4A. In this example, the 135°-component of the right (seeing) eye had a gain of 0.75. At the same time the collinear


A
BINOCULAR 0° CW stimulation
↑ CW
L 45°
R 45°
↑ CCW
L 135°
R 135°
↑ CW
↑ CCW
B
BINOCULAR 0° CCW stimulation
C
MONOCULAR 0° CW stimulation
↑ CW
L 45°
R 45°
↑ CCW
L 135°
R 135°
↑ CW
↑ CCW
D
MONOCULAR 0° CCW stimulation
30°
20 s

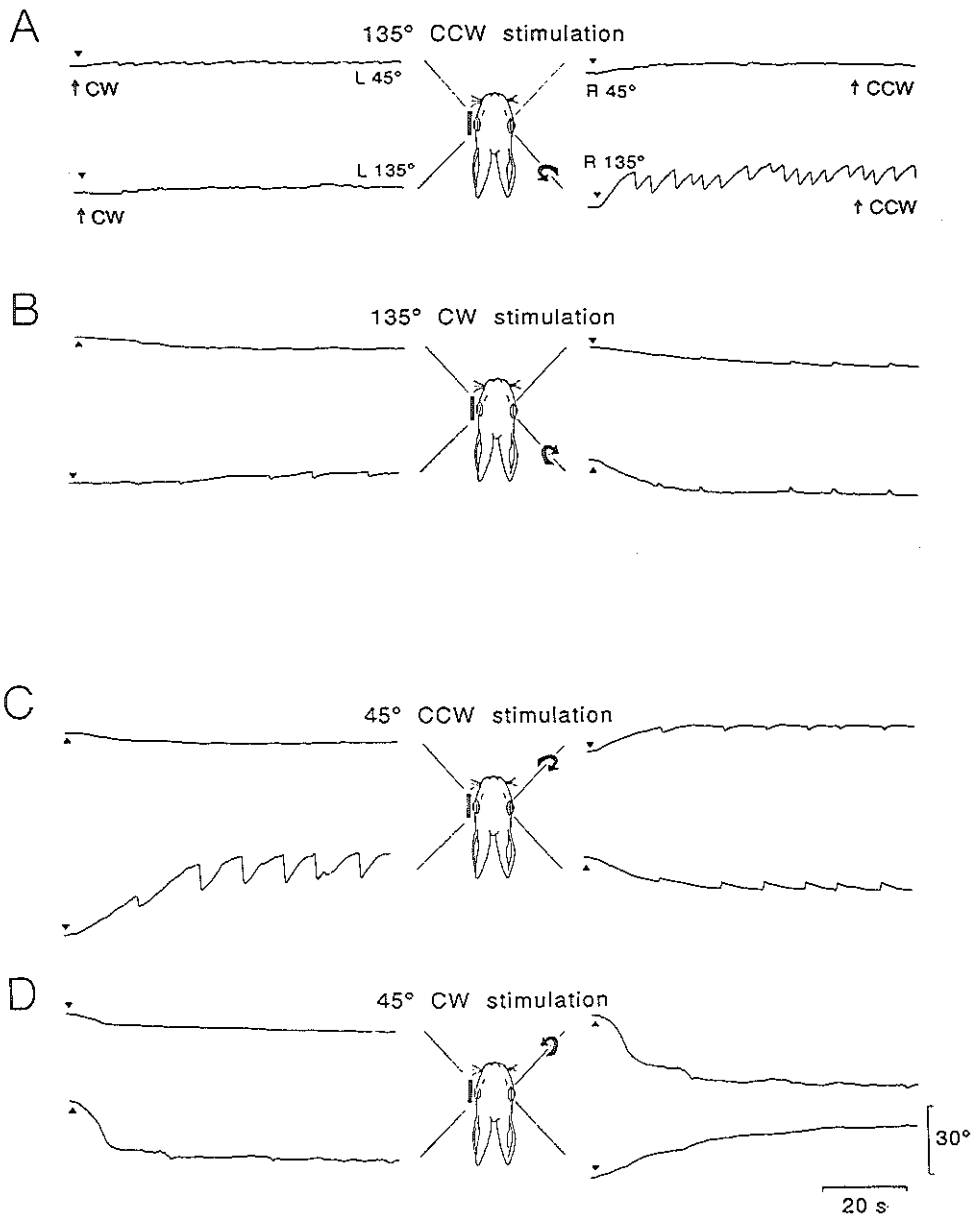
45°-component of the left (covered) eye showed a gain of only 0.02. During CW stimulation about the 135° HA the responses of both eyes were small (Fig. 4B) with gains of 0.10 for the 135°-component of the right, seeing eye and 0.02 for the 45°-component of the left, covered eye.

Even more markedly dissociated eye movements occurred when the right eye was stimulated about its 45° HA in the CCW sense. As depicted in the example of Fig. 4C, the 45°-component of the response of the right, seeing eye had a gain of 0.11 whilst the 135°-component of the left, covered eye showed a much larger response with a gain of 0.64. Thus, in contrast to stimulation about the 135° HA described above, the response of the covered eye was much larger than the response of the seeing eye. The responses of both eyes to CW stimulation about the 45° HA were very small (Fig. 4D), as was the case for CW stimulation about the 135° HA.

Fig. 5 shows the gains of the 45°- and 135°-components of the responses for all monocular stimulus axis orientations, as averaged over the 12 data sets. The responses of the right, seeing eye are shown in the upper panel of Fig. 5. There was a small VA-component at all axis orientations, with an average gain of about 0.06. The gain of the 45°-component of the response was small for all axis orientations, although some modulation was present with maxima during stimulation at 45° CW and 45° CCW (= 225° CW) and minima at 135° CW and 135° CCW (= 315° CW). The 135°-component was much larger. Its gain showed a bimodal distribution with one maximum for CCW stimulation about the 135° HA (average gain 0.40) and a smaller peak at CCW stimulation about the 0° HA (gain 0.22). Minima were found at 45° CW and CCW stimulation. The net response of the seeing eye is thus dominated by its 135°-component, and is maximal with CCW stimulation about the 135° HA.

The lower panel of Fig. 5 shows the data for the covered eye, with the gains of the response components plotted against stimulus-axis orientation relative to the covered eye. In this format one can appreciate that the general response pattern is approximately similar for both the covered and seeing eyes. The 135°-component

Fig. 3 Eye movement responses during constant speed (2°/s) optokinetic stimulation about the 0° HA (roll axis). Recordings of the 45° HA and 135° HA components of both eyes are shown during binocular (A,B) and monocular (C,D) viewing for rotation in CW (A,C) and CCW (B,D) directions. The top view of the rabbit's head in this and all related figures shows the eye rotation components in relation to the 45° and 135° horizontal axes of the recording system. Straight arrows near the eye position records indicate the CW and CCW direction of rotation. Small, curved arrows indicate the direction of the components of the slow-phase eye movement response about each axis. The large, curved arrow identifies the stimulus axis and indicates the direction of stimulus rotation. Note the dominance of the 135°-HA eye rotation components.


A
135° CCW stimulation
↑ CW
L 45°
R 45°
↑ CCW
L 135°
R 135°
↑ CW
↑ CCW
B
135° CW stimulation
C
45° CCW stimulation
D
45° CW stimulation
30°
20 s

dominated the total response with a maximum gain of 0.33 when the stimulation axis was oriented at 135°. The magnitude of the 45°-component of the covered and seeing eye closely resembles that of the concomitantly recorded VA-component.

For both the seeing and covered eye, the direction and magnitude of the net HA responses were reconstructed through vectorial addition of the 45°- and 135°-components shown in Fig. 5. Fig. 6 depicts the calculated gain of the net response and the (reconstructed) orientation of the eye-rotation-axis of the net response in the horizontal plane. To show the differences between response and stimulus axes, each data point is connected to its corresponding stimulus axis by an interrupted line. The right panel of Fig. 6 represents the net responses to optokinetic stimulation about a set of axes presented to the right, seeing eye. The left half of the right polar diagram represents responses to CW rotation, while the right half represents responses to CCW rotation. Opposite directions of drum rotation are thus represented in Fig. 6 by data-points that are connected by interrupted lines to oppositely directed segments of a single stimulation axis. The figure shows directional asymmetries (differences in response gain for CW and CCW stimulation about the same axis), anisotropies (differences in responses to stimulation about different axes), and angular deviations (differences in orientation between stimulus axis and response axis). For the right, seeing eye the mean gain was maximal (0.44) when the drum axis was oriented at 135° and rotated in the CCW direction. Stimulation about the same axis in the CW direction resulted in a much smaller response with an average gain of 0.13. This preference for CCW stimulation was statistically significant ($p < 0.02$; Wilcoxon signed-rank test). The axis of the eye rotation (response axis), elicited by stimulation about the 135° HA, was almost collinear with the stimulus axis. The deviation of the response axis from the stimulus axis was 3° for CW and 6° for CCW rotation. With the drum axis oriented at 45°, the average gains were similar (0.14) during CW and CCW rotation and the response axes showed deviations from the stimulus axis of 31° and 22° for CW and CCW rotation, respectively.

The left panel of Fig. 6 depicts the pattern of the gains and response axis

Fig. 4 Examples of dissociated movements of the two eyes in response to monocular stimulation with constant speed about the 135° HA (A,B) or the 45° HA (C,D) re the seeing (right) eye. CCW stimulation about the 135° HA evoked a regular nystagmus about the corresponding axis for only the seeing eye (A); a response during CW stimulation was almost absent in the steady-state (B). C illustrates a regular nystagmus in the covered eye evoked by CCW stimulation about the 45° HA of the seeing eye. The response of the seeing eye is almost absent in the steady-state. During CW stimulation about the 45° HA of the seeing eye, all of the components saturate after the initial response (D).

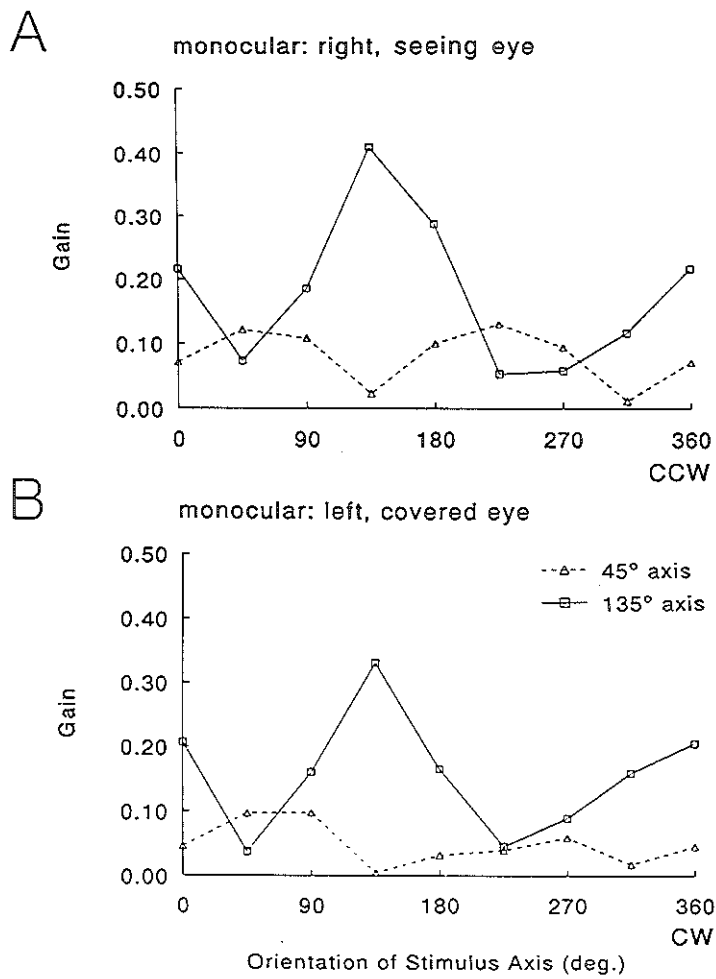


Fig. 5 Average gains (n=12) for the 45° HA and 135° HA response components of the seeing eye (top panel) and the covered eye (lower panel) for constant speed, monocular stimulation, plotted as a function of stimulus axis orientation in the horizontal plane. The abscissa of A represents the axis orientation for CCW stimulation. The abscissa in B represents the axis orientation for CW stimulation. Note that the orientation of the stimulus axis has been represented using a range of 360°. Consequently, a 225° CCW rotation corresponds to a 45° CW rotation. In A the azimuth values increase from 0° toward the seeing eye, while in B they increase from 0° toward the covered eye.

orientations of the left, covered eye as a function of the stimulus axis orientation relative to the left, covered eye. Representation of the data in this format shows clearly that the distribution of the gain as a function of the orientation of the stimulus axis referred to the covered eye is not conjugate with that of the seeing eye. Rather, the pattern for the covered eye is approximately the mirror image of the pattern for the seeing eye.

As was the case for the seeing eye, the response of the covered eye was modulated with stimulus axis orientation. A maximum response was found in the left, covered eye when it was "stimulated" in the CW direction about its 135° HA (gain = 0.33), i.e., when the stimulus was CCW around the 45° HA re the seeing eye. Stimulation in the opposite direction (CCW about the 135° HA of the covered, left eye) elicited a response with a gain of 0.16. This directional asymmetry was statistically significant ($p < 0.05$). The rotation axis of the left, covered eye was also almost collinear with the 135° stimulus axis, with deviations of 1° for CW and 7° for CCW rotation.

With the drum axis positioned at 45° azimuth re the covered eye (135° re the seeing eye), the gain of the covered eye was only 0.1 and 0.06 for CW and CCW rotation, respectively, with deviations of the response-axis from the stimulus-axis by 21° and 49°.

Stimulation of only the right, seeing eye about the 0° HA elicited a response in that eye with an average net gain of 0.30 for CW and 0.23 for CCW stimulation. In other words, the response to upward stimulation was slightly better than to downward stimulation, but this difference was not statistically significant. For both directions of stimulation, however, the response axis deviated considerably from the 0° horizontal stimulus axis. The deviation was 27° and 26° for upward and downward rotation, respectively, and was directed from 180° (or 0°) azimuth towards 135° (or -45°) azimuth. A similar, but mirror-symmetrical pattern was revealed for the left, covered eye, where the average net gain was 0.17 for downward (re the covered eye) stimulation and 0.21 for upward stimulation, with deviations of the response-axis from the stimulus axis of 34° and 21°, respectively. A similar pattern was present in the binocular condition (Fig. 3).

Responses to triangular drum rotation: binocular stimulation

To investigate the characteristics of the initial part of the optokinetic response in more detail, a triangular stimulus motion (2°/s; 20° peak-to-peak; 0.05 Hz) was used. With this stimulus the eyes made relatively small excursions and the saturation that occurred during continuous rotation of the drum at constant speed was avoided. A second advantage of this stimulus was that the limited excursions of the drum during

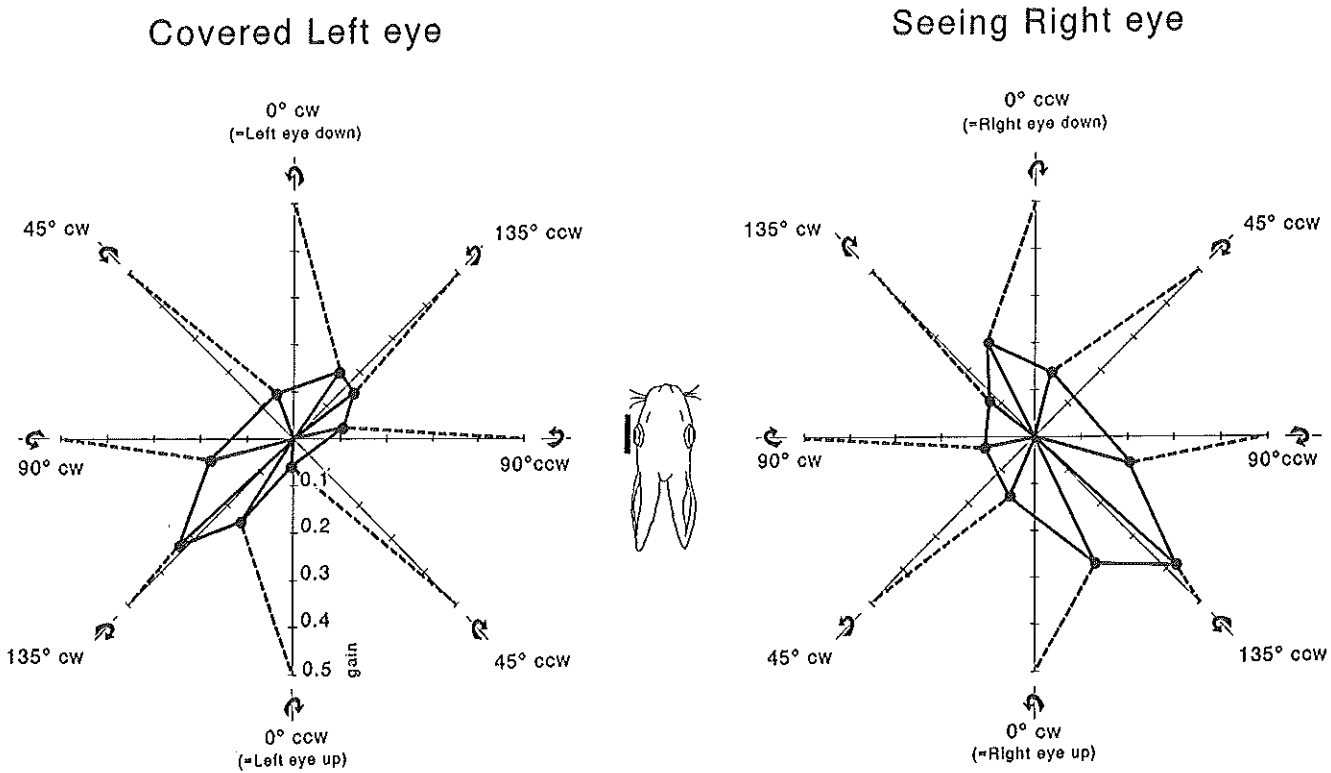

Covered Left eye
0° cw
(=Left eye down)
45° cw
135° ccw
90° cw
90°ccw
135° cw
45° ccw
0° ccw
(=Left eye up)
0.1
0.2
0.3
0.4
0.5
gain
Seeing Right eye
0° ccw
(=Right eye down)
135° cw
45° ccw
90° cw
90°ccw
45° cw
135° ccw
0° cw
(=Right eye up)

Fig. 6 Averaged (n=12) responses to monocular optokinetic stimulation with constant velocity (2°/s), plotted in polar coordinates. Net gains were calculated from the components shown in Fig. 5. Each net gain value is plotted as the length of a line segment orientated along the response axis, which has been connected by an interrupted line to the stimulus axis. The responses of the left, covered eye are shown in the left panel (A); those of the right, seeing eye in the right panel (B). The polar distributions show maximum gain and minimum angular deviation when the stimulus axis is oriented at about 135°.

triangular motion allowed us to cover the open end of the drum, and thus to provide a true full-field visual stimulus over the entire range of horizontal axis orientations during binocular, as well as monocular viewing.

Three rabbits were tested with triangular motion of the drum about horizontal axes under binocular viewing conditions. The recordings of Figs. 7A and B show typical responses of the two eyes during stimulation about the 45° HA and the 135° HA. The angular excursion of the drum was only 10° to each side of the midposition and, as a result, fast phases occurred very rarely. In this example both eyes responded with gains close to unity (0.95) during both 45° HA and 135° HA stimulation.

Fig. 8A depicts the mean gains of the 45°- and the 135°-components and the net gain as a function of the stimulus axis orientation for the binocular viewing condition as measured in 3 rabbits. Because there was no difference between the responses of left and right eye during binocular optokinetic stimulation, the data of the left and right eye were pooled and normalized as if presented to the right eye, resulting in a total of 6 data sets. The net gain of the response and the orientation of the response axis were calculated by vectorial addition of the measured 45°- and 135°- components.

With binocular viewing, the gains of the 45°- and 135°- components showed a systematic modulation as a function of stimulus axis orientation (Fig. 8A). Each gain curve reached a maximum of about 0.8 when the stimulus axis was close to the recorded axis, and a minimum (about 0.1) when the stimulus was orthogonal to the recorded axis. As a result, the net gain of the response as a function of stimulus axis orientation was nearly constant at about 0.9. Furthermore, no systematic directional asymmetries (CW/CCW differences) were encountered in the gains of the slow phase responses for triangular stimulation.

The right panel of Fig. 9 shows the magnitude and angular deviation of the response to binocular optokinetic stimulation as a function of stimulus axis orientation. In addition, each point is connected to the respective orientation of the stimulus axis through the interrupted line.

The responses to binocular triangular stimulation were functionally almost ideal, with gains close to unity for all directions of stimulation and no systematic directional

asymmetries were encountered for stimulation about any of the axes tested. Furthermore, for most directions of stimulation, the response axis was fairly well aligned with the stimulus axis, although some deviation was apparent for stimulation about axes close to the 0° axis (up to 12°). The magnitude of the angular deviation of the response axis from the stimulus axis is summarized in Fig. 10 for binocular as well as monocular viewing conditions.

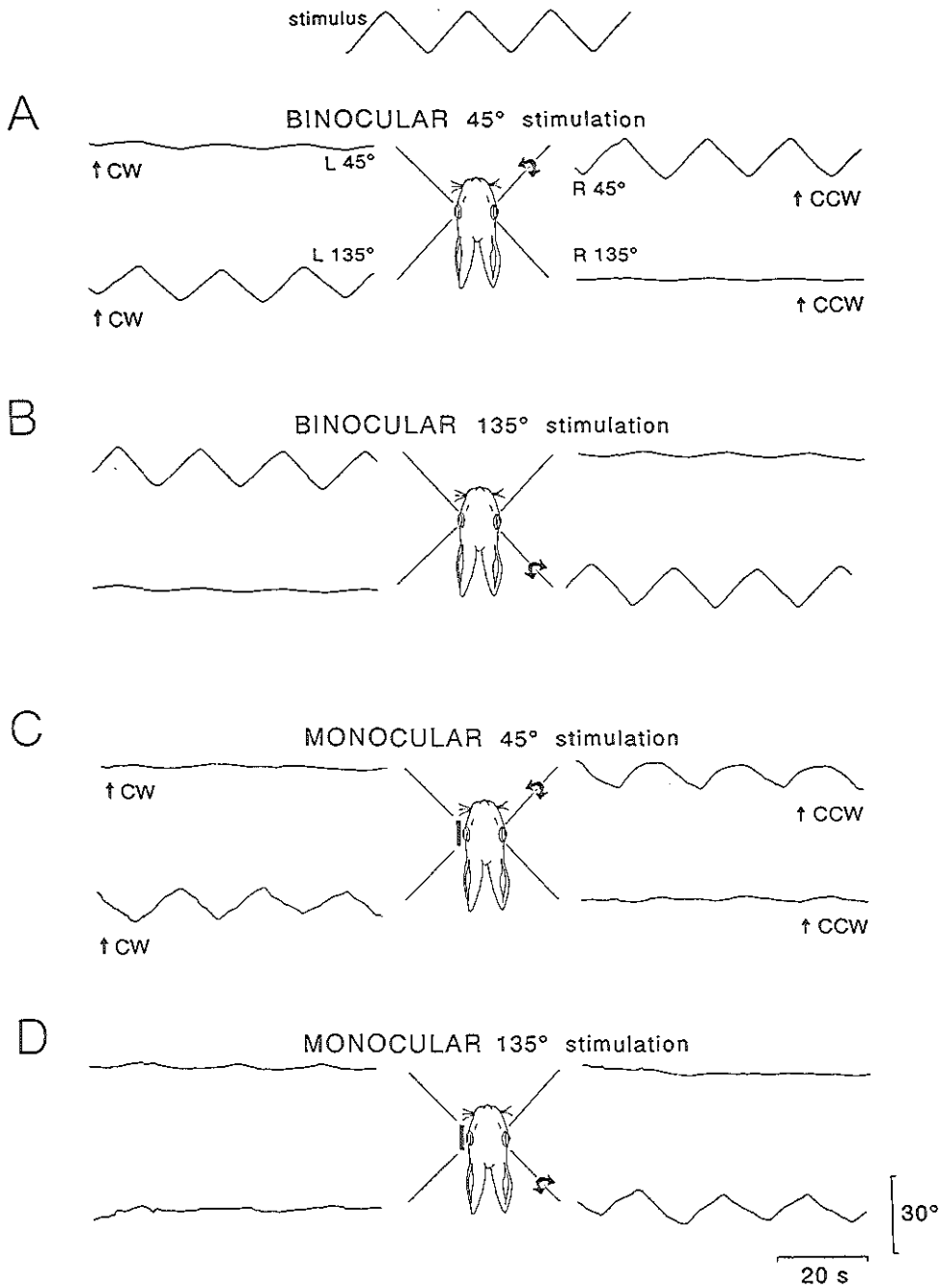
Responses to triangular drum rotation: monocular stimulation

Covering of one eye caused, first of all, a substantial reduction in the optokinetic response to triangular stimulation. The gain of the seeing eye was about 50% of the gain in the binocular condition. For the seeing, right eye stimulation about the 45° HA elicited a response in the 45°-component with a gain of about 0.5. The 135°-component of the seeing eye responded in a similar way to stimulation about the 135° HA (Figs. 7 C and D; right traces). The responses of the left, covered eye, however, were essentially different from the responses of the right, seeing eye, and revealed an anisotropy with respect to stimulus axis orientation. Stimulation of the seeing eye about its 45° HA elicited a nearly conjugate response in the covered eye, with a gain of about 0.5 (Fig. 7C). Thus, the average gain was approximately equal for seeing and covered eye during monocular stimulation about the 45° HA re seeing eye. During stimulation of the seeing eye about its 135° HA, however, the covered eye showed almost no response (Fig. 7D), which resulted in a very disconjugate movement of the two eyes about the 135° HA of the seeing eye.

Although some cases (like the response of the 45° component of the seeing eye in Fig. 7C) showed a directionally asymmetric shape of the slow phase intervals, this did not lead to any systematic trend in the average eye position. As with binocular viewing, no systematic directional asymmetry was found for either the seeing or covered eye with monocular triangular stimulation in the six rabbits tested.

The mean gains of the 45°- and 135°-components and the calculated net gain of the seeing and covered eye during monocular stimulation are shown in Figs. 8 B and C, respectively. The pattern of modulation of the gains of the 45°- and 135°- components and the net gain of the *seeing* eye during monocular stimulation was very similar to that found for binocular viewing, except that all gains were reduced by a factor 2 (Fig. 8B).

Fig. 7 Eye movements in response to triangular stimulus motion (0.05 Hz, 20° peak-to-peak) about the 45° HA (A and C) and 135° HA (B and D) under binocular (A and B) and monocular (C and D) conditions. Notice the conjugate responses, with a gain close to unity, under binocular conditions (A and B), and the anisotropies in the responses during monocular stimulation (C and D).


stimulus
A
BINOCULAR 45° stimulation
↑ CW
L 45°
L 135°
↑ CW
R 45°
↑ CCW
R 135°
↑ CCW
B
BINOCULAR 135° stimulation
C
MONOCULAR 45° stimulation
↑ CW
↑ CW
↑ CCW
↑ CCW
D
MONOCULAR 135° stimulation
30°
20 s

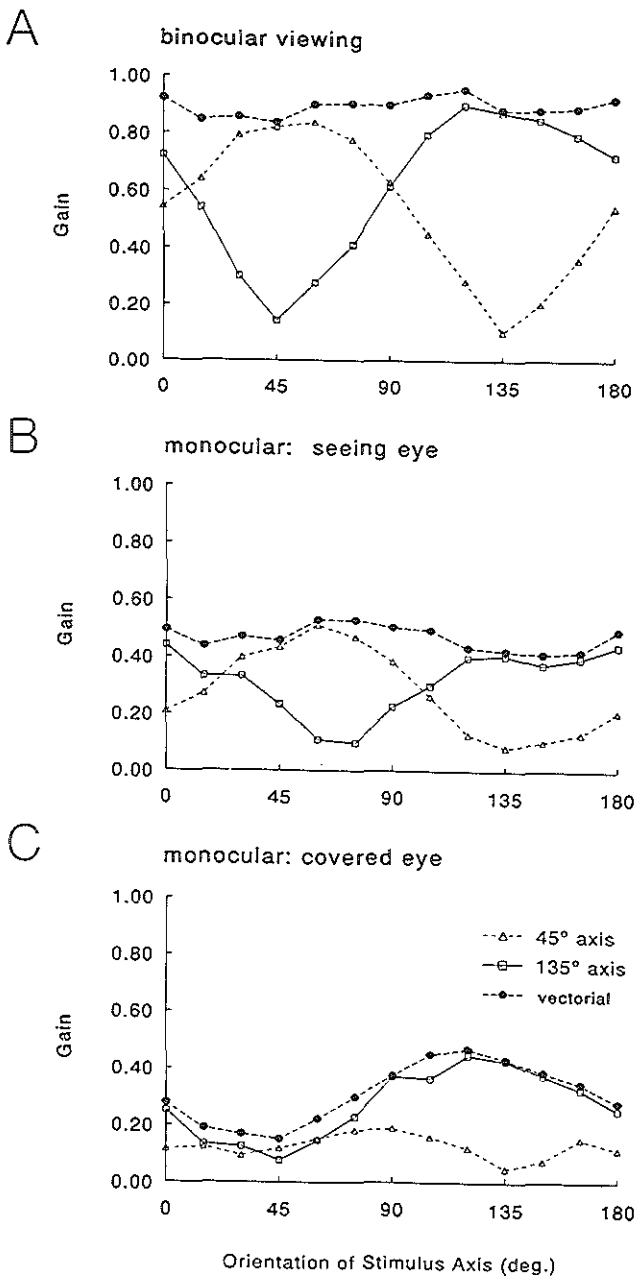


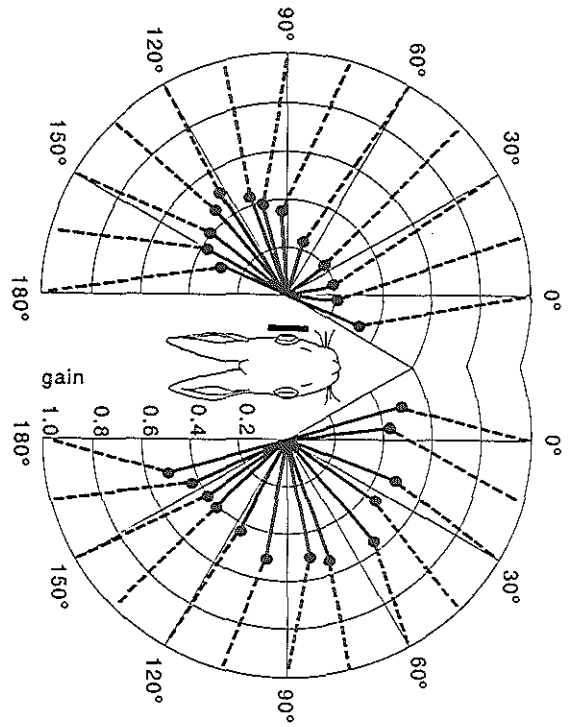
Fig. 8 Average gains of the 45° HA (dashed lines, triangles), 135° HA (solid lines, squares) components and the net gain (dashed lines, circles) as a function of stimulus axis orientation. A: binocular stimulation, with net gains close to unity for all stimulus axis orientations (n=6). B: responses of the seeing eye during monocular stimulation with a net gain of about 0.5 for all orientations of the stimulus axis (n=12). C: Responses of the covered eye with a maximal net gain at about 135° azimuth (n=12).

The gains of the two components were modulated sinusoidally as a function of stimulus axis orientation, with a 90° phase difference and nearly equal amplitudes (of about 0.5) for both curves. As a result, the net gain was of approximately constant magnitude for the entire range of horizontal axes.

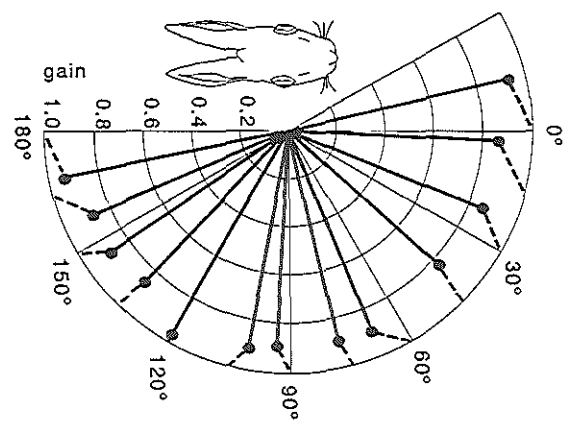
The modulation of the gain of the 135°-component of the *covered* eye as a function of stimulus-axis orientation was similar in direction and magnitude to that of the seeing eye (Fig. 8C). In contrast, the 45°-component of the response of the covered eye was very weak for all orientations of the stimulus axis. The gain of this component showed a minimum of 0.06 at 135° and never exceeded 0.2 for any stimulus axis orientation. As a result of the strong dominance of the 135°-component over the 45°-component, the net gain showed a pattern very similar to that of the dominant 135°-component.

The left panel of Fig. 9 depicts, for monocular stimulation, the net gain as a function of stimulus axis orientation for both the seeing and covered eye. The right, seeing eye responded with a net gain between 0.4 and 0.5 for stimulation about all horizontal axes, and the response axes deviated from the stimulus axis by a maximum of 22° during stimulation about the 15° HA (Figs. 9 and 10). The left, covered eye showed an anisotropic distribution of the gain, which was very different from the seeing eye. The pattern of distribution of the magnitude and angular deviation of the net response can be entirely accounted for by the weakness of the 45° component in the response of the covered eye, as shown in Fig. 8. The net gain, as a function of stimulus axis orientation, showed a modulation dominated by the 135° component, with a maximum around 135° HA and a minimum around 45° HA, re the covered, left eye. As a consequence, the response axis showed a deviation from the stimulus axis towards 135° (or -45°) azimuth for all orientations of the stimulus.

As pointed out before, the anisotropic responses of the covered eye led to strongly disconjugate eye movements when the seeing eye was stimulated about its 135° HA: the responses of the covered eye were very weak. Although stimulation about the 45° HA (re seeing eye) led to responses in the seeing and the covered eye that were equal in gain, the response axes of the two eyes were not collinear. While the response-axis of the seeing eye during stimulation about its 45° HA was displaced in the horizontal plane by 10° in the nasal direction, to an orientation of 35° azimuth, the response axis of the covered eye was oriented at 130° (re the covered eye), resulting in a net misalignment of 15°. A more pronounced misalignment was found with stimulation about the 0° HA. The response axis of the seeing eye was oriented at -17° azimuth (re seeing eye), while the response axis of the covered eye was oriented at -22° (re covered eye; Fig. 9), resulting in a net misalignment 39°.



Monocular viewing



Binocular viewing

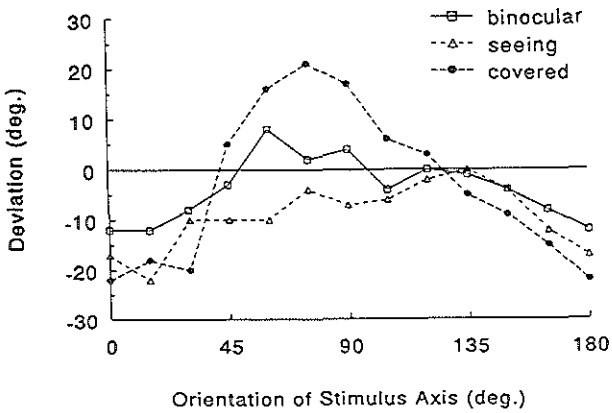


Fig. 10 Summary of the angular deviations of the computed response axes from the stimulus axes during triangular stimulus motion under binocular and monocular conditions. The angular deviation (in degrees) has been plotted as a function of stimulus axis orientation.

Discussion

The function of optokinetic eye movements is to reduce image motion across the retina during head movements. In this respect the optokinetic system works in close synergy with the vestibular reflexes evoked by the activation of the semicircular canals and otoliths. In the rabbit the accessory optic system (AOS) represents retinal signals underlying OKN in a reference frame that is geometrically similar to that of the semicircular canals and the extraocular muscles (Graf and Simpson, 1981; Simpson et al., 1981, 1988; Simpson and Graf, 1981, 1985; Graf et al., 1988; Soodak and Simpson, 1988). The behavior of the 3-D optokinetic eye movement responses described in this paper agrees well with this intrinsic organisation. In particular, the anisotropies of the optokinetic responses are related to the optimal response axes of the visual motion-sensitive cells described for the AOS, inferior olive (IO) and flocculus (Graf et al.,1988; Leonard et al., 1988; Simpson et al., 1988).

Fig. 9 Polar plots showing net gain and angular deviation of average responses to binocular (right panel) and monocular (left panel) triangular stimulation. The gains are plotted as line-segments along the computed response axes, which have been connected to the corresponding stimulus axis by an interrupted line. The right panel shows that the responses have a gain close to unity during binocular viewing. The left panel shows that for all stimulus axis orientations the gain of the seeing (right) eye is nearly uniform (about 0.5). In contrast, the gain of the covered (left) eye is anisotropic with respect to stimulus axis orientation, with a maximum at about 120° azimuth.

The present findings show that in the rabbit the optokinetic responses of each eye alone fall into two main categories: (1) the optokinetic eye

movements evoked by vertical axis (VA) stimulation, and (2) those elicited by rotation of the visual surround about a horizontal axis (HA). The different characteristics of the two categories as revealed by constant velocity stimulation and by triangular stimulation will be discussed below.

Constant velocity stimulation: vertical axis responses

As documented before (for a review see Collewijn, 1991) and confirmed in this study, VA optokinetic responses to constant speed stimulation under binocular conditions are characterized by a slow phase response in the direction of the stimulus motion, frequently and regularly interrupted by fast phases. The response is further characterized by a gradual build-up of the slow phase eye movement (Ter Braak, 1936; Collewijn, 1969, 1991). This velocity build-up, which in the rabbit is limited to about 1°/s² (Collewijn, 1969; Tan et al., 1992), has been attributed to the presence of a velocity storage mechanism (Cohen et al, 1977; Raphan et al., 1979; Raphan and Cohen, 1981, 1985).

Under monocular viewing conditions the VA response shows a directional preference for temporal-to-nasal stimulation. In the preferred direction the eye movements have similar characteristics as during binocular conditions. In the non-preferred direction the slow phase has a low gain, is only occasionally interrupted by resetting saccades, and velocity build-up is lacking. For monocularly elicited horizontal OKN in response to VA stimulation in the preferred direction, there was no improvement in the steady-state gain due to binocular viewing, confirming earlier reports by Collewijn (1969) in the rabbit and by Hess et al., (1985) in the rat. The reason for a lack of improvement may be that although a well-developed bidirectional optokinetic response requires binocular vision, the sensitivity of the VA optokinetic response in one direction is almost exclusively derived from one eye: a situation which leads to little summation for the steady state response. In binocular as well as monocular conditions the movements of both eyes remain essentially conjugate, although small differences (up to about 10%) between the seeing and covered eye can be observed, with the seeing eye moving faster than the indirectly driven eye, as has been reported before (rabbit: Collewijn and Noorduin, 1972b; pigeon: Gioanni, 1988; cat: Hamada, 1986).

Basically, the VA optokinetic responses show the characteristics of a velocity-servo system. In the preferred direction the velocity response of the system has a high gain at velocities below 10°/s, but gain gradually falls off at higher stimulus velocities, although responses have been found even at 120°/s (see Collewijn, 1991). In the non-preferred direction, velocity sensitivity falls off above 1-2°/s, and above 10°/s the response is absent.

Constant velocity stimulation: horizontal axis responses

Optokinetic responses of the rabbit about horizontal axes are, in several respects, less well developed than optokinetic responses about the vertical axis. The velocity of horizontal axis optokinetic responses is, in general, not sustained. Slow phases are characterized by an initial period with a velocity closely matching that of the stimulus, which is followed by a drop in eye velocity as the eyes reach a more eccentric position in the orbit. The eye excursions in the orbit are often larger than 20°, with the eye remaining locked in such eccentric positions until the occurrence of a resetting saccade. Similarly to the monocular VA responses in the non-preferred direction, the eye movements in response to HA-stimulation have no velocity build-up. Another similarity between the HA-responses and the non-preferred VA-responses is that both are maximally sensitive to velocities of 1-2 °/s. Aspects of this general shape of the optokinetic eye movements in response to constant speed drum rotation about a horizontal axis have been described before by Erickson and Barmack (1980) and Collewijn and Noorduin (1972a). Because the velocity of the response is not constant, but falls to zero as the eyes reach their extreme position in the orbit, the gain of the response measured over a longer time depends critically on the occurrence of resetting fast phases. During binocular stimulation, fast phases are predominantly generated about the 135°-axis in response to both directions of drum rotation. During monocular stimulation, the generation of resetting saccades depends on the direction of drum rotation. With the stimulus axis oriented about the 135°-axis of the seeing eye, CCW drum rotation produced an initial response followed by a nystagmus with regularly occurring fast phases, whereas CW drum rotation produced only an initial response followed by the locking of the eye in an extreme position. With the drum axis oriented at 45° re the seeing eye, the response of the seeing eye was relatively weak during CCW drum rotation. The response of the contralateral, covered eye about the (collinear) 135°-axis, however, consisted of a regular nystagmus with well-developed fast phases. In contrast, during CW drum rotation no fast phases occurred, and both eyes deviated to an extreme position.

With regard to the direction of rotation preferences, a similarity exists between the optokinetic responses to monocular HA and VA stimulation. Visual motion about a vertical axis in the temporal-to-nasal direction develops a more pronounced nystagmus with a regular sequence of slow and fast phases than does motion in the nasal-to-temporal direction. As pointed out earlier, the response in the preferred (temporal-to-nasal) direction acts to reduce retinal slip occurring during head rotation that would excite the horizontal canal ipsilateral to the seeing eye. For the HA optokinetic responses a regular nystagmus, likewise, developed exclusively for visual world rotation in the direction corresponding to head rotation that would excite the anterior canals

ipsilateral to the well-responding eye. For the oppositely directed rotation, fast phases were scarce and the eye tended to remain in an eccentric position, analogous to the VA optokinetic response for motion in the non-preferred (nasal-to-temporal) direction.

The rotational axis of the eye movements in response to constant speed HA stimulation is generally not collinear with the stimulus axis. For instance, stimulation about the 0°-(roll)axis, which should ideally induce eye rotations with equal magnitudes about the 45°- and 135°-axes, induces rotations in each eye with a preponderance of the 135°-axis component. Thus, the two eyes are rotating in a nonconjugate way, each about an axis close to its 135°-axis.

The sensitivity to only low velocities, the absence of velocity-storage and the infrequent occurrence of saccades indicate that the HA optokinetic system does not act as a velocity-control system, but as a position-control system. The limited range and bandwidth of the control for constant velocity optokinetic stimulation about horizontal axes imposes no constraints on the physiological value of this control system in eye-position control, because natural head rotations about horizontal axes are limited in extent and, in addition, the otoliths contribute significantly to position control for non-vertical axis rotations (Barmack, 1981; Van der Steen and Collewijn, 1984). When these physiological constraints of head movements are taken into account, the initial slow phase eye movements in response to optokinetic stimulation about any horizontal axis are the most important ones.

Optokinetic responses to triangular drum motion

Triangular drum motion provided a stimulus avoiding the saturation of the optokinetic eye movements which was observed during constant velocity stimulation. The physiological relevance of a stimulus corresponding to a limited range of rotation is underlined by our finding that, under binocular seeing conditions, the gain of the optokinetic response to triangular motion about any horizontal axis is only slightly below unity.

However, a large difference exists between the gain of the optokinetic responses during HA-stimulation under binocular and monocular seeing conditions: a 50% reduction of the gain occurred under monocular conditions. Summation effects as a result of binocular stimulation have been found for VA-stimulation in the goldfish (Easter, 1972) and the pigeon (Gioanni, 1988), but are absent for VA stimulation in the rabbit.

With binocular triangular optokinetic stimulation, no anisotropy or directional preference was encountered and adequate responses of both eyes to stimulation about all horizontal axes were elicited. Dominance of the 135° HA responses of the covered eye appeared with monocular stimulation. Monocular HA stimulation about the 45°

HA of the seeing eye elicited a conjugate movement of the two eyes, while stimulation about the 135° HA of the seeing eye elicited a response of the seeing eye that was substantially greater than that of the covered eye (see Fig. 9).

Similarly as during constant velocity stimulation, during triangular stimulation the alignment between the stimulus-axis and the response-axis is not perfect. The misalignment is maximal for stimulation about the 0°-axis, which corresponds to roll-axis stimulation. Similarly to what was found for constant velocity stimulation, disconjugacy occurs with each eye rotating about an axis shifted towards 135° azimuth. The misalignment between eye rotation axis and stimulus axis is different for monocular and binocular seeing conditions. During binocular seeing the maximal misalignments do not exceed 10°, whereas under monocular conditions the misalignment can amount to up to 39°.

Vertical-axis optokinetic responses in relation to the VOR

Understanding the behavior of the optokinetic eye movement responses requires a discussion of the intrinsic organisation of the pathways conveying optic flow signals and their relation with the VOR pathways. It has long been recognized that the primary vestibular signals from the horizontal semicircular canals carry the integrated acceleration-vector of head rotation about the vertical axis (Fernandez and Goldberg, 1971; Goldberg and Fernandez, 1971; Wilson and Melvill Jones, 1979), thus representing angular velocity, provided that head velocities are not sustained. Insufficient compensation through the VOR results in residual retinal slip, which is sensed by speed- and direction-selective retinal ganglion cells (Oyster, 1968). They provide, in addition to the vestibular sensing of head rotation, a visual measure of self-motion. The signals originating from ganglion cells tuned to low speed horizontal motion are conveyed to the contralateral nucleus of the optic tract (NOT) and the dorsal terminal nucleus (DTN). Cells in these nuclei are excited by temporal-to-nasal motion (Simpson, 1984; Soodak and Simpson, 1988), while inhibition occurs with stimulation in the nasal-to-temporal direction. One of the major projections of the DTN and NOT is to the dorsal cap of Kooy of the inferior olive (IO) (Giolli et al, 1984, 1985; Takeda and Maekawa, 1976), which in turn is the origin of the majority of the climbing-fiber projection to the flocculus. The floccular Purkinje-cells that receive a climbing fiber input selective for horizontal motion send an inhibitory output to target neurons in the medial vestibular nucleus. By modulation of this inhibitory output the flocculus influences the activity of the medial and lateral rectus muscles of predominantly the ipsilateral eye.

Electrical microstimulation of particular zones in the flocculus elicits abduction of the ipsilateral eye, as a result of the increased floccular output inhibiting excitatory

vestibular pathways to the ipsilateral medial rectus and inhibiting inhibitory pathways to the ipsilateral lateral rectus muscle (Ito et al., 1970, 1973, 1977; Simpson et al., 1989; Van der Steen et al., 1991). Under more natural conditions the modulation of the floccular output is achieved by the usually reciprocal modulation of complex and simple spike activities. With monocular optokinetic stimulation, a directional preference exists for motion of the visual surround in the temporal-to-nasal direction relative to the seeing eye. This stimulus direction causes an enhancement of the climbing fiber activity in the flocculus ipsilateral to the seeing eye, with simultaneous suppression of the simple-spike activity (Leonard, 1986; Leonard and Simpson, 1986). The net effect of these changes in activity is a reduced Purkinje-cell activity. The resulting decreased inhibition of the horizontal canal-to-horizontal recti pathway leads to adduction of the seeing eye.

Horizontal axis optokinetic responses in relation to the VOR

One of the prominent findings of the HA-stimulation experiments is that disconjugate eye movements occur with monocular viewing. This behavior can be understood by considering together the intrinsic organisation of the pathways conveying optic flow signals through the flocculus and the selectivity of the flocculus for inhibiting only some of the VOR pathways. Excitation of the anterior canal results in excitation of the ipsilateral superior rectus and the contralateral inferior oblique muscles, whereas excitation of the posterior canal results in excitation of the ipsilateral superior oblique and contralateral inferior rectus muscles. In each case the antagonist muscle is concomitantly inhibited. Given the geometric organisation of the muscles, activation of the anterior canal would lead to a rotation of the ipsilateral eye about an axis close to 135° azimuth and an accompanying rotation of the contralateral eye about an axis close to 45° azimuth. Activation of the posterior canal would result in ipsilateral eye rotation about an axis close to 45° azimuth and in contralateral eye rotation about an axis close to 135° azimuth (Simpson and Graf, 1981).

