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

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Summary. 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 microinjection of the nonselective 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 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 enhances the dynamics of OKN, while velocity storage is shortened.

Key words: Cerebellum – Flocculus – Acetylcholine – Optokinetic – Nystagmus – Afternystagmus

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 immediate 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), a role in the cancelling of vestibular 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).

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

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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; Van der Steen et al. 1991). However, the role of the flocculus in controlling the direct and indirect components of OKN in the rabbit 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; Maioli and Precht 1984). 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 (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, a sudden initial drop in velocity occurs, followed by a gradual further decay of 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.

The cerebellum is subject to several neuromodulatory systems, such as the noradrenergic and cholinergic systems. We reviewed the evidence for a cholinergic innervation, particularly of the flocculus as a part of the archicerebellum, in a previous paper (Tan and Collewijn 1991), in which we demonstrated the enhancing effect of bilateral floccular injection of the nonselective cholinergic agonist carbachol on the optokinetic response to a sinusoidal, low velocity stimulus in the rabbit. 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. 1992).

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, USA) 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 which, in the rabbit, is characteristic for the flocculus proper (Graf et al. 1988). 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 animal was restrained in a bag and secured to a platform. The head bolts were fastened to a frame that was attached to the platform, without intruding in the animal's visual field. 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 s. 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, St. Louis, MO, USA) in 1 μl saline was injected in each flocculus. Starting 20 min after these injections, all OKR measurements were repeated. Control sessions were run with the same stimulus protocol 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 third 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, St. Louis, MO, USA) 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 proper. These findings corroborate that our drug injections were made in the flocculus proper, although they cannot exclude minor diffusion to neighboring areas, such as the ventral paraflocculus.

Results

Basic properties of optokinetic response to binocular constant-velocity stimulation

Typical baseline OKN, elicited during binocular stimulation at 5 and 10°/s, is shown in the upper parts of the 4 panels of Fig. 1, while responses to a stimulus velocity of 60°/s are shown in the upper part of Fig. 2. 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. 1–2), 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. 1–2) 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 and 10°/s OKAN lasted about 10 s (Fig. 1). After stimulation with velocities above 20°/s, the duration of OKAN increased proportionally with stimulus velocity. A secondary after-nystagmus (OKAN II) in the opposite direction of the primary OKN was encountered frequently after stimulus velocities of 60°/s (see e.g. Fig. 2, baseline CCW stimulation).

Figure 3 shows some parameters of the mean baseline responses in 5 rabbits for 5 different stimulus velocities.

