The hypothesis of the uniqueness of the oculomotor neural integrator: direct experimental evidence in the cat

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1. As far as horizontal eye movements are concerned, the well-known hypothesis, not yet experimentally proved, of the common neural integrator states that the eye-position signal is generated by a common network, regardless of the type of versional movement. The aim of this study was to evaluate the validity of this hypothesis by checking whether the sensitivity to eye position of the neurones of the nucleus prepositus hypoglossi (NPH) (the main component of the system integrating the different incoming velocity signals) would be the same regardless of the type of versional movement.

2. The discharge of sixty-five NPH neurones was recorded in the alert cat during spontaneous eye movements made in the light and in response to sinusoidal rotations of the head in complete darkness.

3. For each NPH neurone, the sensitivity to eye position was determined from measurements carried out during intersaccadic fixation. The discharge rate of the studied neurone was plotted against eye position. The slope of the resulting regression line gave the sensitivity (measured during intersaccadic fixation in the light) of the neurone to eye position, which was termed K_f.

4. A new method was developed to measure the sensitivity to eye position (K_v) of neurones during vestibular slow phases. The difficulty came from the fact that, during slow phases, eye velocity and eye position changed simultaneously and that each of those two variables could influence neuronal activity. For each neurone, the instantaneous firing rate was measured each time the eye passed through a given position during any slow phase generated during any vestibulo-ocular reflex. At a given position, the discharge rate of the neurone under study was plotted against the eye velocity. From the resulting linear regression line, two interesting values were obtained: its slope, corresponding to the sensitivity of the neurone to eye velocity, R_v, (at that given eye position) and its 'y'-intercept, $F(0)$, the interpolated firing rate when the eye velocity was zero. This procedure was repeated for different eye positions. The values of $F(0)$ were then plotted against the eye positions. The slope of the resulting regression line gave the sensitivity (measured during vestibular stimulation) of the neurone to eye position, which was termed K_v.

5. The errors on the individual values of K_f and K_v were assessed in order to allow a statistical comparison at the single unit level.

6. We found that, for each of our sixty-five neurones, the sensitivity to eye position measured during intersaccadic fixation in the light was equal to the sensitivity to eye position measured during the vestibulo-ocular reflex (VOR) elicited in complete darkness. We conclude that our results favour the hypothesis of a unique horizontal oculomotor integrator for all versional movements.

The current interest in the control of eye movements emanates from the belief that the oculomotor system is one of the simplest sensorimotor systems (Miles, 1986) and from the fact that the field has been stimulated by a number of intriguing hypotheses. Among the latter are the two hypotheses of Robinson concerning the 'oculomotor neural integrator'.

The first hypothesis postulates the existence of such an integrator (Robinson, 1968; Skavenski & Robinson, 1973; Robinson, 1975), while the second hypothesis concerns its single aspect (Robinson, 1975). Robinson recognized an apparent paradox about oculomotor function: while the motoneurones of the extraocular muscles carry both an eye-velocity and an eye-position signal (Fuchs & Luschei, 1970;

Skavenski & Robinson, 1973; Henn & Cohen, 1973), the movements are driven by command signals that encode only the velocity of the on-going movement. For example, in the vestibulo-ocular reflex (VOR), the vestibular afferents carry information only about head velocity (Melvill Jones & Milsum, 1970; Fernandez & Goldberg, 1971; Blanks, Estes & Markham, 1975), while the discharge rate of the oculomotoneurones is proportional to both eye velocity and eye position (Skavenski & Robinson, 1973; Delgado-Garcia, Del Pozo & Baker, 1986). From this fact, Robinson hypothesized that a mathematical integration was necessary to convert the velocity command signal of the VOR into a position signal, designating the hypothetical circuit the 'oculomotor neural integrator'. For spontaneous movements of the eye (consisting of rapid shifts of gaze, the saccades, separated by periods of fixation), the command originates from the so-called medium-lead burst neurones (Keller, 1974; Henn & Cohen, 1976; Kaneko, Evinger & Fuchs, 1981; Fuchs, Kaneko & Scudder, 1985), whose rate of discharge is roughly proportional to eye velocity (Van Gisbergen, Robinson & Gielen, 1981). While such velocity-encoding command signals may be sufficient to move the eye to a desired position, they cannot maintain the eye in the achieved position. Hence, Robinson proposed that the saccadic command signals also had to be integrated so that fixation of the gaze can be sustained. That first hypothesis of Robinson, concerning the existence of an oculomotor integrator, has been proved experimentally. The major part of the oculomotor neural integrator(s) has (have) been located in the nucleus prepositus hypoglossi (NPH) for horizontal movements (Cheron, Godaux, Laune & Vanderkelen, 1986*b*; Cheron & Godaux, 1987; Cannon & Robinson, 1987; Cheron, Mettens & Godaux, 1992; Mettens, Godaux, Cheron & Galiana, 1994) and in the interstitial nucleus of Cajal for vertical movements (Fukushima, 1987; Crawford, Cadera & Vilis, 1991) and for torsional movements (Crawford *et al.* 1991).