In anatomical and physiological studies on the pathways conveying visual information about self-motion it has been shown that in the flocculus, in addition to VA Purkinje cells, two HA Purkinje cell-classes exist:
(1) A class of Purkinje cells that is best modulated by rotational optic flow presented to the ipsilateral eye at an axis close to 135°azimuth. For the non-dominant (contralateral) eye the preferred axis is oriented at about 45°azimuth. (2) A class of Purkinje cells that is best modulated by optic flow rotating about the contralateral 45°-axis; the ipsilateral eye either provides no input or a nondominant input that is greatest for rotation about an axis between 45° and 90°. The orientation of the dominant preferred axis for the climbing fiber responses of the two classes of HA Purkinje cells

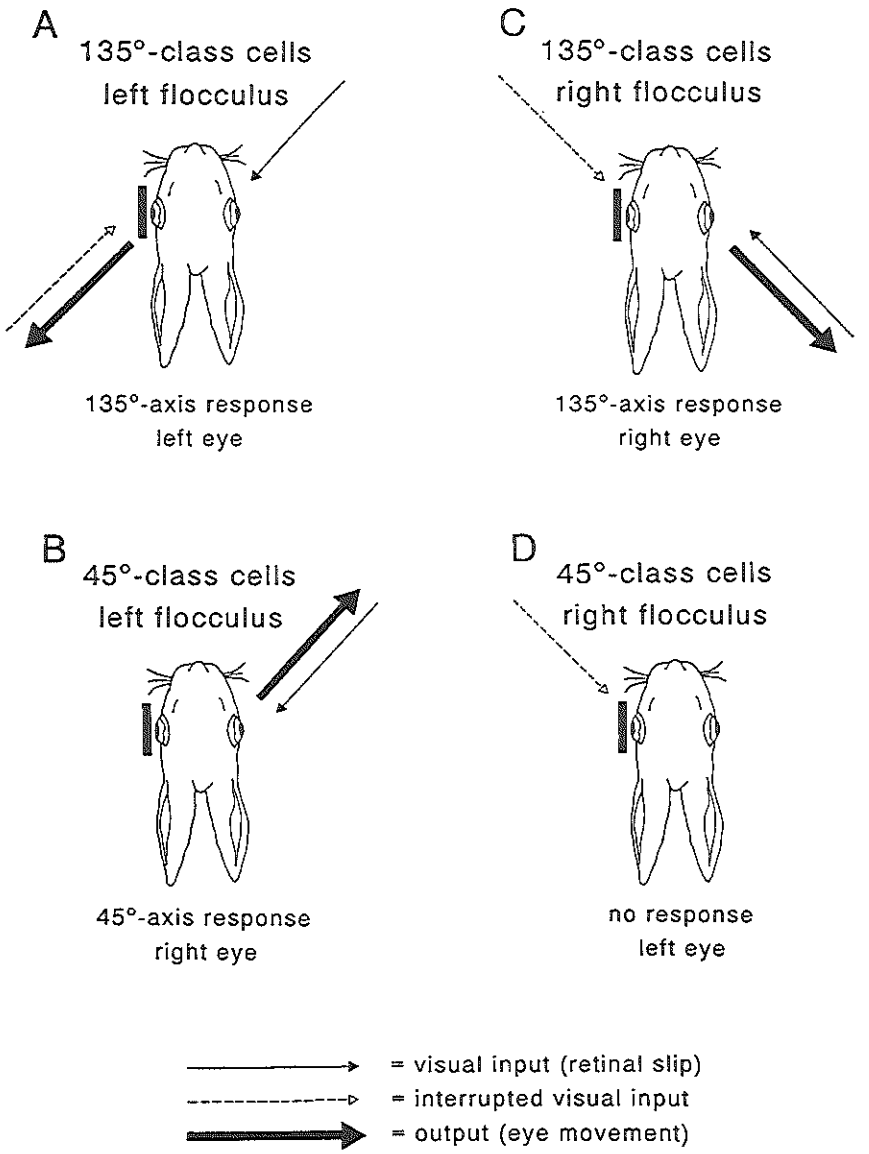
is geometrically in accord with the finding of Ito et al. (1977) that the flocculus directly inhibits the ipsilateral anterior canal VOR pathways, but not the ipsilateral posterior canal pathways. That is, the best response axis of the anterior canal makes an angle of about 135° azimuth on the ipsilateral side and an angle of about 45° azimuth on the contralateral side.

Recently, it has been found that electrical microstimulation in the flocculus likewise elicits predominantly eye movements corresponding to horizontal and anterior canal pathways and not those corresponding to posterior canal pathways (Van der Steen et al., 1991). The floccular control of the ipsilateral anterior canal pathways, but not the ipsilateral posterior canal pathways, is in line with the finding that each flocculus shows preferred horizontal-axes responses collinear with the 135° HA of the ipsilateral eye.

In conclusion, the difference in response pattern for 45° and 135° HA stimulation suggests that the flocculus contralateral to the seeing eye relays optokinetic information to both eyes for 45°-axis stimulation, whereas the flocculus ipsilateral to the seeing eye controls only the ipsilateral seeing eye for 135°-axis stimulation. To account for this arrangement we propose that each optic flow channel controls predominantly the eye that provides its dominant visual input to that eye.

The plausibility of this hypothesis is shown by relating responses of known Purkinje cell classes of the flocculus with the eye movement responses during 45° HA and 135° HA monocular stimulation (Fig. 11). Stimulation of the right, seeing eye about its 45° HA elicits a conjugate response of the two eyes (see Fig. 9). Visual flow caused by this stimulus would be mediated, in part, by the 135°-class of Purkinje cells in the left flocculus (Fig. 11A). With binocular viewing, this class of Purkinje cells would receive a dominant input from the left (ipsilateral) eye and a nondominant input from the right (contralateral) eye. However, since the left eye is covered, only an input from the nondominant, contralateral eye is present (solid input line, Fig. 11A). Since the dominant input of this class of Purkinje cells originates from the currently covered eye, the 45° HA input from the seeing eye influences, according to the hypothesis above, predominantly the covered eye (thick solid line Fig. 11A). The accompanying response of the right, seeing eye is associated with the 45°-class of Purkinje cells located in left flocculus (Fig. 11B), because this Purkinje cell class receives its dominant input from the currently seeing, right eye. Thus, the hypothesis indicates that the conjugate responses of seeing and covered eyes found with 45° HA monocular stimulation are in fact controlled by separate pathways, through different Purkinje cell classes in the flocculus contralateral to the seeing eye.

The hypothesis also accounts for the dissociated responses found for monocular stimulation about the 135° HA. With this stimulus condition, the response of the seeing


MONOCULAR VIEWING
45°-axis stimulation
135°-axis stimulation
A
135°-class cells
left flocculus
135°-axis response
left eye
C
135°-class cells
right flocculus
135°-axis response
right eye
B
45°-class cells
left flocculus
45°-axis response
right eye
D
45°-class cells
right flocculus
no response
left eye
= visual input (retinal slip)
= interrupted visual input
= output (eye movement)

eye is robust, whereas the response of the covered eye is virtually absent (see Fig. 9). In this case, the 135°-class Purkinje cells of the flocculus ipsilateral to the seeing eye would be stimulated through the dominant eye, resulting in a response of predominantly the ipsilateral, seeing eye (Fig. 11C). With 135° HA monocular stimulation of the right eye, no input goes to the Purkinje cell class in the right flocculus that controls the 45° HA component of the left, covered eye, and this eye consequently shows only very little response (Fig. 11D). Thus, the hypothesis nicely links the geometrical pattern of laterality of floccular Purkinje cell responses to rotating optic flow with the responses to monocular, triangular stimulation.

In summary, this study of 3-D optokinetic responses provides support, at the behavioral level, for the idea that in the rabbit the optokinetic system is organized in a reference frame similar to that of the semicircular canals. The organization of the visual input to the optokinetic system is such that each eye is not fully represented on its own side of the body. Therefore, functionally complete responses to optokinetic stimulation rotating about an arbitrary axis are achieved only by the synthesis of the representations derived from the two eyes.

Summary and conclusions

1. Three-dimensional rotations of both eyes were measured in alert rabbits during optokinetic stimulation about axes lying in the horizontal plane or about an earth-vertical axis, with either one or both eyes viewing the stimulus. Optokinetic stimulus speed was 2°/s, either continuous or alternating in polarity (triangular stimulus). In addition to the gains of the responses, the orientations of the response axes, relative to the stimulus axes, were determined.

2. In comparison to the response to constant speed optokinetic stimulation about the vertical axis, the response to constant speed optokinetic stimulation about any horizontal axis was characterized by the lack of a speed build-up and the sporadic fast phases. Slow phase tracking was good as long as the eye was within the central oculomotor range, but deteriorated as eye deviation saturated in eccentric positions. These features suggest that the optokinetic reflex about horizontal axes functions as a position-control system, rather than a velocity-control system.

Fig. 11 Diagram showing the correlation between horizontal axis optokinetic responses and known classes of Purkinje cells in the flocculus, responding to retinal image-slip around the 45° and 135° axes, for the condition of monocular viewing. For further explanation see text.

3. Binocular optokinetic stimulation at constant speed (2°/s) about the roll axis (0° azimuth horizontal axis) elicited disconjugate responses. Although the gain of the response was not significantly different in the two eyes (0.38 for downward and 0.44 for upward stimulation), the response axes of the two eyes differed by as much as 51°.

4. Monocular horizontal axis optokinetic stimulation at constant speed elicited responses that were grossly dissociated between the two eyes. The magnitude of the responses was anisotropic in that it varied with the azimuth direction of the stimulus axis; the maximum for each eye (0.44 for the seeing and 0.33 for the covered eye) was at 135° azimuth. The orientation and direction (sense of rotation) of the optokinetic stimulus eliciting the maximal response for each eye coincided with the optic flow normally associated with the maximal excitation of the corresponding ipsilateral anterior canal. This relation suggests that optokinetic nystagmus in response to constant speed stimulation about horizontal axes is produced by visual interaction with exclusively the anterior canal pathways.

5. Binocular, triangular optokinetic stimulation with small excursions (± 10°), which avoided the saturation problems of constant-speed stimulation, elicited adequate responses without systematic directional asymmetries. Gain was about 0.9 for all stimulus axis orientations in the horizontal plane.

6. During monocular stimulation with triangular stimuli, the initial response of the seeing eye showed a gain of about 0.5 for all orientations of the stimulus axis. The covered eye showed anisotropic responses with a maximum gain of about 0.5 during stimulation of the seeing eye about its 45° axis. In view of the arrangement of the optokinetic neural pathways in three pairs of optic flow channels, this response pattern suggests that each optic-flow channel controls primarily that eye from which it receives its dominant visual input.

7. The anisotropies encountered with monocular triangular stimulation are likely to be the behavioral consequences of an intrinsic optokinetic system that is organized in a reference frame similar to that of the semicircular canals and extra-ocular muscles.

References

BAARSMA, E.A. AND COLLEWIJN, H. Vestibulo-ocular and optokinetic reactions to rotation and their interaction in the rabbit. *J. Physiol. Lond.* 238: 603-625, 1974.

BARMACK, N.H., A comparison of the horizontal and vertical vestibulo-ocular reflexes of the rabbit. *J. Physiol. Lond.* 314: 547-564, 1981.

COHEN, B., MATSUO, V., AND RAPHAN, T. Quantitative analysis of the velocity characteristics of optokinetic nystagmus and optokinetic after-nystagmus. *J. Physiol. Lond.* 270: 321-344, 1977.

COLLEWIJN, H. Optokinetic eye movements in the rabbit: input-output relations. *Vision Res.* 9: 117-

132, 1969.
COLLEWIJN, H. Eye- and head movements in freely moving rabbits. *J. Physiol. Lond.* 266: 471-498, 1977.
COLLEWIJN, H. *The Oculomotor System of the Rabbit and its Plasticity.* Springer, Berlin, 1981.
COLLEWIJN, H. The optokinetic contribution. In: *Eye Movements*, edited by R.H.S. Carpenter. Vol. 8 in "Vision and Visual Disfunction, Macmillan, London, pp. 45-70, 1991.
COLLEWIJN, H. AND NOORDUIN, H. Vertical and torsional optokinetic eye movements in the rabbit. *Pflügers Arch.* 332: 87-95, 1972a.
COLLEWIJN, H. AND NOORDUIN, H. Conjugate and disjunctive optokinetic eye movements in the rabbit, evoked by rotatory and translatory motion. *Pflügers Arch.* 335: 173-185, 1972b.
EASTER, S.S. Pursuit eye movements in goldfish (*Carassius auratus*). *Vision Res.* 12: 673-688, 1972.
ERICKSON, R.G. AND BARMACK, N.H. A comparison of the horizontal and vertical optokinetic reflexes of the rabbit. *Exp. Brain Res.* 40: 448-456, 1980.
FERNANDEZ, C. AND GOLDBERG, J.M. Physiology of peripheral neurons innervating semicircular canals of the squirrel monkey. II. Responses to sinusoidal stimulation and dynamics of the peripheral vestibular system. *J. Neurophysiol.* 34: 661-675, 1971.
GIOANNI, H. Stabilizing gaze reflexes in the pigeon (*Colombia livia*). I. Horizontal and vertical optokinetic eye (OKN) and head (OCR) reflexes. *Exp. Brain Res.* 69: 567-582, 1988.
GIOLLI, R.A., BLANKS, R.H.I., AND TORIGOE, Y. Pretectal and brain stem projections of the medial terminal nucleus of the accessory optic system of the rabbit and rat as studied by anterograde and retrograde neuronal tracing methods. *J. Comp. Neurol.* 227: 228-251, 1984.
GIOLLI, R.A., BLANKS, R.H.I., TORIGOE, Y., AND WILLIAMS, D.D. Projections of medial terminal accessory optic nucleus, ventral tegmental nuclei, and substantia nigra of rabbit and rat as studied by retrograde axonal transport of horseradish peroxidase. *J. Comp. Neurol.* 232: 99-116, 1985.
GOLDBERG, J.M. AND FERNANDEZ, C. Physiology of peripheral neurons innervating semicircular canals of the squirrel monkey. I. Resting discharge and response to constant angular accelerations. *J. Neurophysiol.* 34: 635-660, 1971.
GRAF, W. AND SIMPSON, J.I. The relations between the semicircular canals, the optic axis, and the extraocular muscles in lateral-eyed and frontal-eyed animals. In: *Progress in Oculomotor Research*, edited by A. Fuchs and W. Becker. Elsevier, Amsterdam, pp. 411-420, 1981.
GRAF, W., SIMPSON, J.I., AND LEONARD, C.S. Spatial organization of visual messages of the rabbit's cerebellar flocculus. II. Complex and simple spike responses of Purkinje cells. *J. Neurophysiol.* 60: 2091-2121, 1988.
HAMADA, T. Non-conjugate monocular optokinetic nystagmus in cats. *Vision Res.* 26: 1311-1314, 1986.
HESS, B.J.M., PRECHT, W., REBER, A., AND CAZIN, L. Horizontal optokinetic ocular nystagmus in the pigmented rat. *Neuroscience* 15: 97-107, 1985.
ITO, M., HIGHSTEIN, S.M., AND FUKUDA, J. Cerebellar inhibition of the vestibulo-ocular reflex in rabbit and cat and its blockage by picrotoxin. *Brain Res.* 17: 524-526, 1970.
ITO, M., NISIMARU, N. AND YAMAMOTO, M. Specific neural connections for the cerebellar control of vestibulo-ocular reflexes. *Brain Res.* 60: 238-243, 1973.
ITO, M., NISIMARU, N., AND YAMAMOTO, M. Specific patterns of neuronal connections involved in the control of the rabbit's vestibulo-ocular reflexes by the cerebellar flocculus. *J. Physiol. Lond.* 265:

833-854, 1977.
LEONARD, C.S. *Signal Characteristics of Cerebellar Purkinje Cells in the Rabbit Flocculus During Compensatory Eye Movements* (PhD dissertation). New York: New York University, 1986.
LEONARD, C.S. AND SIMPSON, J.I. Simple spike modulation of floccular Purkinje cells during the reversible blockade of their climbing fiber afferents. In: *Adaptive processes in Visual and Oculomotor Systems*, edited by E.L. Keller and D.S. Zee. Oxford, UK: Pergamon, 1986, pp. 429-434.
LEONARD, C.S. SIMPSON, J.I., AND GRAF, W. Spatial organization of visual messages of the rabbit's cerebellar flocculus. I. Typology of inferior olive neurons of the dorsal cap of Kooy. *J. Neurophysiol.* 60: 2073-2090, 1988.
OYSTER, C.W. The analysis of image motion by the rabbit retina. *J. Physiol.* 199: 613-635, 1968.
OYSTER, C.W., TAKAHASHI, E., AND COLLEWIJN, H. Direction-selective retinal ganglion cells and control of optokinetic nystagmus in the rabbit. *Vision Res.* 12: 183-193, 1972.
PELLIONISZ, A. AND LLINÁS, R. Brain modeling by tensor network theory and computer simulation. The cerebellum: distributed processor for predictive coordination. *Neuroscience* 4: 323-348, 1979.
PELLIONISZ, A. AND LLINÁS, R. Tensorial approach to the geometry of brain function: cerebellar coordination via a metric tensor. *Neuroscience* 5: 1125-1136, 1980.
RAPHAN, T. AND COHEN, B. The role of integration in oculomotor control. In: *Models of Oculomotor Behaviour and Control*, edited by B. Zuber. West Palm Beach, FL: CRC Press, pp.91-109, 1981.
RAPHAN, T. AND COHEN, B. Velocity storage and the ocular response to multidimensional vestibular stimuli. In: *Adaptive Mechanisms in Gaze Control*, edited by A. Berthoz and G. Melvill Jones. Amsterdam: Elsevier, pp. 123-143, 1985.
RAPHAN, T., MATSUO, V., AND COHEN, B. Velocity storage in the vestibulo-ocular reflex arc (VOR). *Exp. Brain Res.* 35: 229-248, 1979.
ROBINSON, D.A. A method of measuring eye movement using a scleral search coil in a magnetic field. *IEEE Trans. Biomed. Electronics* BME-10: 137-145, 1963.
SIMPSON, J.I. The accessory optic system. *Ann. Rev. Neurosci.* 7: 13-41, 1984.
SIMPSON, J.I. AND GRAF, W. Eye muscle geometry and compensatory eye movements in lateral-eyed and frontal-eyed animals. *Ann. NY Acad. Sci.* 374: 20-30, 1981
SIMPSON, J.I. AND GRAF, W. The selection of reference frames by nature and its investigators. In: *Adaptive Mechanisms in Gaze Control*, edited by A. Berthoz and G. Mellvill Jones. Elsevier, Amsterdam, pp. 3-16, 1985.
SIMPSON, J.I., GRAF, W., AND LEONARD, C. The coordinate system of visual climbing fibers to the flocculus. In: *Progress in Oculomotor Research, Developments in Neuroscience*, vol. 12, edited by A. Fuchs and W. Becker. Elsevier, Amsterdam, pp. 475-484, 1981.
SIMPSON, J.I., LEONARD, C.S., AND SOODAK, R.E. The accessory optic system of rabbit. II. Spatial organization of direction selectivity. *J. Neurophysiol.* 60: 2055-2072, 1988.
SIMPSON, J.I, VAN DER STEEN, J., TAN, J. GRAF, W. AND LEONARD, C.S. Representations of ocular rotations in the cerebellar flocculus of the rabbit. In: *Progress in Brain Research*, Vol 80, edited by J.H.J. Allum and M. Hulliger, eds. Elsevier, Amsterdam, pp. 213-223, 1989.
SOODAK, R.E. AND SIMPSON, J.I., The accessory optic system of rabbit. I. Basic visual response properties. *J. Neurophysiol.* 60: 2037-2054, 1988.

TAKEDA, T. AND MAEKAWA, K. The origin of pretecto-olivary tract. A study using the horseradish peroxidase method. *Brain Res.* 117: 319-325, 1976.

TAN, H.S., COLLEWIJN, H., AND VAN DER STEEN, J. Optokinetic nystagmus in the rabbit and its modulation by bilateral microinjection of carbachol in the cerebellar flocculus. Under review, *Exp. Brain Res.*, 1992.

TER BRAAK, J.W.G. Untersuchungen über optokinetischen Nystagmus. *Arch. Néerl. Physiol.* 21: 309-376, 1936.

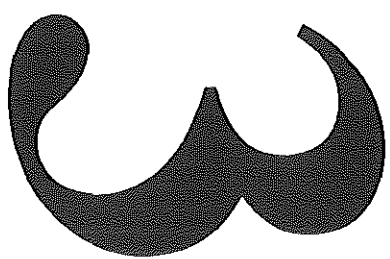
VAN DER STEEN, J. AND COLLEWIJN, H. Ocular stability in the horizontal, frontal and sagittal planes in the rabbit. *Exp. Brain Res.* 56: 263-274, 1984.

VAN DER STEEN, J., SIMPSON, J.I., AND TAN, J. Representation of three-dimensional eye movements in the cerebellar flocculus of the rabbit. In: *Oculomotor Control and Cognitive Processes*, edited by R. Schmid and D. Zambarbieri. Elsevier, Amsterdam, pp. 63-77, 1991.

VAN NEERVEN, J., POMPEIANO, O., COLLEWIJN, H., AND VAN DER STEEN, J. Injections of β-noradrenergic substances in the flocculus of rabbits affect adaptation of the VOR gain. *Exp. Brain Res.* 79: 249-260, 1990.

WALLMAN, J. AND VELEZ, J. Directional asymmetries of optokinetic nystagmus: developmental changes and relation to the accessory optic system and to the vestibular system. J. Neurosci. 5(2): 317-329, 1985.

WILSON, V.J. AND MELVILL JONES, G. *Mammalian Vestibular Physiology.* Plenum, New York, 1979.



Cholinergic modulation of optokinetic and vestibulo-ocular responses: a study with microinjections in the flocculus

Introduction

Despite a large body of evidence that acetylcholine (ACh) and its related enzyme systems are present in the cerebellum, the role of ACh in cerebellar neurotransmission has remained elusive. In the present paper we investigate the possibility that ACh is involved in the regulation of the gain of vestibulo-ocular and optokinetic reflexes by the cerebellar flocculus.

A possible role of ACh as a cerebellar neurotransmitter is suggested by the biochemical demonstration of substantial amounts of ACh (Israel and Whittaker, 1965; Kása et al., 1982), acetylcholinesterase (AChE; Goldberg and McCaman, 1967; Kása and Silver, 1969) and choline-acetyltransferase (ChAT; Goldberg and McCaman, 1967; Kása and Silver, 1969; Hayashi, 1987) in the cerebellum.

More detailed histochemical and immunocytochemical techniques have revealed specific distribution patterns of the cholinergic system between cerebellar lobules, layers, cells and fibers. Although these patterns show considerable species differences, the archicerebellum (lobules IX and X) stand out as a site with high concentrations of AChE and ChAT (Kása and Silver, 1969; Kása et al., 1982; Ojima et al., 1989). Furthermore, ChAT, the more reliable presynaptic marker, has been located in a subpopulation of mossy fiber terminals in the granular layer of the cerebellar cortex (Karen Kan et al., 1978; 1980; Ojima et al., 1989; Illing, 1990). In the rat, the immunohistochemical study of Ojima et al. (1989) showed that ChAT-immunoreactive glomerular rosettes are most numerous in the vermal lobules IX and X and in the flocculus. The mossy fibers in these areas of the cerebellum originate mainly from the vestibular complex (except from the lateral vestibular nucleus) and from the prepositus hypoglossi nucleus (see Epema et al., 1990). In an immunohistochemical double-labelling study, Barmack et al. (1986) demonstrated the cholinergic nature of a subset of secondary vestibulo-cerebellar neurons, located in the caudal half of the medial and descending vestibular nuclei and in the prepositus hypoglossi nucleus and projecting to the cerebellar flocculus and nodulus. Thus, the vestibular complex appears to be a source of cholinergic mossy fibers to the cerebellar flocculus.

In addition to mossy fibers, other cholinergic elements have been encountered in the cerebellar cortex. Thin varicose fibers, closely associated with the Purkinje-cell layer, similar fibers in the molecular layer (rat: Ojima et al., 1989; cat: Illing, 1990) and a subpopulation of Golgi cells (cat: Illing, 1990) were found to be ChAT-immunoreactive, which suggests a cholinergic nature of these structures.

The functionality of the presynaptic cholinergic elements described above is further supported by the identification of muscarinic receptors of the M_2 type (Mash and Potter, 1986; Spencer et al., 1986; Neustadt et al., 1988) and nicotinic receptors (Hunt and Schmidt, 1978; Swanson et al., 1987). The nicotinic receptors appear to be located in the

granular layer in the rat (Swanson et al., 1987). In the rabbit, muscarinic receptors, labelled by [^{3}H]quinuclidinyl benzilate (Neustadt et al., 1988) in lobules IX and X, including the flocculus, were most dense in the Purkinje cell layer; moderate and low densities were present over the granular and molecular layers, respectively. Furthermore, parasagittal columns of very high density were present over the molecular layer of several cerebellar cortical regions, including the vermis and hemispheres of lobules IX and X.

In comparison to these detailed histochemical studies, the physiological actions of acetylcholine in the cerebellum have remained unclear (see also Ito, 1984). Iontophoretic application of ACh has been reported to mildly excite Purkinje cells in the cat (Crawford et al., 1966; McCance and Phillis, 1968), while a more recent study by De la Garza et al. (1987) with pressure-ejected ACh reported inhibition of Purkinje cells in the rat. For granular cells, no effect of ACh was found by Crawford et al. (1966), while excitation was reported by McCance and Phillis (1968). For interneurons, no effect of ACh was found by Crawford et al. (1966), whereas De la Garza et al. (1987) found a strong excitatory effect. Also the reports on the receptor-types involved in the ACh-effects are inconsistent. Crawford et al. (1966) reported that their effects were muscarinic, while McCance and Phillis (1968) found that they were nicotinic. Crepel and Dhanjal (1982) found a slow depolarization of Purkinje cells, accompanied by an increase in membrane resistance, with application of high doses of ACh in slices of lobules IX and X of the rat's cerebellum; this effect was blocked by atropine and thus muscarinic in nature. De la Garza et al. (1987) found that the effects of ACh on Purkinje cells and interneurons were mimicked by nicotine. The inhibitory effects of ACh on Purkinje cells were abolished only by a combination of scopolamine and hexamethonium; thus, they appeared to be mixed muscarinic and nicotinic (ganglion type). The excitatory action of ACh on interneurons, on the other hand, was effectively blocked by curare, a blocker of the neuro-muscular nicotinic receptor type.

In summary, the physiological actions of ACh in the cerebellar cortex are controversial down to the single unit level and it is not even clear that ACh acts as a synaptic transmitter. It has also been suggested that ACh may act as a neuro-modulator (see Ito, 1984). No specific role of the cholinergic system in cerebellar signal-processing has been demonstrated.

The cerebellar flocculus is known to be intimately involved in the control of the gain-characteristics of the optokinetic (OKR) and vestibulo-ocular (VOR) reflexes. In the rabbit, lesioning of the flocculus (Ito et al., 1982; Ito, 1984) or its temporary functional ablation by the intrafloccular microinjection of GABA-agonists (van Neerven et al., 1989) have been reported to result in a substantial decrease in the gain of the OKR and the VOR. Barmack and Pettorossi (1985) found, after unilateral flocculectomy in the rabbit, a long-term reduction in the gain of the OKR, without a reduction in the gain of the

VOR. As the rabbit's vestibulo-cerebellum contains muscarinic receptors (Neustadt et al., 1988) and ChAT-positive mossy fibers, part of which seems to originate from secondary vestibulo-cerebellar neurons in the brainstem vestibular complex (Barmack et al., 1986), we investigated the effects of some cholinergic agonists and antagonists on the floccular control of the gain of the VOR and the OKR.

The present study will show that the intrafloccular micro-injection of carbachol, a potent aselective cholinomimetic agent, produces a very pronounced increase in the gain of the OKR and a distinct increase in the gain of the VOR. Micro-injection of eserine, an AChE inhibitor, produced a similar, but smaller increase, whereas the ACh antagonists mecamylamine and atropine caused a significant decrease in the gain of the OKR. These results suggest a role of acetylcholine in the control of signal-processing in the flocculus.

Methods

General

Young adult, pigmented Dutch belted rabbits (n=9) of either sex were used. All surgical procedures were done under general anesthesia, induced by a mixture of ketamine (Nimatek, 100 mg/ml, AUV, Holland), acepromazine 1% (Vetranquil, 10 mg/ml, Sanofi, France) and xylazine-HCl (Rompun 2%, 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 of xylazine-HCl were given intramuscularly. These initial doses, which maintained an adequate anesthesia for about 1 h, were supplemented as necessary.

Measurement of eye movements

Eye movements were measured with a magnetic induction method with ocular sensor coils in a rotating magnetic field, based on phase-detection as described before (Collewijn, 1977). A major advantage of this method is the absolute angular calibration of the recordings. The rabbits were implanted permanently with scleral search coils and skull screws for fixation of the head. Five windings of stainless steel, teflon coated wire (type AS 632, Cooner, Chatsworth, CA) were woven underneath the conjunctiva and the superior and inferior rectus muscles.

Implantation of guide cannulas

About a week after the implantation of the scleral coil, guide-cannulas, aimed towards the flocculus, were implanted bilaterally on the skull under general anesthesia. The flocculus was localized by electrophysiological recordings with a glass micropipette with a 4 μm tip, filled with 2.0 M NaCl. Floccular Purkinje cells were identified by the

modulation of their complex spike activity, synchronous with horizontally directed movements of a random dot pattern in front of the animal's ipsilateral eye (Simpson et al., 1981; Graf et al., 1988). Once the micropipette was in the desired position, the surrounding guide cannula was attached to the skull. The micropipette was withdrawn after its depth had been marked. For further description of this method, see Van Neerven et al. (1990).

Injected drugs

We used: a) carbachol, an aspecific (muscarinic and nicotinic) cholinergic agonist (carbamylcholine chloride; Sigma, USA; 1 μg/μl); b) eserine, an acetylcholinesterase inhibitor (physostigmine; Sigma, USA; 15 μg/μl); c) mecamylamine, a nicotinic antagonist of the ganglion blocking, hexamethonium type (Sigma, USA; 5 μg/μl); and atropine sulphate, a muscarinic antagonist (Sigma, USA; 5 μg/μl). Each compound was dissolved in saline and adjusted to pH 7.0 - 7.4. The solutions were injected through a stainless steel injection cannula (outer diameter 0.35 mm) which was connected to a Hamilton 1.0 μl syringe, and introduced through the guide cannula to the predetermined depth where the flocculus had been previously localized. In the control experiments, only the solvent (saline) was injected.

Experimental conditions

During each experiment, the rabbit was restrained in a linen bag and tied down on a platform. The head was bolted to a frame which was attached to the platform, but did not intrude into the rabbit's visual field. The platform was surrounded by a concentric, richly patterned drum (diameter 70 cm), which was oscillated sinusoidally around a vertical axis (0.15 Hz, 5 deg peak-to-peak) to elicit the OKR. The VOR could be elicited by oscillation of the platform (with the same parameters) in darkness. In 3 animals, also the synergistic visuo-vestibular responses were tested after carbachol injection by oscillation of the platform within the illuminated, stationary drum. This condition is called "VOR in light".

At the start of each experiment, 3 baseline measurements of the VOR in darkness and the OKR were recorded in a period of 15 min. The mean value of these baseline measurements was normalized to a reference gain value at time t=0, relative to which subsequent changes in gain were evaluated. After the baseline measurements, a bilateral injection with one of the substances was made into the right and left flocculus. A period of 4 min was taken to inject 1.0 μl of the solution. After the injections, five recordings of both the VOR in darkness and the OKR were obtained at intervals of 5 min, followed by 9 further recordings, taken at intervals of 15 min. Each recording lasted 2 min. During the intervening resting periods of 3 and 13 min, respectively, the rabbit was kept stationary on the platform in the lighted, stationary drum.

In 6 rabbits, the effects of injection of all substances (carbachol, eserine, mecamylamine, atropine and saline (as a control) on the OKR and the VOR in darkness were tested in successive sessions. Only one substance was tested in a single session, and successive sessions were separated by intervals of one or several days, to allow complete recovery from the effects of earlier injections. The order in which the drugs were injected varied among rabbits. Three other rabbits were tested in a separate experiment in which, in addition to the VOR in darkness and the OKR, the VOR in light was also measured after carbachol injections.

The entire experiment was computer-driven (Digital Equipment Corporation, PDP 11/73). The data were gathered by a data-acquisition program, which collected in each trial 12 cycles of the sinusoidal motion stimulus at a sample frequency of 102.4 Hz. In subsequent off-line analysis the fast phases of the eye movement response were removed from the record, after which gain and phase of the responses were determined after a fast Fourier transformation.

The effects of the injected substances were tested for statistical significance with a Multiple Analysis of Variance (MANOVA), a test which allows the comparison of several variables in a single group of animals. Because several of the changes observed were especially marked during the first hour, only the first 8 measurements following the injections were subjected to this statistical testing.

Results

Control experiments

The 6 rabbits used in the main study were subjected to an experiment in which saline alone was injected into the flocculi. These experiments served as controls for the effects of the injection of the solvent and of the subsequent measurement procedure on the gains of OKR and VOR. The results are plotted as reference curves (open circles) in Figs. 1 and 4-6. The mean baseline gain values before the saline injections were 0.56 $\pm$ 0.23 for the OKR and 0.80 $\pm$ 0.19 for the VOR. After the injection, these values showed the tendency to rise slightly (by about 0.1) during the first hour, after which this higher level was maintained. We ascribe this basic effect to a mild upward adaptation of the reflexes, due to the repeated optokinetic stimulus. A similar, slight upward trend of VOR and OKR gains during repeated measurements, even without any injection, was also present in the data of Van Neerven et al. (1990; their Figs. 7 and 8). The values obtained in the control experiments served as the reference values in the statistical analysis of the effects of the subsequently injected drugs. The zero value at t=0 min in each of the graphs shown in Figs. 1 and 3-6 represents the average initial value, obtained in the 3 baseline measurements of the VOR in darkness and the OKR before an injection was made.

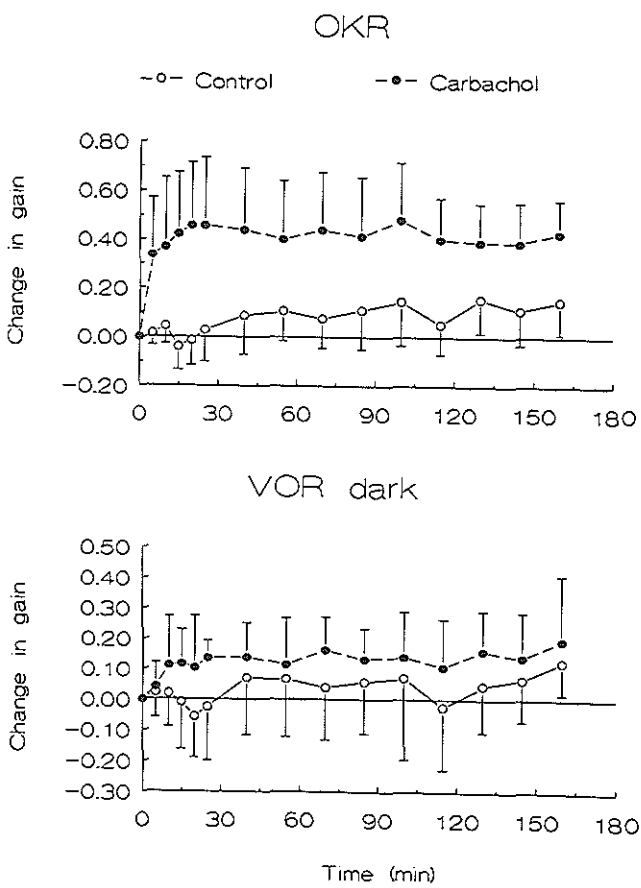


Fig. 1 Time course of the changes in the gain of the OKR (upper panel) and the VOR in darkness (lower panel) in control experiments (floccular injection of saline; open circles) and after bilateral injection of carbachol in the flocculus at time t=0 (closed circles). Mean and standard deviations (bars) of 6 rabbits. The zero level of the ordinate represents the mean baseline gains, measured just prior to the injection; see text for actual values.

Effects of carbachol

The mean baseline gains (± S.D.) of the same 6 rabbits in the sessions in which carbachol was to be injected, were 0.63 ± 0.18 for the OKR and 0.73 ± 0.17 for the VOR. After injection of carbachol, all 6 rabbits showed a very pronounced increase in the gain of the OKR (Fig. 1) and a smaller, but distinct increase in the gain of the VOR in darkness. These increases were evident in the first measurement after the injection and reached a maximum after about 20 min, which lasted throughout the experiment. By the next day, values had returned to baseline levels. At 25 min after the injection, the increase of the gain of the VOR in darkness, averaged over the 6 rabbits, was 0.14 ± 0.06 (S.D.) and the increase of the gain of the OKR was 0.46 ± 0.28 (S.D.). The maximum increase in OKR gain, observed in 1 rabbit, was 0.81. The increases in gain of both the VOR in darkness

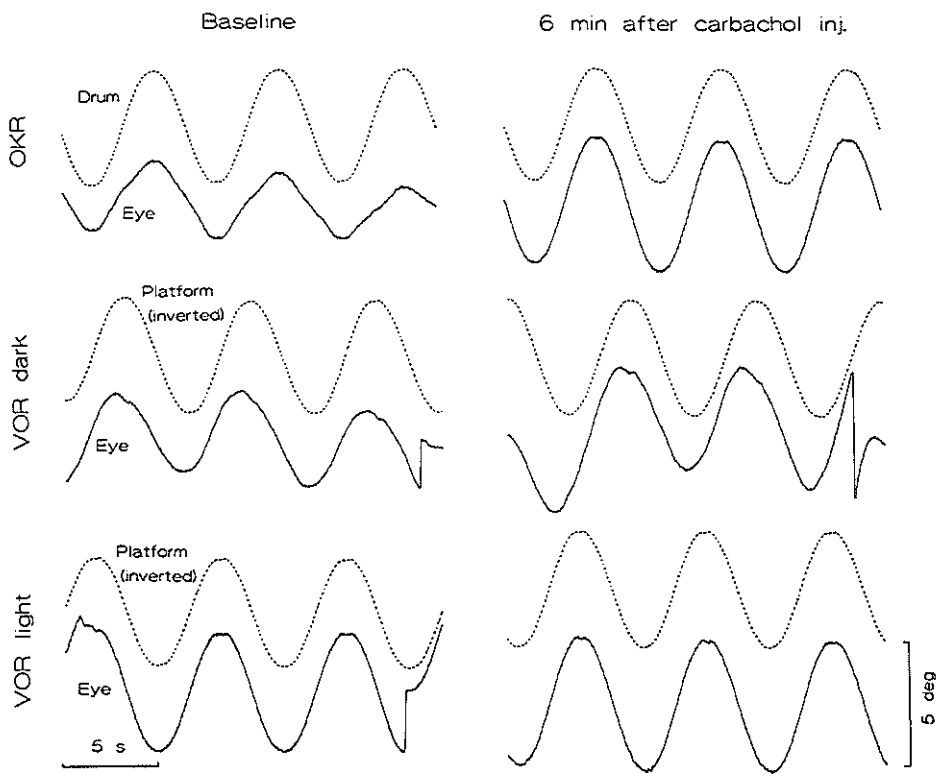


Fig. 2 Representative recordings of horizontal eye movements of a rabbit in response to oscillation of the drum (OKR) or platform (VOR dark; VOR light) at 0.15 Hz, 5 deg (peak-to-peak) in baseline condition and 6 min after the bilateral floccular injection of carbachol. Note the marked enhancement of the OKR and VOR in darkness after the injection.

and the OKR were found to be statistically significant, as shown by low probability levels ($p=0.033$ and $p=0.013$, respectively) in the multiple analysis of variance.

In 3 further rabbits, the course of the VOR in the light was also assessed. The mean baseline values of the gains of OKR, VOR in darkness and VOR in light were 0.52, 0.71 and 1.06, respectively. At 25 min after the injection of carbachol, the gain of the OKR had increased to 0.94; the gain of the VOR in darkness had increased to 0.92 and the gain of the VOR in the light was only slightly changed (gain 1.11). Thus, once again, there was a strong increase in the gain of the OKR and a moderate increase in the gain of the VOR

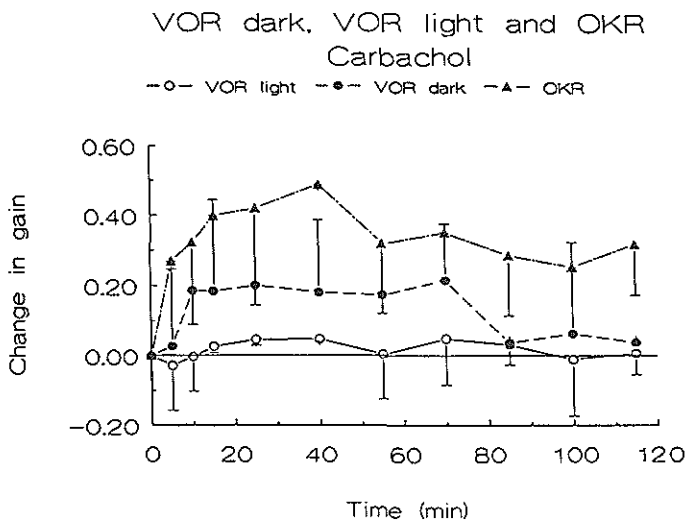


Fig. 3 Time course of the mean changes in gain of the OKR, VOR in darkness and VOR in light after the bilateral floccular injection of carbachol at time t=0 in 3 rabbits.

in darkness, while there was only a slight increase in the gain of the VOR in the light. These changes were already very evident 6 min after the injection of carbachol, as shown in the eye movement recordings in Fig. 2. The complete time-course of the changes in gain for the three types of response is shown in Fig. 3.

The fact that the gains of the VOR in the light and the OKR occasionally exceeded unity is, in hindsight, almost certainly due to a positioning of the rabbit's eyes anterior to the center of rotation of the drum and platform. This would result in visual angles of rotation larger than the nominal rotation of the drum or platform. This error of calibration does not affect any of our conclusions, which only deal with *changes* in gain within single sessions, during which all measuring conditions remained constant.

Effects of eserine

The mean baseline gains in these sessions were 0.73 $\pm$ 0.22 for the OKR and 0.66 $\pm$ 0.18 for the VOR. Shortly after the injection of the AChE inhibitor eserine, the gains of both the VOR in darkness and the OKR showed an increase which lasted for about 30 min (Fig. 4). Similarly as after injection of carbachol, the increase in gain was larger for the OKR than for the VOR in darkness. The increase in the gain of the VOR in darkness at t=15 min was 0.16 $\pm$ 0.20 (S.D.), while the increase in OKR gain was 0.22 $\pm$ 0.14 (S.D.). The increase of the OKR gain was statistically significant at the 5% chance level ($p=0.026$) whereas the increase in gain of the VOR in darkness was not ($p=0.164$).

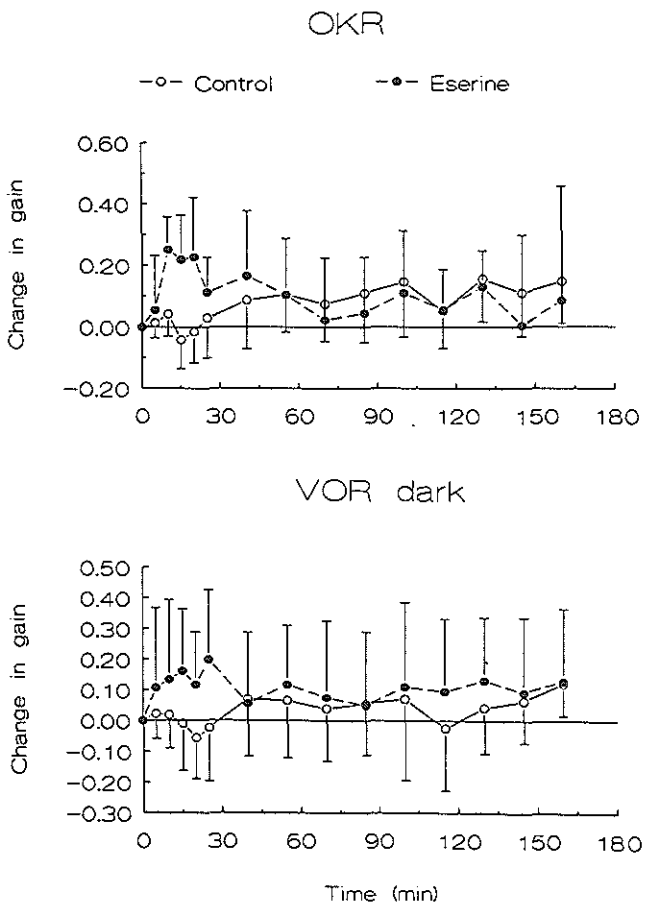


Fig. 4 Time course of the mean changes in gain of the OKR and VOR in darkness after the bilateral floccular injection of eserine in 6 rabbits. Further details as in Fig. 1.

Effects of mecamylamine

The mean baseline gains in these sessions were 0.96 ± 0.26 for the OKR and 0.71 ± 0.19 for the VOR. Injection of the nicotinic, hexamethonium-type antagonist mecamylamine resulted in an immediate decrease in the gains of both the VOR in darkness and the OKR (Fig. 5). The mean change in gain at t=15 min was -0.16 ± 0.17 (S.D.) for the gain of the VOR in darkness and -0.21 ± 0.26 (S.D.) for the gain of the OKR.

This decrease persisted for only about 30 min. In statistical testing, however, these decreases in gain did not reach significance at the 5% level for any of the two reflexes (p=0.187 for the VOR in darkness and 0.072 for the OKR).

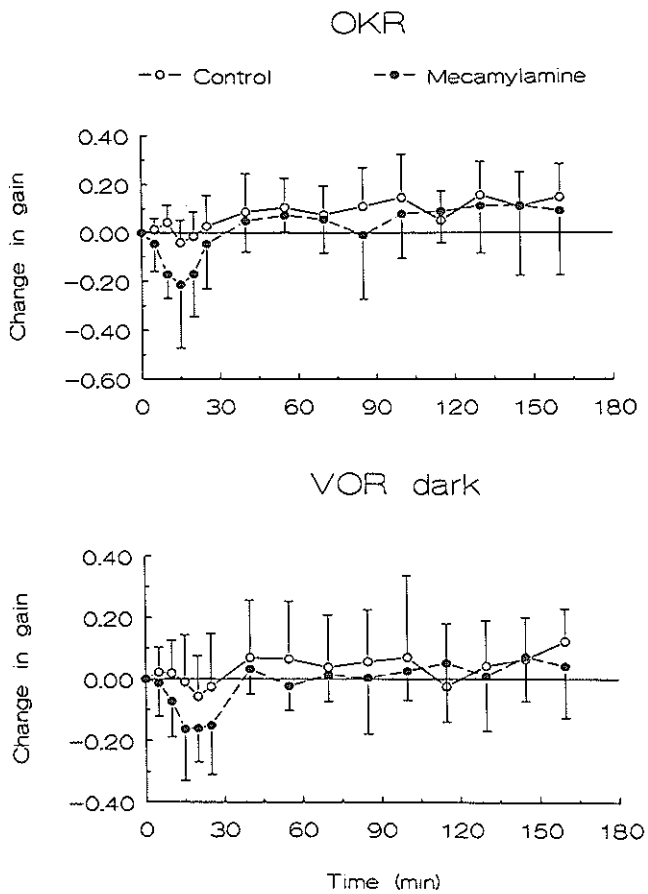


Fig. 5 Time course of the mean changes in gain of the OKR and VOR in darkness after the bilateral injection of mecamylamine in 6 rabbits. Further details as in Fig. 1.

Effects of atropine

The mean baseline gains in these sessions were 0.89 $\pm$ 0.25 for the OKR and 0.69 $\pm$ 0.20 for the VOR. Blockage of the muscarinic receptors in the flocculi by atropine resulted in a slow decrease in the OKR gain (Fig. 6). After this initial decrease, which amounted to -0.18 $\pm$ 0.18 (S.D.) at t=25 min, the OKR gain recovered partially within the first hour but remained lower than the gain in the control experiments during the next 1.5 hrs of testing. In statistical testing, the OKR gain turned out to be significantly lower after atropine injection than after the injection of saline ($p=0.008$). The gain of the VOR in darkness did not appear to be affected by the atropine injection, and did not differ significantly from the control experiment ($p=0.259$).

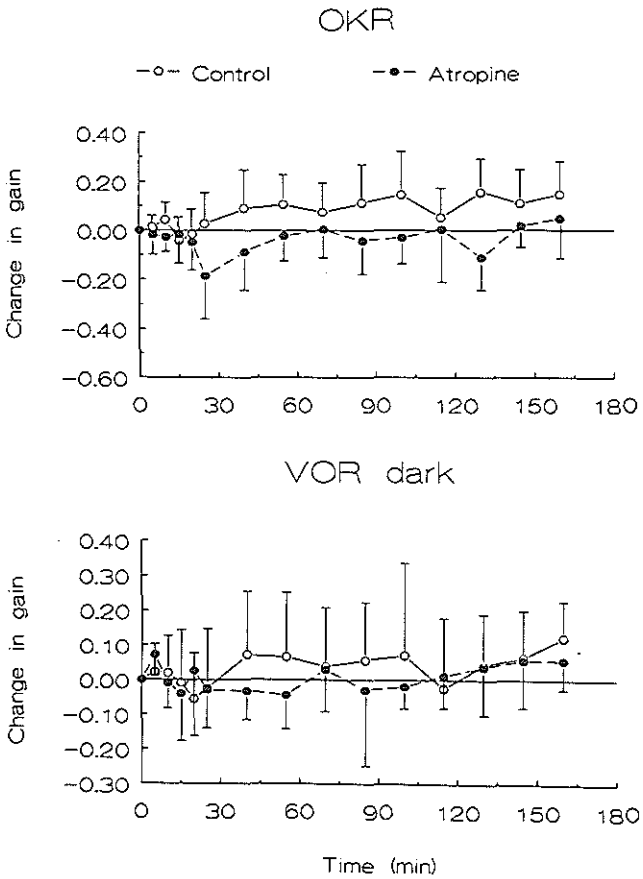


Fig. 6 Time course of the mean changes in gain of the OKR and VOR in darkness after the bilateral injection of atropine sulphate in 6 rabbits. Further details as in Fig. 1.