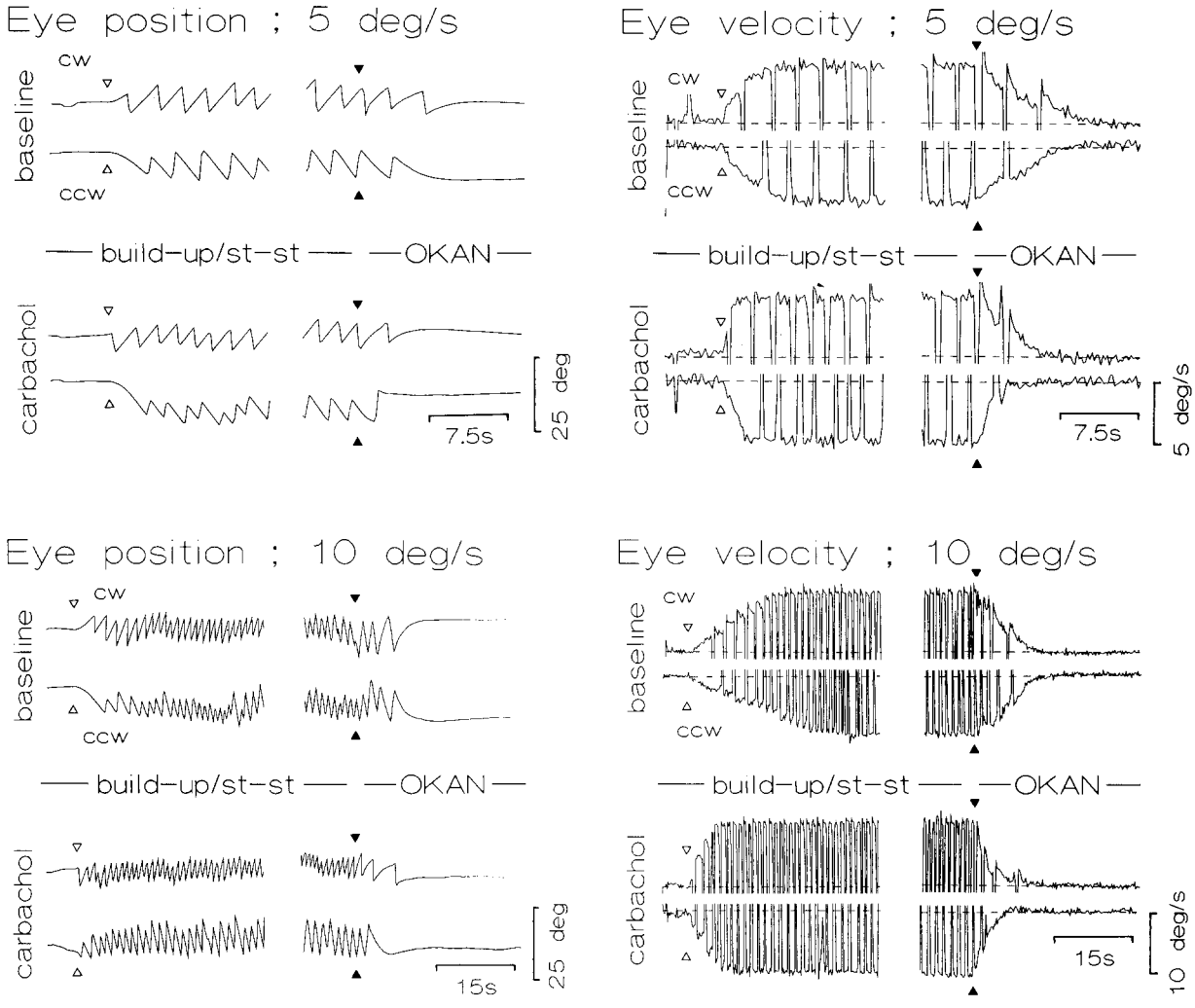

Fig. 1. OKN elicited binocularly by steps in surround-velocity of 5 and 10°/s in either direction (*CW*=right; *CCW*=left). For each stimulus velocity, eye position (*left*) and eye velocity (*right*) are shown as a function of time before and after injection of carbachol, with compressed time-scale for 10°/s stimulus velocity. *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