In his second hypothesis, Robinson proposed that all of the different eye-movement commands are sent, not to separate integrators (one for each particular input) (Fig. 1*A*) but to a single, common integrator (Fig. 1*B*). It is important to realize that, despite the wide acceptance of this hypothesis, there is only indirect empirical evidence to support it. Lesion studies have shown that whenever a lesion was sufficient to disrupt neural integrator function, then every type of horizontal vergence movement (saccade, VOR and optokinetic nystagmus (OKN) in both the cat and the monkey, pursuit in the monkey) displayed the characteristic abnormality expected from loss of the eye-position signal (Crawford *et al.* 1981; Cheron, Gillis & Godaux, 1986*a*; Cheron *et al.* 1986*b*; Cannon & Robinson, 1987; Cheron & Godaux, 1987; Fukushima, 1987; Mettens *et al.* 1992). In fact, the existence of a common integrator would be proved if the relation between firing rate and eye position in the neurones of the integration system did not depend on the type of velocity signal that carried the eye to that position. Restricting our attention to horizontal movements, it must be realized that it is not trivial to test the hypothesis of the common integrator by a direct experimental approach. Indeed, at least one type of horizontal eye movement, the vergence movement, does not share an integrator with the other types of movement. Mays & Porter (1984) found that the eye-position

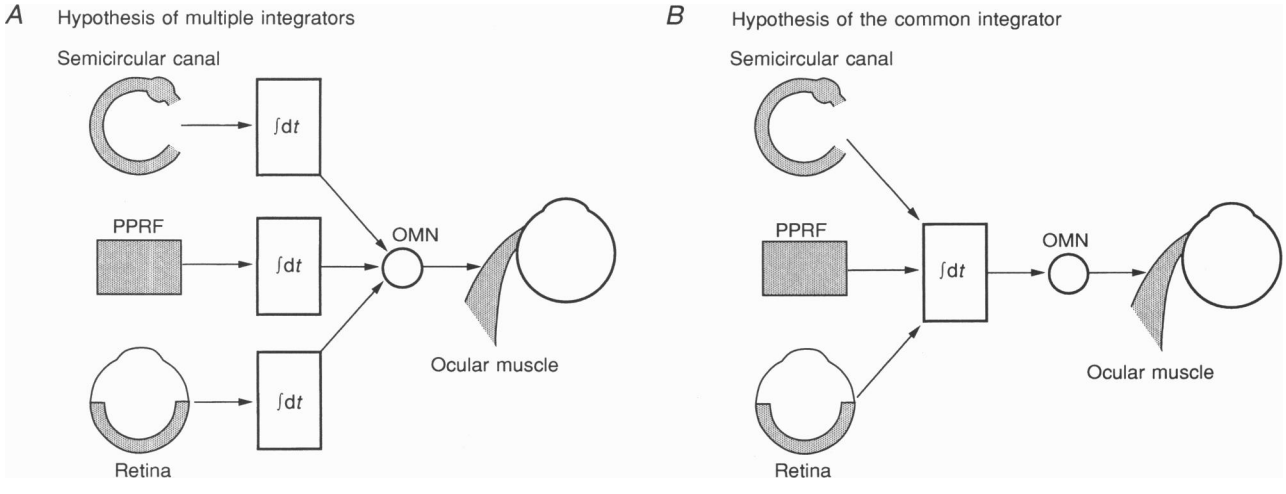

Figure 1. Diagram illustrating the multiple integrators hypothesis (*A*) and the common neural integrator hypothesis (*B*)

In the cat, versional ocular movements can be triggered, individually or in partnership, by (1) a head-velocity signal originating in the semicircular canal, (2) a signal from the saccade generator located in the pontine paramedian reticular formation (PPRF) and (3) an optokinetic signal originating in the retina. Each input might be processed by its own integrator ($\int dt$) (*A*: hypothesis of multiple integrators) or all inputs might be processed by a single, common integrator (*B*: hypothesis of the common neural integrator). OMN, oculomotoneurone.

sensitivity during vergence movements and the eye-position sensitivity during conjugate movements were not matched at the level of individual abducens neurones.

The aim of this paper was to test the common integrator hypothesis by checking whether the neurones of the major component of the horizontal oculomotor integrating system (i.e. the NPH) displayed the same sensitivity to eye position in two different versional movements: the saccades made in the light and the vestibulo-ocular reflex elicited in complete darkness.