Discussion

The present study provides evidence for the functionality of the cholinergic system in the cerebellum. Intraflocculär injection of the aspecific cholinergic agonist carbachol resulted in significant increases in the gain of the OKR and the VOR in darkness. In addition, inhibition of acetylcholinesterase by eserine produced effects on the gains of the OKR and the VOR in darkness that were qualitatively similar to the effects of carbachol injection, suggesting that the receptors stimulated by carbachol are indeed innervated by cholinergic elements and thus functionally significant. This suggestion is further corroborated by the fact that blocking of the cholinergic receptors resulted in a significant lowering of the gains of the OKR. Although an extra-floccular site of these actions due

to diffusion cannot be entirely discounted, the small quantities injected and the immediacy and consistency of the effects argue against this possibility.

The enhancing effect of carbachol on the OKR gain is probably mediated in part through muscarinic receptors, because the OKR gain could be lowered significantly by specific blocking of these receptors with atropine. Although the effect of blocking of nicotinic receptors with mecamylamine did not reach statistical significance, an additional functional role of nicotinic receptors in the control of the dynamics of the OKR gain is suggested by our results. For better insight in the receptor-types involved, specific agonists, and agonist-antagonist combinations, will have to be tested in future experiments. It will be also necessary to establish dosage-response relationships. With all drugs, the effects on the VOR in darkness were smaller than the effects on the OKR. This difference undoubtedly contributed to the levels of statistical significance of the changes in the VOR being lower than those for the OKR.

The finding that the gain of the VOR in the light remained virtually unaffected by carbachol (Figs. 2, 3) has to be interpreted in the context of the high gain level measured for this reflex under baseline conditions (around unity gain), and the nature of the control of compensatory eye movements. A high (close to unity) gain of the VOR (a feed-forward system) in combination with a high (close to unity) gain of the OKR (a negative feed-back system) will automatically result in a close-to-unity gain of the VOR in the light. More specific evidence on any effect of the cholinergic system on the *interaction* between VOR and OKR will require the choice of stimulus conditions for which the baseline gains of the components are relatively low.

The anatomical evidence on the cholinergic innervation of the cerebellum was briefly reviewed in the Introduction. In the rabbit's flocculus, muscarinic receptors are present with high density in the Purkinje cell layer, while moderate densities were found in the granular layer, and low densities in the molecular layer (Neustadt et al., 1988). Nicotinic receptors were localized in the granular layer in the rat (Swanson et al., 1987). In combination with the strong evidence for cholinergic mossy fiber terminals (Karen Kan et al., 1978, 1980; Barmack et al., 1986; Ojima et al., 1989; Illing, 1990) these findings suggest the mossy fiber-granular cell transition as a likely site for the actions of the cholinergic drugs in our present experiments. The nature of this effect is not evident. It is well known that simple spike activity in the rabbit's flocculus is profoundly modulated by optokinetic visual stimuli; this modulation is reciprocal with complex spike activity (Graf et al., 1988). Blocking experiments with GABA-agonists strongly suggest that this floccular activity contributes strongly to the gain of the OKR (Van Neerven et al., 1989). As the depth of optokinetic modulation of simple spike activity is not affected by the reversible blockade of climbing fibers (Leonard and Simpson, 1986), the source of the simple spike modulation has to be a mossy fiber input. We may speculate that this depth

of modulation may be enhanced by the cholinergic system. The origin of the specific mossy fibers carrying an optokinetic signal is unknown. Neither is it known what signals are carried by the cholinergic subpopulation of mossy fibers originating in the vestibular and prepositus nuclei, as described by Barmack (1986). There is no evidence suggesting that the cholinergic and the optokinetically modulated subpopulations of mossy fibers are in any way identical. It seems unlikely that the enhancing effect on the OKR of global stimulation with carbachol is caused by an action on post-synaptic receptors receiving optokinetic signals carried by mossy fibers that use ACh as their transmitter. Such stimulation would more probably upset than improve transmission of specific optokinetic information to the Purkinje cells. It seems more likely that carbachol has a modulatory effect, making the granular cells, or the Purkinje cells, more sensitive to specific signals, carried by other fibers.

One possibility of a modulatory action of ACh in the cerebellum is via interference with the GABA system. Such a mechanism was encountered in the hippocampus (Freund et al., 1988), where ACh was found to cause disinhibition of hippocampal cells by blocking of the hippocampal GABA system. This effect of ACh was found to be mediated by nicotinic receptors. In the cerebellum, GABA is recognized as the neurotransmitter of several types of cerebellar interneurons.

Finally, our present results may be relevant for the mechanism of cholinergic blockers acting against motion sickness. In line with the concept that such action is related to the inhibition of vestibular responses, Pyykkö et al. (1985) found that the muscarinic blocker scopolamine, a potent anti motion-sickness drug, lowered the gains of the VOR in darkness and the OKR in humans. Our results leave the possibility open that these effects could be (partially) mediated through actions on cerebellar circuits.

Summary and conclusions

In spite of a large body of histochemical evidence for a cholinergic system in the cerebellum, particularly in lobules IX and X, the physiological role of such a system has remained obscure. In view of the important role of these same lobules in the control of the vestibulo-ocular (VOR) and optokinetic (OKR) responses, we tested the effect of microinjections of cholinergic (ant)agonists in the flocculus of the rabbit on these reflexes. Very marked effects were found. Bilateral floccular injection of the aspecific cholinergic agonist carbachol raised the gain of the OKR by about 0.46 above the baseline values, while the gain of the VOR in darkness was raised by about 0.14. These effects were statistically significant and persisted for several hours. Similar, but smaller effects were obtained after injection of eserine, an inhibitor of acetylcholinesterase. Thus, the effects

could be produced by increasing the naturally present amount of acetylcholine. Microinjections of the nicotinic blocker mecamylamine reduced the gain of the VOR and OKR, although these effects did not reach statistical significance. The muscarinic blocker atropine significantly reduced the gain of the OKR, but not of the VOR. The present results argue strongly for an important physiological role of the cholinergic system in the cerebellum. Specifically, acetylcholine appears to be involved in the modulation of oculomotor reflexes through the flocculus.

References

Barmack NH, Pettorossi VE (1985) Effects of unilateral lesions of the flocculus on optokinetic and vestibuloocular reflexes of the rabbit. J Neurophysiol 53: 481-496

Barmack NH, Baughman RW, Eckenstein FP, Westcott-Hodson M (1986) Cholinergic projections to the cerebellum. Soc Neurosci Abstr 12: 577

Collewijn H (1977) Eye- and head movements in freely moving rabbits. J Physiol (Lond) 266: 471-498

Crawford JM, Curtis DR, Voorhoeve PE, Wilson VJ (1966) Acetylcholine sensitivity of cerebellar neurones in the cat. J Physiol (Lond) 186: 139-165

Crepel F, Dhanjal SS (1982) Cholinergic mechanisms and neurotransmission in the cerebellum of the rat. An in vitro study. Brain Res 244: 59-68

De la Garza R, Bickford-Wimer PC, Hoffer BJ, Freedman R (1987) Heterogeneity of nicotine actions in the rat cerebellum: an in vivo electrophysiologic study. J Pharmacol Exp Ther 240: 689-695

Epema AH, Gerrits NM, Voogd J (1990) Secondary vestibulocerebellar projections to the flocculus and uvulo-nodular lobule of the rabbit - A study using HRP and double fluorescent tracer techniques. Exp Brain Res 80: 72-82

Freund RK, Jungschaffer DA, Collins AC, Wehner JM (1988) Evidence for modulation of GABAergic neurotransmission by nicotine. Brain Res 453: 215-220

Goldberg AM, McCaman RE (1967) A quantitative microchemichal study of choline acetyltransferase and acetylcholinesterase in the cerebellum of several species. Life Sci 6: 1493-1500

Graf W, Simpson JI, Leonard CS (1988) Spatial organization of visual messages of the rabbit's cerebellar flocculus. II. Complex and simple spike responses of Purkinje cells. J Neurophysiol 60: 2091-2121

Hayashi M (1987) Ontogeny of glutamic acid decarboxylase, tyrosine hydroxylase, choline acetyltransferase, somatostatin and substance P in monkey cerebellum. Dev Brain Res 32: 181-186

Hunt S, Schmidt J (1978) Some observations on the binding patterns of α-bungarotoxin in the central nervous system of the rat. Brain Res 157: 213-232

Illing RB (1990) A subtype of cerebellar Golgi cells may be cholinergic. Brain Res 522: 267-274

Israël M, Whittaker VP (1965) The isolation of mossy fibre endings from the granular layer of the cerebellar cortex. Experientia 21: 325-326

Ito M, Jastreboff PJ, Miyashita Y (1982) Specific effects of unilateral lesions in the flocculus upon eye movements in albino rabbits. Exp Brain Res 45: 233-242

Ito M (1984) The Cerebellum and Neural Control. Raven, New York

Karen Kan KS, Chao LP, Eng LF (1978) Immunohistochemichal localization of choline acetyl-transferase

in rabbit spinal cord and cerebellum. Brain Res 146: 221-229

Karen Kan KS, Chao LP, Forno LS (1980) Immunohistochemical localization of choline acetyl-transferase in the human cerebellum. Brain Res 193: 165-171

Kása P, Silver A (1969) The correlation between choline acetyltransferase and acetylcholinesterase activity in different areas of the cerebellum of rat and guinea pig. J Neurochem 16: 389-396

Kása P, Bánsághy K, Rakonczay Z, Gulya K (1982) Postnatal development of the acetylcholine system in different parts of the rat cerebellum. J Neurochem 39: 1726-1732

Leonard CS, Simpson JI (1986) Simple spike modulation of floccular Purkinje cells during the reversible blockade of their climbing fiber afferents. In Keller EL, Zee DS (eds) Adaptive processes in visual and oculomotor systems. Pergamon, Oxford pp 429-434

Mash DC, Potter LT (1986) Autoradiographic localization of M1 and M2 muscarine receptors in the rat brain. Neuroscience 19: 551-564

McCance I, Phillis JW (1968) Cholinergic mechanisms in the cerebellar cortex. Int J Neuropharmacol 7: 447-462

Neustadt A, Frostholm A, Rotter A (1988) Topographical distribution of muscarinic cholinergic receptors in the cerebellar cortex of the mouse, rat, guinea pig, and rabbit: a species comparison. J Comp Neurol 272: 317-330

Ojima H, Kawajiri SI, Yamasaki T (1989) Cholinergic innervation of the rat cerebellum: qualitative and quantitative analyses of elements immunoreactive to a monoclonal antibody against choline acetyltransferase. J Comp Neurol 290: 41-52

Pyykkö I, Schalén L, Matsuoka I (1985) Transdermally administered scopolamine vs. dimenhydrinate. II. Effect on different types of nystagmus. Acta Otolaryngol 99: 597-604

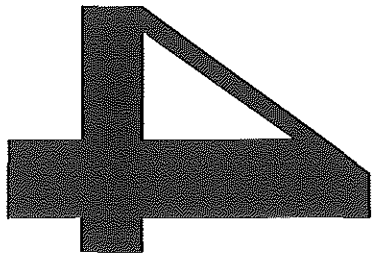
Simpson JI, Graf W, Leonard C (1981) The coordinate system of visual climbing fibers to the flocculus. In Fuchs A, Becker W (eds) Progress in oculomotor research. Elsevier, Amsterdam, pp 475-484

Spencer DG, Horváth E, Traber J (1986) Direct autoradiographic determination of M1 and M2 muscarinic acetylcholine receptor distribution in the rat brain: relation to cholinergic nuclei and projections. Brain Res 380: 59-68

Swanson LW, Simmons DM, Whiting PJ, Lindstrom J (1987) Immunohistochemical localization of neuronal nicotinic receptors in the rodent central nervous system. J Neurosci 7: 3334-3342

Van Neerven J, Pompeiano O, Collewijn H (1989) Depression of the vestibulo-ocular and optokinetic responses by intraflocculer microinjection of GABA-A and GABA-B agonists in the rabbit. Arch Ital Biol 127: 243-263

Van Neerven J, Pompeiano O, Collewijn H, Van der Steen J (1990) Injections of ß-noradrenergic substances in the flocculus of rabbits affect adaptation of the VOR gain. Exp Brain Res 79: 249-260



Cholinergic and noradrenergic stimulation in the flocculus have synergic facilitatory effects on optokinetic responses

Introduction

A functional relationship between the acetylcholine (ACh) and noradrenaline (NA) systems has been encountered in the control of behaviour and neural plasticity in various parts of the brain. For example, the increase in locomotor activity following systemic muscarinic type ACh receptor blocking was potentiated by a general NA depletion, while NA depletion itself had no effect on locomotor activity (Mason and Fibiger, 1979). The effects of more localized manipulation of NA and ACh function in the visual cortex have been described by a study of Bear and Singer (1986). Combined destruction of the NA and ACh innervation of the visual cortex in kittens markedly impaired neural plasticity induced by monocular deprivation, while depletion of either NA or ACh alone did not affect plasticity. A synergistic action of ACh and NA has been proposed in the promotion of a level of excitability in cortical neurones which is consistent with the state of arousal in the sleep-wake cycle (see McCormick, 1989).

Based on electrophysiological studies, a number of mechanisms has been proposed which could account for the functional relationship between the ACh and NA systems. First, various studies have shown interactions at the level of the release of one of these transmitters (see Kuhar et al., 1978 and Vizi et al., 1991). Application of ACh in the locus coeruleus (LC) induced excitation of NA cells, resulting in increased release of NA in the target structures of the LC (Bird and Kuhar, 1977; Eagan and North, 1985). At the level of the target structure, presynaptic cholinergic stimulation of LC terminals also elicited an increase in NA release (Waser, 1975; Westfall, 1974). NA-mediated modification of ACh release has also been reported. NA, applied to cortical slices, was found to reduce evoked release of ACh (Moroni et al. 1983; Vizi 1980), whereas NA release in the septal region induced an increase in ACh turnover in the hippocampus (Costa et al., 1983).

In addition, there is abundant evidence for a convergence and summation of actions of ACh and NA on a common postsynaptic structure. A potent and universal action of ACh is the augmentation of synaptically driven responses of target neurons (Krnjevic et al., 1971). The proposed mechanism underlying this phenomenon is a prevention of the accommodation of spike discharge, which normally occurs after depolarization. This effect can be explained by the well-documented inactivation of the slow afterhyperpolarization (AHP) by ACh (Cole and Nicoll, 1984; Krnjevic et al., 1971). This effect of ACh was observed, amongst others, in hippocampal pyramidal cells (Bernardo and Prince, 1982; Cole and Nicoll, 1984; Madison et al., 1987), neocortical cells (Lamour, 1988; McCormick and Prince, 1986; Schwindt et al., 1988; Sillito and Kemp, 1983A) and cells in the dorsal lateral geniculate nucleus (LGNd) (Sillito and Kemp, 1983B). In synergy with ACh, NA-application caused an increase in excitability of cells to excitatory afferents in cerebellar Purkinje-cells (Freedman et al., 1977), hippocampal pyramidal cells (Madison and Nicoll,

1986), cells of the sensorimotor cortex (Lamour, 1988; Schwindt et al., 1988), and neurons of the LGNd (Rogawski and Aghajanian, 1980). Thus, both ACh and NA modulate the excitability of the postsynaptic structure, and there is evidence that both can affect the properties of a single neuron (Nicoll, 1988; Schwindt et al., 1988). Based on studies on the intracellular mechanism of action of ACh and NA in the hippocampus, Nicoll (Nicoll, 1988) put forward the hypothesis that ACh and NA, through different second messenger systems, block the same Ca^{2+} dependent K^+ channel which is responsible for the slow AHP.

Many studies have focussed on the NA and ACh transmitter systems in the cerebellum. Histochemical studies established that the cerebellum contains measurable, though low, amounts of choline acetyltransferase (ChAT) (Goldberg and McCaman, 1967; Kasa et al., 1982; Ojima et al., 1989) and endogeneous ACh (McIntosh, 1941; Kasa et al., 1982), with appreciable levels of acetylcholinesterase (AChE) (Austin and Phillis, 1965; Kasa and Silver, 1969), suggesting the occurrence of cholinergic transmission in the cerebellum. However, the physiological actions of ACh in the cerebellum have remained largely unclear. Although application of Ach was found to mildly excite Purkinje cells in the cat (Crawford et al., 1966; McCance and Phillis, 1968), a significant amount of cholinergic transmission at the level of the Purkinje cell was regarded as unlikely because synaptic responses of Purkinje cells were unaffected by application of ACh antagonists (Crepel and Dhanjal, 1982; McCance and Phillis, 1968). Moreover, a more recent study (De la Garza et al., 1987) reported inhibition of rat Purkinje cells by pressure-injected ACh. For application of ACh to cerebellar granule cells no effect (Crawford et al., 1966) as well as excitation (McCance and Phillis, 1968) have been reported.

Purkinje cells in the cerebellum are known to receive a direct projection of NA-containing afferents from the nucleus LC (Bloom et al., 1971; Hoffer et al., 1973) and subsequent exploration of the function of this system revealed that noradrenergic stimulation increased the responsiveness of Purkinje cells to excitatory (Freedman et al., 1977) as well as inhibitory (Moises and Woodward, 1980) afferent synaptic activity. Moreover, application of NA selectively was found to augment both excitatory and inhibitory modulation of simple-spike discharge in parafloccular Purkinje cells evoked by visual stimulation.

Little is known about the functionality of the cerebellar NA and ACh systems at the behavioral level. Van Neerven et al. 1990 first used the hypothesised involvement of the cerebellar flocculus in the adaptation of the gain of the vestibulo-ocular reflex (VOR; see ref Ito, 1984) as a model to study the functional significance of the floccular NA system. The results of this study indicated that NA enhanced adaptation of the VOR, in line with its proposed function in plasticity.

In addition to a role in adaptive control of the VOR, the flocculus is thought to

be involved in the on-line control of the VOR and the optokinetic reflex (OKR). Recently, we discovered a strong enhancement of the basic gains of the OKR and, to a lesser extent, the VOR, by intra-floccular micro-injection of the cholinergic agonist carbachol, which suggested a positive modulatory role of ACh in the signal-transmission in the cerebellar flocculus (Tan and Collewijn, 1991). As a next step in our investigations of the neuropharmacology of the flocculus, the present study was conducted to further elucidate the effect of noradrenergic stimulation on the basic gain characteristics of oculomotor reflexes and to test, in analogy with findings in other parts of the brain, for possible interactions between the ACh and NA systems. Specifically, we compared the effects of floccular injection of the ACh agonist carbachol, the β-adrenergic agonist isoproterenol and of conjoint injection of both of these substances on the basic gain of the VOR and the OKR.

Methods

Implantation of guide cannulas

Six pigmented Dutch belted rabbits of either sex were used in this study. In all surgical procedures, the animals were anesthetized with a mixtured of ketamine, acepromazine 1% and xylazine-HCl. The skull above the paramedial lobe of the cerebellum was removed and a chamber of dental acrylic was placed over it. In a subsequent session, the flocculus was localized by electrophysiological recordings with a glass micropipette with a 4 µm tip, filled with 2.0 M NaCl. Floccular Purkinje cells were identified by the modulation of their complex spike activity, synchronous with horizontally directed movements of a random-dot pattern in front of the animal's ipsilateral eye (Graf et al., 1988). Once the micropipette was in the desired position, a surrounding guide-cannula was fixed to the skull and the depth of the micropipette was measured. The exact coordinates of the flocculus relative to the skull were thus determined, enabeling us to make multiple, reproducible injections of different substances into the same area in the flocculus. For a more detailed description of this method see ref Van Neerven et al., 1990.

Injected drugs

We used carbachol (carbamylcholine chloride; Sigma, USA; 1 µg/µl) as an aselective agonist of both muscarinic and nicotinic cholinergic receptors, and isoproterenol (isoproterenol-HCl, Sigma, USA; 16 µg/µl) as an aselective agonist of the β-adrenergic receptors. These compounds were dissolved in saline and the pH was adjusted to 7.0-7.4. Furthermore, a solution of isoproterenol and carbachol (16 µg + 1 µg in 1 µl) was used for the combined application. The solutions were injected through stainless steel injection

cannulas (outer diameter 0.35 mm), connected to a Hamilton 1.0 µl syringe, and introduced through the guide-cannula to the predetermined depth where the flocculus had been previously localized. In the control experiments, only the solvent (saline) was injected.

Measurement of eye movements

For the purpose of eye movement measurement, the animals were permanently implanted with scleral search-coils. Five turns of stainless steel, teflon coated wire (type AS 632, Cooner, Chatsworth, CA) were woven underneath the conjunctiva and the superior, inferior and medial rectus and inferior oblique muscles. In addition, screws were mounted on top of the skull for fixation of the head during the experiments.

Experimental conditions

During the experiment, the rabbit was restrained in a linen bag and tied down onto a platform which could be rotated about an earth-vertical axis. The rabbit's head was bolted to a frame which was attached to the platform, but did not intrude into the rabbit's visual field and positioned in such a way that the intraocular axis was intersected by the rotational axis of the platform. The platform was surrounded by a richly patterned drum (diameter 70 cm) which could be rotated about an earth-vertical axis, collinear with the axis of the platform. For measurement of the OKR, the drum was oscillated sinusoidally. As described earlier (Tan and Collewijn, 1991), during stimulation with a frequency of 0.15 Hz and a peak-to-peak amplitude of 5°, injection of carbachol alone caused an improvement of the gain of the OKR from a baseline value of 0.6 to a value close to unity. Under such conditions, a potentiation of the carbachol-effect would be obscured by the saturation of the gain at 1.0. For this reason we used in the present study a more demanding stimulus. From pilot studies, it appeared that raising the stimulus amplitude from 5° peak-to-peak to 10° peak-to-peak resulted in a gain of the OKR remaining well below unity, even after injection of carbachol.

The VOR in darkness was tested by sinusoidal oscillation of the platform in total darkness, with the same parameters as the drum movement. The combined response of the VOR and the OKR was tested by oscillating the rabbit within the illuminated, stationary drum, and this response will be referred to as the 'VOR in the light'.

Before any injection, 3 sets of baseline measurements were recorded, each set consisting of a measurement of the VOR in the light, the VOR in darkness and the OKR. The mean values of these 3 sets of 3 baseline measurements was normalized to a reference-gain value at time t=0, relative to which subsequent changes in gain were evaluated. After the baseline measurements, a bilateral injection with one of the substances or the mixture was made into the right and left flocculus. Within 30 seconds

after injection, the next set of measurements was started. This was followed by 3 sets of measurements at intervals of 5 min and two further sets of measurements at intervals of 15 min. In total, a time-span of 45 min after injection was covered. One set of recordings took 2 min. During the intervening 3 or 13 min, respectively, the rabbit was kept stationary on the platform within the lighted, stationary drum.

The experiments were computer-driven (DEC, PDP 11/73) and the data were gathered by a data-acquisition program, which collected in each trial 4 cycles of the sinusoidal motion stimulus at a sample frequency of 51.2 Hz. In the subsequent off-line analysis, the fast phases were removed from the eye position recordings, after which gain and phase of the responses were determined by a fast Fourier transformation. The statistical significance of the effects of the injected substances was tested by a Multiple Analysis of Variance (MANOVA), which allows the comparison of several variables in a single group of animals.

Results

The mean (± S.D.) changes in gain after the various injections are shown in Fig. 1. Representative recordings of the OKN in one animal are shown in Fig. 2.

Control experiments

All 6 rabbits were subjected to an experiment in which saline, the solvent used for the substances, was injected into both flocculi. These experiments served to exclude a possible mechanical effect of injection of the solvent, in addition to a possible effect of the measurement procedure on the gains of the OKR and the VOR. The gain-values, obtained in these experiments were used as reference-values in the statistical analysis of the effects of the injected drugs, thereby isolating the specific effect of the applied drug.

The mean baseline-values of the gains in these control experiments were 0.83 ± 0.03 (S.D.) for the VOR in light, 0.67 ± 0.11 (S.D.) for the VOR in darkness and 0.55 ± 0.19 (S.D.) for the OKR. These baseline-values were normalized and are depicted in Fig. 1 as zero-values at t=0. The values obtained after injection of saline are plotted as changes in gain, relative to these average baseline-values. The gain of the VOR in the light showed a small decrease after injection of saline, with a maximum decrease of -0.04 ± 0.02 (S.D.) at 5 min after injection. This decrease was statistically significant ($p=0.006$), in a multiple analysis of variance. The gain of the VOR in darkness also showed a decrease, amounting to -0.07 ± 0.06 (S.D.), but this decrease did not reach statistical significance ($p=0.102$). Injection of saline had no apparent effect on the gain of the OKR, which was confirmed by the absence of statistical significance ($p=0.350$).

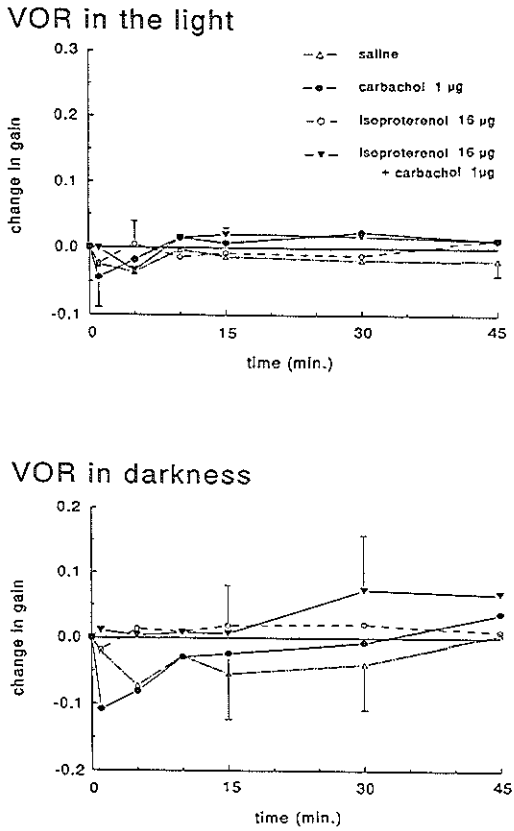


Fig. 1 Mean effects of injections of the different solutions at time = 0 on the time course of the changes in gain, as averaged over 6 rabbits. The upper graph depicts the effects on the VOR in the light, the middle graph shows the time course of the gain of the VOR in darkness and the lower graph represents the effect on the OKR. Bars represent 1 S.D. Baseline gains before the injection are normalized to zero; see text for actual values.

Effects of carbachol injection

Of the three conditions tested, the VOR in the light was least affected by injection of the non-selective cholineric agonist carbachol. The baseline-gain of the VOR in light, averaged over the 6 rabbits tested, was 0.83 ± 0.03 (S.D.) and despite a slight decrease directly after injection of carbachol, the time course of the gain of the VOR in the light after injection of carbachol did not differ significantly from the course of the gain of the control experiment ($p=0.083$).

The baseline-gain of the VOR in darkness was 0.62 ± 0.06 (S.D.). At the first measurement, taken 30 sec after the injection, the gain showed a larger decrease of 0.11 ± 0.09 (S.D.) than after injection of saline but it returned to the control value after 5 minutes. This deviation from the control did not reach statistical significance ($p=0.175$).

The mean baseline-gain of the OKR was 0.53 ± 0.21 (S.D.) and, similar to the gain of the VOR in darkness, showed a decrease amounting to -0.08 ± 0.08 (S.D.) at the first measurement, taken within 30 sec after injection. This decrease was found in 5 of the 6 rabbits tested. Subsequent measurements, however, reflected an increase in the OKR gain, reaching a level of about +0.14 ± 0.11 at about 15 min after injection. Statistical comparison with the control experiment showed that this increase was significant ($p=0.041$).

Effects of isoproterenol injection

The mean baseline-gains in these sessions were 0.83 ± 0.03 (S.D.) for the VOR in the light and 0.66 ± 0.14 (S.D.) for the VOR in darkness. For neither of these conditions, a change in gain was induced by injection of the β-adrenergic agonist isoproterenol; the gain remained at baseline-level throughout the experiment. The time course of the VOR in the light after injection of isoproterenol did not significantly differ from the control experiment ($p=0.172$). Relative to the time course of the gain of the VOR in darkness in the control experiment, which showed a decrease after the injection of saline, there was a slight improvement in the gain of the VOR in darkness immediately after application of isoproterenol, but this was statistically insignificant ($p=0.403$).

The mean baseline-gain of the OKR was 0.45 ± 0.18 (S.D.). After injection of isoproterenol, all 6 rabbits showed an increase in the gain of the OKR. This increase was already manifest in the first measurement after the injection, reaching a maximum of +0.11 ± 0.11 (S.D.) at 30 min after injection. This increase in gain reached statistical significance at the 5% probability-level ($p=0.048$).

Effects of combined carbachol and isoproterenol injection

The mean baseline-gains in these sessions were 0.83 ± 0.02 (S.D.) for the VOR in the light and 0.66 ± 0.07 (S.D.) for the VOR in darkness. Combined injection of the same

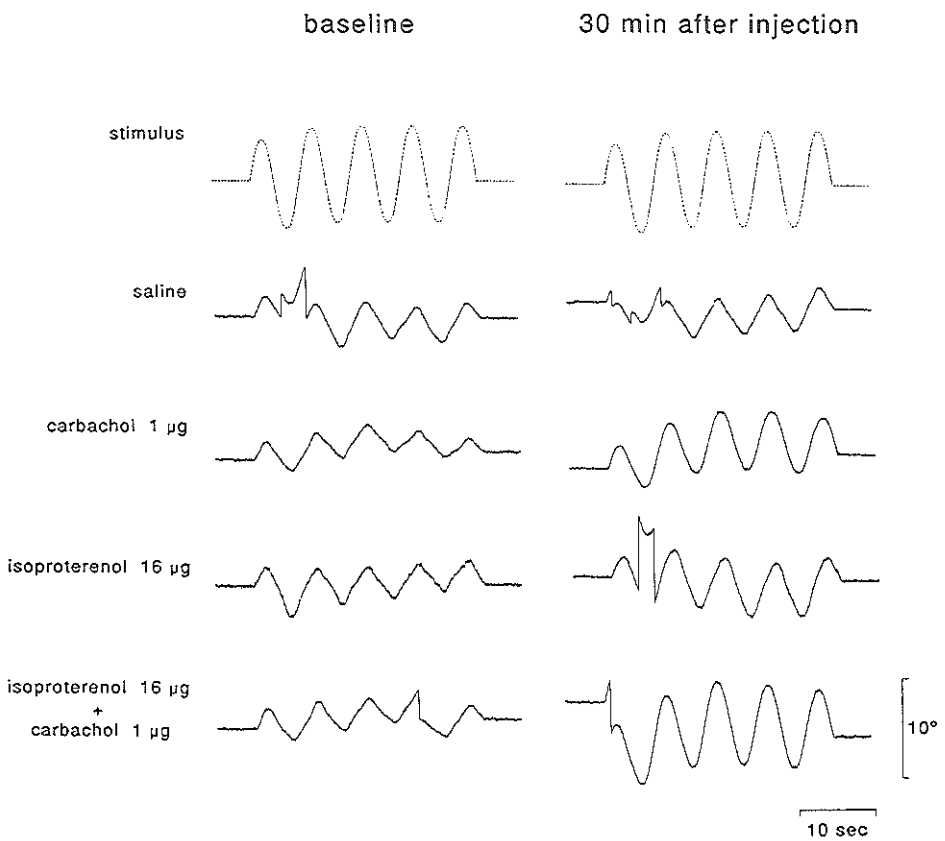


Fig. 2 Recordings of optokinetic responses during the four sessions in one representative animal, showing responses before and 30 min after injection of the 4 solutions. Notice the marked enhancement of the optokinetic response after combined injection of isoproterenol and carbachol.

amounts of carbachol and isoproterenol as used before into both flocculi caused a slight increase in the VOR in the light by a maximum of 0.02 ± 0.01 (S.D.). The time course of the VOR in the light was significantly different from the time course after injection of saline ($p=0.036$). The gain of the VOR in darkness slightly increased by +0.07 ± 0.09 (S.D.), but the increase did not appear until 30 min after the injection and did not reach statistical significance ($p=0.119$).

The OKR showed a mean baseline-gain of 0.47 ± 0.21 (S.D.). Immediately after the injection of the mixture of carbachol and isoproterenol, a pronounced increase in the gain of the OKR was evident. This increase amounted to +0.29 ± 0.16 (S.D.) at 15 min

after injection and continued at a slower rate throughout the further duration of the experiment. This increase was statistically highly significant ($p=0.006$).

Discussion

The present study revealed an expression at the behavioural level of a functional synergy between NA and ACh in the cerebellar flocculus. In addition to the previously described (Tan and Collewijn, 1991) enhancing effect of aselective cholinergic stimulation in the flocculus on the gain of the OKR (+0.14), floccular β-adrenergic stimulation by application of isoproterenol caused a statistically significant increase in the gain of the OKR (+0.11). Moreover, conjoint injection of NA and ACh into the flocculus resulted in an increase (+0.29).

Differently from our earlier study (Tan and Collewijn, 1991), we started measurements of VOR and OKR within 30 sec after injection of the drug, thereby revealing a biphasic time-course of the gain of the OKR following carbachol injection with a decrease in gain immediately after injection, followed by an increase at the second measurement, 5 min after injection, which was sustained during the experiment.

In the same previous study (Tan and Collewijn, 1991), we have found that the gain of the VOR, elicited with a 0.15 Hz, 5 deg peak-to-peak amplitude sinusoidal stimulus, is increased by carbachol injection and very recent experiments have shown that floccular injections of carbachol shorten the time constant of post-rotatory nystagmus (Tan et al., in prep). The absence of effects of carbachol on the VOR in the dark in the present study (0.15 Hz, 10 deg peak-to-peak stimulus) seems to contrast with these observations but this difference may be due to stimulus dependency of the effect on the VOR. The VOR in the light on the other hand, being in fact a combination of the OKR and the VOR in the dark, did show a small but significant increase after injection of isoproterenol and carbachol together.

There is ample evidence for a role of the flocculus in maintaining optimal gain levels of the OKR and, probably, the VOR in the rabbit. Whereas the direct effects of the floccular efference upon the vestibular nuclear cells are inhibitory (Ito and Yoshida, 1964), the net effect of the floccular involvement on the VOR and the OKR is positive, due to the predominantly out-of-phase polarity of Purkinje cell modulation (Graf et al., 1988; Ito, 1984). In agreement with a positive contribution of the flocculus to the oculomotor reflexes, functional or physical ablation of the flocculus of the rabbit results in a decrease in the gain of the OKR and the VOR (Ito et al., 1982; Nagao, 1983; Van Neerven et al., 1989) or of the OKR alone (Barmack and Pettorossi, 1985). From this point of view, the positive modulatory effect of isoproterenol and carbachol on the gain of the OKR, as

found in the present study, can be interpreted as an increase in signal flow through the flocculus.

At the cellular level, NA has been found to increase the responsiveness of Purkinje cells to excitatory (Freedman et al., 1977) but also to inhibitory (Moises and Woodward, 1980) afferent synaptic activities. Thus, we propose that the cellular substrate for the facilitory effect of our floccular injection of the β-adrenergic agonist isoproterenol on the gain of the OKR is a facilitatory effect of isoproterenol on the Purkinje-cell responses. This assumption is further supported by a recent study (Moises et al., 1990), which showed in paraflocculars Purkinje cells that application of NA selectively augments both excitatory and inhibitory patterns of simple spike discharge, evoked by visual stimulation.

A very similar facilitatory action of NA was encountered in the hippocampus, and further research indicated the underlying mechanism to be a prevention of the accommodation of spike discharge that normally occurs during the depolarization by blocking of the slow AHP (Madison and Nicoll, 1986). Based on subsequent studies of the intracellular mechanisms that underly this action, Nicoll (1988) proposed a mechanism in which NA, through β-receptors and subsequent production of cyclic adenosine monophosphate (cAMP), blocks a Ca^{2+} dependent K^+ channel that is responsible for the slow AHP.

Further investigations of the mechanism of the NA effect on cerebellar Purkinje cells revealed that the facilitatory action of NA could be blocked specifically by β-adrenergic antagonists (Moises et al., 1981), in agreement with our present findings, and that this effect is mediated intracellularly by cAMP (Mori-Okamoto and Tatsuno, 1988; Siggins et al., 1973), suggesting a similar mechanism as proposed for hippocampus. Furthermore, a calcium-dependent potassium conductance is found in Purkinje-cells (Llinás and Walton, 1980) which could provide a similar substrate for the ion-current causing a slow AHP in the cerebellum. as encountered in hippocampus.

The synergy between NA and ACh, encountered in the present study, is consistent with a pattern of findings that has emerged from other behavioural and electrophysiological studies in various parts of the brain. In hippocampus and neocortex, one of the most potent actions ACh is a blockade of the slow AHP, through muscarinic type receptors (Bernardo and Prince, 1982; Cole and Nicoll, 1984). As with NA, this blockade is associated with a loss of accommodation of spike discharge, caused by blocking of a Ca^{2+}-dependent K^+ channel, raising the possibillity that NA and ACh both exert their action by blocking of the same ion-channel (Nicoll, 1988).

Recent experiments show that the facilitatory action of carbachol on the OKR is specifically mediated by muscarinic type receptors (Tan and Collewijn, in prep.), similar to the facilitatory action of ACh in hippocampus and neocortex. In the cerebellum, however, electrophysiological studies have only focussed on the direct effect of ACh on

the post-synaptic potential and subsequent changes in discharge rate. A modulatory action of ACh on the excitability of Purkinje cells to afferent signals at the cellular level, as suggested by our behavioural studies, is yet to be proven.

In line with the hypothesis that NA plays a facilitatory role in synaptic plasticity (Gilbert, 1975; Kety, 1970; Watson and McElligot, 1984), generalized depletion of NA by intra-cisternal injection of 6-OHDA abolished adaptation of the VOR (Keller and Smith, 1983; McElligot and Freedman, 1988A). Later (McElligot and Freedman, 1988B), it was reported that depletion of cerebellar NA by injection of 6-OHDA in the coeruleo-cerebellar pathways had the same effect as generalized depletion and conluded that the action of NA on adaptation of the VOR takes place in the cerebellum. Van Neerven et al. further restricted the localization of the NA effect to the cerebellar flocculus by modulation of VOR adaptation using local micro-injections of the β-adrenergic drugs isoproterenol and sotalol. The question arises as to how the effect of NA application on the basic performance of the OKR, found in the present study, relates to the enhancing influence of NA on adaptation of the VOR.

Following the hypothesis that modifiable synapses are located in the cerebellar flocculus, the enhancing effect of floccular noradrenergic stimulation on the adaptation of the VOR would corroborate the proposed function of NA in plasticity. Recent evidence, however, has favoured the brainstem over the flocculus for the location of the modifiable synapses (Lisberger and Pavelko, 1988; Miles and Lisberger, 1981), implying that the NA effect on adaptation would not necessarily have been the result of a direct control of NA in the modification of synaptic transmission. Irrespective of the localization of the modifiable synapses, however, the flocculus is thought to play a crucial role in conveying retinal motion information, which is considered as the error signal subserving the recalibration of the VOR (Miles and Lisbergber, 1981). Thus, in case that the brainstem hypothesis is correct, it is likely that the effect of NA manipulation on VOR adaptation is based on the enhancement of input of the error signal, the optokinetic signal, rather than on a direct effect of NA on synaptic plasticity.

Summary and conclusions

Many behavioural and electrophysiological studies have revealed a functional relationship between acetylcholine (ACh) and noradrenaline (NA), varying from a synergistic action to a negative presynaptic interaction. A recent study (Tan and Collewijn, 1991) showed that injection of the cholinergic agonist carbachol into the flocculi had a pronounced facilitatory effect on the gains of the optokinetic (OKR) and vestibulo-ocular (VOR) reflexes, suggesting a positive modulatory role of the cholinergic system in the flocculus. The present study was undertaken to compare the effects of floccular injection

of the ACh agonist carbachol, the β-adrenergic agonist isoproterenol and a conjoint injection of both of these substances on the basic gain of the VOR and the OKR. Despite a different initial response, injection of carbachol and isoproterenol both significantly raised the gain of the OKR, by 0.14 and 0.11 respectively. Neither of the two substances significantly affected the gain of the VOR in light or darkness. Conjoint injection of the same amounts of carbachol and isoproterenol resulted in an increase in the gain of the OKR by 0.29, and the gain of the VOR in the light was significantly raised by 0.02, wherease the gain of the VOR in darkness was not affected significantly. These results suggest a synergistic and positive modulatory role of ACh and NA in the flocculus, with a positive interaction between these two systems. In analogy with findings in neocortex and hippocampus, both ACh and NA might act by blocking the slow afterhyperpolarization in Purkinje cells.

References

Austin L, Phillis JW (1965) The distribution of cerebellar cholinesterases in several species. J Neurochem 12: 709-727.

Bear MF, Singer W (1986) Modulation of visual cortical plasticity by acetylcholine and noradrenaline. Nature 320: 172-176

Barmack NH, Pettorossi VE (1985) Effects of unilateral lesions of the flocculus on optokinetic and vestibulo-ocular reflexes of the rabbit. J Neurophysiol 53: 481-496

Bernardo LS, Prince DA (1982) Cholinergic excitation of mammalian hippocampal pyramidal cells. Brain Res 249: 315-331

Bird SJ, Kuhar MJ (1977) Iontophoretic application of opiates to the locus coeruleus. Brain Res 122: 523-533

Bloom FE, Hoffer BJ, Siggins GR (1971) Studies on norepinephrine-containing afferents to Purkinje cells of rat cerebellum. I. Localization of the fibers and their synapses. Brain Res 25: 501-522

Cole AE, Nicoll RA (1984) Characterization of a slow cholinergic post-synaptic potential recorded in vitro from rat hippocampal pyramidal cells. J Physiol (Lond) 352: 173-188

Costa E, Panula P, Thompson HK, Cheney DL (1983) The trans-synaptic regulation of the septal-hippocampal cholinergic neurons. Life Sci 32: 165-179

Crawford JM, Curtis DR, Voorhoeve PE, Wilson VJ (1966) Acetylcholine sensitivity of cerebellar neurons in the cat. J Physiol (Lond) 186: 139-165

Crepel F, Dhanjal SS (1982) Cholinergic mechanisms and neurotransmission in the cerebellum of the rat: an in vitro study. Brain Res 244: 59-68

De la Garza R, Bickford-Wimer PC, Hoffer BJ, Freedman R (1987) Heterogeneity of nicotine actions in the rat cerebellum: an in vivo electrophysiologic study. J Pharmacol Exp Ther 240 689-695

Eagan TM, North RA (1985) Acetylcholine acts on m_2-musarinic receptors to excite rat locus coeruleus

neurones. Br J Pharmacol 85: 733-735
Freedman R, Hoffer BJ, Woodward DJ, Puro D (1977) Interaction of norepinephrine with cerebellar activity evoked by mossy and climbing fibers. Exp Neurol 55: 269-288
Gilbert P (1975) How the cerebellum could memorise movements. Nature 254: 688-689
Goldberg AM, McCaman RE (1967) A quantitative microchemical study of choline acetyltransferase and acetylcholinesterase in the cerebellum of several species. Life Sci 6: 1493-1500
Graf W, Simpson JI, Leonard CS (1988) Spatial organisation of visual messages of the rabbit's cerebellar flocculus. II. Complex and simple spike responses of Purkinje cells. J Neurophysiol 60: 2091-2121
Hoffer BJ, Siggins GR, Oliver AP, Bloom FE (1973) Activation of the pathway from locus coeruleus to rat cerebellar Purkinje neurons: pharmacological evidence of noradrenergic central inhibition. J Pharmacol Exp Ther 184: 553-569
Ito M (1984) The Cerebellum and Neural Control. Raven New York 1984
Ito M, Jastreboff PJ, Miyashita Y (1982) Specific effects of unilateral lesions in the flocculus upon eye movements in albino rabbits. Exp Brain Res 45: 233-242
Ito M, Yoshida M (1964) The cerebellar-evoked monosynaptic inhibition of Deiters' neurones. Experientia 20: 515-516
Kása P, Bánsághy Z, Rakonczay Z, Gulya K (1982) Postnatal development of the acetylcholine system in different parts of the the rat cerebellum. J Neurochem 39: 1726-1732
Kása P, Silver A (1969) The correlation between choline acetyltransferase and acetylcholinesterase activity in different areas of the cerebellum of rat and guinea pig. J Neurochem 16: 389-396
Keller EL, Smith MJ (1983) Suppressed visual adaptation of the vestibular-ocular reflex in catecholamine depleted cats. Brain Res 258: 323-327
Kety SS (1970) The biogenic amines in the central nervous system: possible roles in arousal emotion and learning. In FO Schmidt (Ed) The Neurosciences: Second Study Program. Rockefeller University Press, New York pp 324-336
Krnjevic K, Pumain R, Renaud L (1971) The mechanism of excitation by acetylcholine in the cerebral cortex. J Physiol (Lond) 215: 447-465
Kuhar MJ, Atweh SF, Bird SJ (1978) Studies of cholinergic-monoaminergic interactions in the brain. In LL Butcher (Ed) Cholinergic-Monoaminergic Interactions in the Brain. Academic Press, New York
Lamour Y, Dutar P, Jobert A, Dykes RW (1988) An iontophoretic study of single somatosensory neurons in rat granular cortex serving the limbs: a laminar analysis of glutamate and acetylcholine effects on receptive-field properties. J Neurophysiol 60(2): 725-750
Lisberger SG, Pavelko TA (1988) Brain stem neurons in modified pathways for motor learning in the primate vestibulo-ocular reflex. Science 242: 771-773
Llinás R, Walton K (1980) Voltage-dependent conductances in neurons. In CW Cotman G Poste and GL Nicholson (Eds). The Cell Surface and Neuronal Function. Elsevier, Amsterdam pp 87-118
Madison DV, Lancaster B, Nicoll RA (1987) Voltage clamp analysis of cholinergic action in the hippocampus. J Neurosci 7: 783-741
Madison DV, Nicoll RA (1986) Actions of noradrenaline recorded intracellularly in rat hippocampal CA_1 pyramidal neurones in vitro. J Physiol (Lond) 372: 221-244
Mason ST, Fibiger HC (1979) Interaction between noradrenergic and cholinergic systems in the rat brain: behavioural function in locomotor activity. Neuroscience 4: 517-525
McCance I, Phillis JW (1968) Cholinergic mechanisms in the cerebellar cortex. Int J Neuropharmacol 7: 447-462

McCormick DA (1989) Cholinergic and noradrenergic modulation of thalamocortical processing. TINS 12(6): 215-221

McCormick DA, Prince DA (1986) Mechanisms of action of acetylcholine in the guinea-pig cerebral cortex in vitro. J Physiol (Lond) 375: 169-194

McElligot JG, Freedman W (1988) Vestibular-ocular reflex adaptation in cats before and after depletion of norepinephrine. Exp Brain Res 69: 509-521

McElligot JG, Freedman W (1988) Central and cerebellar norepinephrine depletion and vestibulo-ocular reflex (VOR) adaptation. In: Flohr H (Ed). Post-Lesion Neuronal Plasticity. Springer, Berlin pp 661-674

McIntosh FC (1941) The distribution of acetylcholine in the peripheral and the central nervous system. J Physiol (Lond) 99: 436-442

Miles FA, Lisberger SG (1981) Plasticity in the vestibulo-ocular reflex: a new hypothesis. Annu Rev Neurosci 4: 273-299

Moises HC, Burne RA, Woodward DJ (1990) Modification of the visual response properties of cerebellar neurons by norepinephrine. Brain Res 514: 259-275

Moises HC, Waterhouse BD, Woodward DJ (1981) Locus coeruleus stimulation potentiates Purkinje cell responses to afferent input: the climbing fiber system. Brain Res 222: 43-64

Moises HC, Woodward DJ (1980) Potentiation of GABA inhibitory action in the cerebellum by locus coeruleus stimulation. Brain Res 182: 327-344

Mori-Okamoto J, Tatsuno J (1988) Effects of noradrenaline on the responsiveness of cultured cerebellar neurons to excitatory amino acids. Brain Res 448: 259-271

Moroni F, Tanganelli S, Antonelli T, Carla V, Bianchi C, Beani L (1983) Modulation of cortical acetylcholine and γ-aminobutyric acid release in freely moving guinea pigs: effects of clonidine and other adrenergic drugs. J Pharmacol Exp Ther 227: 435-445

Nagao S (1983) Effect of vestibulocerebellar lesions upon dynamical characteristics and adaptation of vestibulo-ocular and optokinetic responses in pigmented rabbits. Exp Brain Res 53: 36-46

Nicoll RA (1988) The coupling of neurotransmitter receptors to ion channels in the brain. Science 241: 545-551

Ojima H, Kawajiri SI, Yamasaki T (1989) Cholinergic innervation of the rat cerebellum: qualitative and quantitative analyses of elements immunoreactive to a monoclonal antibody against choline acetyltransferase. J Comp Neurol 290: 41-52

Rogawski MA, Aghajanian GK (1980) Modulation of lateral geniculate neurone excitability by noradrenaline microiontophoresis or locus coeruleus stimulation. Nature 287: 731-734

Schwindt PC, Spain WJ, Foehring RC, Chubb MC, Crill WE (1988) Slow conductances in neurones from cat sensorimotor cortex in vitro and their role in slow excitability changes. J Neurophysiol 59: 450-467

Siggins GR, Battenberg EF, Hoffer BJ, Bloom FE (1973) Noradrenergic stimulation of cyclic adenosine monophosphate in rat Purkinje neurons: an immunocytochemical study. Science 179: 585-588

Sillito AM, Kemp JA (1983) Cholinergic modulation of the functional organisation of the cat visual cortex. Brain Res 289: 143-155

Sillito AM, Kemp JA, Berardi N (1983) The cholinergic influence on the function of the cat dorsal lateral geniculate nucleus (dLGN). Brain Res 280: 299-307

Tan HS, Collewijn H (1991) Cholinergic modulation of optokinetic and vestibulo-ocular responses: a study with microinjections in the flocculus of the rabbit. Exp Brain Res 85: 475-481

Van Neerven J, Pompeiano O, Collewijn H (1989) Depression of the vestibulo-ocular and optokinetic

responses by intra-floccular micro-injection of GABA-A and GABA-B agonists in the rabbit. Arch Ital Biol 127: 243-263

Van Neerven J, Pompeiano O, Collewijn H, Van der Steen J (1990) Injections of β-noradrenergic substances in the flocculus of rabbits affect adaptation of the VOR gain. Exp Brain Res 79: 249-260

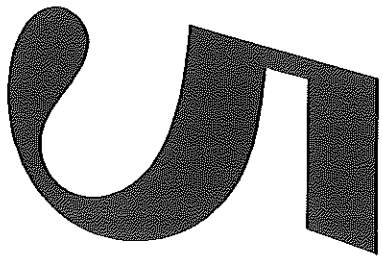
Vizi ES (1980) Modulation of cortical release of acetylcholine by noradrenaline released from nerves arising from the rat locus coeruleus. Neuroscience 5: 2139-2144

Vizi ES, Kiss J, Elenkov IJ (1991) Presynaptic modulation of cholinergic and noradrenergic neurotransmission: interaction between them. NIPS 6: 119-123

Waser PG (1975) Cholinergic Mechanisms. Raven Press, New York

Watson M, McElligot JG (1984) Cerebellar norepinephrine depletion and impaired acquisition of specific locomotor tasks in rats. Brain Res 296: 129-138

Westfall TC (1974) Effect of muscarinic agonists on the release of 3H-norepinephrine and 3H-dopamine from rat brain slices. Life Sci 14: 1441-1452



Muscarinic nature of cholinergic receptors in the cerebellar flocculus involved in the enhancement of the optokinetic response

Introduction

In the cerebellar cortex, cholinergic receptors of both the muscarinic type (m-receptors; Mash and Potter, 1986; Spencer et al., 1986; Neustadt et al., 1988; Alonso et al., 1990) and the nicotinic type (n-receptors; Hunt and Schmidt, 1978; Swanson et al., 1987) have been identified. Both types have also been implicated in cholinergic transmission. Crawford et al. (1966) found an excitatory effect of ACh on Purkinje-cells (P-cells). This effect was slow in onset and could be mimicked by muscarine and not by nicotine, implying transmission by m-type cholinergic receptors. In contrast, McCance and Phillis (1968) denied a direct effect of ACh on P-cells and designated the granule cells as the cholinoceptive cells. The action of nicotine on cerebellar cells was studied more specifically by De la Garza et al. (1987), who differentiated nicotinic receptors further into n_1 and n_2 types. A curare-sensitive (n_2) site was found to mediate excitatory effect of nicotine on cerebellar interneurons, while a hexamethonium-sensitive (n_1) site mediated the inhibitory effect of nicotine on P-cells. Furthermore, presynaptic n_1-type receptors were encountered which enhanced the release of ACh (Lapchak et al., 1989).