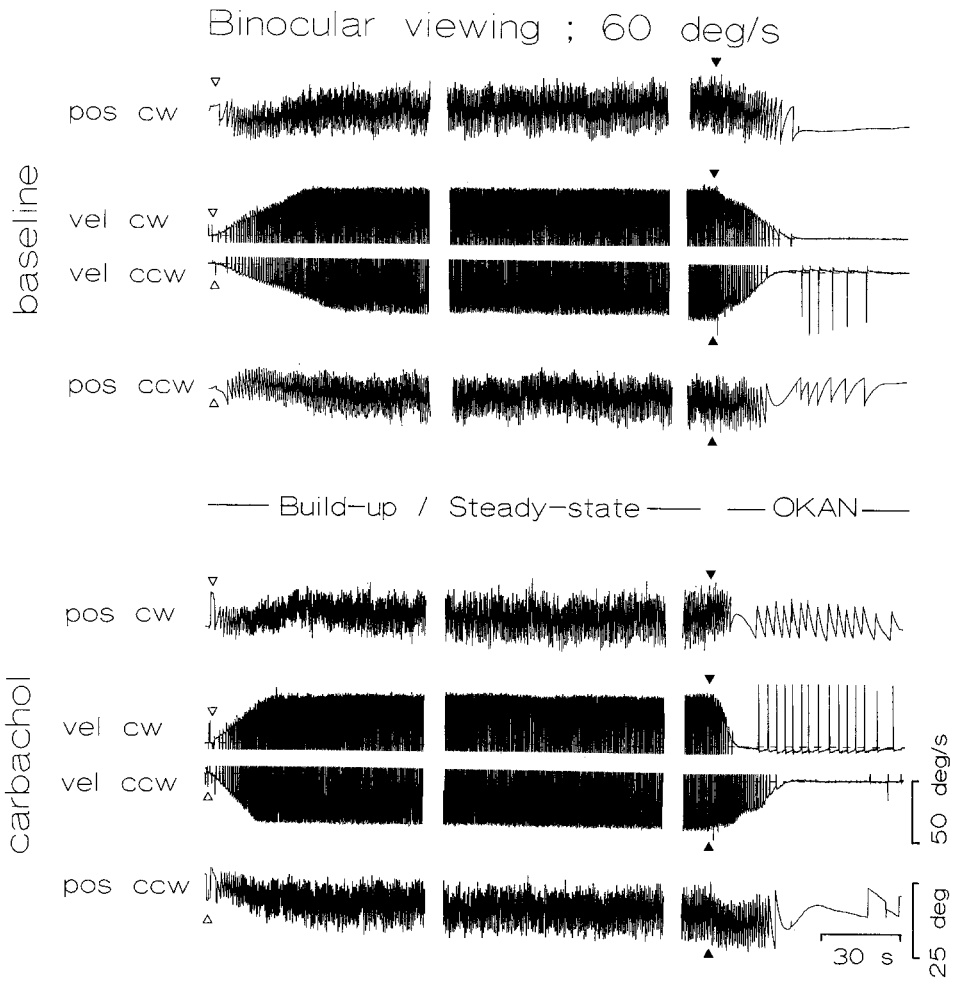

Fig. 2. Eye-position and slow phase velocity of binocularly elicited OKN as a function of time for a stimulus velocity of 60°/s in either direction (*CW* = right; *CCW* = left). *Upper panel* shows response before, lower panel after bilateral injection of carbachol. *Open triangles* indicate the start 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

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. 4, 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. 3). 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 was 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 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. 4). For stimulus velocities exceeding 20°/s, the duration of OKAN increased monotonically with increasing stimulus velocity, up to 24 s after stimulation at 110°/s.

Effect of carbachol on optokinetic responses to binocular stimulation

Injection of carbachol never led to changes in the somatic behavior of the animals. Moreover, no spontaneous nystagmus appeared, not even in darkness. The typical changes in the OKN velocity build-up and OKAN after carbachol are also illustrated in Figs. 1–2 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

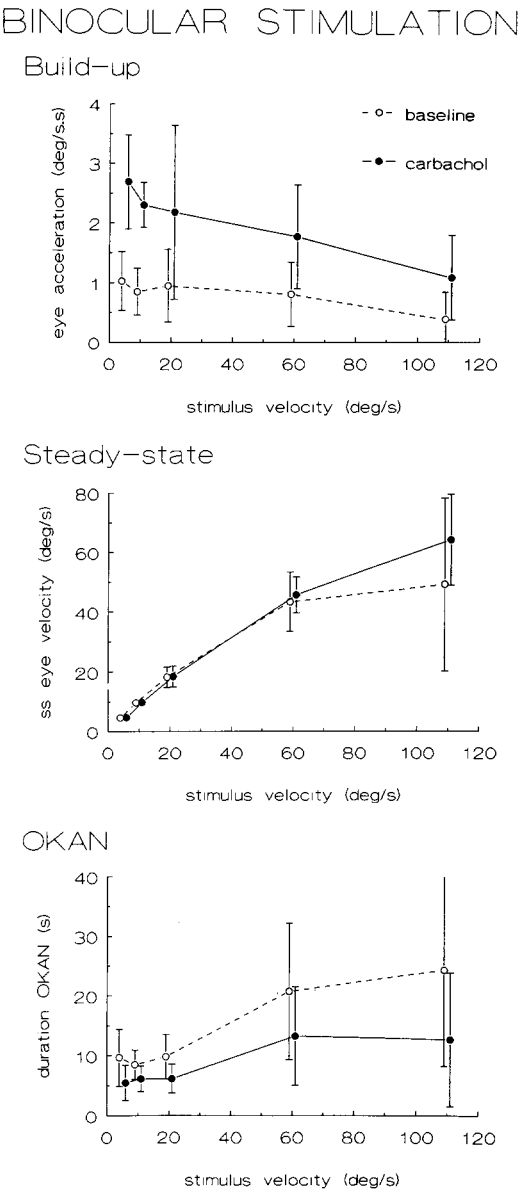

Fig. 3. Mean characteristic parameters of OKN before (*open circles, interrupted lines*) and after (*closed circles, continuous lines*) injection of carbachol as a function of stimulus velocity. Data points and error bars represent means ± standard deviation ($n = 10$). Upper panel depicts mean eye-acceleration 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