METHODS

Surgical procedure

Experimental results were obtained from four male adult cats weighing between 2·5 and 3·5 kg. All of the experiments conformed to the recommendations of the 'Guide for the Care and Use of Laboratory Animals' (DHEW Publication, NIH85-23, 1985). The protocols were approved by the University of Mons-Hainaut Ethical Committee.

All of the cats were prepared for chronic recording of neuronal activities in the brainstem and for chronic recording of eye movements. Under general anaesthesia (3 mg kg^{-1} xylazine (Rompun) from Bayer, Germany, and 20 mg kg^{-1} pentobarbitone (Nembutal) from Ceva, Belgium) and aseptic conditions, cats were fitted with several chronic devices. Scleral search coils were implanted subconjunctivally on both eyes (Judge, Richmond & Chu, 1980). Bipolar silver stimulating electrodes were intracranially implanted on each VIth nerve at its exit from the brainstem (stereotaxic co-ordinates: L = 3·5 mm left or right, P = 1·0 mm) (Berman, 1968). The position of each electrode was adjusted to produce a lateral movement of the ipsilateral eye with a single pulse of 0·1 ms duration and less than 1 mA in intensity. This movement was checked with the aid of an operating microscope (Delgado-Garcia *et al.* 1986). Three screws were cemented to the skull to immobilize the head of the animal during the experimental sessions. A square hole (8 mm side, stereotaxic co-ordinates: L = 4 mm left to 4 mm right, P = 12–20 mm) was made in the skull. This hole allowed exploration of the rostral two-thirds of the NPH thanks to a micromanipulator tilted 30 deg posteriorly. The dura mater was removed and a dental cement chamber constructed around the hole. Between recording sessions, the surface of the cerebellum was protected with a silicone rubber sheet and the chamber sealed with bone wax. Terminal wires from eye coils and stimulating electrodes were attached to a socket cemented to the holding system. Further details of this chronic preparation have been described by Delgado-Garcia *et al.* (1986).

In the post-operative period, drops containing hydrocortisone and antibiotics (Terra-cortril, ocular and auricular suspension; Pfizer) were instilled in both eyes 3 times a day for 7 days. As the animal was placed on a stereotaxic frame during the operation, the same topical medication was also instilled in both auditory meatuses. Oxytetracycline (Hostocycline, long acting; Hoecht, Belgium) was injected intramuscularly on the operation day and during the three following days to prevent infection.

Recording of eye movements

Eye movements were measured using the scleral search coil technique (Fuchs & Robinson, 1966). The measurement system had a bandwidth of 1000 Hz and a sensitivity of 0·25 deg. Calibration was obtained by rotating both magnetic fields ±5 deg around the horizontal and vertical axes with the head of the cat kept still in space. In order to estimate the zero position of the gaze, the vertical and horizontal positions of one eye were sampled at a rate of 10 s^{-1} during spontaneous ocular movements made in the light over a period of 10 min. Zero position was obtained by computing the mean horizontal and vertical positions of the gaze (n = 6000 ocular positions). The level of alertness of the animal was continuously checked by measuring the number of spontaneous saccades over 5 min periods (Delgado-Garcia *et al.* 1986).

Recording of neuronal activities

Eight days after surgery, each animal was trained to accept restraining conditions without stress. Animals were lightly restrained by elastic bandages. After 2 weeks of post-operative recovery, recording sessions of about 3 h per day were carried out once every 2 days, for a maximum of 4 weeks. Each experimental session began by attaching the head of the animal to a holding bar located in the centre of a turntable and placed so that the horizontal semicircular canals were horizontal (nose 21 deg down). Special caution was taken to avoid any discomfort in the animals throughout the experimental sessions. The cranial opening was cleaned with sterile saline and antibiotics. Furthermore, local anaesthetics were used to irrigate the cement chamber to prevent any pain. During the experimental sessions, the animals seldom got excited. When they gave signs of restlessness they were gently caressed by the operator. More frequently, they tended to fall asleep, so that alertness had to be maintained by producing unexpected sounds.

The first recording session of neural activity was carried out in order to localize the abducens (ABD) nucleus. A glass microelectrode (1–5 MΩ impedance at 1000 Hz), attached to a micromanipulator tilted 30 deg posterior, was lowered through the central cranial opening in the direction of one ABD nucleus. The antidromic field potential, evoked by stimulation of the abducens nerve, was used to map out the location of the ABD nucleus. Once identified, the ABD nucleus was systematically used as a landmark to find the NPH. At the stimulation intensity that we used, the animal did not show any sign of discomfort.