The studies cited above recorded in the rostral vermis or did not specifically mention the localization of their recordings within the cerebellum. Biochemical and histochemical studies have indicated that the archicerebellum (the ventral part of lobule IX and lobule X) contains a much larger amount of acetylcholinesterase (AChE) and cholineacetyltransferase (ChAT) than the paleocerebellum and the neocerebellum (Csillik et al., 1963; Kasa and Silver, 1969). This differential distribution of ChAT was confirmed more recently in the rat with a monoclonal antibody, raised against ChAT (Ojima et al., 1989). Furthermore, the archicerebellum of a variety of species has been found to contain the highest density of m-receptors within the cerebellum (Neustadt et al., 1988).

Specific studies of cholinergic effects in the archicerebellum (Crepel and Dhanjal, 1982) showed an action of ACh that was very similar to muscarinic effects on other central neurones. ACh application caused a depolarization of the cell, which increased the sensitivity of cells to a depolarizing input. In the cerebellar P-cells, this action could be blocked by muscarinic antagonists, in a similar way as in the neocortex and hippocampus (see Nicoll, 1988; Halliwel, 1990).

The archicerebellum and specifically the cerebellar flocculus are closely involved in the control of eye movements. In a previous study, the function of the cholinergic system in the control of gaze-stabilizing reflexes by the cerebellar flocculus was investigated (Tan and Collewijn, 1991). Injection of carbachol, an aselective cholinergic agonist, in the flocculus caused a pronounced increase in gain of the optokinetic reflex (OKR), and a smaller increase in the gain of the vestibulo-ocular reflex (VOR). These results suggested a positive modulatory function of the cerebellar cholinergic system,

possibly analogous to similar mechanisms described in hippocampus and neocortex. Due to the aselective nature of carbachol, however, this study did not distinguish between actions of carbachol on muscarinic or nicotinic receptors. The present study was undertaken to make this differentiation.

Methods

Approach of the flocculus

Six pigmented Dutch belted rabbits of either sex were permanently implanted with ocular sensor coils for electromagnetic eye movement recording (Collewijn, 1977) and scull-screws for fixation of the head. The flocculi were localized electrophysiologically on the basis of visually evoked direction-selective complex-spike and simple-spike activity and guide cannulas, aimed towards right and left flocculi, were implanted bilaterally. All surgical procedures were done under general anesthesia, induced by a mixture of ketamine, acepromazine and xylazine. More details of the animal preparation are described in Tan and Collewijn (1991).

Injected drugs

We used carbachol (carbamylcholine chloride; Sigma, U.S.A.; 1 μg/μl) as an aselective agonist of both muscarinic and nicotinic cholinergic receptors; betanechol (carbamyl-β-methyl-choline chloride; Sigma, U.S.A.; 10 μg/μl) as a selective muscarinic agonist and DMPP (1,1-dimethyl-4-phenyl-piperazinium iodide; Aldrich, U.S.A.; 10 μg/μl) as a selective n_1-agonist. These substancess were dissolved in saline and the pH was adjusted to 7.0-7.4. The solutions were injected through stainless steel injection cannulas (outer diameter 0.35 mm), connected to a Hamilton 1.0 μl syringe, and introduced through the guide-cannula to the predetermined depth where the flocculus had been previously localized. In control experiments, only the solvent (saline) was injected.

Experimental conditions

During the experiment, the rabbit was restrained in a linen bag and secured onto a platform which could be rotated about an earth-vertical axis. The rabbit's head was bolted to a frame which was attached to the platform, but did not intrude into the rabbit's visual field and positioned in such a way that the interocular axis was intersected by the rotational axis of the platform. The platform was surrounded by a richly patterned drum (diameter 70 cm) which could be rotated about an earth-vertical axis, collinear with the axis of the platform. For measurement of the OKR, the drum was oscillated sinusoidally with an amplitude of 10° peak-to-peak at 0.15 Hz.

The VOR in darkness was tested by similar sinusoidal oscillation of the platform

in total darkness. The combined response of VOR and OKR ("VOR in the light") was tested by oscillating the rabbit within the illuminated, stationary drum.

Before any injection, 3 sets of baseline measurements were recorded, each consisting of a measurement of the VOR in the light, the VOR in darkness and the OKR. The mean values of these 3 sets of 3 baseline measurements were designated as the reference gain-value at time t=0, relative to which subsequent changes in gain were evaluated. After the baseline measurements, a bilateral injection with one of the substances was made into the right and left flocculus. Within 30 seconds after injection, the next set of measurements was started. This was followed by 3 sets of measurements at intervals of 5 min and two further sets of measurements at intervals of 15 min. In total, a time-span of 45 min after injection was covered. One set of recordings took 2 min. During the intervening 3 or 13 min, respectively, the rabbit was kept stationary on the platform within the lighted, stationary drum. The several drugs used were injected in successive sessions, separated by several days, to allow complete recovery and return to baseline values after a previous session.

The experiments were computer-driven (DEC, PDP 11/73) and the data were gathered by a data-acquisition program, which collected in each trial 4 cycles of the sinusoidal motion stimulus at a sample frequency of 51.2 Hz. In the subsequent off-line analysis, the fast phases were removed from the eye position recordings, after which gain and phase of the responses were determined by a fast Fourier transformation. The statistical significance of the effects of the injected substances was tested by a Multiple Analysis of Variance (MANOVA), which allows the comparison of several variables in a single group of animals.

Results

Fig. 1 shows the mean effects of the various injections on the VOR in the light, the VOR in darkness and the OKR in the six rabbits tested. Changes in gain, relative to the baseline value, are plotted against time after the injection.

Control experiments

Bilateral floccular injections with the solvent, saline (1 μl), were given to reveal any aspecific effects of the injection procedure or the measurement program as such. The gain values obtained in these sessions served as the control-values against which the effects of the injected substances were statistically evaluated.

The average baseline gains in these sessions were 0.81 ± 0.05 (S.D.) for the VOR in the light, 0.62 ± 0.12 (S.D.) for the VOR in darkness, and 0.58 ± 0.15 for the OKR.

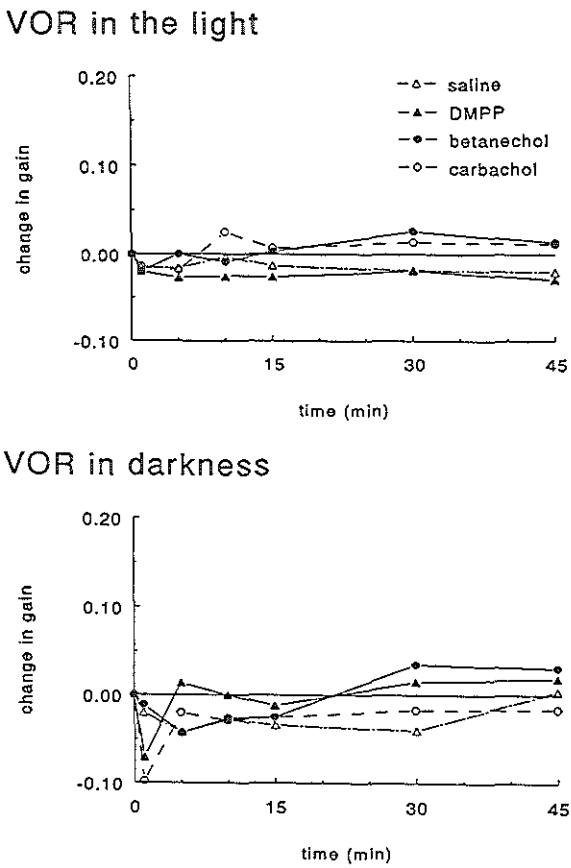


Fig.1 Mean effects of bilateral, floccular injections of the different substances at time = 0 on the gain of oculomotor reflexes as a function of time, averaged over 6 rabbits. The graphs show the effects on the VOR in the light (upper panel), the VOR in darkness (middle panel) and the OKR (lower panel). Baseline gains before the injection are normalized to zero. Standard deviations are given in the text.

These baseline values are depicted in Fig. 1 as zero-values at t=0. The gain values measured after the injection of saline are plotted as changes in gain, relative to these average baseline values, as a function of time after injection. Changes in gain after saline injections were slight and statistically insignificant for the VOR in the light (p=0.463), the VOR in darkness (p=0.275) and the OKR (p=0.837).

Effects of carbachol

The effect of carbachol on the oculomotor reflexes in the present study agrees well with previous investigations (Tan and Collewijn, submitted). The baseline values were 0.81 ± 0.02 (S.D.) for the VOR in the light, 0.58 ± 0.08 for the VOR in darkness and 0.58 ± 0.15 (S.D.) for the OKR. The gain of the VOR in the light remained unaffected by carbachol throughout the session (p=0.534). The gain of the VOR in darkness showed a transient reduction by 0.09 ± 0.11 (S.D.), only in the first measurement, immediately after the injection, but remained statistically unchanged (p=0.375) for the session as a whole. The effect of carbachol on the OKR, however, was very marked. After a small, transient decrease immediately after the injection (resembling the dip in VOR gain in darkness), subsequent measurements showed a strong increase of the mean OKR gain in all rabbits, which was statistically significant (p=0.035). A maximum increase of 0.16 ± 0.06 (S.D.) was reached 15 min after the injection and sustained during the remainder of the session, which lasted for 45 min.

Effects of betanechol

The mean baseline values in these sessions were 0.85 ± 0.03 (S.D.) for the VOR in the light, 0.67 ± 0.07 (S.D.) for the VOR in darkness, and 0.58 ± 0.20 for the OKR. Neither the gain of the VOR in the light, nor the gain of the VOR in darkness were changed significantly by betanechol (p=0.735 and p=0.426, respectively). In contrast, immediately after the injection of betanechol a pronounced increase in the gain of the OKR was evident. Already the first measurement, taken within 30 sec after the injection, showed an increase in the gain of the OKR of 0.05 ± 0.03 (S.D.). The increase reached a maximum of 0.16 ± 0.07 (S.D.) 15 min after the injection; this maximum was sustained throughout the further course of the experiment. The increase was statistically significant (p=0.042), and similar to the increase induced by carbachol.

Effects of DMPP

The mean baseline values in the session in which the n_1-agonist DMPP was injected, were 0.85 ± 0.05 (S.D.) for the VOR in the light, 0.70 ± 0.12 (S.D.) for the VOR in darkness and 0.59 ± 0.23 (S.D.) for the OKR. The injections of DMPP had no significant effect on the gains of the VOR in the light (p=0.712) and the VOR in darkness (p=0.284)

The gain of the VOR in the light never deviated more than 0.03 from the baseline gains. The gain of the VOR in darkness, however, showed a small initial decrease of 0.07 ± 0.06 (S.D.), which resembled the dip induced by carbachol. The mean gain of the OKR initially showed a slight decrease, which turned into an increase at t = 15 min after injection. This increase, however, amounted to only 0.06 ± 0.05 (S.D.). and is unlikely to reflect a real effect, because the post-injection gain values were statistically identical to those in the control experiment ($p=0.910$).

Discussion

The present study shows that the enhancement of OKR and VOR by carbachol, described in previous studies (Tan and Collewijn, 1991; Tan et al., submitted), is most probably relayed by cholinergic receptors of the muscarinic type. Application of the selective muscarinic agonist betanechol strongly increased the gain of the OKR, in the same way as carbachol. The absence of effects after injection of the nicotinic agonist DMPP shows that the carbachol effects were not mediated by nicotinic receptors of the n_1 type. However, no conclusions can be drawn concerning a possible nicotinic contribution through n_2 receptors. Furthermore, the present study did not attempt to differentiate between muscarinic subtypes.

Carbachol induced a transient decrease in the gain of the OKR and VOR in darkness, consistent with previous observations (Tan and Collewijn, submitted). Whereas the increase in gain of the OKR produced by betanechol is similar to carbachol, the initial decrease in gain after carbachol injection was not mimicked by betanechol. This suggests that the initial negative effect is due to stimulation of another type of receptor, possibly nicotinic. Indeed, stimulation of n_1 receptors with DMPP induced a similar transient decrease in the gain of the VOR in darkness.

In a previous study (Tan et al., submitted), we hypothesised that the positive effect of carbachol injection of the oculomotor reflexes is achieved by a mechanism similar to the one revealed in hippocampus and neocortex, i.e., the blockade of slow after-hyperpolarization (see Nicoll, 1988). This would permit higher rates of firing, and thus a deeper modulation of P-cell activity, which would contribute to the gain of the OKR. Important points of similarity between the cholinergic effects in the flocculus and in the hippocampus and neocortex are that the effects are muscarinic (see Nicoll, 1988; Halliwell, 1990) and that they are mimicked by β-noradrenergic stimulation (Nicoll, 1988; Tan and Collewijn, submitted).

Localization of muscarinic receptors in the cerebellar cortex

It has been demonstrated that cerebellar cortical microvessels and capillaries contain muscarinic receptors (Estrada et al 1983; Grammas et al 1983). Investigation of the distribution of muscarinic receptors in the cerebellar cortex of the mutant mouse showed that even in spite of the absence of various cell types, including granule, Purkinje, and Golgi cells, no corresponding decreases in receptor density were observed, suggesting that muscarinic receptors in the cerebellum are localized on vascular, but not neuronal elements. For the archicerebellum of the rabbit, however, there is specific evidence in favour of a neuronal localization of m-receptors. Neustad et al. (1988) have studied the laminar and lobular distribution of muscarinic receptors. They demonstrated a considerable species variability in the distribution of muscarinic receptors in mouse, rat, guinea pig and rabbit cerebellar cortex. In all species, the archicerebellum contained the highest density of muscarinic receptors. Specifically in the rabbit, a narrow band of high density was seen over the P-cell layer. Importantly, the interlaminar and interlobular differences in distribution of muscarinic binding site density were not accompanied by corresponding increases in regional density of microvessels and capillaries. The higher density of muscarinic binding sites encountered in the archicerebellum, thus, reflects the presence of neuronal receptors in that structure. More specifically, in the rabbit (but not in the mouse, rat and guinea pig) the density in the molecular layer, granule cell layer and white matter was similar in the archicerebellum and the neo- and paleocerebellum, but the density in the P-cell layer was about twice as high in the archicerebellum than in the neo- and paleocerebellum. This suggests that the neuronal muscarinic receptors in the rabbit flocculus are localized indeed primarily on P-cells; a condition which would, in principle, allow for the kind of cholinergic actions postulated above.

Summary and conclusions

In a previous study, intrafloccular micro-injection of the aselective cholinergic agonist carbachol was found to enhance the optokinetic reflex, OKR (Tan and Collewijn, 1991). Histochemical and physiological studies have identified cholinergic receptors of the muscarinic as well as nicotinic type in the cerebellar cortex, and both have been implicated in cholinergic transmission. The present study was undertaken to elucidate the receptor type involved in the control of OKR. For that purpose, effects of injections of the nicotinic n_1-agonist DMPP on the OKR and vestibulo-ocular reflex (VOR) were compared with injections of the muscarinic agonist betanechol and the aselective cholinergic agonist carbachol. Injection of betanechol mimicked the enhancement of the OKR by carbachol, while DMPP had no effect. We conclude that muscarinic receptors are involved in the

positive modulatory action of the cholinergic system in the cerebellar flocculus.

References

Alonso, R., Didier, M., and Soubrie, P., [^{3}H]*N*-Methylscopolamine binding studies reveal M_2 and M_3 muscarinic receptor subtypes on cerebellar granule cells in primary culture, *J. Neurochem.* 55 (1990) 334-337.

Collewijn, H., Eye- and head movements in freely moving rabbits, *J. Physiol. (Lond.)*, 266 (1977) 471-498.

Crawford, J.M., Curtis, D.R., Voorhoeve, P.E. and Wilson, V.J., Acetylcholine sensitivity of cerebellar neurons in the cat, *J. Physiol. (Lond.)*, 186 (1966) 139-165.

Crepel, F. and Dhanjal, S.S., Cholinergic mechanisms and neurotransmission in the cerebellum of the rat: an in vitro study, *Brain Research*, 244 (1982) 59-68.

Csillik, B., Joo, F., and Kasa, P., Cholinesterase activity of archicerebellar mossy fibre apparatuses, *J. Histochem. Cytochem.*, 11 (1963) 113-114.

De la Garza, R., Bickford-Wimer, P.C., Hoffer, B.J. and Freedman, R., Heterogeneity of nicotine actions in the rat cerebellum: an in vivo electrophysiologic study, *J. Pharmacol. Exp. Ther.*, 240 (1987) 689-695.

Estrada, C., Hamel, E., and Krause, D.N., Biochemical evidence for cholinergic innervation of intracerebral blood vessels, *Brain Research*, 266 (1983) 261-270.

Grammas, P., Diglio, C.A., Marks, B.H., Giacomelli, F., and Wiener, J., Identification of muscarinic receptors in rat cerebral cortical microvessels. *J. Neurochem.*, 40 (1983) 645-651.

Halliwel, J.V., Physiological mechanisms of cholinergic action in the hippocampus, *Prog. Brain Res.*, 84 (1990) 255-272.

Hunt, S. and Schmidt, J., Some observations on the binding patterns of α-bungarotoxin in the central nervous system of the rat, *Brain Research*, 522 (1978) 213-232.

Kása, P. and Silver, A., The correlation between choline acetyltransferase and acetylcholinesterase activity in different areas of the cerebellum of rat and guinea pig, *J. Neurochem.*, 16 (1969) 389-396.

Lapchak, P.A., Araujo, D.M., Quirion, R., and Collier, B., Presynaptic cholinergic mechanisms in the rat cerebellum: evidence for nicotinic, but not muscarinic autoreceptors, *J. Neurochem.*, 53 (1989) 1843-1851.

Mash, D.C. and Potter, L.T., Autoradiagraphic localization of M_1 and M_2 muscarine receptors in the rat brain, *Neuroscience*, 19 (1986) 551-564.

McCance, I. and Phillis, J.W., Cholinergic mechanisms in the cerebellar cortex, *Int. J. Neuropharmacol.*, 7 (1968) 447-462.

Neustadt, A., Frostholm, A., and Rotter, A., On the cellular localization of cerebellar muscarinic receptors: An autoradiographic analysis of weaver, reeler, Purkinje cell degeneration and staggerer mice, *Brain Res. Bull.*, 20 (1988) 163-172.

Neustadt, A., Frostholm, A., and Rotter, A., Topographical distribution of muscarinic cholinergic receptors in the cerebellar cortex of the mouse, rat, guinea pig, and rabbit: a species comparison, *J. Comp. Neurol.*, 272 (1988) 317-330.

Nicoll, R.A., The coupling of neurotransmitter receptors to ion channels in the brain, *Science*, 241 (1988) 545-551.

Ojima, H., Kawajiri, S.I., Yamasaki, T., Cholinergic innervation of the rat cerebellum: qualitative and

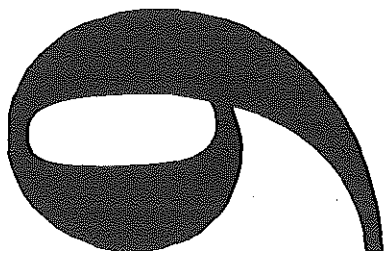
quantitative analyses of elements immunoreactive to a monoclonal antibody against choline acetyltransferase, *J. Comp. Neurol.*, 290 (1989) 41-52.

Spencer, D.G., Horváth, , E., and Traber, J., Direct autoradiographic determination of M_1 and M_2 muscarinic acetylcholine receptor distribution in the rat brain: relation to cholinergic nuclei and projections. *Brain Research*, 380 (1986) 59-68.

Swanson, L.W., Simmons, D.M., Whiting, P.J., and Lindstrom, J., Immunohistochemical localization of neuronal nicotinic receptors in the rodent central nervous system. *J. Neurosci.*, 7 (1987) 3334-3342.

Tan, H.S. and Collewijn, H., Cholinergic modulation of optokinetic and vestibulo-ocular responses: a study with microinjections in the flocculus of the rabbit, *Exp. Brain Res.*, 85 (1991) 475-481.

Tan, H.S. and Collewijn, H., Cholinergic and noradrenergic stimulation in the rabbit flocculus have synergistic facilitatory effects on optokinetic responses, Submitted to *Brain Research.*



Optokinetic nystagmus and its modulation by bilateral microinjection of carbachol in the cerebellar flocculus

Introduction

Steady rotation of the visual surroundings around an animal elicits, via the optokinetic reflex (OKR), a regular pattern of tracking eye movements (slow phases) and resettings (fast phases), called optokinetic nystagmus (OKN). In the rabbit, the slow phase velocity of OKN shows a very limited direct rise in velocity (not exceeding a few degrees per second) at the onset of stimulation (Collewijn, 1969). This "direct response" is followed by an "indirect" response, showing a gradual build-up of slow-phase eye velocity, as first described by Ter Braak (1936), until a steady-state is reached. Upon termination of the stimulus by sudden darkness, nystagmus continues for some time as optokinetic afternystagmus (OKAN; Ter Braak, 1936; Collewijn, 1969). From the beginning (Ter Braak, 1936, Mowrer, 1937; Jung, 1948), a role in the cancelling of post-rotatory nystagmus (PRN), following a step of rotation, has been attributed to gradual build-up and OKAN. The gradual build-up and OKAN are often described as the charging and discharging of a central velocity-storage mechanism, resembling a central integrator that is possibly shared with the vestibular system (Collewijn, 1972; Cohen et al., 1977; Robinson, 1977; Raphan et al., 1979; Raphan and Cohen, 1985).

Intimate involvement of the cerebellar flocculus with the OKR has been demonstrated in the rabbit by lesions (Ito et al., 1982; Nagao, 1983; Barmack and Pettorossi, 1985) and by temporary inhibition of signal-transmission due to floccular injection of GABA-agonists (Van Neerven et al., 1989). Furthermore, electrophysiological recording (Graf et. al, 1988) showed Purkinje-cell activity corresponding with retinal image-slip, and the role of the flocculus in oculomotor control was further corroborated by the discovery that smooth eye movements could be elicited by electrical micro-stimulation in the flocculus (Dufossé et al., 1977; Nagao et al., 1985; Van der Steen et al., 1991). However, the role of the flocculus in controlling the direct and indirect components of OKN has not yet been investigated.

The involvement of the flocculus in the OKR has also been established in other species. The cat's OKN is very similar to that of the rabbit (Evinger and Fuchs, 1978; Donaghy, 1980; Maioli and Precht, 1984; see Collewijn, 1985). Flocculectomy in the cat reduced OKR gain (Keller and Precht, 1979), but the charging and discharging characteristics of OKN were not specifically studied. In the monkey, build-up occurs in conjunction with a well-developed direct response (Komatsuzaki et al., 1969; Igarashi et al., 1977; Cohen et al., 1977; Lisberger et al., 1981), which is capable of reaching eye velocities close to 100°/s within a second. Higher velocities are built up only gradually. After termination of stimulation by the onset of darkness, an immediate initial drop in velocity occurs, followed by a gradual decaying OKAN. Recordings in the flocculus showed activity of floccular cells during OKAN, related to eye velocity (Waespe and Henn, 1981).

However, the main effect of flocculectomy in the monkey appeared to be an almost total abolition of the direct component of the optokinetic response without a change in the discharge characteristics of OKAN (Zee et al., 1981; Waespe et al., 1983) and PRN (Zee et al., 1981), disproving floccular involvement in velocity-storage. Due to the grossly different properties of monkey and rabbit OKN, however, extrapolation of data on flocculectomy in the monkey to the rabbit is perilous.

We previously demonstrated in the rabbit an enhancing effect of bilateral floccular injection of the aselective cholinergic agonist carbachol on the optokinetic response to a sinusoidal, low velocity stimulus (Tan and Collewijn, 1991). The present study was initiated to assess the effects of such injections on the build-up rate of OKN and the duration of OKAN, both storage-dependent parameters of OKN. Preliminary results have been presented elsewhere (Collewijn et al., in press).

Methods

Animal preparation

We used 5 young adult, pigmented Dutch belted rabbits of either sex. They were permanently implanted with scleral sensor coils for eye movement recording and scull-screws for fixation of the head. Five windings of stainless steel, teflon-coated wire (type AS 632, Cooner Chatsworth, CA) were woven underneath the conjunctiva and the superior, inferior and medial rectus muscles and inferior oblique muscle. Eye movements were measured with the magnetic induction method, based on phase detection, with absolute angular calibration of the recordings (Collewijn, 1977). Both flocculi were localized electrophysiologically on the basis of visually induced direction-selective complex-spike and simple-spike activity (Graf et al., 1988) and cannulas were implanted bilaterally to guide the injection (for further details see Van Neerven et al., 1989). All surgical procedures were done under general anaesthesia, induced by a mixture of ketamine, acepromazine and xylazine.

Experimental conditions and data analysis

The rabbit remained stationary throughout the experiment and was surrounded by a drum (diameter 70 cm), lined inside with a random-dot pattern, which was rotated at constant speed about an earth-vertical axis. Drum motion was started in darkness and once its velocity was stable, the light was turned on. Build-up of OKN was recorded for 40 s. After a steady state was reached, a second measurement was started. After 10 s, the light was turned off and optokinetic afternystagmus (OKAN) was recorded during the following 30 sec. Stimulation was applied in either direction (clockwise, CW = rightward and

counterclockwise, CCW = leftward) at three speeds: 5, 10 and 30°/s. These stimuli were presented three times under three different viewing conditions: binocular viewing, and monocular viewing of left or right eye. After baseline measurements had been obtained for all of these conditions, 1 μg of carbachol (Sigma, U.S.A) in 1 μl saline was injected in each flocculus. Starting 20 min after these injections, all OKR measurements were repeated. Control sessions were run on 3 animals with bilateral injection of 1 μl of saline (the solvent) only. The effect of carbachol was also tested at higher velocities (20, 60 and 110°/s) in a separate session in all 5 rabbits. To record the entire period of build-up and OKAN, these measurements had to be longer (160 s for build-up and 80 s for the OKAN). These high speeds were only applied during binocular viewing.

The experiments were computer-controlled (DEC, PDP 11/73). The position signal of the left eye was stored by a data-acquisition program with a sample frequency of 51.2 Hz. In the subsequent off-line analysis, slow-phase eye velocity was calculated and plotted as a function of time. From these plots, steady-state velocity and duration of OKAN were manually determined. The consistently linear shape of velocity build-up allowed calculation of eye acceleration as the tangent of the angle of a manually fitted line through the plotted slow-phase eye velocity data points. The statistical significance of the effects of carbachol was tested with a Multiple Analysis of Variance (MANOVA), which allows the comparison of several variables in a single group of animals.

Histological verification

Although the flocculus had already been carefully localized under electrophysiological guidance, an additional verification of the localization of the injection site was done in three rabbits at the end of their series of experiments. With the same injection technique used for the injections of carbachol, 1 μl of a 0.2% solution of kainic acid (Sigma, U.S.A) was injected bilaterally. After 2 weeks survival, the animals were deeply anaesthetized and perfused from the left cardiac ventricle with saline and 10% formalin. After sectioning and staining of the brainstem and cerebellum, the injection site showed degeneration of cells. In all cases, the site was localized within, and restricted to the flocculus.

Results

Basic properties of optokinetic response to binocular constant-velocity stimulation

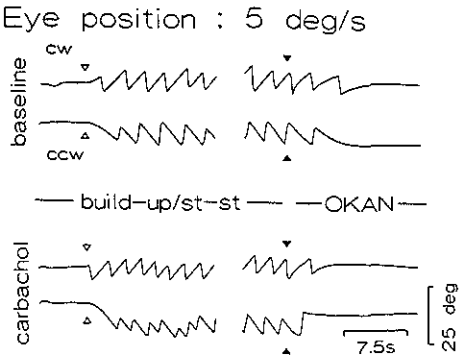
Typical baseline OKN, elicited during binocular stimulation at 5, 10 and 20°/s, is shown in the upper parts of the 6 panels of Fig. 1, while responses to stimulus velocities of 60 and 110°/s are shown in the upper parts of Figs. 2 and 3. An immediate slow-phase

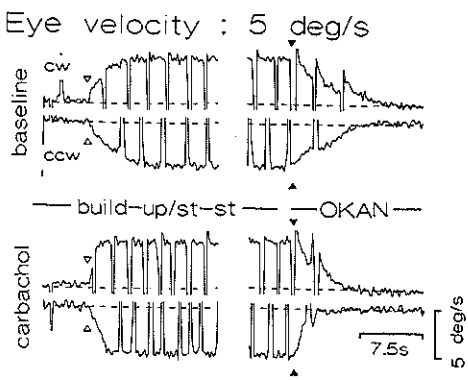
response of 1-2°/s occurred after the light was turned on (marked by open triangles), after which slow-phase velocity was built up to higher levels gradually. For all stimulus velocities tested, this build-up followed an approximately linear course, until a steady velocity was reached and maintained throughout the period of stimulation. The steady-state gain was about 0.9 for stimulus velocities up to 20°/s but decreased for higher stimulus velocities. The time it took to reach steady state increased almost proportionally with stimulus velocity (Figs. 1-3), implying a fixed value of eye-acceleration during build-up for all stimulus speeds.

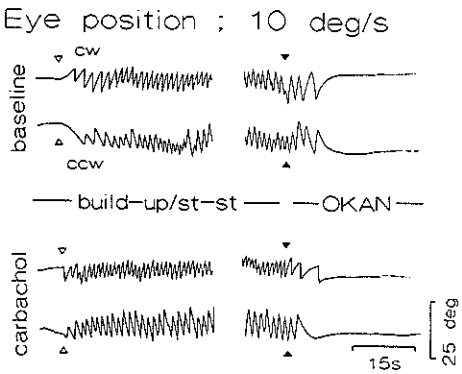
The light was turned off (as marked by the solid triangles in Figs. 1-3) after a steady-state OKN velocity had been maintained for some time. From that instant, the nystagmus continued as OKAN, with an approximately linear decay of eye velocity for all stimulus velocities examined. Following stimulation at speeds of 5, 10 and 20°/s, OKAN lasted about 10 s (Fig. 1). After stimulation with velocities above 20°/s, the duration of OKAN increased proportionally with stimulus velocity. In the rather extreme case shown in Fig. 3, OKAN after CCW stimulation at 110°/s even lasted for more than 70 s. A secondary afternystagmus (OKAN II) in the opposite direction of the primary OKN was encountered frequently after stimulus velocities of 60 and 110°/s (see e.g. Fig. 2, baseline CCW stimulation).

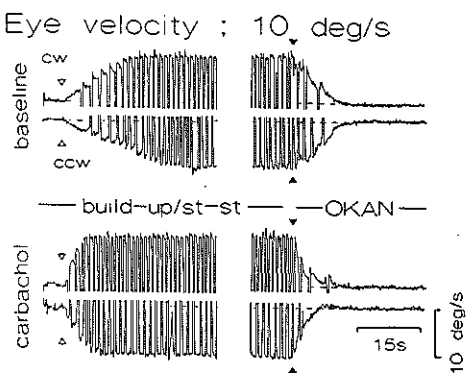
Fig. 4 shows some parameters of the mean baseline responses in 5 rabbits for 5 different stimulus velocities. Mean values are given for averaged responses to binocular stimulation in either direction. The upper panel shows the average build-up rate, expressed as mean eye-acceleration. Acceleration had a constant value of about 1°/s² for velocities up to 60°/s. This is shown in a different way in Fig. 5, in which average velocity-profiles of OKN elicited by different stimulus velocities are plotted. The responses show equal build-up acceleration for the different stimulus velocities, leading to an increase in duration of the velocity build-up to a steady state, proportional to stimulus velocity. For a stimulus velocity of 110°/s, however, the mean acceleration of the response dropped to 0.5°/s² (Fig. 4). After the stage of velocity build-up, a steady-state velocity was always sustained for stimulus velocities up to 60°/s; 2 out of 10 cases examined showed an irregular response to stimulation at 110°/s. The mean steady-state gain is about 0.9 up to 20°/s, but saturated at approximately 50°/s for higher stimulus velocities.

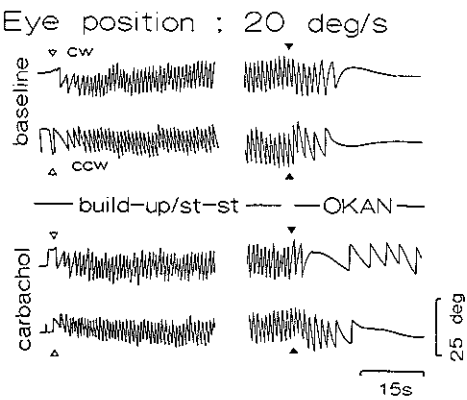
The analysis of OKAN was only performed in cases a with well-developed steady-state OKN. Consequently, the 2 out of 10 cases which showed poor responses to stimulation at 110°/s were omitted. On average, the duration of OKAN had a constant value of about 10 s for stimulus velocities up to 20°/s (see also Fig. 5). For stimulus velocities exceeding 20 °/s, the duration of OKAN increased proportionally to stimulus velocity, up to 24 s after stimulation at 110°/s.

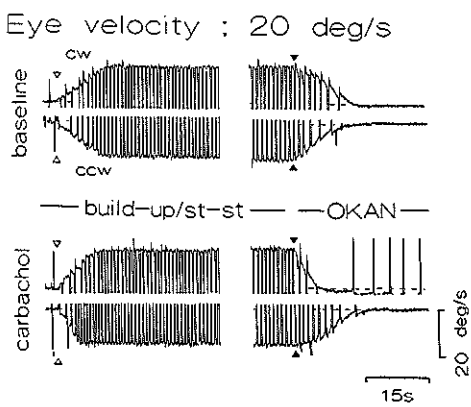

Eye position : 5 deg/s
cw
baseline
ccw
build-up/st-st
OKAN
carbachol
7.5s
25 deg


Eye velocity : 5 deg/s
cw
baseline
ccw
build-up/st-st
OKAN
carbachol
7.5s
5 deg/s


Eye position : 10 deg/s
cw
baseline
ccw
build-up/st-st
OKAN
carbachol
15s
25 deg


Eye velocity : 10 deg/s
cw
baseline
ccw
build-up/st-st
OKAN
carbachol
15s
10 deg/s


Eye position : 20 deg/s
cw
baseline
ccw
build-up/st-st
OKAN
carbachol
15s
25 deg


Eye velocity : 20 deg/s
cw
baseline
ccw
build-up/st-st
OKAN
carbachol
15s
20 deg/s

Effect of carbachol on optokinetic responses to binocular stimulation

Injection of carbachol never led to changes in the behavior of the animals. Moreover, no spontaneous nystagmus appeared, even not in darkness. The typical changes in the OKN velocity build-up and OKAN after carbachol are also illustrated in Figs. 1-3 for eye-position and slow-phase eye-velocity as a function of time. The most pronounced effect of bilateral floccular injection of 1 μg of carbachol was a strong acceleration of the velocity build-up. This is clearly visible in the velocity profiles as a shortening of the build-up phase. In contrast, steady-state responses were not affected by carbachol.

Even for a stimulus of 5°/s, the enhancement of the build-up by carbachol was distinct. In the case illustrated in Fig. 1, peak-velocity was attained after about 6 s in the baseline condition, and within 2-3 s after injection of carbachol into the flocculus. Acceleration had risen from about 0.72°/s² in the baseline condition to about 2.15°/s² after carbachol injection. Before the injection, build-up was slow during optokinetic stimulation at 10°/s, especially for CCW rotation, with an acceleration of about 0.65°/s². Steady-state velocities were reached after 20-25 s and were equal in either direction. After the carbachol injection, similar steady-state velocities as before injection were attained within 5-10 s, with mean accelerations of about 2.19°/s². Despite this enhanced build-up, there was no change in the level of the steady-state velocity. Similar changes were found for higher velocities. For a stimulus velocity of 60°/s (Fig. 2) the average acceleration was about 1.2°/s² before injection, taking about 36 sec to reach a steady-state velocity of about 50°/s. After carbachol, equal steady-state velocities were reached within less than 20 s with an acceleration of about 2.7°/s². The response to stimulation at 110°/s was very irregular. CW stimulation induced a build-up of velocity in the first 15 s, followed by a decay, after which velocity was built up gradually once again to a velocity of 40°/s. A steady state, however, was not reached because stimulation was terminated before the build-up stage had ended. Build-up was initially totally absent during stimulation in the CCW direction, but suddenly started after 80 s, to reach a steady state velocity of about 60°/s.

Furthermore, there was a clear shortening of the OKAN, which parallelled to some extent the shortening of the build-up. Before injection, OKAN typically lasted for about

Fig. 1 OKN elicited binocularly by steps in surround-velocity of 5, 10 and 20°/s in either direction (CW = right; CCW = left). For each stimulus velocity, eye position (left) and eye velocity (right) are shown as a function of time before and after injection of carbachol, with compressed time-scale for 10 and 20°/s stimulus velocities. Open triangles indicate the start of optokinetic stimulation; closed triangles indicate the time that the lights were turned off and eye movements continued as OKAN. Horizontal dashed lines in the right panels indicate zero velocity; fast phase velocities have been truncated. Notice the faster build-up after injection of carbachol, compared to the baseline conditions.

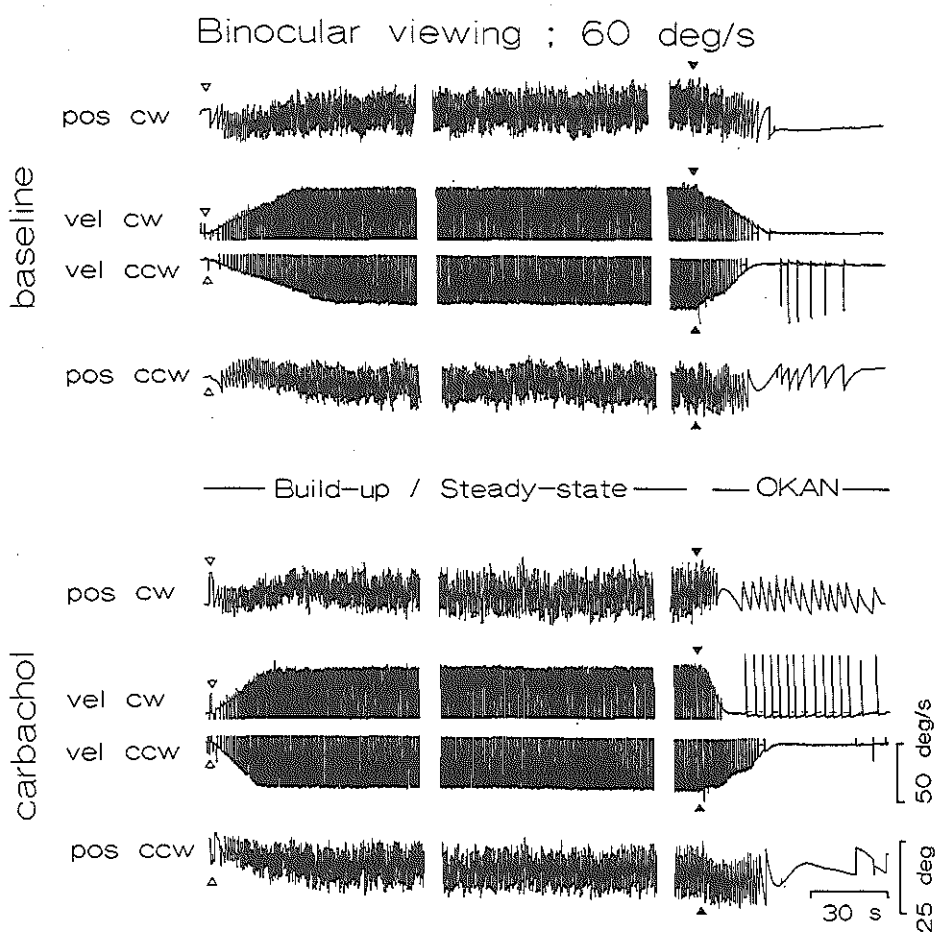


Fig. 2 Eye-position and slow phase velocity of binocularly elicited OKN as a function of time for a stimulus velocity of 60°/s in either direction (CW = right; CCW = left). Upper panel shows response before, lower panel after bilateral injection of carbachol. Open triangles indicate the start of optokinetic stimulation; closed triangles indicate the time that the lights were turned off and eye movements continued as OKAN. Horizontal dashed lines indicate zero velocity; fast phase velocities have been truncated. Notice the faster build-up after injection of carbachol, compared to the baseline conditions, and OKAN II in the baseline CCW and carbachol CW conditions.

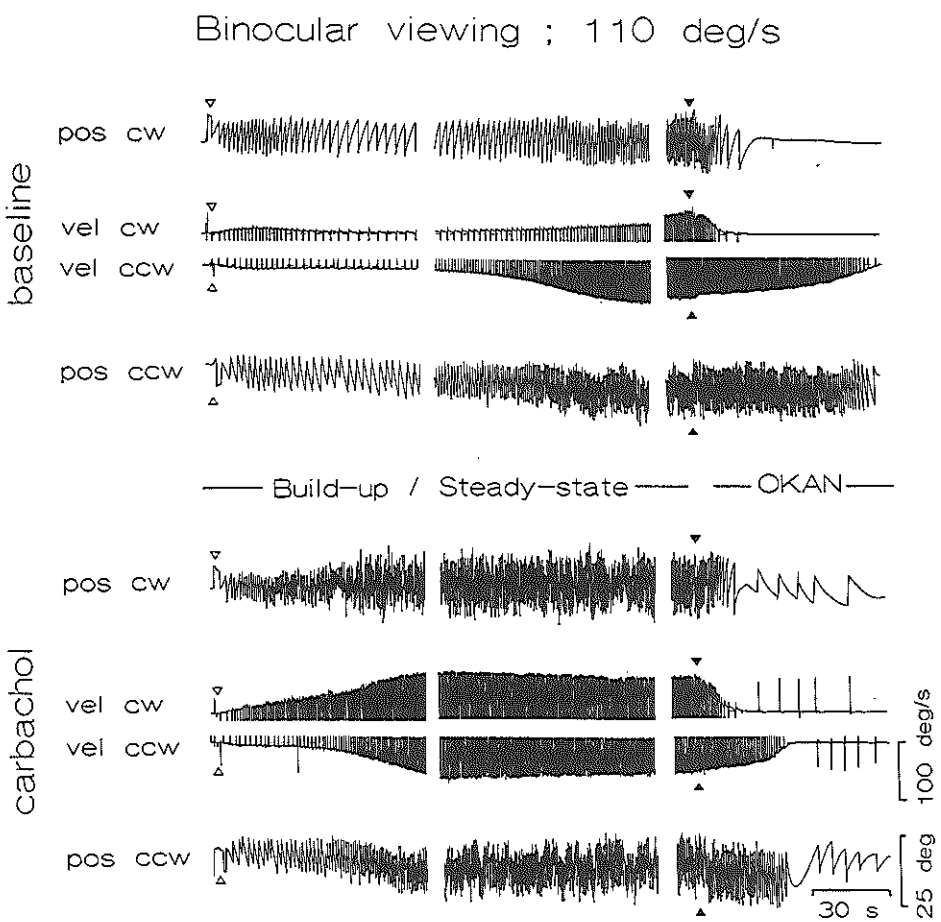


Fig. 3 Eye-position and slow phase velocity of binocularly elicited OKN as a function of time for a stimulus velocity of 110°/s in either direction (CW = right; CCW = left). Upper panel shows response before, lower panel after bilateral injection of carbachol. Open triangles indicate the start of optokinetic stimulation; closed triangles indicate the time that the lights were turned off and eye movements continued as OKAN. Horizontal dashed lines indicate zero velocity; fast phase velocities have been truncated. Notice the faster build-up after injection of carbachol, compared to the baseline conditions.

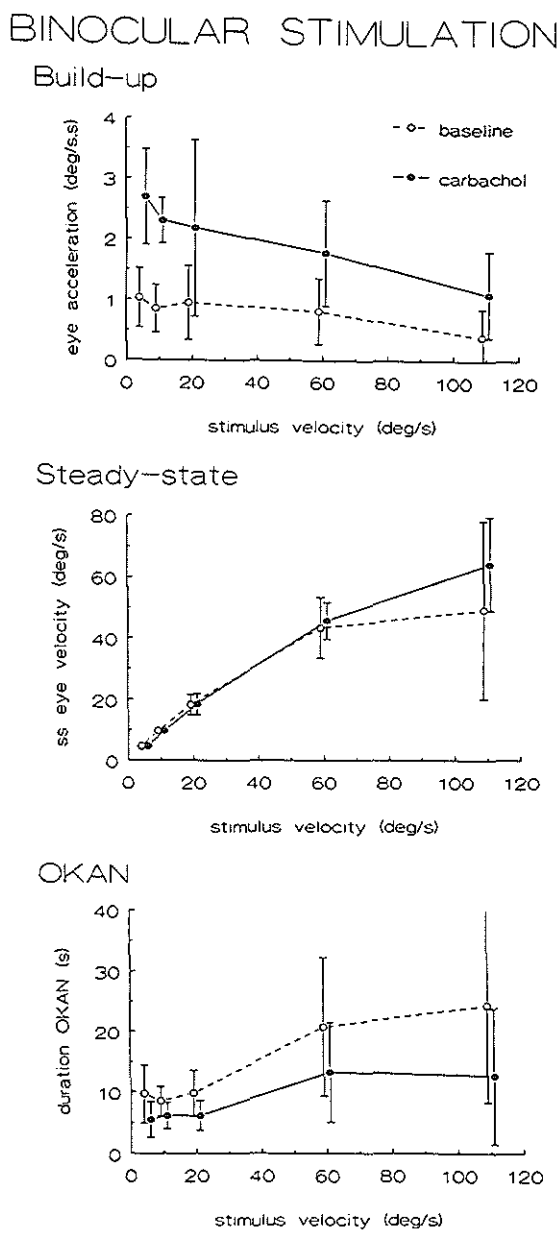


Fig. 4 Mean characteristic parameters of OKN before (open circles, interrupted lines) and after (closed circles, continuous lines) injection of carbachol as a function of stimulus velocity. Data points and error bars represent means ± standard deviation (n=10). Upper panel depicts mean eye-acceleration during build-up, middle panel shows steady-state velocity and lower panel shows duration of OKAN. Carbachol significantly enhances average eye-acceleration during build-up for all stimulus velocities tested, while duration of OKAN is shortened significantly. Steady state velocity is increased only at 110°/s stimulus velocity.