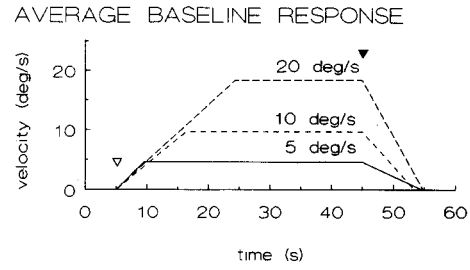

Fig. 4. Plots of average OKN velocity during build-up to steady-state and during OKAN as a function of time, based on data depicted in Fig. 4. For stimulation at 5, 10 and 20°/s, eye-acceleration during build-up has a fixed value, resulting in proportionally increasing duration of build-up with stimulus 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

was slow during optokinetic stimulation at 10°/s, especially for CCW rotation, with an acceleration of about 0.65°/s^2. 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°/s^2. 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°/s^2 before injection, taking about 36 s 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°/s^2.

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 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 60°/s in the rabbit illustrated in Fig. 2.

The mean effects of carbachol on the build-up of slow-phase velocity in the 5 rabbits tested are summarized in Fig. 3. 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. 1–2).

Steady-state velocities of OKN were not affected significantly by carbachol ($p = 0.073$; Fig. 3, 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. 3. 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

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).

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. 5.

The upper traces of each panel in Fig. 6 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 baseline values of the responses to monocular stimulation are shown in Figs. 7 and 8.

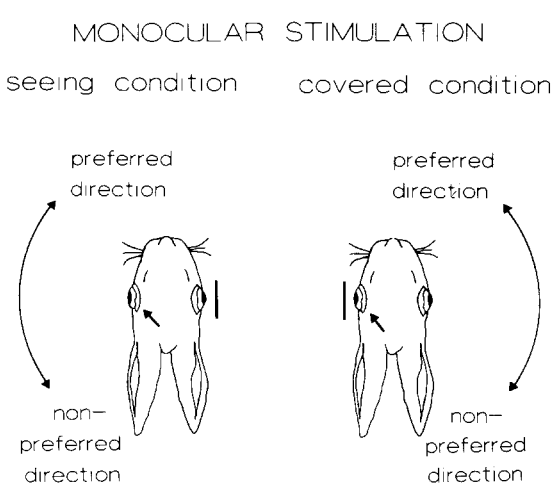

Fig. 5. Diagram explaining terminology for stimulus conditions during monocular stimulation as employed in Figs. 8–10 and in the text. Only responses of the left eye were recorded, but since monocular stimulation was delivered to left and right eye, it is possible to differentiate between seeing and 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 the concomitant responses of the viewing and the covered eye during monocular stimulation

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. 6 to Fig. 1, and Fig. 7 to Fig. 3). 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. 7 and 3) 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. 8) 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

The velocity profiles of the responses of the left eye under both the seeing as well as the covered condition are depicted in Fig. 6. 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. Due to unknown reasons, such a strong effect on the response in the non-preferred direction, however, was encountered in only 2 out of the 5 rabbits. Although this makes this finding somewhat preliminary, it should be noticed that similar vigorous responses in the non-preferred direction were never seen before the carbachol injections. Notice also that, in spite of the appearance of a regular build-up in the non-preferred direction in the example of Fig. 6, 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.