10 s (Fig. 1) but carbachol shortened the decay, sometimes to less than 5 s (see e.g. the example for stimulation at 5°/s CW, Fig. 1). Although the examples for 5 and 10°/s stimulus velocity display fairly symmetrical enhancement of build-up and decay, asymmetry between these effects of carbachol was frequently found, as shown for OKAN following stimulation at 20-110°/s in the rabbit illustrated in Figs. 1-3.

The mean effects of carbachol on the build-up of slow-phase velocity in the 5 rabbits tested are summarized in Fig. 4. The mean baseline value of eye-acceleration for velocities from 5 to 60°/s was about 1°/s^2, with an acceleration of 0.6°/s^2 for stimulation at 110°/s. After injection of carbachol, build-up acceleration was increased to about 2.6°/s^2 for 5-20°/s stimulus-velocity, and to progressively lower values for higher stimulus velocities. This increase in build-up acceleration was statistically significant ($p < 0.001$). For all velocities, the general shape of the build-up remained unchanged. In particular, the linear increase of velocity as a function of time (i.e. constant acceleration) was maintained; only the time course of build-up was compressed by carbachol (see examples in Figs. 1-3).

Steady-state velocities of OKN were not affected significantly by carbachol ($p=0.073$; Fig. 4, middle panel). Up to 20°/s the steady-state gain was already high before injection (about 0.9) and therefore not expected to increase substantially. Without exception, all rabbits showed well-developed steady-state OKN for velocities up to 50°/s before and after injection of carbachol. At 110°/s, however, 2 out of 5 rabbits had a very poor response for stimulation in one direction in the baseline condition. Injection of carbachol had a distinct effect at this high end of the velocity range, with an average increase in gain of 0.14. The two rabbits that did not develop a steady-state OKN in one direction before injection showed a convincing steady-state OKN after carbachol application.

Due to lack of steady-state OKN in the baseline experiments, these same two cases were rejected from the analysis of OKAN. The average OKAN-durations of the remaining eight cases are depicted in the lower panel of Fig 4. Carbachol induced a statistically significant increase in deceleration for all velocities tested ($p=0.045$). The effect was most pronounced at stimulus velocities of 60 and 110°/s, for which OKAN-duration was decreased from about 20 s to about 10 s.

Basic responses to monocular optokinetic stimulation

The upper part of Fig. 6 shows the typical eye movement responses elicited by monocular stimulation in both directions. Monocularly elicited OKR in the rabbit and other lateral-eyed animals is well known to be asymmetric, with a strong preference for stimulus motion in the temporal-to-nasal (anterior) direction (Ter Braak, 1936; Collewijn, 1969). The response in the temporal-to-nasal direction, indicated in Fig. 6 as "preferred", showed a regular nystagmus with steadily increasing slow phase velocity to steady-state,

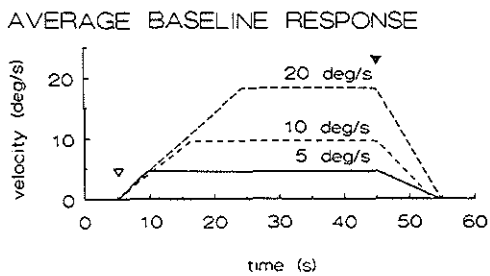


Fig. 5 Plots of average OKN velocity during build-up to steady-state and during OKAN as a function of time, based on data depicted in Fig. 4. For stimulation at 5, 10 and 20°/s, eye-acceleration during build-up has a fixed value, resulting in proportionally increasing duration of build-up with stimulus velocity. In contrast, OKAN shows a fixed duration. Open triangle marks time that lights are turned on; solid triangle marks time that lights are turned off.

followed by an afternystagmus after the lights were extinguished. In contrast, the response in the opposite ("non-preferred") direction showed a very poor response, with only incidental slow tracking.

In each rabbit, the optokinetic response was measured first with the left eye viewing and the right eye covered, and then with the right eye viewing and the left eye covered. Since only the responses of the left eye were measured, the first measurement will be referred to as the "seeing condition", while the right-eye-viewing condition will be referred to as the "covered condition". These conventions are shown schematically in Fig. 7.

The upper traces of each panel in Fig. 8 show typical baseline responses to monocular stimulation. Responses of the left eye with stimulation of only the left eye (seeing condition), are shown in the left panels and responses of the left eye with the right eye viewing (covered condition), are shown in the right panels. The mean basline values of the responses to monocular stimulation are shown in Figs. 9 and 10.

The responses in the seeing condition to monocular optokinetic stimulation in the *preferred, temporal-to-nasal direction* showed similarly shaped velocity profiles as obtained with binocular stimulation: a linear build-up of velocity until steady-state velocity was reached, and a linear decay of velocity during OKAN after the lights were turned off. There were, however, characteristic differences with the binocular viewing condition, revealing a deterioration of the response in the monocular situation. Accelerations were typically only about 0.5°/s^2, compared to about 1°/s^2 in the binocular viewing condition (compare Fig. 8 to Fig. 1, and Fig. 9 to Fig. 4). This difference was statistically significant ($p=0.036$). Steady-state gains were also lower than in the binocular viewing condition: values reached about 0.8 for the lowest stimulus velocities (5 and 10°/s) but declined markedly even at 30°/s (compare middle panels of Figs. 9 and 4) although this difference did not reach statistical significance ($p=0.097$). Furthermore, there was a tendency for the responses in the covered eye to be slightly inferior to those of the seeing eye in terms of

acceleration and steady-state gain. This tendency has been described previously (Collewijn and Noorduin, 1972).

Thus, responses to monocular stimulation, even in the preferred direction, showed a somewhat degraded build-up, compared to the binocular viewing condition. However, the decay-rates of the velocities during OKAN were identical in the monocular and binocular conditions ($p=0.476$), suggesting that viewing conditions affected the input to the storage system, but not its intrinsic properties.

In the *non-preferred direction* (Fig. 10) the baseline response was very poor, as shown by the lack of build-up and the virtual absence of a steady state nystagmus. For this reason, no attempt was made to quantify OKAN.

Effects of carbachol on responses to monocular stimulation

Fig. 6 shows eye position recordings obtained with monocular stimulation at 10°/s before and after injection. In the preferred direction, bilateral injection of carbachol affected monocularly elicited OKN in a similar way as described above for the binocular viewing condition. Build-up in the preferred direction was accelerated, while OKAN duration was slightly shortened. The response in the non-preferred direction showed a dramatic improvement after injection of carbachol.

The velocity profiles of the responses of the left eye under both the seeing as well

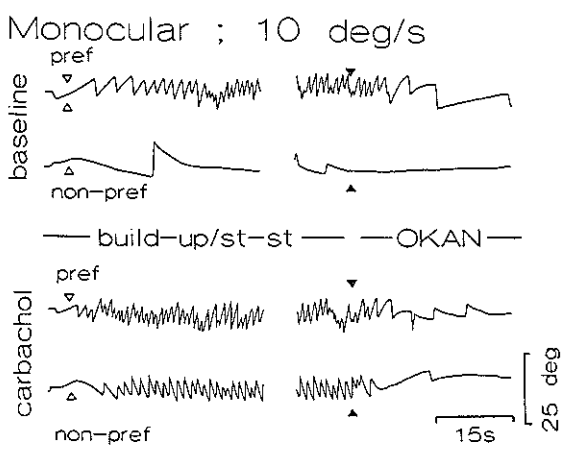


Fig. 6 Eye position recordings during monocular stimulation at 10°/s in the preferred and the non-preferred direction. Open triangles indicate the start of optokinetic stimulation; closed triangles indicate the time that the lights were turned off and eye movements continued as OKAN. Upper panel shows response before, lower panel after bilateral injection of carbachol. Notice the accelerated velocity build-up in the preferred direction after injection and the appearance of a regular nystagmus with build-up of velocity in the non-preferred direction. The corresponding velocity plots are shown in Fig. 8 middle left panel.

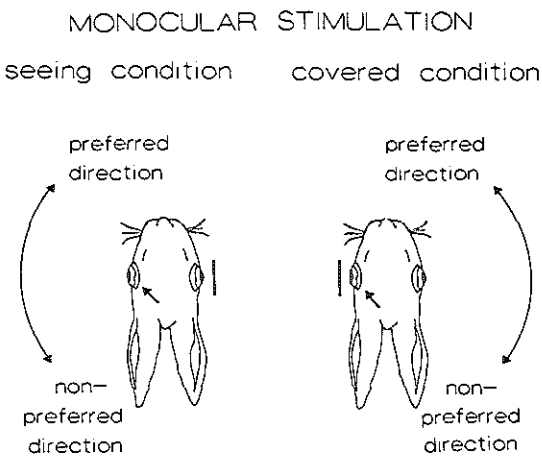
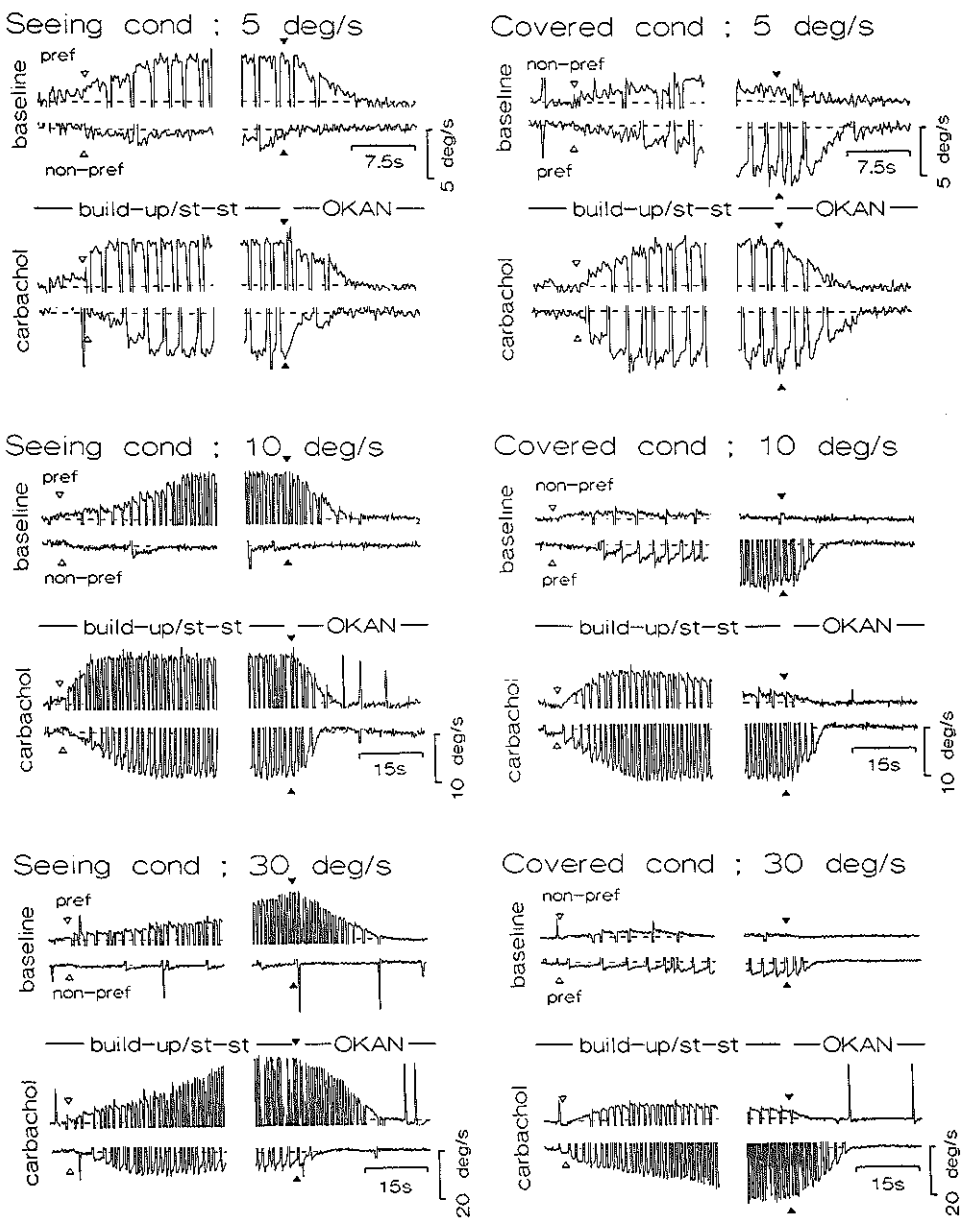


Fig. 7 Diagram explaining terminology for stimulus conditions during monocular stimulation as employed in Figs. 8-10 and in the text. Only responses of the left eye were recorded, but since monocular stimulation was delivered to left and right eye, it is possible to differentiate between seeing and covered condition. Discounting the possibility that injections were consistently more effective in the similar (left or right) flocculus, the responses under seeing and covered conditions can be interpreted as simultaneous responses of the viewing and the covered eye during monocular stimulation.

as the covered condition are depicted in Fig. 8. Injection of carbachol strongly accelerated velocity build-up for all velocities tested. In the *preferred direction*, steady-state velocities remained unchanged for stimulation at 5 and 10°/s, but increased for 30°/s. Carbachol induced a rapid build-up of slow-phase velocity in the *non-preferred direction*, while such a build-up was entirely absent in the baseline condition. Such a strong effect on the response in the non-preferred direction, however, was only encountered in 2 out of the 5 rabbits. Notice that, in spite of the appearance of a regular build-up in the non-preferred direction in the example of Fig. 8, velocity was not maintained at a steady level, but in fact decreased, so that the testing of OKAN was preceded by a less than maximum response velocity.

The mean effects of carbachol on the monocularly elicited OKR in the preferred direction are shown in Fig. 9. With stimulation at 5°/s in the seeing condition, the

Fig. 8 OKN elicited monocularly by steps in surround-velocity of 5, 10 and 30°/s under seeing (left panels) and covered (right panels) condition in the preferred (temporal-to-nasal) and the non-preferred direction (nasal-to-temporal). Slow phase velocity is plotted as a function of time before and after injection of carbachol. Open triangles indicate the start of optokinetic stimulation; closed triangles indicate the time that the lights were turned off and eye movements continued as OKAN. Horizontal dashed lines indicate zero velocity; fast phase velocities have been truncated. Notice the faster build-up after injection of carbachol in the preferred direction, compared to the baseline conditions and the appearance of nystagmus and velocity build-up in the non-preferred direction.


Seeing cond ; 5 deg/s
Covered cond ; 5 deg/s
Seeing cond ; 10 deg/s
Covered cond ; 10 deg/s
Seeing cond ; 30 deg/s
Covered cond ; 30 deg/s
baseline
carbachol
pref
non-pref
build-up/st-st
OKAN
7.5s
15s
5 deg/s
10 deg/s
20 deg/s

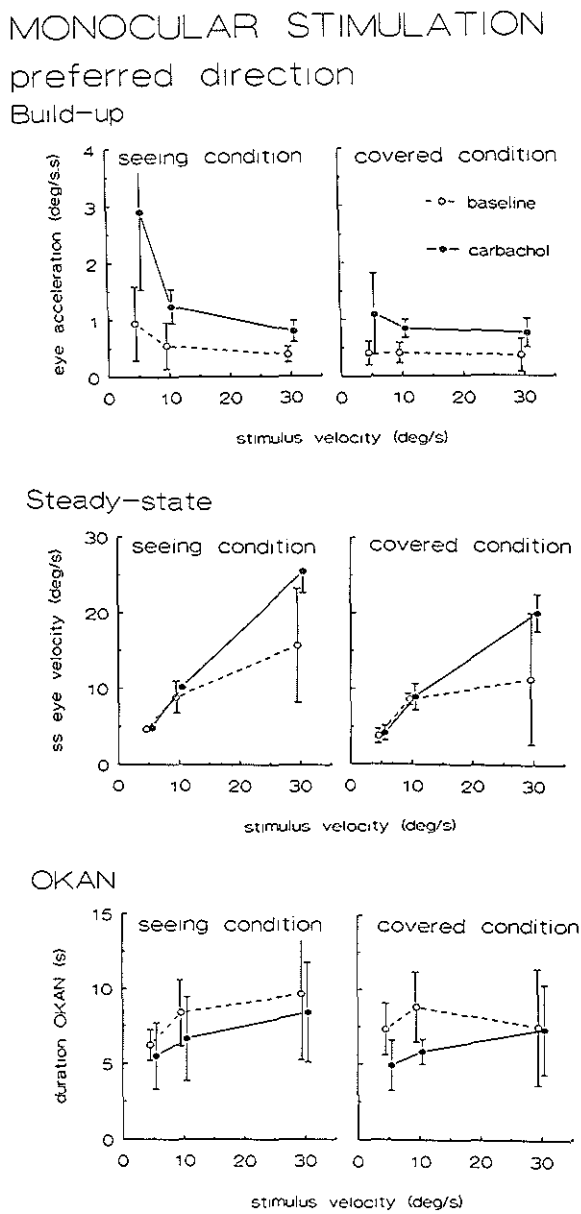


Fig. 9 Mean values of parameters of OKN and OKAN elicited by monocular stimulation in the preferred direction, before (open circles, interrupted lines) and after (closed circles, continuous lines) injection of carbachol as a function of stimulus velocity under seeing (left panels) and covered (right panels) condition. Data points and error bars represent means ± standard deviation (n=5). Upper panel depicts mean eye-acceleration during build-up, middle panel shows steady-state velocity and lower panel shows duration of OKAN. Carbachol significantly enhances average eye-acceleration during build-up for all stimulus velocities tested. Steady-state velocity is increased only at 30°/s stimulus velocity in both seeing and covered conditions.

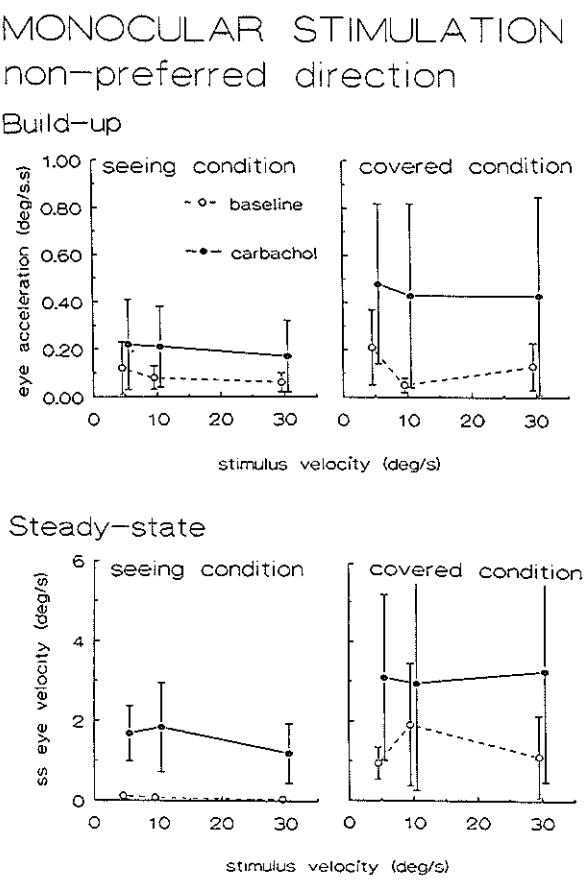


Fig. 10 Mean values of parameters of OKN elicited by monocular stimulation in the non-preferred direction, before (open circles, interrupted lines) and after (closed circles, continuous lines) injection of carbachol as a function of stimulus velocity under seeing (left panels) and covered (right panels) condition. Data points and error bars represent means ± standard deviation (n=5). Upper panel depicts mean eye-acceleration during build-up and lower panel shows steady-state velocity. Eye-acceleration during build-up and steady-state velocity are enhanced by carbachol for all stimulus velocities tested.

enhancement of the build-up induced by carbachol was relatively large, and acceleration reached a value of 2.9°/s², which was even slightly higher than after carbachol injection under binocular viewing conditions. The increase was less pronounced in the covered condition, in which acceleration was increased from 0.41 to 1.10°/s². At the higher velocities of 10 and 30°/s, effects in the seeing and in the covered condition were comparable. Statistical significance of the carbachol effect was only reached for the seeing condition ($p=0.036$ for the seeing and $p=0.074$ for the covered condition). In both the seeing and covered condition, the steady-state velocities were unchanged for 5 and 10°/s but increased for the 30°/s stimulus. These effects on steady-state responses were, however, not statistically significant for either the seeing ($p=0.164$) or the covered condition ($p=0.314$). Carbachol induced some shortening of OKAN (Fig. 9, lower panels) although the induced

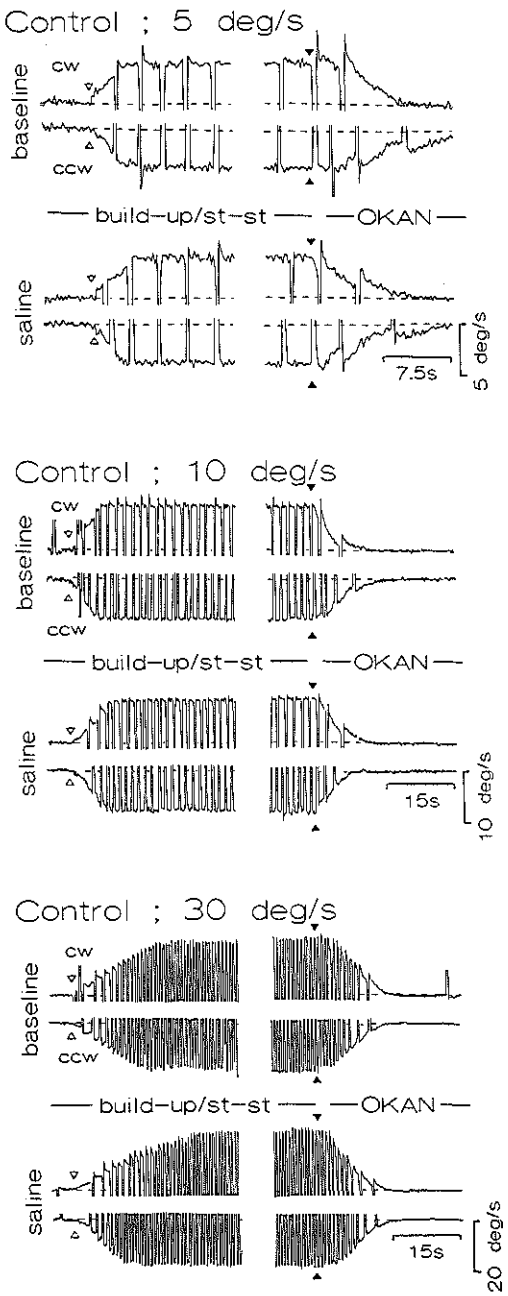


Fig. 11 Typical example of a control experiment, conducted to determine the specificity of the carbachol effects. Slow phase velocity of OKN, binocularly elicited by steps in surround-velocity of 5, 10 and 30°/s is plotted as a function of time before and after injection of saline. Open triangles indicate the start of optokinetic stimulation; closed triangles indicate the time that the lights were turned off and eye movements continued as OKAN. Horizontal dashed lines indicate zero velocity; fast phase velocities have been truncated. Saline had no effect on build-up, steady state or OKAN.

changes were not statistically significant (p=0.342 for the seeing and 0.513 for the covered condition).

Mean effects for monocular stimulation in the *non-preferred* (temporal) direction are shown in Fig. 10. Build-up was enhanced by carbachol, reaching reasonable mean accelerations of 0.2 °/s² for the seeing and even 0.4 °/s² for the covered condition, although these increases were not statistically significant (p=0.254 and 0.085 for seeing and covered condition, respectively), due to the fact that only 2 out of 5 rabbits showed this effect. Nonetheless, the eventually reached steady-state velocity saturated at low levels (2-3°/s) and differentiation of the response between this group of stimulus velocities (5-30 deg/s) remained virtually absent (Fig. 10, lower panels). The relatively modest effect of carbachol on steady-state velocity is partly due to the decrease in response after a good initial build-up (see Fig. 8), resulting in relatively low velocities during the last 10 s of stimulation, prior to extinguishing of the lights, which period was used to determine steady-state velocity. No attempt was made to assess OKAN after monocular stimulation in the non-preferred direction, as only a minority of the cases showed a reliable steady-state response, even after injection of carbachol.

It should be emphasized that, although OKN was monocularly elicited, floccular injections with carbachol were bilateral in all cases.

Control experiments

The specificity of the effects of carbachol on the time course of OKN velocity build-up was verified by bilateral floccular injections with the same volume (1µl) of the solvent (saline) only. As illustrated in the examples of Fig. 11 and the mean values in Fig. 12, such injections had no effect at all on OKN, neither during the build-up nor during the OKAN phase. Thus, the acceleration of build-up may be considered as specific for carbachol, and unrelated to the injection procedure as such, or the sequence in time of the recordings. This is further corroborated by the absence of statistical significance of effects of saline on build-up rate (p=0.734), steady-state velocity (p=0.534), or OKAN duration (p=0.635).

Discussion

Basic properties of binocular OKN

OKN, elicited by prolonged optokinetic stimulation, shows a gradual build-up of eye velocity as described earlier (Ter Braak, 1936; Collewijn, 1969). This gradual velocity build-up is believed to correspond to the "indirect" optokinetic response in the monkey (Cohen et al., 1977). Whereas in the monkey the time-course of velocity build-up during the indirect optokinetic response can be fitted by an exponential function (Cohen et al.,

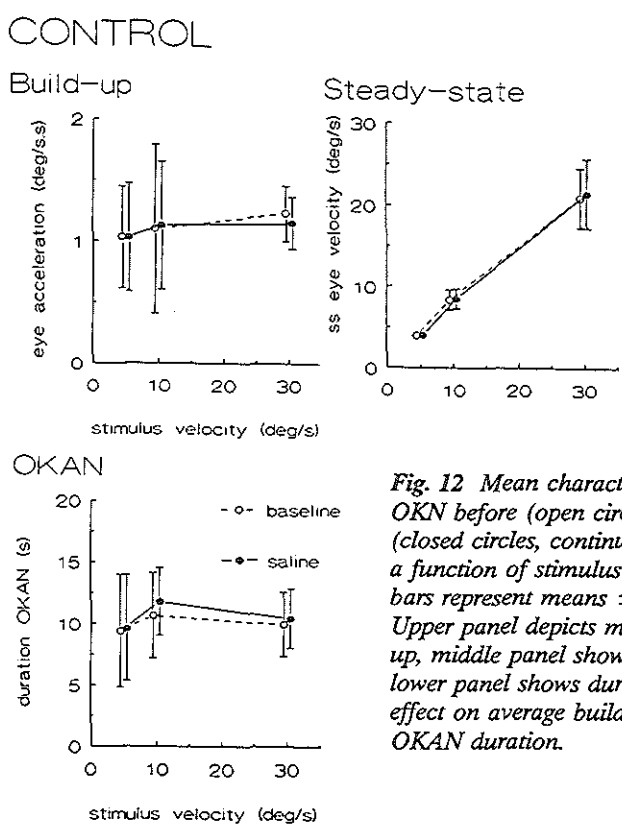


Fig. 12 Mean characteristic parameters of binocular OKN before (open circles, interrupted lines) and after (closed circles, continuous lines) injection of saline as a function of stimulus velocity. Data points and error bars represent means ± standard deviation (n=6). Upper panel depicts mean eye-acceleration during build-up, middle panel shows steady-state velocity and lower panel shows duration of OKAN. Saline had no effect on average build-up rate, steady-state velocity or OKAN duration.

1977), the present results show a typical linear time-course, in analogy with the linear time-course of OKAN (Collewijn et al., 1980). Collewijn (1969) found a maximal acceleration of 0.4°/s² for constant velocity stimulation in rabbit, whereas the average acceleration during velocity build-up encountered in the present study was 1°/s². This higher acceleration value is possibly related to the superior response to a random-dot stimulus, compared to a striped pattern, used in the study of Collewijn (1969; see Dubois and Collewijn, 1979). In addition, the present study demonstrated that eye acceleration during build-up is fixed for all stimulus velocities up to 60°/s.

This constant acceleration, resulting in a the build-up proportional to stimulus velocity, reflects non-linearities in the charging of the storage integrator. The acceleration-limitation of OKN build-up, encountered in the present study, is in line with the results of Maioli and Precht (1984) in the cat who showed that the gain of the OKR, elicited by a sinusoidal stimulus, correlates best with maximal stimulus acceleration. A second non-

linearity is imposed on the optokinetic response by limitations in retinal image-slip detection. Such limitations were encountered during electrophysiological studies (Oyster et al., 1972; Hoffmann and Schoppmann, 1981) and inferred from studies of OKN which showed dependency of open-loop gain on retinal slip velocity (Dubois and Collewijn, 1979; Maioli and Precht, 1984). It is conceivable, though, that the two non-linearities are related.

Steady-state velocity shows a nearly unity gain for stimulus velocities up to about 20°/s. At higher velocities, the steady-state velocity saturates at about 50°/s as described earlier by Collewijn (1969). For velocities up to 20°/s, OKAN is characterized by a fixed duration of about 10 s. Thus, in contrast to velocity build-up, OKAN can be interpreted as the discharge of a velocity-storage system behaving similarly to a leaky integrator (Collewijn, 1972; Demer, 1981; Lisberger et al., 1981; Waespe et al., 1983) although it should be stressed that the time course of velocity decay during OKAN is linear, rather than exponential (Collewijn et al., 1980). A similar disparate velocity-dependence of build-up and OKAN has been described earlier in the cat (Maioli and Precht, 1984). The difference between the charging and discharging of the velocity storage integrator, however, does not contradict a unitary storage mechanism since it could reflect a difference in behavior of the mechanism under closed-loop and open-loop condition. Build-up will be affected by changes in retinal image-slip velocity due to the decreasing difference between eye and stimulus velocity, while discharge during OKAN in darkness is not affected by retinal image-slip.

Monocular versus binocular OKN

Although studies in goldfish (Easter, 1972), pigeon (Gioanni, 1988), and human (Van den Berg and Collewijn, 1988) described slightly better optokinetic responses during binocular viewing than during monocular stimulation, no advantage of binocular viewing was previously encountered in rabbit (Collewijn, 1969) and rat (Hess et al. 1985). A possible explanation for a resemblance between the binocular response and the monocular response in the nasal direction would be that, due to the strong naso-temporal asymmetry, the sensitivity in one direction is almost exclusively derived from one eye with, consequently, little summation of input from both eyes (Collewijn, 1991). The present study, however, revealed distinct differences between binocular and monocular stimulation in the nasal direction. Steady-state OKN velocity saturates at about 50°/s for binocular stimulation, while for monocular stimulation in the nasal direction saturation is reached for velocities well below this value (approximately 15-20°/s, see Fig. 9). Moreover, build-up acceleration in the nasal direction during monocular stimulation in the preferred direction had only about half the value encountered during binocular stimulation. The differences in build-up acceleration and steady state eye-velocity between binocular and nasally directed monocular stimulation suggest that, during binocular viewing in the higher velocity

range, a substantial contribution is made by the temporally stimulated fellow eye. This is difficult to reconcile with the poor response to monocular stimulation in the non-preferred direction, which is not able to deal with surround velocities higher than a few degrees/sec, while a response is generally absent to stimulation in the range of 10°/s and higher (Collewijn, 1969; Collewijn and Holstege, 1984; see also Fig. 10 of the present paper). Our results could be due to a mutual potentiation of the inputs from each eye during build-up of OKN in binocular viewing. An interesting neurophysiological parallel is formed by the finding of Graf et al. (1988) that in some Purkinje-cells, the increase in simple-spike activity to binocular optokinetic stimulation was substantially greater than the sum of the simple spike increases obtained with monocular stimulation of each eye alone.

Effects of carbachol on binocular OKN

In a recent, related study, injection of carbachol in the rabbit's cerebellar flocculi raised the gain of the optokinetic response to sinusoidal stimulation dramatically (Tan and Collewijn, 1991). In view of the positive contribution of signal flow through the flocculus on the OKR as suggested by the decreased performance of OKR after floccular lesions and by electrophysiological data, carbachol is expected to act by increasing the signal transmission through the flocculus. We propose, in analogy with effects of ACh in other parts of the brain (hippocampus: Bernardo and Prince, 1982; Cole and Nicoll, 1984; neocortex: McCormick and Prince, 1986; Schwindt et al., 1988), that carbachol specifically acts by blocking of the slow afterhyperpolarization, thus increasing the excitability of neurons to afferent input.

One of the effects of injection of carbachol on the response to prolonged optokinetic stimulation is a strong enhancement of velocity build-up. Eye acceleration during build-up showed an increase from 1 to 2.5°/s^2. This effect on velocity build-up could underlie the facilitation of the tracking of an oscillatory stimulus motion as described in a previous paper (Tan and Collewijn, 1991). This supposition can be supported more quantitatively. The sinusoidal optokinetic stimulus (f=0.15 Hz, A=2.5 deg), used in the previous study, contained maximum velocities ($A\omega$) of 2.36 °/s and maximum accelerations ($A\omega^2$) of 2.22 °/s^2. In the baseline condition, an acceleration of 1 °/s^2 is typical for constant stimulus velocities of 5-60 °/s. It is clear that this maximum acceleration would be a limiting factor in the tracking of a sinusoidal motion with peak accelerations of 2.22 °/s^2. Under the assumption of approximately linear behavior, the gain would be about 1.0/2.2 = 0.45. After carbachol, peak acceleration (Fig. 4) was increased to about 2.5 °/s^2, sufficient to cover the acceleration of the sinusoidal stimulus, and to allow a nearly unity gain. For a stimulus amplitude of 5 deg (f=0.15 Hz), injection of carbachol induced an increase in the gain of the OKR (Tan and Collewijn, submitted), without reaching unity gain. The maximum acceleration of this stimulus was 4.44°/s^2, too high to be overcome by the optokinetic

system, even after carbachol injection. Thus, the gain for sinusoidal optokinetic stimulation after carbachol injection in previous studies agrees well with the maximum eye-acceleration attained during build-up of OKN in response to constant-velocity stimuli. Moreover, the amount of increase in gain of the response to sinusoidal stimuli can be predicted from the increase in eye-acceleration during build-up.

Steady-state OKN velocity in response to stimulation at lower velocities showed very high gains (close to unity) and in the context of the role of the OKR as a negative feed-back loop that functions to cancel retinal slip, the gain is not expected to rise above unity following carbachol injection. At higher velocities, though, gain is below unity and some effect of carbachol is visible. For binocular optokinetic stimulation, a clear increase in gain was found for OKN elicited by stimulation at 110°.

Although a more rapid build-up need not necessarily be accompanied by a shortening of OKAN, as argued by Zee et al. (1981), this was the case in the present study. For the entire range of stimulus velocities tested, duration of OKAN was shortened from 10 s to about 6 s for stimulus velocities up to 60°/s. The equal accelerating effect of carbachol on velocity-charging during build-up and velocity-decay during OKAN supports the hypothesis that they are manifestations of a single, common central storage mechanism (Collewijn, 1972).

Effects of carbachol on monocular OKN

With monocular stimulation in the *preferred* direction, the increase was equally strong as for binocular stimulation in the seeing condition for a stimulus velocity of 5°/s but weaker for higher stimulus velocities. In the covered condition, the effect was weak throughout the entire velocity-range examined. This suggests that with monocular stimulation at a velocity of 5°/s in the preferred direction, the build-up of the response of the stimulated eye is more accelerated by carbachol than the build-up of the response of the covered eye.

During monocular stimulation in the preferred direction, the baseline gain of steady-state OKN is already low (0.5) for a stimulus velocity of 30°/s. Injection of carbachol strongly enhances this response, increasing the steady-state gain to 0.9. Thus, at lower velocities, the effect of carbachol on steady-state OKN is masked by the close-to-unity gain of this response, while a facilitatory effect of carbachol is displayed as soon as the response becomes inadequate with increasing demands (higher stimulus velocities).

The enhancing effect of carbachol on the velocity build-up during monocular stimulation in the *non-preferred* direction in 2 out of 5 animals is remarkable. It suggests that the optokinetic system is potentially capable of compensating retinal slip in the non-preferred direction. This potential is reflected at the cellular level in the sensitivity of accessory optic system (Soodak and Simpson, 1988), the nuclear of the optic tract

(Collewijn, 1975; Hoffmann and Schoppmann, 1975), the inferior olive (Leonard et al., 1988) and floccular Purkinje-cells (Leonard, 1986) to monocular stimulation in either nasal and temporal direction. Our findings suggest that despite the presence of bidirectional monocular optokinetic information in the brain, the response in the temporal direction is deliberately kept at a low level during everyday life, possibly by lowering the gain at the level of the flocculus. A possible reason for this could be that during forward locomotion, both eyes are stimulated in the temporal direction. As the optokinetic system uses retinal image-slip to compensate for self-rotation, rather than translation, this temporally directed input would be inappropriate, and therefore overruled by the brain. An argument against this assumption, however, is the inability of the brain to adapt the response adequately to very drastic changes in requirements as imposed by early monocular enucleation (Collewijn and Holstege, 1984).

Floccular control of OKN

Lesioning has been the classical approach in studies of floccular function. In rabbit, the effect of flocculectomy (Ito et al., 1982; Nagao, 1983) and temporary functional ablation of the flocculus by localized injection of GABA-agonists (Van Neerven et al., 1989) on the OKR is a decrease in the gain of the response to sinusoidal stimulation. A decrease in the steady-state response to prolonged stimulation, for the entire range of stimulus velocities tested, was encountered by Barmack and Pettorossi (1985). However, these studies did not look into effects of flocculectomy on velocity build-up and OKAN. Since, in rabbit, optokinetic responses to stimuli higher than a few degrees per second are believed to be produced by velocity storage alone and not by the direct response, these results based on inactivation of the flocculus would suggest involvement of the flocculus in velocity storage.

In the monkey, the optokinetic response consists of a direct and an indirect component (Cohen et al., 1977). During prolonged optokinetic stimulation, the direct component is responsible for the initial rapid rise in eye velocity, and the indirect component for the subsequent slow rise of velocity and OKAN. The indirect component is believed to be produced by the velocity-storage mechanism, and thus equivalent to the optokinetic response as encountered in the rabbit. Lesioning of the flocculus in monkey indisputably results in abolition of the direct response (Zee et al., 1981; Waespe et al., 1983). The effects on the indirect pathway subserving velocity storage is, however, hard to interpret because of the possibility of covariation of effects on direct and indirect pathways. Lesions made by Zee et al. (1981) resulted, in addition to abolition of direct pathway, in a nearly doubling of the rise-time of the response. Waespe et al. (1983) found essentially the same results, but corrected for remaining retinal slip velocity and concluded that the response was not affected. In either interpretation, though, the rather implausible

assumption is made that the direct and indirect mechanisms do not interact.

In the monkey, OKAN, PRN and visual-vestibular interaction, assessed with steps in velocity, remained unaffected after flocculus lesions, suggesting that the primate flocculus is not involved in velocity storage (Zee et al., 1981; Waespe et al., 1983). In the present study, we did encounter effects of floccular injection of carbachol on OKAN duration, while data from a following study (Tan et al., in prep) suggest that also the duration of PRN is shortened by carbachol injection. Our results provide evidence for floccular control of the storage-integrator's charging and discharging rates.

Summary and conclusions

1. In the alert, pigmented rabbit, eye movements were recorded during optokinetic nystagmus (OKN) and during optokinetic afternystagmus (OKAN). These responses were elicited by steps in surround-velocity ranging from 5-110°/s during binocular as well as monocular viewing.
2. In the baseline condition, OKN showed an approximately linear build-up of eye velocity to a steady-state, followed by a linear decay of eye velocity during OKAN after the lights were turned off. Build-up during binocular viewing was characterized by a constant, maximum eye-acceleration (about 1°/s^2) for stimulus velocities up to 60°/s. OKAN, instead, was characterized by a fixed duration (about 10 s) for stimulus velocities up to 20°/s. Steady-state eye velocity saturated at about 50°/s.
3. Monocular stimulation in the preferred (nasal) direction elicited a build-up that was on average twice as slow as during binocular stimulation. Steady-state velocity during monocular stimulation saturated at about 20°/s. OKAN was of equal duration as during binocular stimulation. In the non-preferred direction, a very irregular nystagmus was elicited without velocity build-up. The stronger response to binocular stimulation, compared to the responses under monocular viewing condition in either nasal and temporal direction suggests potentiation of the signals of either eye during binocular viewing.
4. OKN and OKAN were re-assessed after intra-floccular micro-injection of the aselective cholinergic agonist carbachol. In the binocular viewing condition, eye-acceleration during build-up was strongly enhanced from 1°/s^2 before to 2.5°/s^2 after injection. The saturation level of steady-state eye velocity was also increased, from 50°/s before to more than 60°/s after carbachol. The duration of OKAN, however, was shortened from 10 s before to 6 s after injection. The response to monocular stimulation in the preferred direction revealed similar changes.
5. The response to monocular stimulation in the non-preferred direction was strongly

enhanced by carbachol in 2 out of 5 rabbits. After the injection, a regular nystagmus could be elicited with a build-up to a steady state, which saturated at about 10°/s. This result suggests that the optokinetic system is potentially capable of responding adequately to temporally directed retinal slip. Apparently, this response is normally kept at a low level to prevent inappropriate optokinetic responses during forward locomotion.

6. The flocculus appears to be involved in the control of the dynamics of OKN in the rabbit. Cholinergic mechanisms affect the floccular control of the rate at which slow-phase velocity can be built up and the rate of decay of eye velocity during OKAN. Cholinergic stimulation of the flocculus thus shifts the dynamics of OKN to a more direct response, while velocity storage is shortened.

References

Barmack NH, Pettorossi VE (1985) Effects of unilateral lesions of the flocculus on optokinetic and vestibuloocular reflexes of the rabbit. J Neurophysiol 53: 481-496

Bernardo LS, Prince DA (1982) Cholinergic excitation of mammalian, hippocampal pyramidal cells. Brain Res 249: 315-331

Cohen B, Matsuo V, Raphan T (1977) Quantitative analysis of the velocity characteristics of optokinetic nystagmus and optokinetic after-nystagmus. J Physiol (Lond) 270: 321-344

Cole AE, Nicoll RA (1984) Characterization of a slow cholinergic post-synaptic potential recorded in vitro from rat hippocampal pyramidal cells. J Physiol (Lond) 352: 173-188

Collewijn H (1969) Optokinetic eye movements in the rabbit: input-output relations. Vision Res 9: 117-132

Collewijn H (1972) An analog model of the rabbit's optokinetic system. Brain Res 36: 71-88

Collewijn H (1975) Direction-selective units in the rabbit's nucleus of the optic tract. Brain Res 100: 489-508

Collewijn H (1977) Eye- and head movements in freely moving rabbits. J Physiol 266: 471-498

Collewijn H (1985) Integration of adaptive changes of the optokinetic reflex, pursuit and the vestibulo-ocular reflex. In: Berthoz A, Melvill Jones G (eds), Adaptive Mechanisms in Gaze Control. Amsterdam: Elsevier, pp 51-69

Collewijn H (1991) The optokinetic contribution. In: Carpenter RHS (ed), Eye Movements. Vol. 8 in "Vision and Visual Disfunction", Macmillan Press, pp 45-70

Collewijn H, Holstege G (1984) Effects of neonatal and late unilateral enucleation on optokinetic responses and optic nerve projections in the rabbit. Exp Brain Res 57: 138-150

Collewijn H, Winterson BJ, Van der Steen J (1980) Post-rotatory nystagmus and optokinetic after-nystagmys in the rabbit: linear rather than exponential decay. Exp Brain Res 40: 330-338

Collewijn H, Tan HS, Van der Steen J (1992) Enhancement of optokinetic and vestibulo-ocular responses in the rabbit by cholinergic stimulation of the flocculus. Ann NY Acad Sci, In press

Demer JL (1981) The variable gain element of the vestibulo-ocular reflex is common to the optokinetic system of the cat. Brain Res 229: 1-13

Donaghy M (1980) The contrast sensitivity, spatial resolution and velocity tuning of the cat's optokinetic

reflex. J Physiol (Lond) 300: 353-365
Dubois MFW, Collewijn H (1979) The optokinetic reactions of the rabbit: relation to the visual streak. Vision Res 19: 9-17
Dufossé M, Ito M, Miyashita Y (1977) Functional localization in the rabbit's cerebellar flocculus determined in relationship with eye movemnets. Neurosci Lett 5: 273-277
Easter SS (1972) Pursuit eye movements in goldfish (Carassius auratus). Vision Res 12: 673-688
Evinger C, Fuchs AF (1978) Saccadic, smooth pursuit, and optokinetic eye movements of the trained cat. J Physiol (Lond) 285: 209-229
Gioanni H (1988) Stabilizing gaze reflexes in the pigeon (Columbia livia). I. Horizontal and vertical optokinetic eye (OKN) and head (OCR) reflexes. Exp Brain Res 69: 567-582
Graf W, Simpson JI, Leonard CS (1988) Spatial organization of visual messages of the rabbit's cerebellar flocculus. II. Complex and simple spike responses of Purkinje cells. J Neurophysiol 60: 2091-2121
Hess BJM, Precht W, Reber A, Cazin L (1985) Horizontal optokinetic nystagmus in the pigmented rat. Neuroscience 15: 97-107
Hoffmann K-P, Schoppmann A (1975) Retinal input to direction selective cells in the nucleus tractus opticus of the cat. Brain Res 99: 359-366
Hoffmann K-P, Schoppmann A (1981) A quantitative analysis of the direction-specific response of neurons in the cat's nucleus of the optic tract. Exp Brain Res 42: 146-157
Igarashi M, Takahashi M, Homick JL (1977) Optokinetic nsytagmus and vestibular stimulation in squirrel monkey model. Arch Oto-Rhino-Laryngol 218: 115-121
Ito M, Jastreboff PJ, Miyashita Y (1982) Specific effects of unilateral lesions in the flocculus upon eye movements in albino rabbits. Exp Brain Res 45: 233-242
Jung R (1948) Die Registrierung des postrotatorischen und optokinetischen Nystagmus und die optisch-vestibuläre Integration beim Menschen. Acta Otolaryngol 36: 199-202
Keller EL, Precht W (1979) Visual-vestibular responses in vestibular nucleus neurons in the intact and cerebellectomized, alert cat. Neuroscience 4: 1599-1613
Komatsuzaki A, Harris HE, Alpert J, Cohen B (1969) Horizontal nystagmus of rhesus monkeys. Acta Oto-laryngologica 67: 535-551
Leonard CS (1986) Signal Characteristics of Cerebellar Purkinje Cells in the Rabbit Flocculus During Compensatory Eye Movements (PhD Dissertation). New York: New York University
Leonard CS, Simpson JI, Graf W (1988) Spatial organization of visual messages of the rabbit's cerebellar flocculus. I. Typology of inferior olive neurons of the dorsal cap of Kooy. J Neurophysiol 60: 2073-2090
Lisberger SG, Miles FA, Optican LM, Eighmy BB (1981) Optokinetic responses in monkey: underlying mechanisms and their sensitivity to long-term adaptive changes in vestibuloocular reflex. J Neurophysiol 45: 869-890
Maioli C, Precht W (1984) The horizontal optokinetic nystagmus in the cat. Exp Brain Res 55: 494-506
McCormick DA, Prince DA (1986) Mechanisms of action of acetylcholine in the guinea-pig cerebral cortex in vitro. J Physiol (Lond) 375: 169-194
Mowrer OH (1937) The influence of vision during bodily rotation upon the duration of post-rotational vestibular nystagmus. Acta Otolaryngol 25: 351-364
Nagao S (1983) Effects of vestibulocerebellar lesions upon dynamic characteristics and adaptation of vestibulo-ocular and optokinetic responses in pigmented rabbits. Exp Brain Res 53: 36-46
Nagao S, Ito M, Karachot L (1985) Eye fields in the cerebellar flocculus of pigmented rabbits determined with local electrical stimulation. Neurosci Res 3: 39-51

Oyster CW, Takahashi E, Collewijn H (1972) Direction-selective retinal ganglion cells and control of optokinetic nystagmus in the rabbit. Vision Res 12: 183-193

Raphan T, Matsuo V, Cohen B (1979) Velocity storage in the vestibulo-ocular reflex arc (VOR). Exp Brain Res 35: 229-248

Raphan T, Cohen B (1985) Velocity storage and the ocular response to multidimensional vestibular stimuli. In: Berthoz A, Melvill Jones G (eds), Adaptive Mechanisms in Gaze Control. Amsterdam: Elsevier, pp 123-143

Robinson DA (1977) Linear addition of optokinetic and vestibular signals in the vestibular nucleus. Exp Brain Res 30: 447-450

Schwindt PC, Spain WJ, Foehring RS, Chubb MC, Crill WE (1988) Slow conductances in neurones from cat sensorimotor cortex in vitro and their role in slow excitability changes. J Neurophysiol 59: 450-467

Soodak RE, Simpson JI (1988) The accessory optic system of rabbit. I. Basic visual response properties. J Neurophysiol 60: 2037-2054

Takemori S, Cohen B (1974) Loss of visual suppression of vestibular nystagmus after flocculus lesions. Brain Res 72: 213-224

Tan HS, Collewijn H (1991) Cholinergic modulation of optokinetic and vestibulo-ocular responses: a study with microinjections in the flocculus of the rabbit. Exp Brain Res 85: 475-481

Tan HS, Collewijn H. Cholinergic and noradrenergic stimulation in the rabbit flocculus have synergistic facilitatory effects on optokinetic responses. submitted to Brain Res

Ter Braak JWG (1936) Untersuchungen über optokinetischen Nystagmus. Arch Néerl Physiol 21: 309-376

Van den Berg AV, Collewijn H (1988) Directional asymmetries of human optokinetic nystagmus. Exp Brain Res 70: 597-604

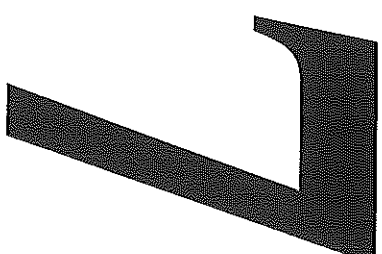
Van der Steen J, Simpson JI, Tan J (1991) Representation of three-dimensional eye movements in the cerebellar flocculus of the rabbit. In: Schmidt R, Zambarbieri D, Oculomotor Control and Cognitive Processes, Elseviers Science Publishers B.V. (North Holland), pp 63-77

Van Neerven J, Pompeiano O, Collewijn H (1989) Depression of the vestibulo-ocular and optokinetic response by intraflocculuar microinjection of GABA-A and GABA-B agonists in the rabbit. Arch Ital Biol 127: 243-263

Waespe W, Cohen B, Raphan T (1983) Role of the flocculus and paraflocculus in optokinetic nystagmus and visual-vestibular interactions: effects of lesions. Exp Brain Res 50: 9-33

Waespe W, Henn V (1981) Visual-vestibular interaction in the flocculus of the alert monkey. II. Purkinje cell activity. Exp Brain Res 43: 349-360

Zee DS, Yamazaki A, Butler PH, Güçer G (1981) Effects of ablation of flocculus and paraflocculus on eye movements in primate. J Neurophysiol 46: 878-899



Unilateral cholinergic stimulation of the cerebellar flocculus: symmetric effects on optokinetic responses

Introduction

There is general agreement about the involvement of the cerebellar flocculus in the execution of the optokinetic reflex (OKN). Anatomical and electrophysiological studies have identified pathways that convey retinal image-slip information, the input-signal for the OKN, from the retinal ganglion cells to the flocculus. Slip-information is imparted to the flocculus by both mossy and climbing fibers, with convergence of these signals at the Purkinje-cell (P-cell). The P-cells relay this image-slip information to the vestibular complex. Although the output of P-cells to vestibular nuclear neurons is inhibitory, in line with the general nature of P-cell output (Ito et al., 1964; Ito and Yoshida, 1966; Eccles et al., 1967), the net effect of floccular activity on the OKN is positive. This is due to the polarity of modulation of P-cell activity, which is predominantly out-of-phase with the polarity of vestibular nuclear neuron firing behavior (Ito et al., 1977), resulting in a net enhancement of vestibular nuclear modulation. Accordingly, inactivation of the flocculus consistently causes a decrease in the performance of OKN in man (Dichgans et al., 1978), monkey (Takemori and Cohen, 1974; Igarashi et al., 1977; Zee et al., 1981; Waespe et al., 1983), cat (Keller and Precht, 1979) and rabbit (Ito et al., 1982; Nagao, 1983; Barmack and Pettorossi, 1985; Van Neerven et al., 1989). Furthermore, smooth eye movements can be elicited by electrical micro-stimulation in the flocculus (Dufossé et al., 1977; Van der Steen et al., 1991).

In preceding studies, bilateral floccular microinjections with the aselective cholinergic agonist carbachol greatly improved optokinetic responses in the rabbit. Such injections increased the gain of the OKN to a sinusoidal motion stimulus from 0.6 to 1.0 (Tan and Collewijn, 1991) while, in response to a step in velocity, the build-up of slow-phase velocity was accelerated from $1°/s^2$ in the baseline condition to $2.5°/s^2$ after injection for stimulation in either direction (Tan et al., 1992a). In view of the positive influence of the floccular output on the OKN, the most probable mechanism of the action of carbachol is an increase in signal-flow through the flocculus. We postulated, in analogy to the action of ACh in hippocampus (Bernardo and Prince, 1982; Cole and Nicoll, 1984) and neocortex (Krnjevic et al., 1971; McCormick and Prince, 1986; Schwindt et al., 1988) that carbachol exerts this facilitatory effect by acting postsynaptically on P-cells as a positive modulator (Tan et al., 1992a). In this scenario, carbachol would act by increasing the sensitivity of P-cells to incoming signals during optokinetic tracking and thus enhance modulation during OKN.

The proposed mechanism for the positive modulatory action of ACh in cortex and hippocampus is blockade of the slow afterhyperpolarization (sAHP) (Madison and Nicoll, 1984). The sAHP is generated by Ca influx during the action potentials, and increases strongly with the number of spikes (Lancaster and Adams, 1986). It tends to suppress

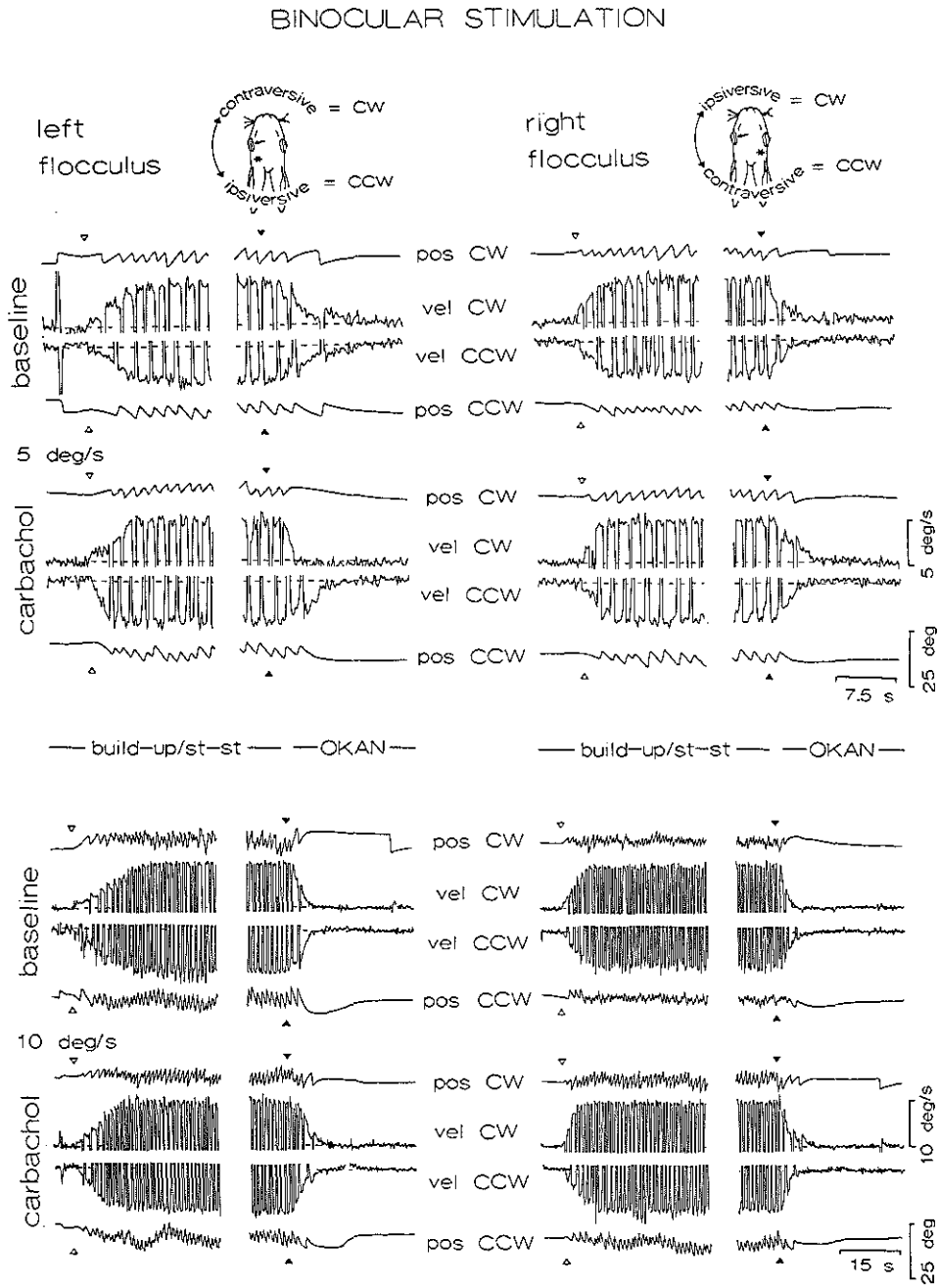
further discharge by the shunting of depolarizing inputs. It is obvious that such a mechanism poses an upper limit to the number of spikes that can be generated in a period of time. Blocking of the sAHP would therefore be expected to shift the maximal firing frequency to a higher level, thereby enhancing the depth of modulation in the excitatory phase. On the other hand, the depth of modulation in the inhibitory direction will be relatively unaffected. Considering the directional specificity of floccular output, such an asymmetric effect at the cellular level of the flocculus could express itself at the behavioral level as a directionally asymmetric effect on optokinetic responses.

In the context of the reciprocity in polarity of directional coding of optokinetic information between the left and the right flocculus, bilateral injection of carbachol, as employed in the previous studies, would result in directionally symmetrical modulation of the OKN in any case. An asymmetric effect could, however, become manifest when carbachol would be applied to only one flocculus. The present study was undertaken to assess the laterality of effects of such a unilateral injection of carbachol.

Methods

The present experiments were run on five rabbits which were permanently implanted with ocular sensor-coils for eye movement recording and scull-screws for fixation of the head. The flocculi were localized electrophysiologically on the basis of visually evoked direction-selective complex-spike and simple-spike activity and guide cannulas, aimed towards right and left flocculi, were implanted bilaterally. All surgical procedures were done under general anesthesia, induced by a mixture of ketamine, acepromazine and xylazine. More details of the animal preparation are described in Tan et al. (1992a).

The rabbit remained stationary throughout the experiment and was surrounded by a drum (diameter 70cm), lined on the inside with a random-dot pattern. The drum was rotated at a constant speed about an earth-vertical axis, passing through the midpoint between the eyes, to elicit steady-state optokinetic nystagmus (OKN). Drum rotation was started in darkness and as soon as the drum velocity was stable, the light was turned on. Build-up of OKN was recorded for a period of 40 s. After a steady state had been reached, a second measurement was made, during which the lights were extinguished after 10 s and optokinetic afternystagmus (OKAN) was recorded during the following 30 sec. Stimulation was applied in either direction (clockwise, CW = rightward and counterclockwise, CCW = leftward) at three drum-rotation speeds: 5, 10 and 30°/s. These stimuli were presented three times under three different viewing conditions: binocular viewing, and monocular viewing with the left or right eye. After baseline measurements


BINOCULAR STIMULATION
left
flocculus
contraversive = CW
ipsiversive = CCW
right
flocculus
ipsiversive = CW
contraversive = CCW
baseline
pos CW
vel CW
vel CCW
pos CCW
5 deg/s
carbachol
5 deg/s
25 deg
7.5 s
build-up/st-st
OKAN
10 deg/s
15 s

had been obtained for all of these conditions, 1 μl of a 1 g/l solution of carbachol in saline was injected in one of the flocculi. Starting 20 min after the injection, all OKN measurements were repeated. In a subsequent session, with at least 24 hr intervening, the experiment was repeated with injection of carbachol in the other flocculus. Control sessions were run on three animals with injection of 1 μl of saline (the solvent) only in one of the flocculi, using the same procedure. After the final session, three animals received an injection of kainic acid in both flocculi for the histological verification of the location of the injections (see Tan et al., 1992a). The injection sites revealed degeneration of cortical cells which was in all cases localized in, and restricted to the flocculus.

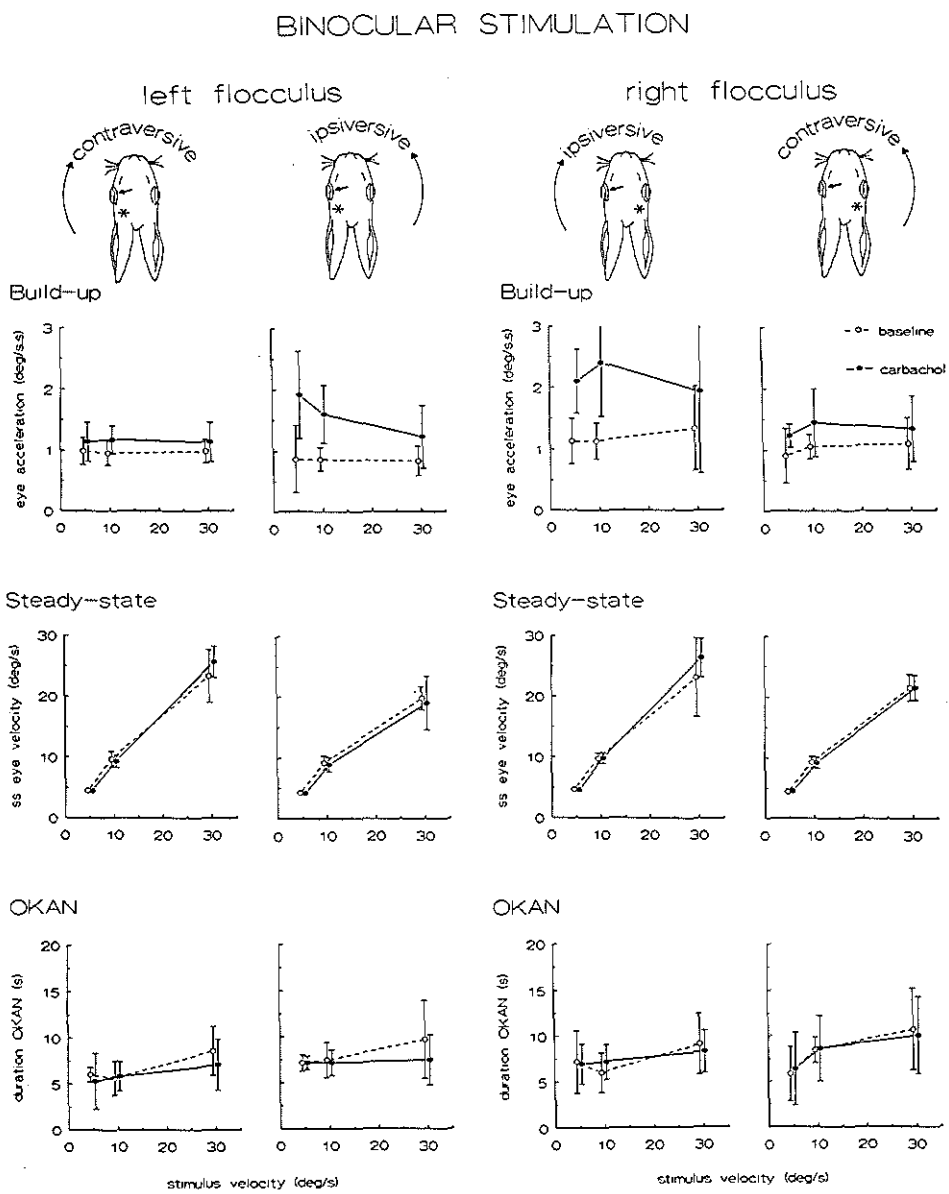
The experiments were computer-controlled (DEC, PDP 11/73). The position signal of the left eye was stored by a data-acquisition program at a sample frequency of 51.2 Hz. In the subsequent off-line analysis, eye velocity was calculated and plotted as a function of time for the assessment of OKN. The statistical significance of the effects of the injections was tested with a Multiple Analysis of Variance (MANOVA), which allows the comparison of several variables in a single group of animals.

Results

Effects on responses to binocular stimulation

Fig. 1 shows position and velocity plots of OKN, elicited by binocular stimulation at velocities of 5 and 10°/s. The response typically showed an initial, gradual build-up of slow-phase eye velocity, as described earlier (Ter Braak, 1936; Collewijn, 1969), which followed an approximately linear time course. The eye acceleration had a constant value of approximately 1°/s², so that the duration of the velocity build-up was a linear function of stimulus velocity, as demonstrated in Fig. 1 (notice the different time scales for the responses to 5 and 10°/s). Eye velocity eventually reached a maximum, approximating the stimulus velocity, which was maintained throughout the period of stimulation.

Fig. 1 Eye-position and slow phase velocity of binocularly elicited OKN as a function of time for stimulation in either direction (CW = right; CCW = left). The head-cartoons on top show the direction of ipsiversive and contraversive stimuli, relative to the injected flocculus (marked by asterisk); movements of the left eye were recorded (arrow). Upper panels show response to 5°/s, lower panels show the response to 10°/s. Left and right panels depict effects of injection of carbachol in the left and the right flocculus, respectively. Open triangles indicate the start of optokinetic stimulation; filled triangles indicate the time that the lights were turned off and eye movements continued as OKAN. Horizontal dashed lines in the velocity panels indicate zero velocity; fast phase velocities have been truncated. Notice the faster build-up after injection of carbachol for ipsiversive stimulation.


BINOCULAR STIMULATION
left flocculus
right flocculus
contraversive
ipsiversive
ipsiversive
contraversive
Build-up
Build-up
baseline
carbachol
eye acceleration (deg/s.s)
Steady-state
Steady-state
ss eye velocity (deg/s)
OKAN
OKAN
duration OKAN (s)
stimulus velocity (deg/s)
stimulus velocity (deg/s)

In the present experiments, only responses of the left eye were recorded. The response to each stimulus direction was tested before and after injection of carbachol in the left flocculus (Fig. 1, left panels) and in the right flocculus (Fig. 1, right panels). The terms "ipsilateral" and "contralateral" will be used to indicate the side of the injected flocculus relative to the recorded (left) eye. The terms "ipsiversive" and "contraversive" will be used to denote the direction of the drum rotation towards and away from the injected flocculus.

The injections of carbachol never led to changes in the behavior of the rabbits and no spontaneous nystagmus occurred. Even in darkness, gaze was stable after the injection. Unilateral application of carbachol had an asymmetric effect on OKN. The response to stimulation of the left eye in its nasal direction (CW) was tested before and after injection of carbachol in the left and right flocculus. Injection of carbachol in the left, ipsilateral flocculus did not affect the response to CW optokinetic stimulation. When the right, contralateral flocculus was injected, however, acceleration during build-up was increased by carbachol from $1°/s^2$ to $2°/s^2$ for stimulation at 5°/s, and from $1.3°/s^2$ to $2.5°/s^2$ for stimulation at 10°/s. In the first condition, the stimulus was directed away from the injected flocculus (contraversive), while in the latter condition, the stimulus was directed towards the injected flocculus (ipsiversive).

Carbachol affected the response of the left eye to stimulation in the temporal direction (CCW) in a way symmetrical to the response to stimulation in the nasal direction (CW). When the injection was made into the left, ipsilateral flocculus, the CCW stimulus was ipsiversive (Fig. 1, left panels). In this condition, the acceleration during build-up of the response was enhanced from $0.68°/s^2$ to $2.5°/s^2$ at a stimulus speed of 5°/s and from $0.79°/s^2$ to $1.38°/s^2$ at 10°/s. When carbachol was injected into the right, contralateral flocculus (Fig. 1, right panels), the CCW was contraversive, and no effects was evident. Thus, during binocular viewing, unilateral injection of carbachol in the flocculus enhanced build-up of ipsiversive OKN of both eyes, while contraversive OKN was not affected.

After a period of steady-state eye velocity, the lights were turned off (as marked by solid triangles) and the nystagmus continued as OKAN. In the rabbit shown in Fig. 1

Fig. 2 Means (points) and standard deviations (bars) of 10 measurements of characteristic parameters of binocularly elicited OKN before (open circles, interrupted lines) and after (filled circles, continuous lines) injection of carbachol, as a function of stimulus velocity. In the head-cartoons on top, large arrows indicate stimulus direction; "ipsiversive" and "contraversive" indicate the stimulus direction relative to the injected flocculus (indicated by asterisks). Upper panels depict mean eye-acceleration during build-up; middle panels show steady-state velocity; lower panels show duration of OKAN. Left columns show effects of injection in the left flocculus; right columns show injections in right flocculus. Notice the faster build-up after injection during ipsiversive stimulation.

the duration of OKAN was about 4-8 s in the baseline condition. Despite some isolated changes (e.g. left flocculus injection, 5°/s CW stimulation in Fig. 1) no consistent differences between OKAN durations before and after injection were found in any of the four tested conditions.

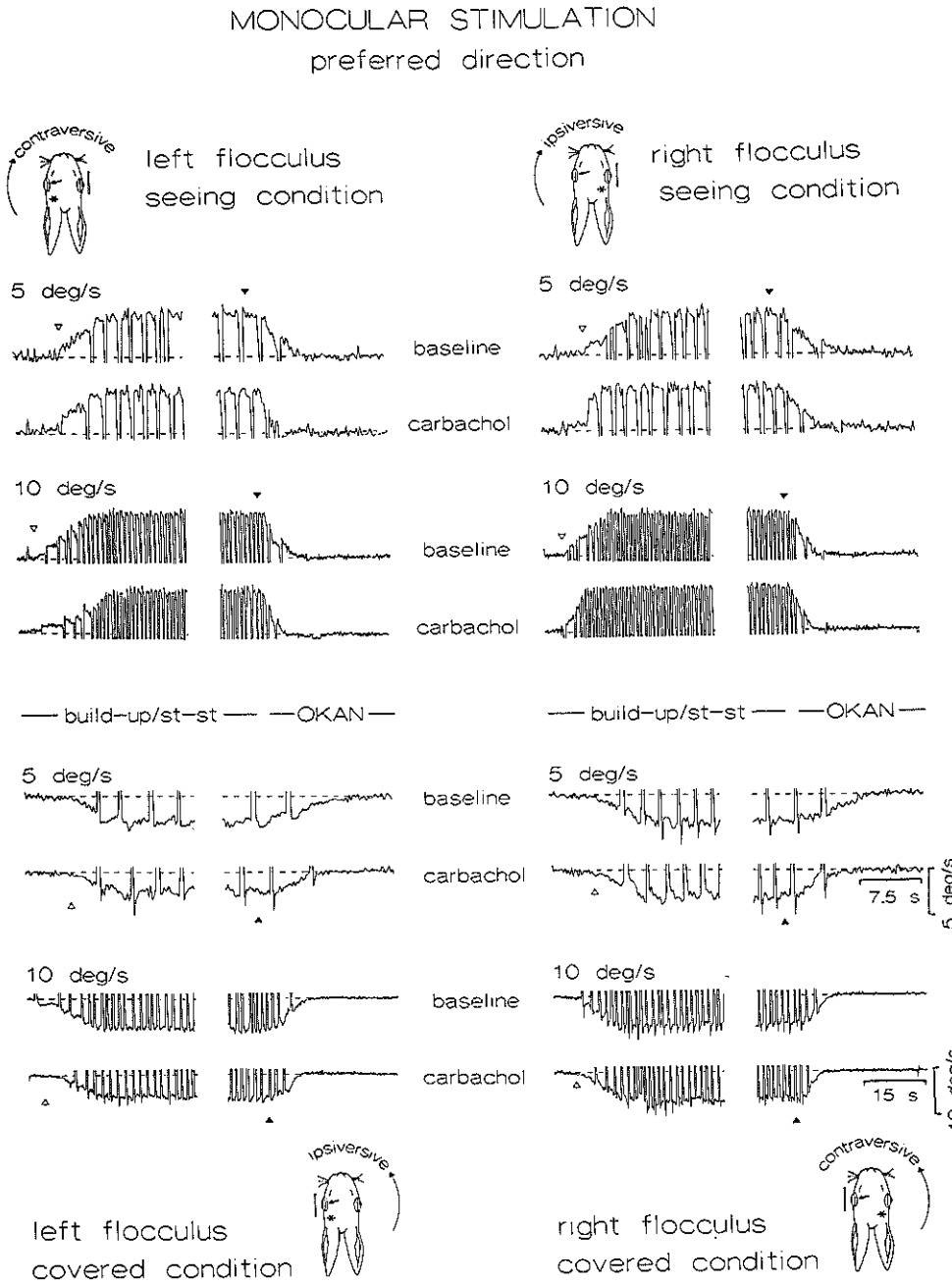
Characteristic parameters of the responses, averaged over the five rabbits, are shown in Fig. 2. The four columns of Fig. 2 depict the effects of carbachol for the four different stimulus conditions, depicted schematically on top. In all four conditions tested, the baseline value of eye acceleration during build-up was about 1°/s² for all stimulus velocities (Fig. 2, upper row). Build-up was enhanced only for ipsiversive stimulation (Fig. 2, columns 2 and 3). The response of the eye contralateral to the injected flocculus to ipsiversive stimulation increased significantly ($p=0.025$) from about 1°/s² before to about 2°/s² after injection (Fig. 2, column 3). Acceleration of the ipsilateral eye in response to ipsiversive stimulation was increased slightly less, but still significantly ($p=0.034$) from 1°/s² to about 1.5°/s² (Fig. 2, column 2). The difference between the effects on the responses of the ipsilateral and contralateral eye was not significant at a 5% probability level ($p=0.074$). With contraversive stimulation, no statistically significant effects appeared after injection of carbachol in either the ipsilateral (Fig. 1, column 1) or contralateral (Fig. 1, column 4) eye ($p=0.538$ and $p=0.486$, respectively).

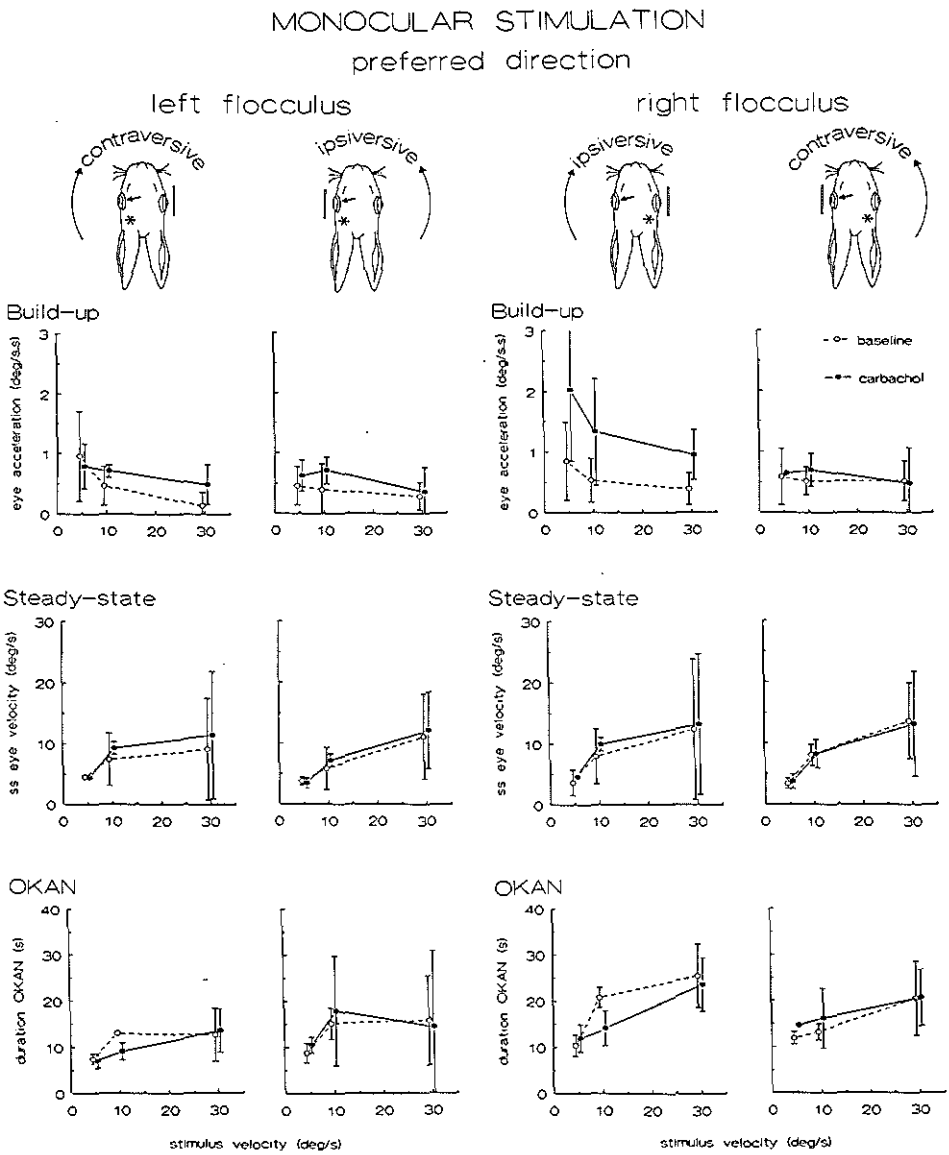
The middle row of graphs in Fig. 2 depicts steady-state eye velocities in the four stimulus conditions, before and after injection of carbachol. It is clear that in none of the conditions carbachol changed this parameter of OKN. Similarly, OKAN duration was not affected by carbachol in any of the stimulus conditions (Fig. 2, lower row of graphs).

Effects on responses to monocular stimulation

Monocularly elicited OKN in lateral-eyed species is known to be asymmetric, with a strong preference for stimulation in the temporal-to-nasal direction (rabbit: Ter Braak, 1936, Collewijn, 1969; rat: Hess et al., 1985; goldfish: Easter, 1972; pigeon: Gioanni et al., 1988). The shape of OKN elicited by monocular stimulation in the preferred direction strongly resembles binocularly elicited OKN. The response is characterized by a gradual, linear build-up of velocity until steady-state velocity is reached, although the acceleration

Fig. 3 Slow phase eye velocity of monocularly elicited OKN as a function of time for stimulation in the preferred direction. Upper panels show responses of the left eye in the seeing condition, lower panels show the responses of the left eye in the covered condition . Left and right panels depict effects of injection of carbachol in the left and the right flocculus (indicated by asterisks). Further conventions as in Fig. 1. Notice the faster build-up after injection of carbachol during ipsiversive, but not contraversive, stimulation in the seeing condition (upper right panels).


MONOCULAR STIMULATION
preferred direction
contraversive
left flocculus
seeing condition
ipsiversive
right flocculus
seeing condition
5 deg/s
baseline
carbachol
10 deg/s
baseline
carbachol
build-up/st-st
OKAN
5 deg/s
baseline
carbachol
7.5 s
5 deg/s
10 deg/s
baseline
carbachol
15 s
10 deg/s
ipsiversive
left flocculus
covered condition
contraversive
right flocculus
covered condition


MONOCULAR STIMULATION
preferred direction
left flocculus
right flocculus
contraversive
ipsiversive
ipsiversive
contraversive
Build-up
Build-up
baseline
carbachol
eye acceleration (deg/s/s)
Steady-state
Steady-state
ss eye velocity (deg/s)
OKAN
OKAN
duration OKAN (s)
stimulus velocity (deg/s)
stimulus velocity (deg/s)

during monocular stimulation is clearly lower than during binocular stimulation (compare Fig. 4 to Fig. 2; see also Tan et al., 1992a). When the lights are turned off, OKN continues as OKAN, which, similar to the binocular condition, is characterized by an approximately linear decay of eye velocity. Stimulation in the opposite, nasal-to-temporal direction elicits only poor responses.

Typical examples of OKN and OKAN during stimulation in the preferred direction, before and after injection of carbachol, are shown in Fig. 3. In each rabbit, the optokinetic response was measured first with only the left eye viewing, and subsequently with only the right eye viewing. Because only the responses of the left eye were recorded, the responses obtained with the left eye viewing will be referred to as the "seeing condition", while the right-eye-viewing condition will be referred to as the "covered condition". The responses under these two viewing conditions were tested with injection of carbachol in either flocculus, leading to a total of four stimulus conditions.

Velocity plots of contraversive and ipsiversive OKN under the seeing condition are shown in the upper panels of Fig. 3. It is clear that, similar to what was found for binocular viewing, carbachol had different effects on OKN in the two directions. Contraversive OKN was not affected by the injection of carbachol (Fig. 3, upper left panel) while ipsiversive build-up of OKN was enhanced, with an acceleration of 0.75°/s^2 before and 3.0°/s^2 after injection (Fig. 3, upper right panel). Steady-state gain and OKAN, however, were not affected in either direction.

The responses under the covered condition are shown in the lower panels of Fig. 3. Neither ipsiversive, nor contraversive OKN or OKAN were changed by carbachol.

The responses to stimulation in the non-preferred direction were also tested. However, this stimulus elicited a very poor and irregular nystagmus without significant build-up of velocity and the response was not improved by injection of carbachol.

Mean values of eye acceleration during build-up, steady-state eye velocity and duration of OKAN, elicited by monocular stimulation in the preferred direction, are depicted in Fig. 4. The rabbit-heads on top indicate the stimulus condition of each column. As reflected in the examples of Fig. 3, no changes occurred under the covered condition

Fig. 4 Means and standard deviations (n=10) of characteristic parameters of monocularly elicited OKN before (open circles, interrupted lines) and after (filled circles, continuous lines) injection of carbachol as a function of stimulus velocity. Conventions as in Fig. 2. Upper panels depict mean eye-acceleration during build-up; middle panels show steady-state velocity and lower panels show duration of OKAN. Left columns show effects of injection in the left flocculus, right columns show injections in right flocculus (indicated by asterisks). Notice the faster build-up after injection for ipsiversive stimulation in the seeing condition (upper row, third column).

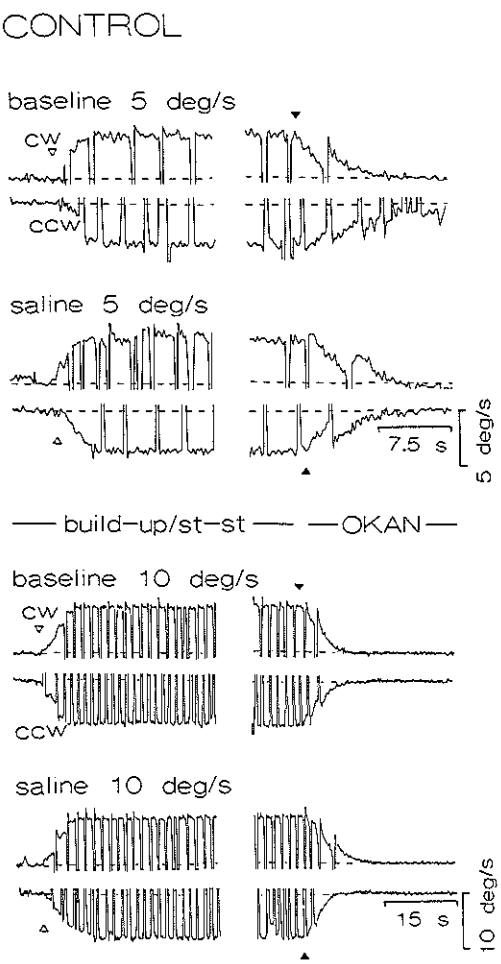


Fig. 5 Typical example of a control experiment, conducted to determine the specificity of the carbachol effects. Slow phase velocity of binocularly elicited by CW and CCW steps in surround-velocity of 5 and 10°/s is plotted as a function of time before and after injection of saline. Open triangles indicate the start of optokinetic stimulation; closed triangles indicate the time that the lights were turned off and eye movements continued as OKAN. Horizontal dashed lines indicate zero velocity; fast phase velocities have been truncated. Saline had no effect on build-up, steady state or OKAN.

(Fig. 4, columns 2 and 4). Eye-acceleration during build-up remained unchanged after injection of carbachol when stimulated in the ipsilateral or contralateral direction ($p=0.345$ and $p=0.612$, respectively). Steady-state eye velocity was also unchanged ($p=0.194$ and $p=0.783$ for the ipsilaterally and contralaterally directed stimulus, respectively). Duration of OKAN also remained at baseline-level after injection of carbachol with p-values of 0.758 and 0.685 for stimulation in the ipsi- and contralateral direction, respectively.

In the seeing condition, build-up of ipsiversive OKN was markedly accelerated, with a statistically significant increase in acceleration from about 0.5°/s² in the baseline condition to more than 1°/s² after injection of carbachol ($p=0.037$). In contrast, contraversive build-

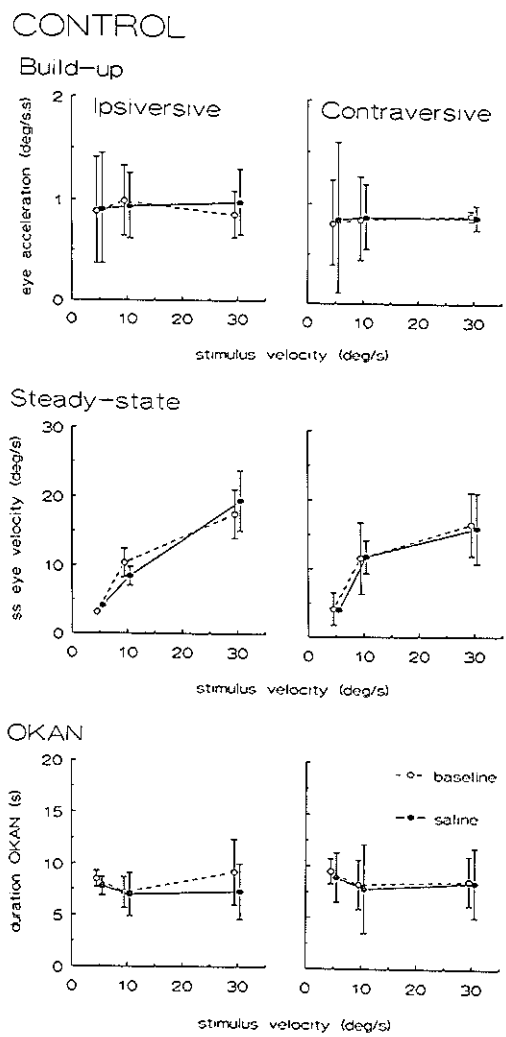


Fig. 6 Mean of characteristic parameters of OKN before (open circles, interrupted lines) and after (closed circles, continuous lines) injection of saline as a function of stimulus velocity. Data points and error bars represent mean ± standard deviation (n=6). Upper panel depicts mean eye-acceleration during build-up, middle panel shows steady-state velocity and lower panel shows duration of OKAN. Left and right column show effects on ipsiversive and contraversive OKN, respectively. Saline had no effect on average build-up rate, steady-state velocity or OKAN duration in either stimulus direction.

up (column 4) was not affected significantly by carbachol (p=0.385).

Control experiments

Fig. 5 shows typical OKN and OKAN, elicited by binocular stimulation, before and after unilateral injection of saline. These control experiments were carried out to assess the specificity of effects of carbachol on the time course of OKN. Unilateral injection of saline had no effect on OKN or OKAN, evoked by stimulation at 5 or 10°/s. Fig 6 shows

the mean parameters of OKN and OKAN as tested in three rabbits before and after unilateral injection of saline. The data from experiments with injection in the left or right flocculus were pooled. No statistically significant changes were found during ipsi- or contraversive stimulation in build-up ($p=0.645$ for ipsi- and $p=0.463$ for contraversive stimulation), steady-state ($p=0.734$; $p=0.534$) or OKAN ($p=0.475$; $p=0.264$). Thus, the effects of carbachol on build-up of OKN can be considered as specific.

Discussion

Unidirectional enhancement of OKN

The main finding of the present study is the asymmetric effect of a unilateral floccular injection of carbachol on the build-up of optokinetic nystagmus. During binocular as well as monocular viewing, build-up of OKN with the slow phase ipsiversive to the injected flocculus was accelerated by carbachol, while contraversive OKN was unaffected.

It is important to note that injection of carbachol in one flocculus was never followed by the occurrence of spontaneous nystagmus. This discounts the possibility that the effects on OKN were based on a left/right imbalance, which occurs after a unilateral flocculectomy (Barmack and Pettorossi, 1985). Another argument against imbalance as a cause of the effects on OKN is our finding that despite the enhanced build-up in one direction, the response in the opposite direction was unchanged. In case of a left/right bias, the enhancement of the response in one direction would be accompanied by a lowering of the response in the opposite direction. Thus, the effect should not be interpreted as a change in the balance between the tonic influence of the flocculi, but rather as a genuine enhancement of the response in one direction.

Relationship between the unidirectional enhancement of OKN and the directional coding of optokinetic information in the flocculus

Understanding the mechanism by which floccular injection of carbachol exerts its direction-specific influence on OKN at the cellular level requires knowledge about the directional coding of optokinetic information in the flocculus. More specifically, we would like to correlate the stimulus conditions under which the behavioral effects occur with floccular P-cell behavior under similar stimulus conditions.

The P-cell of the cerebellar flocculus is supplied with OKN-related input by two afferent systems: a climbing fiber system, arising from the inferior olive (IO) and a mossy fiber system, carrying information from a variety of oculomotor regions in the brainstem. Discharges of climbing fibers occur at a low frequency (about 1 Hz) and each discharge elicits a complex spike response in the P-cell. In contrast, mossy fibers discharge at a high

rate. Mossy fiber activity is relayed via granule cells and their parallel fibers to the P-cells, in which simple-spike discharges are induced at a frequency of up to about 100 Hz.

Due to its high discharge rate, it is the simple-spike activity that forms the effective output of the P-cell. The effect of P-cell discharge on its target structures is inhibitory (Ito et al., 1964; Eccles et al., 1967). However, the polarity of modulation of simple spikes in response to optokinetic stimulation is out of phase with the polarity of discharge of the vestibular target neurons (Ito et al., 1977), so that the P-cell output contributes positively to the modulation-depth of vestibular neuronal discharge, and thereby to OKN. Accordingly, the positive effect of carbachol is probably based on enhanced modulation of the simple-spike activity of the P-cell.

Although complex spike activity with its extremely low rate of discharge is not likely to contribute directly to tracking eye movements, there is evidence that the climbing fiber input can modulate the discharge of simple spikes (Granit and Phillips, 1956; Ebner and Bloedel, 1981). This would be a mechanism by which the optokinetic information carried by the climbing fibers could contribute to the generation of compensatory eye movements. It has been demonstrated, however, that the silencing of IO neurons by local micro-injection of lidocain lowers the level of spontaneous activity of simple-spikes but does not affect the depth of simple-spike modulation in response to optokinetic stimulation (Leonard, 1986; Leonard and Simpson, 1986). This finding proves that the interaction of climbing-fiber input with simple-spike activity is not the driving cause of the modulation of the simple spikes during optokinetic stimulation. As a consequence, the effective simple-spike output of the P-cell (and flocculus) must be driven mainly by parallel fiber activity. Therefore, carbachol most probably changes simple-spike discharge of the P-cell, by affecting the information transfer at the parallel fiber/P-cell synapses.

In response to ipsiversive optokinetic stimulation, the simple-spike firing rate of P-cells is increased as a result of increased activity of parallel fibers, while inhibition of simple-spike activity as a result of a lowering of the parallel fiber input coincides with contraversive motion of the visual surrounds. Consequently, the enhancement of specifically ipsiversive optokinetic responses suggests that carbachol enhances modulation of simple spike activity only in the excitatory phase.

sAHP-block as a possible basis for unidirectional enhancement of OKN

Bilateral floccular injection of carbachol improved the optokinetic response to a sinusoidal stimulus (Tan and Collewijn, 1991) and accelerated build-up of OKN in either direction (Tan et al., 1992a). A possible mechanism for this positive modulatory effect is the blockade of the slow afterhyperpolarization (sAHP) in the P-cell. Such a mechanism for the action of ACh was previously described in hippocampus (Madison and Nicoll, 1984).

The sAHP poses an upper limit to the spike frequency, as explained in the introduction. By blocking the sAHP of floccular P-cells, carbachol would increase the number of simple spikes in the P-cells in the injected flocculus in response to excitatory inputs. The excitatory inputs are known to encode ipsiversive OKN-related signals (Graf et al., 1988) and an increase in simple-spike activity is indeed related to ipsiversive optokinetic tracking, as suggested by electrical stimulation studies (Dufossé et al., 1977; Van der Steen et al., 1991). More specifically, our findings predict that the rate of rise in simple-spike discharge is enhanced, because carbachol accelerates the build-up of ipsiversive OKN, not its steady-state velocity.

The blocking of the sAHP would not have as much effect on the changes in the modulation rate during the phase of decreasing activity in parallel fibers and P-cells, because the sAHP would be less effective as a limiting factor during the lowering of the spike frequency. The decreasing phase of parallel fiber modulation causes a decrease in the simple-spike activity of the P-cell and is known to encode contraversive optokinetic tracking (Graf et al., 1988). Thus, the preferential effect of unilateral injection of carbachol on ipsiversive OKN could be explained by an asymmetrical effect of a carbachol-induced blockade of accommodation on the driving rate of P-cells in response to increases and decreases in parallel fiber input to the injected flocculus.

Laterality of floccular control

In the present study, eye movement responses were measured only in the left eye. Discounting the possibility that injections were consistently more effective in one similar (left or right) flocculus of all rabbits tested, or that systematic asymmetries exist between responses of left and right eye, the effect of carbachol on the build-up of the response to ipsiversive binocular stimulation is stronger in the nasally moving eye than in the temporally moving eye. An even stronger disconjugate effect of carbachol was revealed in the monocular viewing condition. During ispiversive stimulation, the build-up of the response of the contralateral, seeing eye was accelerated, whereas the response of the ipsilateral, covered eye was unaffected.

As explained above, the direction-specific effect of carbachol may be caused by an asymmetric effect of carbachol on a basically symmetrically organized flow of directional information through the flocculus. In contrast, the disconjugate effect of carbachol suggests asymmetries in the floccular control of movements of the two eyes. There is no electrophysiological evidence for such a disjunctive control by the flocculus. Each flocculus receives retinal image-slip information from both eyes, while electrical micro-stimulation elicits horizontal smooth deflections of both eyes (Dufossé et al., 1977; Van der Steen et al., 1991). Our observations on the asymmetrical control of the two eyes during monocular stimulation are, however, in line with previous evidence on disjunctive behavior during

monocular optokinetic stimulation in the rabbit (Collewijn and Noorduin, 1972).

Dissociation between changes in build-up and OKAN after unilateral injections

After bilateral floccular injections with carbachol, the acceleration of OKN build-up was increased, while the duration of OKAN was significantly shortened (Tan et al., 1992a). As will de reported separately (Tan et al., 1992b), also the duration of post-rotatory vestibular nystagmus is significantly shortened after bilateral floccular injection with carbachol. Remarkably, after the present unilateral injections, OKAN-duration remained entirely unaffected, despite accelerations of the build-up in the ipsiversive direction that were similar in magnitude as after bilateral injection. This observation suggests that there is no rigid coupling between the time course of charge and discharge of velocity storage.

Summary and conclusions

In previous work, we demonstrated an acceleration of the build-up of slow-phase velocity of optokinetic responses (OKN) after bilateral floccular injection of the aselective cholinergic agonist carbachol (Tan and Collewijn, 1991; Tan et al., 1992a). In the present study we investigated the effects of unilateral floccular injections of carbachol. Such unilateral injections specifically enhanced the build-up of OKN slow-phase velocity in the direction towards the injected flocculus (ipsiversive). During binocular optokinetic stimulation, this enhancement was expressed in the motion of both eyes. Acceleration of the eye contralateral to the injected flocculus increased from $1°/s^2$ to about $2°/s^2$, while the acceleration of the ipsilateral eye increased from $1°/s^2$ to about $1.5°/s^2$. In contrast, build-up of contraversive OKN was unchanged. No changes were found in the steady-state OKN and optokinetic afternystagmus (OKAN). Monocular optokinetic stimulation was only effective in the nasal direction and the effects of unilateral injection of carbachol were disconjugate. Ipsiversive OKN was enhanced only in the contralateral, seeing eye, while the response of the ipsilateral, covered eye was unchanged. We hypothesize that the directionally specific effect of unilateral cholinergic floccular stimulation on OKN is due to enhancement of predominantly the excitatory phase of modulation of the Purkinje cell's simple-spike activity by carbachol, without a marked effect of carbachol on the inhibitory phase of simple-spike modulation.

References

Barmack NH, Pettorossi VE (1985) Effects of unilateral lesions of the flocculus on optokinetic and vestibuloocular reflexes in the rabbit. J Neurophysiol 53: 481-496

Bernardo LS, Prince DA (1982) Cholinergic excitation of mammalian, hippocampal pyramidal cells. Brain Res 249: 315-331

Cole AE, Nicoll RA (1984) Characterization of a slow cholinergic post-synaptic potential recorded in vitro from rat hippocampal pyramidal cells. J Physiol (Lond) 352: 173-188

Collewijn H (1969) Optokinetic eye movements in the rabbit: input-output relations. Vision Res 9: 117-132

Collewijn H, Noorduin H (1972) Conjugate and disjunctive optokinetic eye movements in the rabbit, evoked by rotatory and translatory motion. Pflügers Arch 335: 173-185.