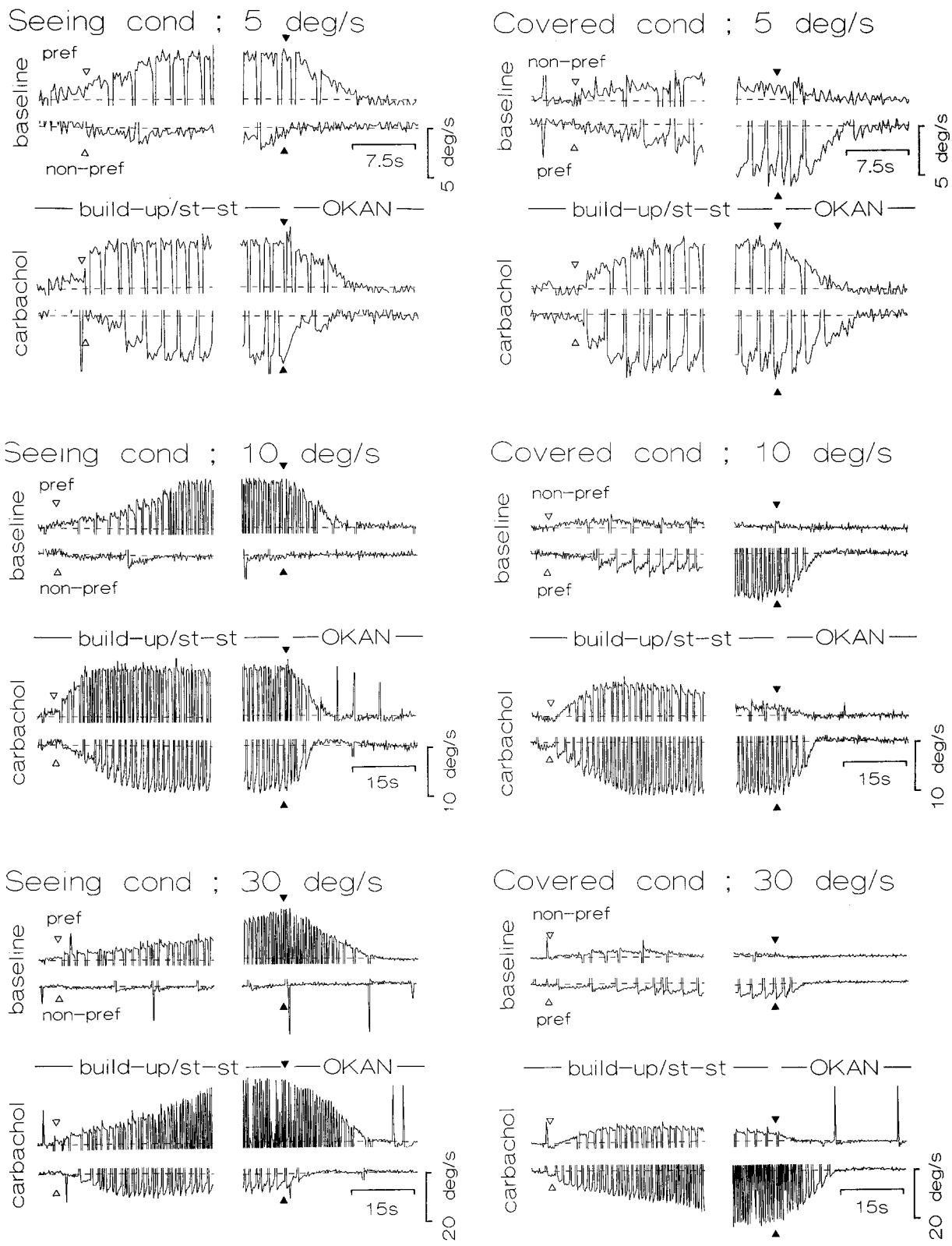

Fig. 6. OKN elicited monocularly by steps in surround-velocity of 5, 10 and 30°/s under seeing (*left panels*) and covered (*right panels*) condition in the preferred (temporal-to-nasal) and the non-preferred direction (nasal-to-temporal). Slow phase velocity is plotted as a function of time before and 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