Dichgans J, von Reutern GM, Rommelt U (1978) Impaired suppression of vestibular nystagmus by fixation in cerebellar and non-cerebellar patients. Arch Psychiatr Nervenkr 266: 183-199

Dufossé M, Ito M, Miyashita Y (1977) Functional localization in the rabbit's cerebellar flocculus determined in relationship with eye movements. Neurosci Lett 5: 273-277

Easter SS (1972) Pursuit eye movements in goldfish (Carassius auratus). Vision Res 12: 673-688

Ebner TJ, Bloedel JR (1981) Temporal patterning in simple spike discharge of Purkinje cells and its relationship to climbing fiber activity. J Neurophysiol 45: 933-947

Eccles JC, Ito M, Szentágothai J (1967) The Cerebellum as a Neuronal Machine. New York: Springer

Gioanni H (1988) Stabilizing gaze reflexes in the pigeon (Columbia livia). I. Horizontal and vertical optokinetic eye (OKN) and head (OCR) reflexes. Exp Brain Res 69: 567-582

Graf W, Simpson JI, Leonard CS (1988) Spatial organisation of visual messages of the rabbit's cerebellar flocculus. II. Complex and simple spike responses of Purkinje cells. J Neurophysiol 60: 2091-2121

Granit R, Phillips CG (1956) Excitatory and inhibitory processes acting upon individual Purkinje cells of the cerebellum in cats. J Physiol (Lond) 133: 520-547

Hess BJM, Precht W, Reber A, Cazin L (1985) Horizontal optokinetic nystagmus in the pigmented rat. Neuroscience 15: 97-107

Igarashi M, Takahashi M, Homick JL (1977) Optokinetic nystagmus and vestibular stimulation in squirrel monkey model. Arch Oto-Rhino-Laryngol 218: 115-121

Ito M, Jastreboff PJ, Miyashita Y (1979) Adaptive modification of the rabbit's horizontal vestibulo-ocular reflex during sustained vestibular and optokinetic stimulation. Exp Brain Res 37: 17-30

Ito M, Jastreboff PJ, Miyashita Y (1982) Specific effects of unilateral lesions in the flocculus upon eye movements in albino rabbits. Exp Brain Res 45: 233-242

Ito M, Nisimaru N, Yamamoto M (1977) Specific patterns of neuronal connexions involved in the control of rabbit's vestibulo-ocular reflexes by the cerebellar flocculus. J Physiol (Lond) 265: 833-854

Ito M, Yoshida M (1966) The origin of cerebellar-induced inhibition of Deiters' neurones. I. Monosynaptic initiation of the inhibitory postsynaptic potentials. Exp Brain Res 2: 330-349

Ito M, Yoshida M, Obata K (1964) Monosynaptic inhibition of the intracerebellar nuclei induced from the cerebellar cortex. Experientia 20: 575-576

Keller EL, Precht W (1979) Visual-vestibular responses in vestibular nucleus neurons in the intact and cerebellectomized, alert cat. Neuroscience 4: 1599-1613

Krnjevic K, Pumain R, Renaud L (1971) The mechanism of excitation by acetylcholine in the cerebral cortex. J Physiol (Lond) 215: 447-465

Leonard CS (1986) Signal Characteristics of Cerebellar Purkinje Cells in the Rabbit Flocculus During Compensatory Eye Movements (PhD Dissertation). New York: New York University

Leonard CS, Simpson JI (1986) Simple spike modulation of floccular Purkinje cells during the reversible blockade of their climbing fiber afferents. In: Keller EL, Zee DS (eds) Adaptive Processes in Visual and Oculomotor Systems. Oxford, UK: Pergamon, pp 429-434

Madison DV, Nicoll RA (1984) Control of the repetitive discharge of rat CA1 pyramidal neurones in vitro. J Physiol (Lond) 354: 319-331

McCormick DA, Prince DA (1986) Mechanisms of action of acetylcholine in the guinea-pig cerebral cortex in vitro. J Physiol (Lond) 375: 169-194

Nagao S (1983) Effects of vestibulocerebellar lesions upon dynamic characteristics and adaptation of vestibulo-ocular and optokinetic responses in pigmented rabbits. Exp Brain Res 53: 36-46

Schwindt PC, Spain WJ, Foehring RS, Chubb MC, Crill WE (1988) Slow conductances in neurones from cat sensorimotor cortex in vitro and their role in slow excitability changes. J Neurophysiol 59: 450-467

Takemori S, Cohen B (1974) Loss of visual suppression of vestibular nystagmus after flocculus lesions. Brain Res 72: 213-224

Tan HS, Collewijn H (1991) Cholinergic modulation of optokinetic and vestibulo-ocular responses: a study with microinjections in the flocculus of the rabbit. Exp Brain Res 85: 475-481

Tan HS, Collewijn H. (1992) Cholinergic and noradrenergic stimulation in the rabbit flocculus have synergistic facilitatory effects on optokinetic responses. Submitted to Brain Res

Tan HS, Collewijn H, Van der Steen J. (1992a) Optokinetic nystagmus in the rabbit and its modulation by bilateral microinjection of carbachol in the cerebellar flocculus. Submitted to Exp Brain Res

Tan HS, Collewijn H, Van der Steen J. (1992b) Shortening of per-rotatory nystagmus by micro-injection of carbachol in the rabbit's cerebellar flocculus. Submitted to Exp Brain Res

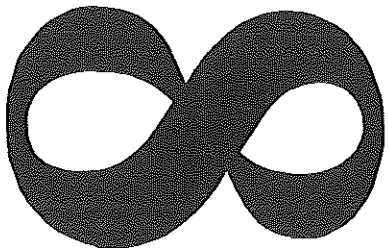
Ter Braak JWG (1936) Untersuchungen über optokinetischen Nystagmus. Arch Néerl Physiol 21: 309-376

Van der Steen J, Simpson JI, Tan J (1991) Representation of three-dimensional eye movements in the cerebellar flocculus of the rabbit. In: Schmidt R, Zambarbieri D, Oculomotor Control and Cognitive Processes, Amsterdam: Elsevier, pp 63-77

Van Neerven J, Pompeiano O, Collewijn H (1989) Depression of the vestibulo-ocular and optokinetic response by intraflocculuar microinjection of GABA-A and GABA-B agonists in the rabbit. Arch Ital Biol 127: 243-263

Waespe W, Cohen B, Raphan T (1983) Role of the flocculus and paraflocculus in optokinetic nystagmus and visual-vestibular interactions: effects of lesions. Exp Brain Res 50: 9-33

Zee DS, Yamazaki A, Butler PH, Güçer G (1981) Effects of ablation of flocculus and paraflocculus on eye movements in primate. J Neurophysiol 46: 878-899



Schortening of per-rotatory nystagmus by micro-injection of carbachol in the cerebellar flocculus

Introduction

Stabilization of the eyes in space during perturbations of head position is achieved by the synergistic action of the vestibulo-ocular reflex (VOR) and the optokinetic reflex (OKR). Whereas the semicircular canals sense fast angular displacements of the head, the optokinetic system is especially sensitive to slow motion of the entire visual image over the retina. In response to a step in head velocity in the light, gaze is accurately stabilized, independently of the duration of the step. Artificial separation of OKR and VOR has revealed that this is due to the complementary action of vestibular and optokinetic response components, both of which are time-dependent in a reciprocal way (Ter Braak, 1936). The isolated response of the VOR, measured by subjecting the animal to a step in rotatory velocity in darkness, consists of a step in slow-phase eye velocity, approaching the magnitude of the stimulus, followed by a gradual decay to zero with a time constant of 15-28 s in the monkey (Raphan et al., 1979) and about 20 s in the cat (Robinson, 1976). This response has originally been related to the mechanical properties of the cupula-endolymph system (Steinhausen, 1933). Direct assessment of the time constant of the response of the primary vestibular afferents to a step in velocity, however, revealed a cupular time constant in the range of 5.7 s in the monkey (Fernandez and Goldberg, 1971) and 4 s in the cat (Blanks et al., 1975); much shorter than the time-constant of the behavioral response. This difference has led to the hypothesis that the time constant of the primary vestibular signal is prolonged by a central neural velocity-storage mechanism (Raphan et al., 1979; Raphan and Cohen, 1985).

In response to a velocity-step of the visual surrounds, while the animal remains stationary, an isolated optokinetic response is elicited. The shape of this optokinetic response is reciprocal, as a function of time, to the vestibulo-ocular response and characterized by a slow build-up of optokinetic nystagmus (OKN), followed by optokinetic afternystagmus (OKAN) after the lights have been turned off, in the same direction as the preceding OKN (Ter Braak, 1936; Collewijn, 1969). Because the duration of OKAN matches the duration of PRN in monkey (Raphan et al., 1979) and rabbit (Collewijn et al., 1980) and because the two after-responses are opposite in direction, they cancel each other when rotation of the animal occurs in the light (Ter Braak, 1936; Mowrer, 1937; Jung, 1948). The tight functional linkage and similarity of optokinetic and vestibular afternystagmus have inspired investigators to propose a common mechanism for the production of the two phenomena (Robinson, 1977; Raphan et al., 1979). There is, however, also some contrary evidence, suggesting that they are separate mechanisms: in certain experiments on habituation of the VOR to steps in velocity shortening of the time constant of the VOR was not accompanied by a shortening of the OKAN, which led to the conclusion that the VOR and the OKR do not share a common velocity storage

mechanism (Skavenski et al., 1981).

Agreement exists on the involvement of the flocculus in the execution of the OKR. Floccular P-cells fire in response to optokinetic stimulation, while flocculectomy invariably decreases the gain of the OKR permanently in the rabbit (Ito et al., 1982; Nagao, 1983; Barmack and Pettorossi, 1985). The optokinetic response of the rabbit is believed to correspond to the "indirect" optokinetic response in monkey. Indeed, flocculectomy in the monkey reduces the indirect optokinetic response (Zee et al., 1981; Waespe et al., 1983). However, the effect of lesioning on the VOR, described by these studies is inconsistent. Inactivation of the flocculus did not affect (Barmack and Pettorossi, 1985; Zee et al., 1981; Waespe et al., 1983) or decreased (Ito et al., 1982; Nagao, 1983; Van Neerven et al., 1989) the gain of the VOR.

Flocculectomy in the monkey changed neither OKAN nor PRN, arguing against involvement of the flocculus in the velocity storage process (Zee et al., 1981; Waespe et al., 1983). In the rabbit, the effects of flocculectomy on optokinetic and vestibular responses to velocity-steps have not been investigated.

In an earlier study, we described the effects of bilateral floccular micro-injection of the aselective cholinergic agonist carbachol on optokinetic and vestibular responses. Carbachol had a pronounced, positive effect on the optokinetic response to a sinusoidally moving drum, and induced a weaker, but statistically significant increase in the gain of the VOR (Tan and Collewijn, 1991). These results suggest involvement of the flocculus in OKR, as well as the VOR. The build-up of OKN after a step in velocity of an optokinetic drum was accelerated and the duration of OKAN was significantly shortened, suggesting involvement of the flocculus in velocity storage (Tan et al., 1992). As an extension to this line of investigation we tested the effect of floccular injection of carbachol on the duration of vestibular afternystagmus in the present study.

Methods

Animal preparation

A first assessment of baseline PRN was done on the five rabbits which had been used in our previous study on OKN and OKAN (Tan et al., 1992) but had never undergone vestibular stimulation with velocity steps. These experiments served to evaluate the vestibular responses to different stimulus velocities. Subsequently, the effect of carbachol on PRN was studied in five different, naive rabbits. Each animal was permanently implanted with ocular sensor coils for eye movement recording and scull-screws for fixation of the head. The flocculi were localized electrophysiologically on the basis of visually evoked direction-selective complex-spike and simple-spike activity and

guide cannulas, aimed towards right and left flocculi, were implanted bilaterally. All surgical procedures were done under general anesthesia, induced by a mixture of ketamine, acepromazine and xylazine. More details of the animal preparation are described in Tan et al. (1992).

Experimental conditions and data analysis

The experiments were carried out in a light-proof room to provide complete darkness during the experiments. The animal was strapped onto a platform which was attached to a vestibular chair. This chair was mounted on a turntable with hydraulic bearings, which was driven by a high-torque hydraulic motor, controlled by a flow-control servo-valve. Velocity feedback was provided by a tacho-generator. The head of the rabbit was rigidly connected to the platform with the head bolts. Eye movements were recorded using the scleral search coil in an a.c. magnetic field according to the technique of Robinson (1963). The field coils were attached to the platform. The turntable was rotated

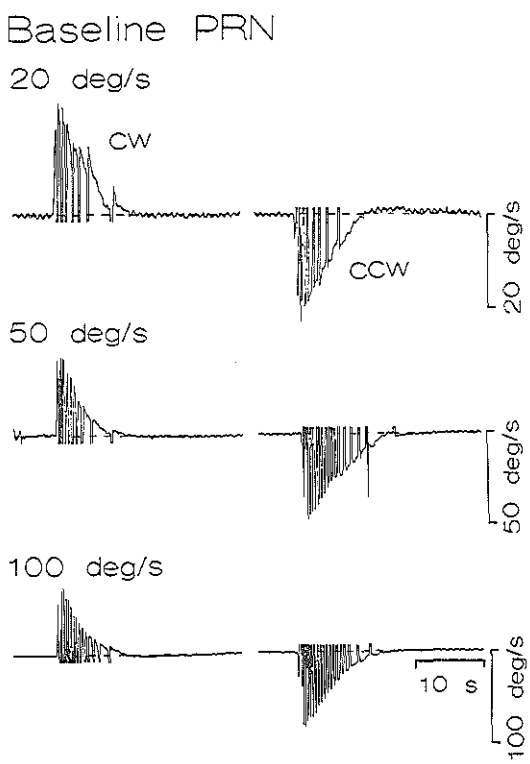


Fig. 1 Typical examples of baseline perrotatory nystagmus (PRN) in response to vestibular stimulation with velocity steps (in darkness) of 20, 50 and 100°/s in the CW (right) and CCW (left) directions. Eye velocity is shown as a function of time; fast phase components have been truncated.

in darkness at a constant velocity. Per-rotatory nystagmus (PRN) was elicited by a sudden change in the direction of chair rotation, resulting in a velocity step. For the assessment of baseline characteristics of PRN, the rabbits underwent two sets of measurements, each consisting of velocity steps of 20, 50 and 100°/s peak-to-peak in the left and right direction. The steps were applied with intervals of 1 min.

For the assessment of the effects of floccular injection of carbachol, a different procedure was used. A set of measurements consisted of 10 velocity-steps, alternating in left and right direction. Because velocity steps of 20°/s elicited reliable and consistent PRN in the experiments done to evaluate baseline responses, these were used in the carbachol experiments. After a set of baseline measurements was taken, the rabbit received a bilateral floccular injection of a saline solution as a control experiment. A rest of 20 min was interposed before we recorded a second set of measurements, after which an injection of carbachol (1μg in 1 μl; Sigma, USA) was made. A third and last set of measurements was taken 20 min after the injections of carbachol.

The position signal of the left eye was stored by a data-acquisition program at a sample-frequency of 51.2 Hz. In the subsequent off-line analysis, slow-phase eye velocity was calculated and plotted as a function of time. From these plots, peak velocity and duration of PRN were determined. The statistical significance of the effects of carbachol was tested with a Multiple Analysis of Variance (MANOVA), which allows the comparison of several variables in a single group of animals.

Results

Basic characteristics of PRN

Fig. 1 shows typical examples of PRN, elicited by rotational velocity-steps at speeds of 20, 50 and 100°/s in left and right direction. The typical response to a velocity step consists of an initial jump in slow-phase eye velocity to a peak, followed by a gradual decay. The maximum velocity and duration of PRN after each step were measured. For each stimulus velocity, the parameters of PRN, elicited in the left and right direction were pooled and averaged over the five rabbits tested. These mean values are shown in Fig. 2.

The average peak gain of a response to a 20°/s step was 0.86 ± 0.06 (S.D.). As shown in the examples of Fig. 1, gain decreased with increasing stimulus velocity. The 50°/s step elicited a response with a mean peak gain of 0.77 ± 0.07 (S.D.), while the 100°/s step was followed by a response with a mean peak gain of 0.64 ± 0.07 (S.D.). Thus, lower peak-gains were encountered after larger velocity-steps.

In the response to the 20°/s step, the initial jump was followed by a linear decay in slow-phase eye velocity, which lasted on average for 13.4 s ± 3.9 s (S.D.) before zero

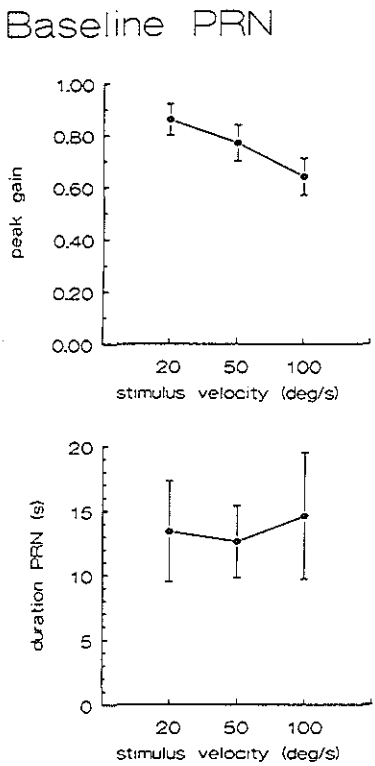


Fig. 2 Means and S.D. of characteristic parameters of PRN (n=5) elicited by velocity steps of different magnitudes (abscissa). Upper graph shows peak-gain of PRN; lower graph shows duration.

velocity was reached. The duration of PRN was similar in response to all stimulus velocities, as illustrated by the example of Fig. 1. Steps of 50°/s and 100°/s elicited PRN with average durations of 12.6 s ± 2.8 s (S.D.) and 14.6 s ± 4.9 s (S.D.). However, after the higher velocity steps the decay of slow phase velocity was sometimes very irregular, deviating from the typical monotonous decay.

The response to steps of 20°/s was consistent, with high peak gains, followed by a invariably linear decay in slow phase velocity. Therefore, this amplitude was used for the carbachol experiments.

Effects of saline and carbachol on PRN

For these experiments, five naive rabbits were used. For each set of measurements, 10 steps of 20°/s (5 pairs of leftward and rightward steps) were applied to elicit a PRN. Typical examples of leftward and rightward eye movement responses are shown in Fig. 3. The mean peak-gain of the 5 subsequent duplets of left and rightward PRN was averaged

over 5 rabbits (10 responses per point) and represented in Fig. 4 by 5 successive data point. A similar procedure was followed for the duration of the PRN.

Injection of saline had no effect on PRN. As illustrated in the examples of Fig. 3, neither maximum gain, nor duration of the PRN were changed. The average values of Fig. 4 show a similar pattern. There was, on average, no significant difference in maximal gain ($p=0.418$) or duration ($p=0.441$) between the 5 pairs of steps before and the 5 pairs of steps after injection of saline. No statistical significance was encountered for either peak-gain ($p=0.418$) or duration of PRN ($p=0.441$).

However, throughout the cluster of "baseline" and "saline" measurements, the maximal gain values show a slight downward trend in the course of the experiments. The average gain was 0.91 ± 0.13 (S.D.) for the first pair of steps during the baseline measurements, decreasing to 0.75 ± 0.15 (S.D.) for the last pair of steps during the post-saline measurements. There was also a slight downward trend for the duration of PRN. The duration was shortened from 12.8 s ± 1.0 s (S.D.) for the first pair of PRN's, during the baseline measurements, to 11.7 s ± 2.2 s (S.D.) for the last pair of PRN's measured after the saline injection. These downward trends of duration and peak amplitude of PRN

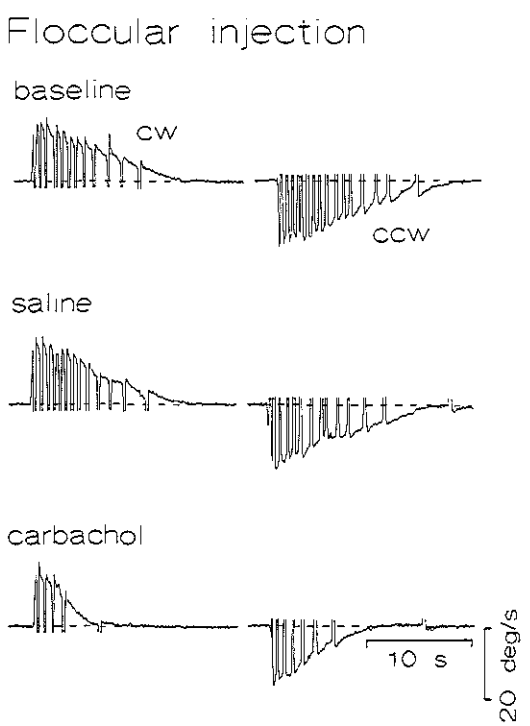


Fig. 3 Typical examples, taken from the same rabbit, of PRN elicited by velocity steps of 20°/s in either direction in the baseline condition (upper panels), after saline injection (middle panels) and after carbachol injection (lower panels). Conventions as in Fig. 1. Notice the shortening of PRN after bilateral injection of carbachol.

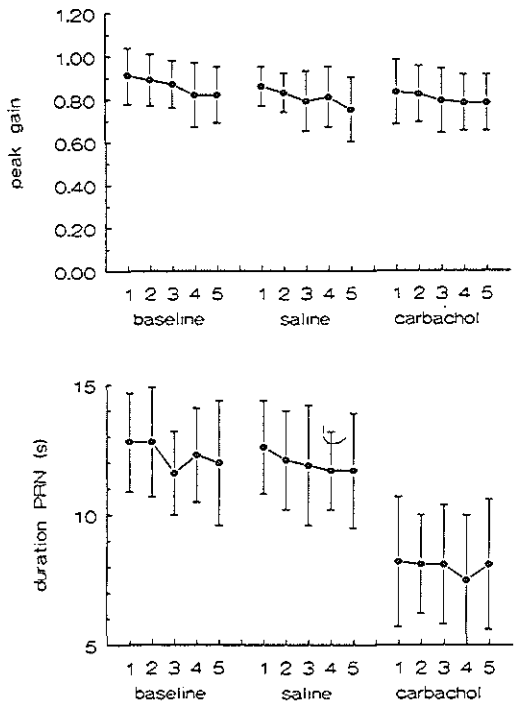


Fig. 4 Means and S.D. of characteristic parameters of PRN (n=5) in the baseline condition, after injection of saline, and after carbachol injection. Each data point represents the mean value for a pair of immediately successive steps (left and rightward), averaged over 5 rabbits. The five successive dots in a cluster represent the successive five pairs of measurements taken in one condition. Upper graph shows peak-gains in the three conditions; successive values only show a modest tendency to habituation. Lower graph shows duration of PRN. Notice that the duration is reduced by about a factor 2 after the injection of carbachol.

are most probably ascribable to habituation of the VOR due to the repeated testing.

Immediately following the post-saline measurements, carbachol was injected bilaterally in the flocculi. Examples of measurements taken after carbachol injection are shown in Fig. 3. Compared with the response before injection, the peak-gain was unchanged. The decay of eye velocity of PRN was still linear, but much faster than before the carbachol injection. As a result, the duration of PRN was shortened from about 15 s before to about 8 s after carbachol injection. This decrease was statistically significant ($p=0.001$). The mean values of the peak gains retained a constant value of about 0.80 after injection of carbachol (Fig. 4) and accordingly, peak-gains before and after carbachol were not significantly different ($p=0.621$). The downward trend of the peak-gain, which started in the baseline measurements, continued very moderately after injection of carbachol, reaching an end value of about 0.78. This habituation of gain appeared to be a continuous process, which was unaffected by any of the injections. Despite a similar slight downward trend of the duration of PRN in the course of the measurements, the decrease after the

injection of carbachol was clearly specific, because there was a marked discontinuity between the durations before and after the injection of carbachol.

Discussion

Basic characteristics of PRN

Vestibular stimulation by steps in angular velocity resulted in PRN with characteristics that agree with the description by Collewijn et al. (1980). The response to a velocity step consisted of an immediate jump to peak slow-phase velocity, followed by a gradual decay of slow-phase velocity to zero, which can be best fitted by a straight line. In line with previous observations in rabbit (Collewijn et al., 1980) and monkey (Raphan et al., 1979), the shape and average duration of PRN coincide very well with those of OKAN, measured in the same group of rabbits in an earlier study (Tan et al., 1992). This similarity suggest that OKAN and PRN are produced by the same velocity-storage mechanism. An alternative explanation for the similarity between PRN and OKAN would be behavioral matching of two independent systems.

Effects of carbachol

The main finding of the present study is a statistically significant shortening of the duration of PRN after bilateral floccular micro-injection of the cholinergic agonist carbachol. After injection, similar peak velocities were attained as before injection, followed by a steeper, but still linear, decline of velocity.

With repeated exposure of an animal to vestibular steps, the maximum amplitude gradually decreases, while the duration of PRN is gradually shortened. This is the well-known phenomenon of "habituation" (for review see Schmid and Jeannerod, 1985). Often repeated exposure may even drive the time constant of PRN towards the time constant of the canals. The measurements in the present study were done in a way that allowed differentiation between changes due to habituation and genuine effects of carbachol on PRN. Testing of PRN was done in series of 10 steps, taken with intervals of 1 min. Habituation would be reflected as a downward trend in the duration of PRN in the course of these measurements. Such a trend was present, but small compared to the marked shortening that occurred after carbachol injection. The decrease in duration of PRN after carbachol injection is therefore clearly independent of habituation.

The shortening of duration of PRN is quantitatively similar to the shortening of OKAN, described in a previous study (Tan et al., 1992). In both cases the duration of afternystagmus was reduced by about a factor 2, suggesting common control of vestibular and optokinetic afternystagmus by the flocculus. On the basis of results of lesion studies,

a role of the flocculus in velocity-storage has been denied in the monkey (Zee et al, 1981; Waespe et al., 1983). The role of the flocculus in velocity-storage in the rabbit has not been investigated previously. The modification of PRN and OKAN by our bilateral floccular injections of carbachol show that in the rabbit such a role exists.

Similar, bilateral injections strongly increase the maximal eye acceleration in response to optokinetic stimulation (Tan et al., 1992) and, to a lesser degree, enhance the response to low-velocity sinusoidal vestibular stimulation (Tan and Collewijn, 1991). This may lead to a more accurate stabilization of the eye in space during normal behavior. Such improvement may be especially important during daylight time, because especially the dynamic response of the optokinetic response is improved. There is, however, no obvious functional purpose for a shortening of OKAN and PRN. Since after rotation in the light OKAN and PRN would cancel each other, shortening of both components would not change anything. Possibly, a long time constant of responses to rotation is more relevant during nighttime, when optokinetic control is very limited and conservation of orientation may critically depend on the storage of rotational velocities, sensed by the canals. In this respect, we might speculate that the cholinergic control of velocity-storage follows a day-night rhythm. It would be of interest to follow up the old observations by Collewijn and Kleinschmidt (1975), suggesting a circadian rhythm of the gain of the VOR in darkness, with higher values being measured during nighttime.

Electrophysiological correlation

Electrophysiological recordings in the cerebellar flocculus have demonstrated modulation of P-cell activity in response to vestibular stimulation. Ito (1972, 1977) proposed a model in which the floccular vestibular pathway serves as a feed-forward side-path of sensory vestibular signals to a brainstem vestibular pathway. Recordings in the monkey floccular area, however, have identified eye movement related signals, in addition to vestibular sensory signals (Miles and Fuller, 1975; Lisberger and Fuchs, 1978; Miles et al., 1980).

In a recent study in rabbit, Purkinje cells showed activity related to eye movements during vestibular stimulation, but absence of head-velocity information (Leonard, 1986; Leonard and Simpson, 1984, 1985). If pure sensory vestibular modulation is indeed absent in the flocculus, then the effect of floccular injection of carbachol on vestibular responses must be due to a modification of the eye movement signals.

During optokinetic stimulation, P-cell activity encodes both sensory retinal image-slip and corollary eye movement signals (Leonard, 1986). Eye movement-related activity of P-cells was also encountered in the monkey during OKAN (Waespe and Henn, 1981). The enhancing effect of injection of carbachol on the build-up of slow-phase eye velocity during OKN could be the result of modification of the response to sensory input and/or

corollary input. During OKAN, retinal input is absent, and floccular activity is expected to encode only eye movements. The modification of OKAN by floccular injection of carbachol could, thus, be caused by a modification in the transfer of the eye movement corollary signal through the flocculus, analogous to PRN.

The eye movement related signal encodes eye velocity and eye position during all types of eye movements: optokinetic, vestibular but also fast phases. It is therefore considered an accurate motor corollary of eye movements. It has been proposed (Leonard, 1986) that this corollary information in the flocculus is part of a positive feedback loop. Such a positive feedback loop would increase the performance of the oculomotor system, especially in the low frequency range. In addition, such a loop would tend to maintain its own activity, and could therefore act as an integrator; this type of mechanism has been proposed by Robinson (1977). In view of the indistinguishable effect of carbachol on PRN and OKAN, the corollary eye movement signal would indeed be a likely target for modification by carbachol, because it is associated similarly with both optokinetic and vestibular responses.

Summary and conclusions

It has been proposed that one common velocity storage mechanism is responsible for the prolongation of post-rotatory vestibular nystagmus (PRN) beyond the duration of the change in firing-frequency of primary vestibular fibers in response to a step in velocity, and for the production of optokinetic afternystagmus. In a previous study, bilateral injection of the aselective cholinergic agonist carbachol in the flocculus shortened the duration of build-up of optokinetic nystagmus (OKN) and the duration of OKAN, suggesting floccular involvement in velocity storage (Tan et al., submitted). In extension to that study of OKN, the present study assesses the effects of floccular carbachol on PRN. Our results show that injection of carbachol shortens the duration of PRN from about 13 s to about 8 s; a finding which supports a common velocity-storage mechanism for optokinetic and vestibular signals. We propose that the indistinguishable effects of carbachol on OKAN and PRN are due to modification of the transmission of an oculomotor corollary signal, which has been identified electrophysiologically in the flocculus.

References

Barmack NH, Pettorossi VE (1985) Effects of unilateral lesions of the flocculus on optokinetic and vestibuloocular reflexes of the rabbit. J Neurophysiol 53: 481-496

Blanks RHI, Estes MS, Markham CH (1975) Physiologic characteristics of vestibular first order canal neurons in the cat. II. Response to constant angular acceleration. J Neurophysiol 38: 1250-1268

Collewijn H (1969) Optokinetic eye movements in the rabbit: input-output relations. Vision Res 9: 117-132

Collewijn H, Kleinschmidt HJ (1975) Vestibulo-ocular and optokinetic reactions in the rabbit: changes during 24 hours of normal and abnormal interaction. In: Lennerstrand G, Bach-y-Rita P (eds), Basic Mechanisms of Ocular Motility and their Clinical Implications. Oxford: Pergamon, pp 477-483

Collewijn H, Winterson BJ, Van der Steen J (1980) Post-rotatory nystagmus and optokinetic after-nystagmus in the rabbit: linear rather than exponential decay. Exp Brain Res 40: 330-338

Fernández C and Goldberg JM (1971) Physiology of peripheral neurons innervating semicircular canals of the squirrel monkey. II. Response to sinusoidal stimulation and dynamics of peripheral vestibular system. J Neurophysiol 34: 661-675

Ito M (1972) Neural design of the cerebellar motor control system. Brain Res 40: 81-84

Ito M (1977) Neuronal events in the cerebellar flocculus associated with an adaptive modification of the vestibulo-ocular reflex of the rabbit. In: Baker R, Berthoz A (eds) Control of Gaze by Brain Stem Neurons, Amsterdam: Elsevier, pp 391-398

Ito M, Jastreboff PJ, Miyashita Y (1982) Specific effects of unilateral lesions in the flocculus upon eye movements in albino rabbits. Exp Brain Res 45: 233-242

Jung R (1948) Die Registrierung des postrotatorischen und optokinetischen Nystagmus und die optisch-vestibuläre Integration beim Menschen. Acta Otolaryngol [Stockh] 36: 199-202

Leonard CS (1986) Signal Characteristics of Cerebellar Purkinje Cells in the Rabbit Flocculus During Compensatory Eye Movements (PhD Dissertation). New York: New York University

Leonard CS, Simpson JI (1984) Purkinje cell activity in the flocculus of the alert rabbit during natural visual and vestibular stimulation. Soc Neurosci Abstr 10: 538

Leonard CS, Simpson JI (1985) Purkinje cell activity in the flocculus of the alert rabbit during natural visual and vestibular stimulation. Part 2. Soc Neurosci Abstr 11: 1033

Lisberger SG, Fuchs AF (1978) Role of the primate flocculus during rapid behavioural modification of vestibuloocular reflex. I. Purkinje cell activity during visually guided horizontal smooth-pursuit eye movements and passive rotation. J Neurophysiol 41: 733-777

Miles FA, Fuller JH (1975) Visual tracking and the primate flocculus. Science 189: 1000-1002

Miles FA, Fuller JH, Braitman DJ, Dow BM (1980) Long-term adaptive changes in primate vestibuloocular reflex. III. Electrophysiological observations in flocculus of normal monkeys. J Neurophysiol 43: 1437-1476

Mowrer OH (1937) The influence of vision during bodily rotation upon the duration of post-rotational vestibular nystagmus. Acta Otolaryngol [Stockh] 25: 351-364

Nagao S (1983) Effects of vestibulocerebellar lesions upon dynamic characteristics and adaptation of vestibulo-ocular and optokinetic responses in pigmented rabbits. Exp Brain Res 53: 36-46

Raphan T, Matsuo V, Cohen B (1979) Velocity storage in the vestibulo-ocular reflex arc (VOR). Exp Brain Res 35: 229-248

Raphan T, Cohen B (1985) Velocity storage and the ocular response to multidimensional vestibular stimuli. In: Berthoz A, Melvill Jones G (eds), Adaptive Mechanisms in Gaze Control. Amsterdam: Elsevier, pp 123-143

Robinson DA (1963) A method of measuring eye movement using a scleral search coil in a magnetic field. IEEE Trans Biomed Electron BME-10: 137-145

Robinson DA (1976) Adaptive gain control of the vestibulo-ocular reflex by the cerebellum. J Neurophysiol 39: 954-969

Robinson DA (1977) Vestibular and optokinetic symbiosis: an example of explaining by modelling. In: Baker R, Berthoz A (eds), Control of Gaze by Brain Stem Neurons. Amsterdam: Elsevier, pp 49-58

Schmid R, Jeannerod M (1985) Vestibular habituation: an adaptive process? In: Berthoz A, Melvill Jones G (eds), Adaptive Mechanisms in Gaze Control. Amsterdam: Elsevier, pp 113-122

Skavenski AA, Blair SM, Westheimer G (1981) The effect of habituating vestibular and optokinetic nystagmus on each other. J Neurosci 1: 351-357

Steinhausen W (1933) Über die Beobachtung der Cupula in den Bogengangsampullen des Labyrinths des lebenden Hechts. Pflügers Arch 232: 500-512

Tan HS, Collewijn H (1991) Cholinergic modulation of optokinetic and vestibulo-ocular responses: a study with microinjections in the flocculus of the rabbit. Exp Brain Res 85: 475-481

Tan HS, Collewijn H, Van der Steen J (1992) Optokinetic nystagmus in the rabbit and its modulation by bilateral microinjection of carbachol in the cerebellar flocculus. Submitted to Exp Brain Res

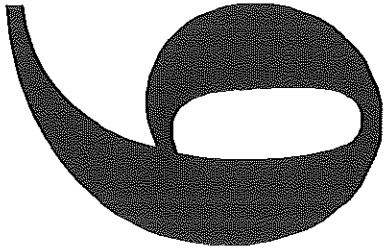
Ter Braak JWG (1936) Untersuchungen über optokinetischen Nystagmus. Arch Néerl Physiol 21: 309-376

Van Neerven J, Pompeiano O, Collewijn H (1989) Depression of the vestibulo-ocular and optokinetic response by intraflocculur microinjection of GABA-A and GABA-B agonists in the rabbit. Arch Ital Biol 127: 243-263

Waespe W, Cohen B, Raphan T (1983) Role of the flocculus and paraflocculus in optokinetic nystagmus and visual-vestibular interactions: effects of lesions. Exp Brain Res 50: 9-33

Waespe W, Henn V (1981) Visual-vestibular interaction in the flocculus of the alert monkey. II. Purkinje cell activity. Exp Brain Res 43: 349-360

Zee DS, Yamazaki A, Butler PH, Güçer G (1981) Effects of ablation of flocculus and paraflocculus on eye movements in primate. J Neurophysiol 46: 878-899



General discussion and synthesis

Each preceding chapter contains a thorough discussion of the data presented in it. To avoid unnecessary overlap, the present and final chapter will not attempt a comprehensive discussion of these data but, rather, a discussion of some unifying points of interest.

3-D Optokinetic responses: functional considerations

An important conclusion from Chapter 2 is that the poor response of the OKR to constant velocity drum rotation about horizontal axes (HA), compared to the responses about the vertical axis (VA), is not simply caused by a low gain, but by differences in the structure of the optokinetic responses of VA and HA OKN. Stimulation about the VA elicited a regular nystagmus with eye excursions that never exceeded 15° before interruption by a fast phase. During binocular stimulation about the 0° HA, however, eye excursions were often larger than 20°, while intervals between fast phases exceeded 10 s. During these long slow phase intervals, eye velocity seemed appropriate initially, but deteriorated as the eye got locked in an extreme, eccentric position. Indeed, a periodical optokinetic stimulus with a small amplitude (10°), that could be tracked by the eyes without a need for fast phases, elicited a very good response about the 0° HA, with a high gain of about 0.9. The major limitation of the OKR about horizontal axes, thus, seems to be the lack of a regular and timely occurrence of fast phases.

The significance of this lack of fast phases in the optokinetic response about the 0° HA should be interpreted in the context of functional demands. In the normal situation, the OKR, the VOR and the otolith-ocular reflex (OOR) work conjointly to stabilize gaze about the 0° HA. In analogy with the lack of fast phases in the optokinetic response about the 0° HA, fast phases were also uncommon in the response to combined vestibular and (dynamical) otolith stimulation about the 0° HA (Barmack 1981, Van der Steen and Collewijn, 1984). While observing spontaneous behavior of freely moving rabbits, Van der Steen and Collewijn (1984) noticed that rotations of the head in roll (0° HA) were very limited, rarely exceeding 20° from the straight-up position. Gaze stabilization within this range does not require fast resettings and, as a consequence, eye stabilization about the 0° HA during everyday life will not be affected by the lack of fast phases during head motion. Moreover, in the light of the specialized function of the combined eye-stabilizing reflexes about the 0° HA in keeping the visual streak of the retina aimed at the horizon, fast phases would have an adverse effect as they would only carry the visual streak away from the horizon. For this reason, fast resettings about the 0° HA even seem to be unwanted under physiological conditions.

In contrast with the limited head rotations about the 0° HA, head movements of

very large amplitudes were encountered about the vertical axis in spontaneously behaving rabbits (Van der Steen and Collewijn, 1984). As gaze stabilization is possible only within a limited (physical) range of eye positions in the orbit, this *position stabilization* is sacrificed in favor of an optimal *velocity stabilization* during these large head movements. Velocity stabilization is achieved by interposition of fast resettings, which serve to maintain the eye within the physical limits of the orbit. Taking into account the organisation of the rabbit retina, with a concentration of retinal ganglion cells in a horizontal visual streak rather than a fovea, the departure from absolute position stabilization during rotation about the vertical axis will not significantly impair vision, as it would during 0° HA rotation.

Absence of "velocity storage" in non-horizontal OKN

In the rabbit, the gain of the optokinetic responses to monocular optokinetic constant-speed stimulation about the entire range of horizontal axes was anisotropically distributed (Chapter 2). There was a maximum response to visual world rotation occurring with excitation of the anterior canal, ipsilateral to the stimulated eye. This maximum gain, however, was still low (about 0.5) and was not built up during the course of stimulation. Also with binocular optokinetic stimulation, the gain was low and no build-up was present. These findings suggest that, in the rabbit, a "velocity storage mechanism" is absent for non-horizontal OKN, implying differences in the processing of horizontal and non-horizontal optokinetic signals.

Slip of the image over the retina is sensed by directional and velocity-sensitive ganglion cells. Two classes of retinal ganglion cells have been identified on the basis of different response properties: "on" and "on-off" cells (Oyster et al., 1968). The "on" class responds to low-velocity slip; the "on-off" class is sensitive to higher velocities of retinal slip.

From the retina, directionally- and velocity-specific slip information is differentially relayed to separate sites in the pretectum. A major recipient of retinal ganglion cell afferents is the accessory optic system (AOS; see Simpson, 1984). Electrophysiological investigations of the AOS (Simpson et al., 1988) have revealed that the three directions of retinal slip are represented in separate nuclei of the AOS. The medial terminal nucleus (MTN) contains neurons that are preferentially excited by upward and somewhat posterior movement. Lateral terminal nucleus (LTN) neurons were excited best by downward and somewhat posterior, while dorsal terminal nucleus (DTN) neurons were best modulated by horizontal movement, posterior to anterior (temporal-to-nasal) movement in the visual field being the preferred excitatory direction. Common to all nuclei of the AOS is

sensitivity only to low-speed retinal slip information. The velocity range coincides with the velocity-sensitivity of the on-type of retinal ganglion cells.

A second important recipient of retinal ganglion cell afferents is the nucleus of the optic tract (NOT; Hoffmann and Schoppmann, 1975; Collewijn, 1975). The neurons in the NOT have excitatory preferences for mainly horizontal, temporal-to-nasal movement of large patterned stimuli (rabbit: Collewijn, 1975, Simpson et al., 1979; cat, Hoffmann and Schoppmann, 1975), similar to the best response direction of the DTN neurons (see Simpson, 1984). A subset of NOT neurons is specifically sensitive to low speeds, while other NOT neurons exhibit an optimal response at speeds higher than those preferred by dorsal terminal nucleus neurons (Collewijn, 1975; Simpson, 1979), suggesting that information from "on" type as well as "on-off" type retinal ganglion cells must converge upon the NOT.

In conclusion, the AOS represents low-velocity retinal slip in three dimensions, while the NOT represents mainly high velocity, horizontal retinal slip. It is possible that this difference in processing of horizontal and non-horizontal optokinetic responses is the basis for the difference in velocity storage. More specifically, the high-velocity pathway through the NOT could be the sole input to the storage integrator and the lack of a high-velocity pathway for non-horizontal optokinetic signals could underlie the absence of storage in non-horizontal optokinetic responses.

Localization of effects of injections

The method used to inject into flocculus is a reliable one. The flocculus was localized initially under the guidance of electrophysiological recordings. In the first of these kinds of experiments, done by Van Neerven (1990) and Van Neerven et al. (1989, 1990), histological verification after the experiments reconfirmed localization of the injections into the flocculus. Although we did not verify all injections histologically, the ones we did check using injections of kainic acid invariably showed that injections were placed into the flocculus without any spread to neighbouring structures.

Despite localization of injections into the flocculus, it is still possible that an injected substance, by diffusion, has reached other structures. Immediately bordering the flocculus is the paraflocculus, the function of which is unknown. Far more remote, oculomotor-related areas, e.g. the caudal vermis, could theoretically be reached via the liquor. It is of importance, however, to keep in mind that the concentration of the substance in brain tissue decreases considerably with increasing distance from the injection site. Pilot investigations have shown that injection of lower concentrations of carbachol induced smaller effects. Injection of a solution of carbachol at one tenth of the standard

concentration used in all experiments even had no effect at all. Thus, the concentration employed in our studies is within a reasonable range and the effect of the injection is not expected to extend over a large area. Moreover, the fact that every single animal consistently had increased optokinetic responses after the injection is a strong argument for localization of the effect of carbachol within a short distance of the injection spot.

Another important issue in the interpretation of the effects in the context of diffusional processes is the timing of the effects. Diffusion of substances through brain tissue takes some time to occur. Whereas in the study described in Chapter 3 a first measurement was taken 5 min after injection, in the studies in Chapters 4 and 6 measurements were taken immediately after injection, with a delay of only 30 sec. This "fast procedure" revealed an initial decrease at the first measurement, followed by an increase in the second and subsequent measurements. This bimodal effect could reflect a delay of the excitatory action of carbachol, and would imply remoteness of the action site of carbachol from the flocculus. However, injections of betanechol or isoprotenerol both increased the gain of the OKR immediately, discounting that explanation. The bimodal effect, therefore, seems specific for carbachol, and could be caused by an inhibitory action of carbachol due to an initially high concentration and/or action via different cholinergic receptors.

The mechanism of action of carbachol in the cerebellar flocculus: an hypothesis

ACh has a potent and longlasting effect on pyramidal neurons in the neocortex and in the hippocampus (Madison and Nicoll, 1984; McCormick and Prince, 1986; Halliwel, 1986, 1990). The effect consists in a positive modulation of the excitability of the postsynaptic neuron. The mechanism by which ACh exerts this positive effect is a reduction of at least two potassium currents in the postsynaptic cell: the I_M (M-current) and the I_{sAHP} (sAHP-current). The muscarinic sensitive current I_M (hence its name) is a voltage-dependent potassium current that is slowly activated by depolarization of the membrane potential, positive to -65 mV. The activation occurs in the course of tens of milliseconds. Such a potassium current would hyperpolarize the cell. The role of the I_M, therefore, can be seen as a stabilizing mechanism that opposes depolarization, controlling, in particular, the unstable membrane behavior conferred by persistent inward currents. Reduction of the size of the I_M by ACh should increase the tendency of the postsynaptic cell to fire repetitively in response to other depolarizing inputs, without producing much cell depolarization by itself. In this view, the cholinergic system would be a universal, enabling device, allowing the cells to respond more briskly to more conventional synaptic

excitation.

The other potassium current is the I_{sAHP}. The sAHP plays a major role in the slowing of the action potential discharge rate (accommodation of adaptation) that typically occurs during a long-duration depolarization (Madison and Nicoll, 1984). The sAHP is voltage insensitive. It is produced by a hyperpolarizing current, brought about by the accumulation of intracellular Ca^{2+} ions during a series of action potentials. The result of blocking of the sAHP is that many more action potentials are evoked by the same depolarizing current pulse. Thus, the effect of ACh through blockade of the I_{sAHP} is a positive modulation of ongoing activity in the postsynaptic cell.

Thus, ACh-induced reductions in I_M and I_{AHP} selectively enhance trains of EPSPs by facilitating those excitatory inputs that bring the membrane potential near firing threshold. Inhibitory PSPs or isolated EPSPs will be less affected by reductions in these currents (especially I_{AHP}).

We have, in this thesis, collected some arguments that support our hypothesis, put forward in chapter 4, that a similar mechanism is responsible for the action of carbachol in the cerebellar flocculus on the oculomotor reflexes.

In the first place, the effect is a positive modulatory one. There is ample evidence for a role of the flocculus in maintaining optimal gain levels of the OKR and, probably, the VOR in the rabbit. Whereas the direct effects of the floccular efference upon the vestibular nucleus cells are inhibitory, the net effect of the floccular involvement on the VOR and the OKR is positive. This follows, amongst others, from studies in which functional of physical ablation of the flocculus of the rabbit results in a decrease in the gain of the OKR and the VOR (Ito et al, 1982; Nagao, 1983; Van Neerven et al., 1989) or of the OKR alone (Barmack and Pettorossi, 1985). From this point of view, the positive modulatory effect of carbachol on the gain of the OKR and the VOR should be understood as an increase in signal flow through the flocculus.

Secondly, the positive modulatory effect of carbachol on the optokinetic response was found to be mediated by muscarinic type cholinergic receptors, because floccular injection of betanechol mimicked the effect of carbachol (Chapter 5). Muscarinic receptors are also involved in the modulatory effect of ACh on hippocampal neurons. Moreover, the underlying Ca^{2+} dependent K^+ currents underlying the modulatory effect were specifically affected by muscarinic and not nicotinic stimulation.

Thirdly, injection of isoproterenol, a specific β-noradrenergic agonist, mimicked the effect of carbachol (Chapter 4). The sAHP in hippocampal neurons could be blocked by muscarinic stimulation as well as β-noradrenergic receptor stimulation (Madison and Nicoll, 1986) and Nicoll (1988) proposed that this synergy is based on NA and ACh acting on the same Ca^{2+} dependent K^+ channel.

Fourthly and finally, the sAHP is generated by Ca^{2+} influx during action potentials,

and increases strongly with the number of spikes (see Nicoll, 1988). It suppresses further discharge by shunting depolarizing inputs. It is obvious that such a mechanism poses an upper limit to the number of spikes that can be generated in a period of time. Blocking of the sAHP would therefore be expected to shift the maximal firing frequency to a higher level, thereby enhancing the depth of modulation in the excitatory phase. On the other hand, the depth of modulation in the inhibitory direction will be relatively unaffected. Considering the directional specificity of floccular output, such an asymmetric effect at the cellular level of the flocculus could express itself at the behavioral level as a directionally asymmetric effect on optokinetic responses. Indeed, *Chapter 7* describes an asymmetric effect on OKN which can be explained as exclusive enhancement of the excitatory phase of firing modulation of cerebellar neurons.

The cell type involved in the action of carbachol is unknown, but a reasonable guess can surely be made. Neustadt et al. (1988) have found that the highest density of muscarinic binding-sites in the rabbit archicerebellum is present in the Purkinje-cell layer. At the cellular level, a positive modulatory effect of noradrenaline was found in the Purkinje cell that was similar as in hippocampal neurons. This suggests that the Purkinje cell membrane features a Ca^{2+} dependent K^+ channel with resulting sAHP that could be involved in the effects of carbachol. Furthermore, an effect of acetylcholine, very similar to the effect of blocking of the I_M in hippocampal cells, was encountered recently in the Purkinje-cell. These results all point to the Purkinje cell as a likely target of carbachol in the present studies.

Cerebellar cholinergic elements

Obviously, a prerequisite for a functional significance of the floccular cholinergic system is the presence of cholinergic fibers, together with cholinoceptive elements.

Extrinsic cholinergic input

ChAT is an enzyme, involved in the synthesis of ACh and is thought to be a reliable marker for cholinergic elements. Immunohistochemical studies using markers for ChAT have identified a number of ChAT-positive structures in the cerebellum. There is evidence for both an extrinsic and an intrinsic supply of ACh in the cerebellum. As an example of a system originating outside the cerebellum, some mossy fibers were found to be cholinergic in studies using ChAT markers (rat: Ojima et al, 1989; cat: Illing, 1990). More specifically, also a subset of mossy fibers projecting to the flocculus was found to express ChAT (Barmack et al., 1986). McCance and Phillis (1968) found results suggesting that a proportion of mossy fibers to the cerebellum is cholinergic, and that these fibers

originate from the pons and the midbrain. However, stimulation of various structures in the medulla failed to provide evidence for a major cholinergic input from this area in another study (Crawford et al., 1966). Similar negative results were encountered by Crepel and Dhanjal (1982).

Another extrinsic source was identified by ChAT markers. Ojima et al encountered varicose fibers just beneath and within the P-cell layer and in the lower part of the molecular layer of the cerebellar cortex. A filigree of beaded fibers was encountered in the molecular and granular layer (Illing, 1990). Despite their difference in localization, the two types of fibers described by the two studies could represent the same fiber system.

Since the filigree fibers could not be traced to the immunoreactive perikarya in the cerebellar cortex, they must be of extrinsic origin. A diffuse cholinergic projection of extrinsic origin is known to pervade various midbrain and forebrain structures (Wainer and Rye, 1984). The origin of this projection is the mesopontine brainstem, pedunculopontine tegmental nucleus, lateral dorsal tegmental nucleus. It is tempting to assume that the filigree plexus encountered in the cerebellum is part of these widespread modulatory projections.

Very recent investigations of the ChAT activity in the flocculus of rat, rabbit, cat, and monkey (Barmack et al., 1992a, b, c), however, revealed considerable species differences. Whereas ChAT activity was high in the flocculus of rat and monkey, ChAT activity was low in the rabbit flocculus. The rabbit was the only animal that lacked the filigree of small fibers adjacent to the Purkinje cell. The only ChAT positive elements in the rabbit's floccular cortex were mossy fibers, originating from the vestibular nuclei. These results argue against cholinergic transmission at the level of the Purkinje cells.

Intrinsic cholinergic input

An intrinsic cerebellar source of ACh could be the Golgi cell, as was suggested by previous histochemical studies (Kása and Silver, 1969; McCance and Phillis, 1968). This could account for the AChE activity remaining after surgical isolation of the cerebellum. Using a monoclonal antibody against ChAT, this was confirmed in one study in the cat (Illing 1990), but denied in a number of other studies in man (Karen Kan et al., 1980), rabbit (Karen Kan et al., 1978), cat (Kimura et al., 1981) and rat (Ojima et al., 1989).

Cerebellar cholinoceptive elements

Physiological studies have led to conflicting conclusions as to which neuronal types in the cerebellum are cholinoceptive. The Purkinje cell, which responds to iontophoreticallly applied ACh or its agonists, after a very slow onset, by prolonged

duration of firing, has been proposed to be cholinoceptive (Curtis and Crawford, 1965; Crawford et al., 1966). This possibility has been questioned by McCance and Phillis (68), however, because ACh fails to excite Purkinje cell dendrites. However, they did not exclude the possibility of ACh receptors on Purkinje cell somata. The findings by Ojima et al. (1989) of varicose fibers, immunoreactive to a monoclonal antibody to ChAT just beneath or in the P-cell layer, is suggestive of innervation of the P-cell somata. The autoradiographic demonstration of a high level of muscarinic ACh receptors in the Purkinje cell layer (Neustadt et al., 1988) in the rabbit, but not in the molecular layer, is consistent with this assumption.

Ojima et al. (1989) also found ChAT immunoreactivity within mossy fiber rosettes. Since granule cells and Golgi cells are known to be postsynaptic to mossy fibers (Palay and Chan-Palay, 1974) this suggests that granule cells are cholinoceptive. A number of physiological studies have indicated that granule cells are excited in response to iontophoretically applied ACh (McCance and Phillis, 68). The receptors on these cells were found to be nicotinic in type, so that our effects probably were not mediated by granule cells. However, no information concerning actions of ACh on Golgi cells is available.

Function of the floccular cholinergic system

As explained above, the effect of cholinergic stimulation in the cerebellar flocculus has many similarities to the effect of ACh applied in other parts of the brain, such as hippocampus and neocortex. Whereas some functions of cholinergic transmission have been formulated in those other parts of the brain, we do not have a single clue about the function of ACh in the cerebellum. In order to obtain a sensible explanation of the cholinergic system of flocculus in functional terms, we should consider hypotheses of functions of the cholinergic systems in neocortex and hippocampus.

The cholinergic system of the forebrain has been under intensive investigation and results emerging from these studies have led to several proposals concerning the function of this system. Since the discovery of electroencephalography (EEG) it has been known that the frequency and amplitude of electrical potentials generated by the forebrain vary with the animals's state of arousal (see Brazier, 1980). During sleep, synchronous rhythmical activity of cortical and thalamic neurons was encountered, while during periods of waking and alertness, this activity is desynchronized (Steriade and Deschênes, 1984). This is probably due to complicated processing in these structures, occurring during waking and alertness. It was found that such a desynchronization of the EEG could be elicited by stimulation in areas in the brainstem (Moruzzi and Magoun, 1949). This finding

led to the proposal that the excitability of neurons in the forebrain is controlled by an ascending activating system, originating from the brainstem.

Two systems that are involved in this ascending control are the cholinergic system, coming from the pedunculopontine and lateral dorsal tegmental nuclei in the brainstem and the basal magnocellular nuclei in the basal forebrain (see Mesulam, 1990) and the noradrenergic system, originating from the locus coeruleus (Lindvall and Björklund, 1984). These cholinergic and noradrenergic neurons discharge at a slow, regular rate during sleep, which becomes faster as the animal becomes more alert (Aston-Jones and Bloom, 1981; Lamour et al., 1986). Furthermore, the effects of stimulation in these areas on neurons in the forebrain are slow in onset, long in duration, and consist of a modulation of the excitability of the postsynaptic neuron. The mechanism of this potent modulation is explained above. The potent positive action of these systems on the excitability of forebrain, but also other, neurons, have led to implication of these systems in the regulation of a large number of behaviors and behavioral deficits, including Alzheimer's disease, epilepsy, sleep-wake cycles, and cognition.

It is not inconceivable that the flocculus, too, is under the influence of such a general arousal system. In fact, increased alertness in rabbit, monitored by EEG and induced by vibration and noise, was found to enhance the performance of the optokinetic response (Pyykkö et al., 1982). The same alerting procedures also enhanced the build-up of slow phase velocity of OKN, similarly as described in Chapter 6 and 7 (Magnusson, 1986). We are tempted to propose that the floccular cholinergic system is involved in the mediation of these effects.

Clinical implications

Motion sickness

Motion sickness has since long attracted much attention (Tayler and Bard, 1949; Money, 1970), more recently especially in relation to astronautics. Transdermal administration of the centrally active, selective muscarinic receptor antagonist scopolamine is known to be effective against motion sickness (see Brand and Perry, 1966; Wood and Graybiel, 1970; Graybiel et al., 1975; Takeda et al., 1989; Pyykkö et al., 1985a).

A possible mechanism of the development of motion sickness is the occurrence of conflicts between vestibular and visual signals (Reason, 1970; Reason and Brand, 1975; Watt, 1987). Interestingly, Shupak et al. (1990) revealed that the VOR gain is a predictive parameter for the susceptibility to motion sickness in humans: the lower the VOR gain, the less the susceptibility to motion sickness. Indeed, Pyykkö et al. (1985b) found that scopolamine administration in man lowered the gains of the VOR in darkness and of the

OKR, suggesting that the amelioration of symptoms of motion sickness by scopolamine could be based on a lowering of the sensitivity of the brain to visual and vestibular signals.

On the basis of previous findings that scopolamine causes a specific inhibition of post-synaptic potentials in the neurons of the vestibular nuclei, Pyykkö et al. (1985a, b) suggested that the effects of scopolamine occur in the vestibular nuclei. Assessment of changes in the gains of the VOR or the OKR, induced by local application of cholinergic substances into the vestibular nuclei has, to our knowledge, not yet been performed. In the light of our present results, the possibility has to be considered that the action of scopolamine could be mediated through direct actions on cerebellar circuits.

Cerebellar ataxia

Hereditary ataxia is caused by a group of degenerative cerebellar diseases of unknown pathophysiology that have in common ataxia as a prominent sign (see Refsum and Skre, 1978). The neuropathology is mainly characterized by spinocerebellar degeneration. Among the many signs of hereditary ataxia are oculomotor abnormalities such as nystagmus, impaired smooth pursuit, saccadic dysmetria and defective visual suppression of vestibular nystagmus (Zee et al., 1976). All these features could be mimicked by flocculectomy (Zee et al., 1980).

Recent studies have suggested a biochemical deficit as the basis of the ataxia. Abnormalities of pyruvate metabolism have been identified in patients with hereditary ataxias (Blass, 1979; Kark and Rodriguez-Budelli, 1979) and Friedreich's ataxia (Blass et al., 1976; Kark and Rodriguez-Budelli, 1979; Barbeau, 1982). Such a disturbance of the pyruvate metabolism interferes with the synthesis of ACh (Blass, 1979). Accordingly, clinical studies have demonstrated that systemical physostigmine, a centrally active cholinesterase inhibitor, ameliorates symptoms in patients with hereditary ataxias (Kark et al., 1977; Perlman et al., 1980).

Interestingly, physostigmine was found to counteract the disturbed visual suppression of vestibular nystagmus in ataxia patients (Tijssen et al., 1985). This suggests that a reduced synthesis of ACh, caused by a defect in the pyruvate metabolism, may result in a disturbance of visual-vestibular interaction and could account for the improvement of visual fixation by physostigmine.

The findings of the studies described in the present thesis leave open the possibility that the cerebellar cholinergic system is the target of the systemically administered physostigmine. Although the possibility of a defect of the cerebellar cholinergic system as the primary cause is very unlikely, considering the relatively weak effect of physostigmine on the ataxia, it can not be ruled out. A more likely explanation, however, could be that physostigmine causes a diffuse improvement of the impaired signal transfer through the cerebellum, by enhancing the effects of ACh in the cerebellar cortex.

Final remarks

The revelation of a robust action of carbachol in the flocculus promotes the cerebellar flocculus as a focus of future studies of the cerebellar cholinergic system. Extra-, but especially intracellular recording studies in the cerebellar flocculus are needed to expose the mechanisms of action of carbachol. Moreover, would it be interesting to elucidate the postsynaptic cholinoceptive structures in the floccular cortex of the rabbit. Studies of this kind have actually started or are about to take place. The validity of the speculative part of the present thesis will, thus, in the very near future be judged upon.

References

Aston-Jones G, Bloom FE (1981) Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. J Neurosci 1: 876-886

Barbeau (1982) Pathophysiology of Friedreich's ataxia. In: Matthews WB, Glaser GH (eds), Recent advances in Clinical Neurology, New York, Churchill Livingstone, pp 125-147

Barmack NH (1981) A comparison of the horizontal and vertical vestibulo-ocular reflexes of the rabbit. J Physiol (Lond) 314: 547-564

Barmack NH, Baughman RW, Eckstein FP (1992a) Cholinergic innervation of the cerebellum of the rat by secondary vestibular afferents. NY Acad Sci, In press

Barmack NH, Baughman RW, Eckstein FP (1992b) Cholinergic innervation of the cerebellum of rat, rabbit, cat and monkey as revealed by choline acetyltransferase activity and immunohistochemistry. J Comp Neurol, In press

Barmack NH, Baughman RW, Eckstein FP, Shojaku H (1992c) Secondary vestibular cholinergic projection to the cerebellum of rabbit and rat as revealed by choline acetyltransferase immunohistichemsitry, retrograde and orthograde tracers. J Comp Neurol, In press

Barmack NH, Baughman RW, Eckenstein FP, Westcott-Hodson M. (1986) Cholinergic projections to the cerebellum. Soc Neurosci Abstr 12: 577

Barmack NH, Pettorossi VE (1985) Effects of unilateral lesions of the flocculus on optokinetic and vestibuloocular reflexes of the rabbit. J Neurophysiol 53: 481-496

Blass JP (1979) Disorders of pyruvate metabolism. Neurology (NY) 29: 280-286

Blass JP, Kark RAP, Menon NK (1976) Low activities of the pyruvate and oxuglutarate dehydrogenase complexes in five patients with Friedreich's ataxia. New Eng J Med 295: 62-67

Brand JJ, Perry WLM (1966) Drugs used in motion sickness. Pharmacol Rev 18: 895-924

Brazier MAB (1980) Trails leading to the concept of the ascending reticular system - the state of knowledge before 1949. In: Hobson JA, Brazier MAB (eds), The Reticular Formation Revisited, New York, Raven, pp 143-153

Collewijn H (1975) Direction selective units in the rabbit's nucleus of the optic tract. Brain Res 100: 489-508

Crawford JM, Curtis DR, Voorhoeve PE, Wilson VJ (1966) Acetylcholine sensitivity of cerebellar

neurones in the cat. J Physiol (Lond) 186: 139-165
Crepel F, Dhanjal SS (1982) Cholinergic mechanisms and neurotransmission in the cerebellum of the rat. An in vitro study. Brain Res 244: 59-68
Curtis DR, Crawford JM (1965) Acetylcholine sensitivity of cerebellar neurones. Nature (Lond) 206: 516-517
Graybiel A, Wood CD, Knepton J et al (1975) Human assay of anti-motion sickness drugs. Aviat Space Environ Med 46: 1107-1118
Halliwel JV (1986) M-current in human neurons. Neurosci Lett 67: 1-6
Halliwel JV (1990) Physiological mechanisms of cholinergic action in the hippocampus. Progr Brain Res 84: 255-272.
Hoffmann KP, Schoppmann A (1975) Retinal input to direction-selective cells in the nucleus tractus opticus in the cat. Brain Res 99: 359-366
Illing RB (1990) A subtype of cerebellar Golgi cells may be cholinergic. Brain Res 522: 267-274
Ito M, Jastreboff PJ, Miyashita Y (1982) Specific effects of unilateral lesions in the flocculus upon eye movements in albino rabbits. Exp Brain Res 45: 233-242
Karen Kan KS, Chao LP, Eng LF (1978) Immunohistochemical localization of choline acetyl-transferase in rabbit spinal cord and cerebellum. Brain Res 146: 221-229
Karen Kan KS, Chao LP, Forno LS (1980) Immunohistochemical localization of choline acetyl-transferase in the human cerebellum. Brain Res 193: 165-171
Kark RAP, Blass JP, Spence MA (1977) Physostigmine in familial ataxias. Neurology (Minneap) 27: 70-72
Kark RAP, Rodriguez-Budelli M (1979) Pyruvate dehydrogenase deficiency in spinocerebellar degenerations. Neurology (Minneap) 29: 126-131
Kása P, Silver A (1969) The correlation between choline acetyltransferase and acetylcholinesterase activity in different areas of the cerebellum of rat and guinea pig. J Neurochem 16: 389-396
Kimura H, McGeer PL, Peng JH, McGeer EG (1981) The central cholinergic system studied by choline acetyltransferase immunohistochemistry in the cat. J Comp Neurol 200: 151-201
Lamour Y, Dutar P, Rascol O, Jobert A (1986) Basal forebrain neurons projecting to the rat frontoparietal cortex: electrophysiological and pharmacological properties. Brain Res 362: 122-131
Lindvall O, Björklund A (1984) Basal forebrain neurons projecting to the rat frontoparietal cortex: electrophysiological and pharmacological properties. In: Descarries I, Reader TA, Jasper HH (eds) Monoamine Innervation of Cerebral Cortex. pp 9-40, Alan R. Liss
Madison DV, Nicoll RA (1984) Control of repetitive discharge of rat CA 1 pyramidal neurons in vitro. J Physiol (Lond) 354: 319-331
Madison DV, Nicoll RA (1986) Actions of noradrenaline recorded intracellularly in rat hippocampal CA 1 neurones in vitro. J Physiol (Lond) 372: 221-244
Magnusson M (1986) Effect of alertness on the vestibulo-ocular reflex and on the slow rise in optokinetic nystagmus in rabbits. Am J Otolaryngol 7: 353-359
McCance I, Phillis JW (1968) Cholinergic mechanisms in the cerebellar cortex. Int J Neuropharmacol 7: 447-462
McCormick DA, Prince DA (1986) Mechanisms of action of acetylcholine in the guinea-pig cerebral cortex in vitro. J Physiol (Lond) 375: 169-194
Mesulam M-M (1990) Human brain cholinergic pathways. Progr Brain Res 84: 231-242
Money KE (1970) Motion sickness. Physiol Rev 50: 1-39
Moruzzi G, Magoun HW (1949) Brain stem reticular formation and activation of the EEG.

Electroencephalogr Clin Neurophysiol 1: 455-473

Nagao S (1983) Effects of vestibulocerebellar lesions upon dynamic characteristics and adaptation of vestibulo-ocular and optokinetic responses in pigmented rabbits. Exp Brain Res 53: 36-46

Neustadt A, Frostholm A, Rotter A (1988) Topographical distribution of muscarinic cholinergic receptors in the cerebellar cortex of the mouse, rat, guinea pig, and rabbit: a species comparison. J Comp Neurol 272: 317-330

Nicoll RA (1988) The coupling of neurotransmitter receptors to ion channels in the brain. Science 241: 545-551.

Ojima H, Kawajiri SI, Yamasaki T (1989) Cholinergic innervation of the rat cerebellum: qualitative and quantitative analyses of elements immunoreactive to a monoclonal antibody against choline acetyltransferase. J Comp Neurol 290: 41-52

Oyster CW (1968) The analysis of image motion by the rabbit retina. J Physiol (Lond) 199: 613-635

Palay SL, Chan-Palay V (1974) Cerebellar Cortex. New York, Springer

Perlman SL, Kark RAP, Schlecter B, Budelli MR Youkeles L (1980) Preliminary studies of oral physostigmine in the long-term management of inherited ataxias. Neurology (Minneap) 30: 381-382

Pyykkö I, Matsuoka I, Ito S, Hinoki M (1982) Enhancement of eye motor response and electrical brain activity during noise and vibration in rabbits. Otolaryngol Head Neck Surg 90: 130-138

Pyykkö I, Schalén L, Jäntti V (1985a) Transdermally administered scopolamine vs. demenhydrate. I. Effect on nausea and vertigo in experimentally induced motion sickness. Acta Otolaryngol (Stockh) 99: 588-596

Pyykkö I, Schalén L, Matsuoka I (1985b) Transdermally administered scopolamine vs. demenhydrate. II. Effect on different types of nystagmus. Acta Otolaryngol (Stockh) 99: 597-604

Reason JT (1970) Motion sickness: a special case of sensory rearrangement. Adv Sci 26: 386-393

Reason JT, Brand JJ (eds)(1975) Motion sickness. London: Academic Press

Refsum S, Skre H (1978) Neurological approaches to the inherited ataxias. In: Kark RAP, Rosenberg RN, Schut LJ (eds). Advances in Neurology 21: 1-12

Shupak A, Spitzer O, Kerem D, Mendelowitz N, Gordon C, Melamed Y (1990) Vestibulo-ocular reflex as a parameter of seasickness susceptibility. Ann Otol Rhinol Laryngol 99: 131-136

Simpson JI (1984) The accessory optic system. Ann Rev Neurosci 7: 13-41

Simpson JI, Leonard CS, Soodak RE (1988) The accessory optic system of rabbit. II. Spatial organization of direction selectivity. J Neurophysiol 60: 2055-2072

Simpson JI, Soodak RE, Hess R (1979) The accessory optic system and its relation to the vestibulocerebellum. In: Granit R, Pompeiano O (eds), Reflex Control of Posture and Movement. Progr Brain Res 50: 715-724

Steriade M, Deschênes M (1984) The thalamus as a neuronal oscillator. Brain Res Rev 8: 1-63

Takeda N, Morita M, Hasegawa S, Kubo T, Matsunaga T (1989) Neurochemical mechanisms of motion sickness. Am J Otolaryngol 10: 351-359

Tayler DB, Bard P (1949) Motion sickness. Physiol Rev 29: 311-369

Tijssen CC, Endtz LJ, Goor C (1985) The influence of physostigmine on visual-vestibular interaction in hereditary ataxias. J Neurol Neurosurg Psychiatry 48: 977-981

Van der Steen J, Collewijn H (1984) Ocular stability in the horizontal, frontal and sagittal planes in the rabbit. Exp Brain Res 56: 263-274

Van Neerven J (1990) Visuo-vestibular interactions in the rabbit: the role of the flocculus and its monoaminergic inputs. Doctoral thesis, Erasmus University Rotterdam

Van Neerven J, Pompeiano O, Collewijn H (1989) Depression of the vestibulo-ocular and optokinetic responses by intrafloccular microinjection of GABA-A and GABA-B agonists in the rabbit. Arch Ital Biol 127: 243-263

Van Neerven J, Pompeiano O, Collewijn H, Van der Steen J (1990) Injections of β-noradrenergic substances in the flocculus of rabbits affect adaptation of the VOR gain. Exp Brain Res 79: 249-260

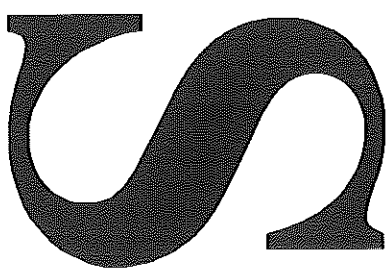
Wainer BH, Rye DB (1984) Retrograde horseradish peroxidase tracing combined with localization of choline acetlytransferase immunoreactivity. J Histochem Cytochem 32: 439-443

Watt DGD (1987) The vestibulo-ocular reflex and its possible roles in space motion sickness. Aviat Space Environ Med 58: A170-174

Wood CD, Graybiel A (1970) A theory of motion sickness based on pharmacologic reaction. Clin Pharmacol Ther 11: 621-629

Zee DS, Yee RD, Cogan DC, Robinson DA, King Engel W (1976) Ocular motor abnormalities in hereditary cerebellar ataxia. Brain 99: 207-234

Zee DS, Yamazaki A, Butler PH, Güçer G (1981) Effects of ablation of flocculus and paraflocculus on eye movements in primate. J Neurophysiol 46: 878-899



Summary
Samenvatting

Summary

Chapter 1 gives a general introduction to the significance of compensatory eye movements as a model of sensorimotor integration; the particular position of the cerebellar flocculus in the control of this behavior; and the possible role of cholinergic mechanisms in the tuning of this control, which is the main topic of this thesis.

Chapter 2 describes a study of three-dimensional eye movements in rabbits during optokinetic stimulation about axes lying in the horizontal plane or about an earth-vertical axis, with either one or both eyes viewing the stimulus. Optokinetic stimulus-speed was 2°/s, either continuous or alternating in polarity (triangular stimulus). In addition to the gains of the responses, the orientations of the response-axes, relative to the stimulus-axes, were determined. In comparison to the response to constant-speed optokinetic stimulation about the vertical axis, the response to constant speed optokinetic stimulation about any horizontal axis was characterized by the lack of a velocity build-up and the sporadic occurrence of fast phases. Slow phase tracking was good as long as the eye was within the central oculomotor range, but deteriorated as eye deviation saturated in eccentric positions. These features suggest that the optokinetic reflex about horizontal axes functions as a position-control system, rather than a velocity-control system.

Binocular optokinetic stimulation at constant-speed (2°/s) about the roll-axis (0° azimuth horizontal axis) elicited disjunctive responses. Although the gain of the response was not significantly different in the two eyes (0.38 for downward and 0.44 for upward stimulation), the response-axes of the two eyes differed by as much as 51°. Monocular horizontal-axis optokinetic stimulation at constant speed elicited responses that were grossly dissociated between the two eyes. The magnitude of the responses was anisotropic in that it varied with the azimuth-direction of the stimulus-axis; the maxima for each eye (0.44 for the seeing eye and 0.33 for the covered eye) was at 135° azimuth. The orientation and direction (sense of rotation) of the optokinetic stimulus eliciting the maximal response for each eye coincided with the optic flow normally associated with the maximal excitation of the corresponding ipsilateral anterior canal. This relation suggests that optokinetic nystagmus in response to constant-speed stimulation about horizontal axes is produced by visual interaction with exclusively the anterior canal-pathways.

Binocular, triangular optokinetic stimulation with small excursions (± 10°), which avoided the saturation problems of constant-speed stimulation, elicited adequate responses without systematic directional asymmetries. Gain was about 0.9 for all stimulus-axis orientations in the horizontal plane. During monocular stimulation with triangular stimuli, the initial response of the seeing eye showed a gain of about 0.5 for all orientations of the stimulus-axis. The covered eye showed anisotropic responses with a maximum gain of

about 0.5 during stimulation of the seeing eye about its 45° axis. In view of the arrangement of the optokinetic neural pathways in three pairs of optic flow channels, this response pattern suggests that each optic-flow channel controls primarily that eye from which it receives its dominant visual input.

The anisotropies encountered with monocular triangular stimulation are probably the behavioral consequences of an intrinsic optokinetic system that is organized in a reference frame, similar to that of the semicircular canals and extra-ocular muscles.

The following chapters describe investigations of the role of the floccular cholinergic system. In spite of a large body of histochemical evidence for a cholinergic system in the cerebellum, particularly the archicerebellum, the physiological role of this system has remained obscure. The important role of the archicerebellum, and especially the flocculus, in the control of the vestibulo-ocular (VOR) and optokinetic (OKR) reflexes provides us with a behavioral model to investigate the role of the cerebellar cholinergic system in the control of eye movements.

The first of these studies is described in *Chapter 3*, which deals with responses tot sinusoidal motion in the horizontal plane. Cholinergic stimulation by local intrafloccular injection of the aspecific cholinergic agonist carbachol increased the gain of the OKR by about 0.46 above the baseline values, while the gain of the VOR in darkness was raised by about 0.14. These effects were statistically significant and persisted for several hours. Similar, but smaller effects were obtained after injection of eserine, an inhibitor of acetylcholinesterase. Thus, the effects could be produced by increasing the naturally present amount of acetylcholine. Microinjections of the nicotinic blocker mecamylamine reduced the gain of the VOR and OKR but these effects did not reach statistical significance. The muscarinic blocker atropine significantly reduced the gain of the OKR, but not of the VOR. These results argue strongly for an important physiological role of the cholinergic system in the cerebellum.

Many behavioral and electrophysiological studies have revealed a functional relationship between acetylcholine (ACh) and noradrenaline (NA), varying from synergistic, postsynaptic action to presynaptic interaction. Whereas Chapter 3 showed that carbachol had a positive modulatory effect in the flocculus, the study described in *Chapter 4* was undertaken to compare the effects of floccular injection of carbachol, the β-adrenergic agonist isoproterenol and the conjoint injection of both of these substances. Despite a different initial response, injection of carbachol and isoproterenol both significantly raised the gain of the OKR, by 0.14 and 0.11 respectively. Neither of the two substances significantly affected the gain of the VOR in light or darkness. Conjoint

injection of the same amounts of carbachol and isoproterenol resulted in an increase in the gain of the OKR by 0.29, suggesting a potentiation between the cholinergic and the noradrenergic effects. Neither the VOR in the light, nor the VOR in darkness was affected significantly. These results suggest a synergistic and positive modulatory role of ACh and NA in the flocculus, with a positive interaction between these two systems. In analogy with comparable findings in neocortex and hippocampus, both ACh and NA might act by blocking the slow afterhyperpolarization in Purkinje cells.

Histochemical and physiological studies have identified muscarinic as well as nicotinic type cholinergic receptors in the cerebellar cortex, and both have been implicated in cholinergic transmission. The study described in *Chapter 5* was undertaken to elucidate the receptor type involved in the cholinergic enhancement of the OKR. For that purpose, effects of injections of the nicotinic agonist DMPP (n_i) were compared with injections of the muscarinic agonist betanechol. Injection of betanechol mimicked the enhancement of the OKR by carbachol, while DMPP had no effect at all. We conclude that muscarinic receptors are involved in the positive modulatory action of the cholinergic system in the cerebellar flocculus.

Chapter 6 describes optokinetic nystagmus (OKN) and optokinetic afternystagmus (OKAN), and their modulation by bilateral floccular injection of carbachol. OKN was elicited by steps in surround-velocity ranging from 5-110°/s during binocular as well as monocular viewing. In the baseline condition, OKN showed an approximately linear build-up of eye velocity to a steady-state, followed by a linear decay of eye velocity during OKAN after the lights were turned off. Build-up during binocular viewing was characterized by a constant, maximum eye-acceleration (about 1°/s^2) for stimulus velocities up to 60°/s. OKAN, instead, was characterized by a fixed duration (about 10 s) for stimulus velocities up to 20°/s. Steady-state eye velocity saturated at about 50°/s. Monocular stimulation in the preferred (nasal) direction elicited a build-up that was on average twice as slow as during binocular stimulation. Steady-state velocity during monocular stimulation saturated at about 20°/s. OKAN was of equal duration as during binocular stimulation. In the non-preferred direction, a very irregular nystagmus was elicited without velocity build-up. The stronger response to binocular stimulation, compared to the responses under monocular viewing condition in either nasal and temporal direction suggests potentiation of the signals of either eye during binocular viewing.

OKN and OKAN were re-assessed after intra-floccular micro-injection of the aselective cholinergic agonist carbachol. In the binocular viewing condition, eye-acceleration during build-up was strongly enhanced from 1°/s^2 before to 2.5°/s^2 after injection. The saturation level of steady-state eye velocity was also increased, from 50°/s

before to more than 60°/s after carbachol. The duration of OKAN, however, was shortened from 10 s before to 6 s after injection. The response to monocular stimulation in the preferred direction revealed similar changes. The response to monocular stimulation in the non-preferred direction was strongly enhanced by carbachol in 2 out of 5 rabbits. After the injection, a regular nystagmus could be elicited with a build-up to a steady state, which saturated at about 10°/s. This result suggests that the optokinetic system is potentially capable of responding adequately to temporally directed retinal slip. Apparently, this response is normally kept at a low level to prevent inappropriate optokinetic responses during forward locomotion.

In conclusion, the flocculus appears to be involved in the control of the dynamics of OKN in the rabbit. Cholinergic mechanisms affect the floccular control of the rate at which slow-phase velocity can be built up and the rate of decay of eye velocity during OKAN. Cholinergic stimulation of the flocculus thus shifts the dynamics of OKN to a more direct response, while velocity storage is shortened.

In extension to the demonstration of the enhancement of the optokinetic responses by bilateral floccular injection of the aselective cholinergic agonist carbachol, as described in Chapters 3 and 6, *Chapter 7* presents the results of investigations of the effect of unilateral injections. Unilateral injection enhanced the build-up of OKN in both eyes, elicited by binocular stimulation in the direction towards the injected flocculus (ipsiversive). Acceleration of the contralateral eye (re injected flocculus) increased from 1°/s^2 to about 2°/s^2, while the acceleration of the ipsilateral eye increased from 1°/s^2 to about 1.5°/s^2. In contrast, build-up of contraversive OKN was unchanged. No changes were found in the steady-state OKN and OKAN. Unilateral injection had a disjunctive effect on OKN, elicited by monocular stimulation in the preferred direction. Ipsiversive OKN was enhanced only in the contralateral, seeing eye, while the response of the covered eye was unchanged. We propose that the directionally specific effect of unilateral injection on OKN is due to enhancement of only the excitatory phase of modulation of the P-cell's simple-spike activity by carbachol, without an effect of carbachol on the inhibitory phase of simple-spike modulation.

It has been proposed that the same velocity storage mechanism is responsible for the elongation of the duration of post-rotatory nystagmus (PRN), relative to the duration of firing of primary vestibular fibers in response to a step in velocity and for the production of optokinetic afternystagmus. In extension to the study of OKN in Chapters 6 and 7, the study described in *Chapter 8* was undertaken to assess the effects of floccular carbachol on PRN. The results show that injection of carbachol shortens the duration of PRN from about 13 s to about 8 s, arguing for a common storage mechanism for optokinetic and vestibular signals. It is possible that the indistinguishable effect of

carbachol on OKAN and PRN is due to modification of an oculomotor corollary signal that has been identified electrophysiologically in the flocculus.

A synthesis of the various findings and their significance is given in *Chapter 9*, in which it is attempted to interpret the current findings in the broader context of the general activating actions of cholinergic brain systems, and in which possible clinical implications are discussed, particularly in relation to motion sickness and cerebellar ataxia.

A general conclusion is, that floccular visuo-oculomotor control is a fruitful model for the further neurophysiological and pharmacological study of cholinergic and other modulatory mechanisms in the brain.

Samenvatting

Hoofdstuk 1 introduceert het belang van reflectoire oogbewegingen als gedragsmodel van sensori-motore integratie en de positie die de cerebellaire flocculus, en in het bijzonder het flocculaire cholinerge systeem hierbij inneemt. Onderzocht worden effecten op de optokinetische reflex (OKR) en de vestibulo-oculaire reflex (VOR).

Hoofdstuk 2 beschrijft een studie van drie-dimensionale oogbewegingen in het konijn, opgewekt door optokinetische stimulatie rond assen in een horizontaal vlak en rond een verticale as. Van de opgewekte oogbewegingen werden gain en afwijking van de respons-as ten opzichte van de stimulus-as bepaald. De respons op optokinetische stimulatie rond horizontale assen met een constante snelheid werd gekenmerkt door afwezigheid van snelheidsopbouw van de langzame fase en het slechts sporadisch optreden van snelle fases. De volgbeweging van de ogen was adequaat zolang het oog zich in het centrale gebied van de orbita bevond maar verslechterde naarmate het oog excentrisch werd gedreven. Deze kenmerken suggereren dat de optokinetische reflex rond horizontale assen een positie stabilisatie binnen een beperkt bereik beoogt, en niet een snelheids-stabilisatie. Binoculaire optokinetische stimulatie met een constante snelheid rond een sagittale as (0° horizontale as) leidde tot disconjugate oogbewegingen. Hoewel de gain van de reacties van de twee ogen niet significant verschilde (0.38 voor opwaartse en 0.44 voor neerwaartse stimulatie) lagen de respons assen van de twee ogen maar liefst 51° uit elkaar.

Monoculaire optokinetische stimuli met constante snelheid rond horizontale assen leidde tot gedissocieerde responsen van de twee ogen. De gains van de horizontale-as responsen vertoonde een anisotrope verdeling, met een maximum gain voor elk oog (0.44 voor het ziende en 0.33 voor het afgedekte oog) bij stimulatie rond de 135° horizontale as. De oriëntatie en richting van deze optimale stimulus komt overeen met excitatie van het ipsilaterale voorste verticale halfcirkelvormige kanaal.

Het gebruik van driehoekvormige stimulatie met kleine excursies (± 10°) vermeed de saturatie problemen en leidde onder binoculaire condities tot optimale responsen (gain van 0.9) rond elke horizontale as, zonder richtingsvoorkeuren. Monoculaire driehoek stimulatie veroorzaakte in het ziende oog een respons met een gain van 0.5 voor elke oriëntatie van de stimulus as in het horizontale vlak. Het afgedekte oog vertoonde echter anisotrope responsen met een maximale gain van 0.5 tijdens stimulatie van het ziende oog rond zijn 45° horizontale as. Dit patroon suggereert, tegen de achtergrond van drie geïdentificeerde paren van kanalen van optokinetische informatie, dat elk kanaal vooral dat oog controleert waarvan het zijn dominante input ontvangt.

De anisotropiën in de monoculaire respons zijn waarschijnlijk het gevolg van een

intrinsieke organisatie van het optokinetische systeem dat lijkt op dat van de halfcirkelvormige kanalen en oogspieren.

De volgende hoofdstukken beschrijven onderzoek naar de rol van het cholinerge systeem in de flocculus. Ondanks vele histochemische bewijzen voor de aanwezigheid van een cholinerg systeem in het cerebellum, vooral in het archicerebellum, is de fysiologische rol hiervan onduidelijk. De belangrijke rol van het archicerebellum in de controle van de vestibulo-oculaire en optokinetische reflexen maakt dit systeem tot een geschikt gedragsmodel voor onderzoek van de rol van het cerebellaire cholinerge systeem.

De eerste van deze serie van studies wordt behandeld in *Hoofdstuk 3*. Cholinerge stimulatie door middel van lokale injectie van de aselectieve cholinerge agonist carbachol verhoogde de gain van de OKR ten opzichte van de baseline met 0.46, terwijl de gain van de VOR met 0.14 steeg. Beide effecten bleken statistisch significant en hielden tot enkele uren na injectie stand. Een kleinere stijging van de gains werd bereikt met injectie van eserine, een acetylcholinesterase remmer. Injectie van de nicotine blokker mecamylamine verlaagde de gains, doch niet significant. De muscarine blokker atropine verlaagde de gain van de OKR significant, maar had geen effect op de VOR. Deze resultaten pleiten sterk voor een belangrijke rol van het flocculaire cholinerge systeem.

Gedragsstudies en electrofysiologische studies hebben een functioneel verband tussen acetylcholine (ACh) en noradrenaline (NA) gevonden dat varieert van postsynaptische synergische werking tot presynaptische interactie. Waar hoofdstuk 3 aantoonde dat carbachol een sterk positief modulatoire werking had in de flocculus, wordt in *Hoofdstuk 4* een onderzoek beschreven waarin de effecten van carbachol met die van de β-adrenerge agonist isoproterenol en een combinatie van de twee farmaca worden vergeleken. Ondanks een afwijkende initiële respons zorgden zowel carbachol als isoproterenol voor een stijging van de gain van de OKR met respectievelijk 0.14 en 0.11. Geen van beide stoffen veranderde echter iets aan de gain van de VOR in het donker of licht. Carbachol en isoproterenol, gecombineerd in een oplossing, verhoogde de gain van de OKR met 0.29, hetgeen een potentiatie van de afzonderlijke effecten van de twee stoffen impliceert. De gains van de VOR in licht en donker werden geen van beide veranderd. Deze resultaten impliceren een synergistische, positieve werking van ACh en NA in de flocculus, met een positieve interactie tussen de twee. In analogie met vergelijkbare bevindingen in neocortex en hippocampus is het mogelijke mechanisme van de werking van ACh en NA in de flocculus een blokkade van de langzame na-hyperpolarisatie van de Purkinje cellen.

Histochemisch en fysiologisch onderzoek hebben zowel muscarine als nicotine receptoren in de cerebellaire cortex geidentificeerd en aan beide is een rol toebedacht in cholinerge transmissie. De studie beschreven in *Hoofdstuk 5* is ondernomen ter bepaling van het receptor-type dat betrokken is in de cholinerge versterking van de OKR. Hiertoe werden de effecten van de nicotine agonist DMPP (n_1) en de muscarine agonist betanechol vergeleken. Injectie van betanechol versterkte evenals carbachol de OKR, terwijl DMPP geen effect sorteerde. Hieruit wordt geconcludeerd dat muscarine receptoren verantwoordelijk zijn voor het positieve modulatoire effect van het cholinerge systeem in de flocculus cerebelli.

Hoofdstuk 6 behandelt optokinetische nystagmus (OKN) en optokinetische na-nystagmus (OKAN) en de modulatie hiervan door bilaterale flocculaire injectie van carbachol. OKN werd opgewekt met snelheids-stappen van een optokinetische trommel, varierend van 5 tot 110°/s, onder binoculaire en monoculaire omstandigheden. Voor de injectie toonde de OKN een ongeveer lineaire opbouw van de snelheid van de langzame fase totdat een " steady-state" werd bereikt, een maximale snelheid die werd aangehouden. Nadat de lichten waren uitgedaan volgde een lineaire afbouw van de snelheid, de optokinetische na-nystagmus. De opbouw tijdens binoculair opgewekte OKN was gekenmerkt door een constante, maximale acceleratie van 1°/s² voor stimulus snelheden tot 60°/s. De OKAN had echter een constante duur (ongeveer 10 s) voor stimulus snelheden tot 20°/s. De maximum snelheid (steady-state) was 50°/s. Monoculaire stimulatie in de voorkeursrichting (nasaal) leidde tot OKN met een snelheidsopbouw die ongeveer twee keer zo traag was. De maximale snelheid was 20°/s, maar de OKAN was van gelijke duur als by binoculaire stimulatie. In de niet-voorkeurs richting verscheen een irregulaire nystagmus, zonder snelheidsopbouw. De betere respons in de voorkeursrichting tijdens binoculaire stimulatie, vergeleken met monoculaire stimulatie, suggereert dat potentiatie optreedt van de signalen uit beide retina's tijdens binoculaire stimulatie.

OKN en OKAN werden opnieuw getest na injectie van carbachol in beide flocculi. Met binoculaire stimulatie bleek de acceleratie van de snelheidsopbouw verhoogd van 1°/s² naar 2.5°/s². De maximale snelheid was eveneens verhoogd van 50°/s tot 60°/s. De lengte van OKAN toonde een verkorting van 10 s naar 6 s. De monoculair opgewekte OKN en OKAN in de voorkeursrichting ondergingen dezelfde veranderingen. Monoculaire stimulatie in de niet-voorkeurs richting was verbeterd na injectie in 2 van de 5 geteste konijnen. In deze konijnen verscheen een regulaire nystagmus met lineaire opbouw, een maximale snelheid van 10°/s en een lineaire OKAN waar deze eerst afwezig waren. Het laatste resultaat suggereert dat een monoculaire optokinetische respons in de niet-voorkeurs richting latent aanwezig is en dat die respons expres laag gehouden wordt om inadequate responsen tijdens voorwaartse bewegingen te verhinderen.

Samenvattend blijkt de flocculus een rol te spelen in de controle van de tijdsafhankelijke respons van de OKR. Het cholinerge systeem in de flocculus lijkt de opbouw van OKN en lengte van OKAN te controleren. Cholinerge stimulatie in de flocculus veroorzaakt een versterking van de "directe" optokinetische respons, samen met een versnelling van het "velocity storage" mechanisme.

Als vervolg op het vorige hoofdstuk beschrijft *Hoofdstuk 7* het effect van unilaterale injecties van carbachol in de flocculus. Een dergelijke unilaterale injectie versterkt de opbouw van OKN in de richting van de geïnjiceerde flocculus (ipsiversief) van 1°/s² tot 2°/s², terwijl de OKN in de andere richting (contraversief) onveranderd blijft. De injecties hadden echter geen effect op de maximum snelheid en de duur van de OKAN. De respons op monoculaire stimulatie in de voorkeurs-richting vertoonde eveneens uitsluitend een versterkte opbouw indien de stimulus ipsiversief gericht was. Dit effect was echter alleen zichtbaar in het oog contralateraal van de flocculus. Het richtings-specifieke effect van de unilaterale injecties is in overeenstemming met een effect van carbachol op uitsluitend de excitatoire fase van modulatie van de "simple-spike" activiteit van de Purkinje cel.

Verondersteld wordt dat het "velocity storage" mechanisme verantwoordelijk is voor zowel OKAN als voor de verlenging van de per-rotatoire nystagmus (PRN) ten opzichte van de duur van primaire vestibulaire activiteit na rotatie met constante snelheid. *Hoofdstuk 8* rapporteert gegevens van een studie van de effecten van flocculaire injectie van carbachol op PRN. Carbachol bleek, evenals voor OKAN, ook de afbouw van PRN te versnellen. De duur van PRN verkortte van 13 s naar 8 s. Dit resultaat suggereert de aanwezigheid van een gezamenlijke "velocity storage" mechanisme voor optokinetische en vestibulaire signalen. Een mogelijk aangrijpingspunt van carbachol zou de oogbewegings "efference copy" zijn die met electrofysiologische studies in de flocculus geidentificeerd is.

Een synthese van de bevindingen wordt gevormd in *Hoofdstuk 9*, met een poging tot interpretatie van de gegevens in relatie tot de gegeneraliseerde activerende invloed van cholinerge transmissie in de hersenen. Verder worden enkele klinische implicaties besproken, specifiek met betrekking tot bewegings- of wagenziekte en cerebellaire ataxie.

Een algemene conclusie is dat de controle van de oogbewegingsreflexen door de flocculus een uitermate geschikt model lijkt voor verder neurofysiologisch en farmacologisch als ook anatomisch onderzoek naar het cerebellaire cholinerge systeem en andere algemene modulatoire mechanismen in de hersenen.

Dankwoord

Hiermee sluit ik een enerverende en uiterst leerzame periode af. Velen hebben op eigen wijze en in verschillende mate bijgedragen aan de totstandkoming van dit werk. Aan al deze mensen ben ik veel dank verschuldigd. Enkelen wil ik hier met name noemen.

Op de eerste plaats uiteraard Han Collewijn en Hans van der Steen; dit is ons proefschrift, het product van onze plezierige samenwerking. Han ben ik zeer dankbaar voor zijn nimmer aflatende vertrouwen in deze hele onderneming. Zijn kundige en intensieve begeleiding stelt zelfs de grootste chaoot in staat wetenschappelijk onderzoek te verrichten. Hans heb ik het in deze periode ook heel moeilijk gemaakt. Maar al te vaak moest hij zijn eigen bezigheden verlaten om mij voor ieder technisch en theoretisch akkefietje te hulp te schieten. Wat ik van jullie beide geleerd heb zal ik altijd meedragen, wat de toekomst ook moge brengen.

Prof. van Hof ben ik zeer erkentelijk voor zijn enthousiaste belangstelling en zijn zeer belangrijke steun achter de schermen. De andere leden van de promotiecommissie, Prof. Voogd en Prof. Saxena dank ik voor de beoordeling van het manuscript.

Suzan Markestijn dank ik voor de ontelbare klusjes die ze voor me opgeknapt heeft en Jan van der Kuil voor de vrolijke noot en zijn nauwe betrokkenheid bij mijn onderzoek. Ik ben trots dat beiden het paranimfschap op zich hebben willen nemen.

It has been a pleasure and privilege to work with Prof. Jerry Simpson. His contribution to the study described in chapter 2 has been extensive at every level and it didn't hurt that he did it all with a good sense of humor.

Ik wil ook Nico Gerrits noemen die me met zijn aanstekelijk enthousiasme voor het vak indertijd zelfs van het nut van een flocculus wist te overtuigen. En Judith van Neerven, die me in het begin alle operatie-, meet- en injectie-technieken heeft bijgebracht.

De afdeling Anatomie dank ik voor het gebruik van hun fotografische faciliteiten en voor de hulp bij de uitvoering van de histologische controle. De afdeling KNO voor gebruik van hun draaistoel en Ing. R. Maas in het bijzonder voor de technische hulp daarbij. Dr. Th. Stijnen voor statistische raad. Henk Dronk voor de verzorging van mijn konijntjes. En verder alle medewerkers van de afdeling Fysiologie I voor de fijne tijd die ik daar heb gehad.

Tot slot het leven naast het proefschrift: Continu stond een team klaar om mij na het werk op te vangen. Allereerst mijn ouders die ik voor altijd dankbaar zal zijn voor de vrijheid die ze me gegeven hebben om te doen waar ik me prettig bij voelde. Dhr. en mevr. Burghouwt dank ik voor hun oprechte belangstelling, de gezelligheid en de vele vitamientjes die voor het schrijven onontbeerlijk zijn geweest.

Maar bijzondere dank ben ik verschuldigd aan jou, Marian. Jij was en bent mijn grote steun en toeverlaat en hebt meer voor dit werk betekend dan je wel denkt. Samen zijn we er echter doorheen gekomen en samen met jou hoop ik mijn verdere leven te mogen vervolgen.

Curriculum Vitae

Hendra Tan (roepnaam Stevie) werd op 9 september 1968 te Jakarta (Indonesië) geboren. Het middelbaar onderwijs aan het Koningin Wilhelmina Lyceum te Oostburg (Zeeland) rondde hij in 1986 af met het behalen van het diploma "ongedeeld VWO". Aansluitend werd de studie geneeskunde aan de Erasmus Universiteit Rotterdam aangevangen waarvan hij het propedeuse gedeelte in 1987 behaalde (cum laude).

In het tweede jaar van zijn studie werd zijn belangstelling getrokken door de neuroanatomie en begon hij onder leiding van Dr. N.M. Gerrits op de afdeling Anatomie een studie naar "Collateralisatie van vestibulo-cerebellaire verbindingen". In het derde jaar verrichte hij daarnaast onderzoek naar "3-Dimensionale OKN in het konijn" op de afdeling Fysiologie I onder leiding van Dr. J. van der Steen, voortgezet door een studie naar "Farmacologische beinvloeding van oogbewegingsreflexen in de cerebellaire flocculus" onder leiding van Prof. Dr. H. Collewijn.

Zijn Rotterdamse bezigheden werden in 1990 voor 4 maanden onderbroken door een onderzoeks-stage in Baltimore (USA) in het kader van een studenten-uitwisseling tussen de EUR en de Johns Hopkins University. Daar onderzocht hij onder begeleiding van Dr. D.S. Zee "Context-specifieke adaptatie van de VOR bij mensen". Na terugkomst werd het onderzoek aan de afdeling Fysiologie afgerond en bewerkte hij dit proefschrift. In januari 1992 behaalde hij het doctoraal examen geneeskunde.