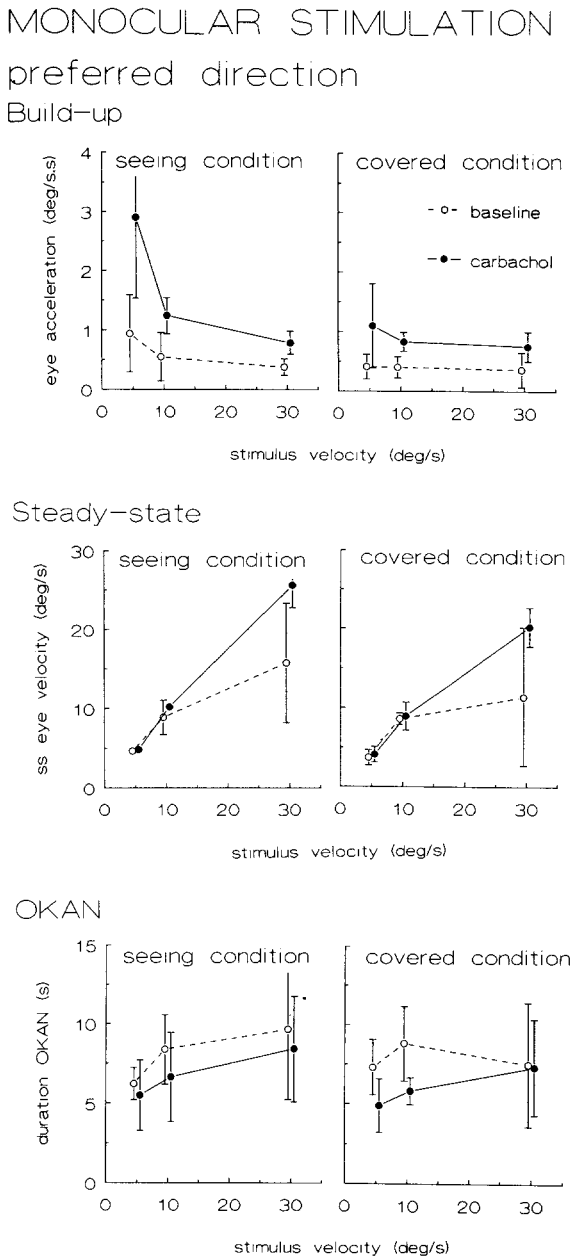

Fig. 7. Mean values of parameters of OKN and OKAN elicited by monocular stimulation in the preferred direction, before (*open circles, interrupted lines*) and after (*closed circles, continuous lines*) injection of carbachol as a function of stimulus velocity under seeing (*left panels*) and covered (*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

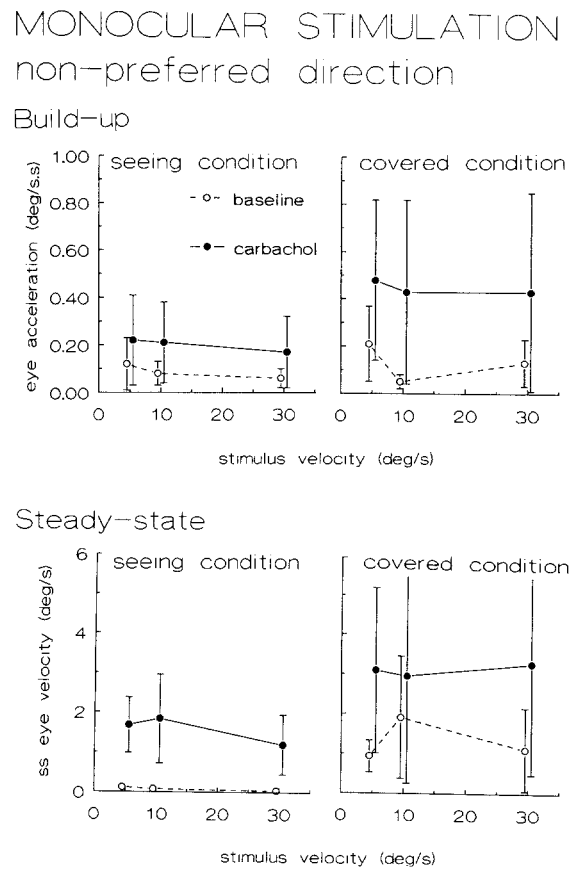

Fig. 8. Mean values of parameters of OKN elicited by monocular stimulation in the non-preferred direction, before (*open circles, interrupted lines*) and after (*closed circles, continuous lines*) injection of carbachol as a function of stimulus velocity under seeing (*left panels*) and covered (*right 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

The mean effects of carbachol on the monocularly elicited OKR in the preferred direction are shown in Fig. 7. With stimulation at 5°/s in the seeing condition, the 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. 7, lower panels) although the induced 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. 8. 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°/s) remained virtually absent (Fig. 8, 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. 6), 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 (1 μl) of the solvent (saline) only. As illustrated in Fig. 9 such injections had no effect at all on OKN, neither during build-up nor during OKAN. 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 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 ($p=0.635$).

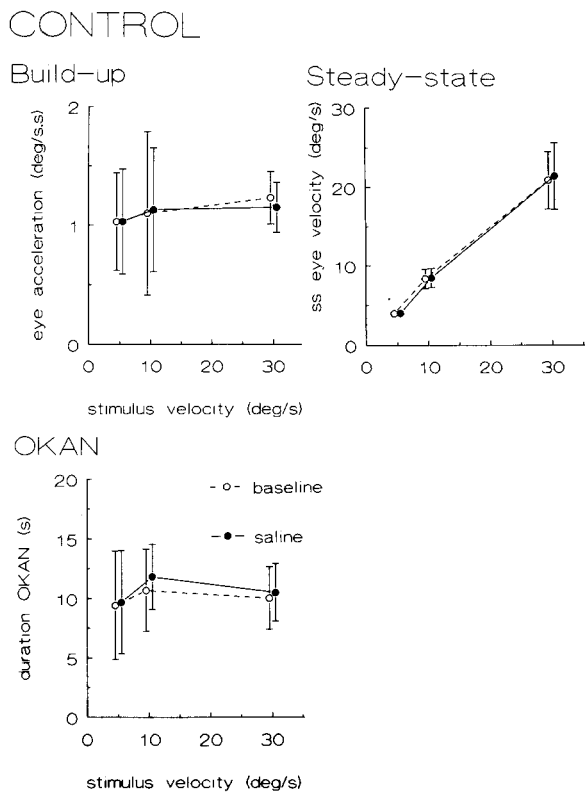

Fig. 9. Mean characteristic parameters of binocular OKN before (*open circles, interrupted lines*) and after (*closed circles, continuous lines*) injection of saline as a function of stimulus velocity. Data points and error bars represent means ± standard deviation ($n=6$). Upper panel depicts mean eye-acceleration 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

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. 1977), the present results show a typical linear time-course, in analogy with the linear time-course of OKAN decay (Collewijn et al. 1980). Collewijn (1969) found a maximal acceleration of 0.4°/s^2 for constant velocity stimulation in rabbit, whereas the average acceleration during velocity build-up encountered in the present study was 1°/s^2. 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 reflects non-linearities in the charging of the storage integrator; it may be related to 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).

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; 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 duration 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) and pigeon (Gioanni 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. 7). 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. 8 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). 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 1992), 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°/s.

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, because it is never seen in uninjected animals. 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 nucleus 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. submitted to Exp Brain Res) 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.

Mechanisms of action of carbachol

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 activity on the VOR and the OKR is positive. This is due to the polarity of the modulation of the simple spike activity of floccular Purkinje cells, as was first pointed out by Ghelarducci et al. (1975). In out-of-phase Purkinje cells, which are the dominant type in the rabbit's flocculus (Dufossé et al. 1978), simple spike activity is enhanced during contraversive head rotation. Correspondingly, floccular Purkinje cells sensitive to vertical-axis visual rotation-stimuli typically show increased simple spike activity during ipsiversive rotation of an optokinetic stimulus (Graf et al. 1988). Both of these types of sensory input are associated with contraversive head rotation in stationary surroundings, which causes inhibition of the ipsilateral medial vestibular nucleus as a result of reciprocal changes in the activities of the two horizontal canals; an effect which is reinforced by the concomitant visual input through incompletely known pathways. The result of the decreased activity in the medial vestibular nucleus is an ipsiversive, compensatory, smooth eye movement. Shifts in activity in the flocculus and medial vestibular nucleus opposite to the ones described above occur during ipsiversive head rotation. Thus, there

is a reciprocity between the upward and downward modulation of the activity of floccular Purkinje cells and the ipsilateral medial vestibular nuclear cells, to which they project, during natural, synergic vestibular and optokinetic stimulation. This reciprocity, in combination with the inhibitory nature of the flocculo-vestibular projections, results in the fact that enhancement of the natural modulation of floccular Purkinje cells enhances the modulation of vestibular nuclear neurons and, consequently, enhances the compensatory eye movements. Correspondingly, electrical microstimulation of the flocculus, simulating enhanced Purkinje cell activity, which is normally associated with contraversive head rotation, induces an ipsilateral smooth eye movement (Dufossé et al. 1977; Van der Steen et al. 1991). Furthermore, the relations described above account for the decrease in the gain of OKN after functional or physical ablation of the flocculi.

In view of the positive contribution of signal flow through the flocculus on the OKR as described above, 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; neocortex: McCormick and Prince 1986), that carbachol specifically acts by augmentation of synaptically driven responses of target neurons. 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 (see Nicoll 1988).

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