To record neuronal activity in the brainstem, a glass micropipette was lowered through the cerebellum to reach first our landmark (the ABD nucleus) and then the NPH. The activity of each NPH neurone was recorded (1) during spontaneous eye movements in the light, and (2) during a set of sinusoidal vestibulo-ocular reflexes in complete darkness. The VOR was elicited by submitting the animal to horizontal sinusoidal rotations about the vertical axis at 0·1 Hz. Four amplitudes were used: ±10, ±20, ±30 and ±40 deg.

Data analysis: general procedure

Neuronal activity, horizontal eye position, vertical eye position and angular velocity of the turntable were analysed off-line on PC/486 clones, after storage on disk from FM tape-recordings. The horizontal and vertical eye-position signals and the table-velocity signal were sampled at 100 Hz and smoothed in three steps. The ordinate value of each point was readjusted by a least-squares fit of that point and its neighbours on a second-order polynomial (Baland *et al.* 1987). This procedure was carried out three times. Four neighbouring points were included in the first two runs, two in the third run.

The VOR induced by a sinusoidal rotation of the turntable consists of slow phases separated by quick resetting phases. The identification of the slow phases was performed automatically, using an algorithm developed by Baland, Godaux & Cheron (1987). Smoothed eye movement data were scanned for the occurrences of

sharp peaks. A sharp peak corresponds to the boundary between two successive phases. First of all, the algorithm detected critical points as points where the derivative from the left and the derivative from the right are of different signs. When both derivatives from the left and the right were nearly zero (in fact, smaller than a threshold value), the critical point was either a horizontal top or a horizontal valley, otherwise it was a sharp peak. The slow phases were identified as those corresponding (by a least-squares fit) to segments of sinusoid shifted along the ordinate axis. This procedure has been described in detail by Baland *et al.* (1987).

Unitary activity of the recorded neurones was amplified and transferred to a window discriminator. The time axis of this signal was divided into intervals 250 μs wide. During each interval, the presence or absence of an action potential was checked. For each occurrence of an action potential, the instantaneous firing rate was calculated as the inverse of the interspike interval. Finally, the instantaneous firing rate corresponding to the sampled eye signals (every 10 ms) was obtained by first-order interpolation.

Histology

After completion of recording sessions, small electrolytic lesions (0·05–0·20 mA for 15 s) were made using a tungsten microelectrode along some tracks directed to the nucleus prepositus hypoglossi (NPH). Then the animals were deeply anaesthetized with sodium pentobarbitone (45–50 mg kg^{-1}) and transcardially perfused with 0·9% saline followed by 10% formaldehyde. The anatomic location of each brainstem unit was established on 20 μm frontal sections stained with Cresyl Fast Violet by combining histological location of the electrolytical lesions and micrometer readings.

RESULTS

Location and general behaviour of the neurones

Sixty-five neurones were recorded. Their precise locations are shown in Fig. 2.

Spontaneous eye movements consisted of rapid saccades followed by fixation intervals. All the studied NPH neurones had either a burst-tonic or a tonic discharge pattern. During fixation between saccades, all the studied neurones maintained a steady firing rate that increased as the cat fixated increasingly eccentric positions in one direction (referred to as the on-direction). The on-direction was ipsilateral with respect to the recording side in forty-four neurones (out ot 65, 67·6%) and contralateral with respect to the recording side in twenty-one neurones (out of 65, 32·3%). Burst-tonic units (55 out of 65 neurones, 84·5%), in addition to modifying their discharge in relation to eye position, exhibited a burst of action potentials for saccades made in the on-direction and paused during saccades made in the opposite direction. The behaviour of such a burst-tonic unit is illustrated in Fig. 3*A*. Tonic units (10 out of 65 neurones, 15·3%) only modified their discharge in relation to eye position and exhibited neither bursts nor pauses during saccades.

When the head was rotated sinusoidally, the compensatory eye movement was primarily sinusoidal, but was interrupted repeatedly by fast movements in the direction of head rotation. The whole curve of eye movement as a function of time thus had a sawtooth appearance consisting of slow phases in the compensatory direction and quick phases in the anticompensatory direction. During the VOR, all the sixty-five NPH neurones modulated their firing rate. All the units with a burst-tonic behaviour during spontaneous eye movements showed bursts for quick phases in one direction and pauses for quick phases in the opposite direction (Fig. 3*B*). Following Duensing & Schaefer (1958), units were referred to as type I units when they increased their discharge during head rotation toward the recording side and as type II units when they increased their discharge during head rotation in the opposite direction. All the units which increased their firing rate during spontaneous movements directed toward the recording side behaved as type II units during the VOR. Conversely, all the units which increased their firing rate during spontaneous movements directed away from the recording side behaved as type I units during the VOR.

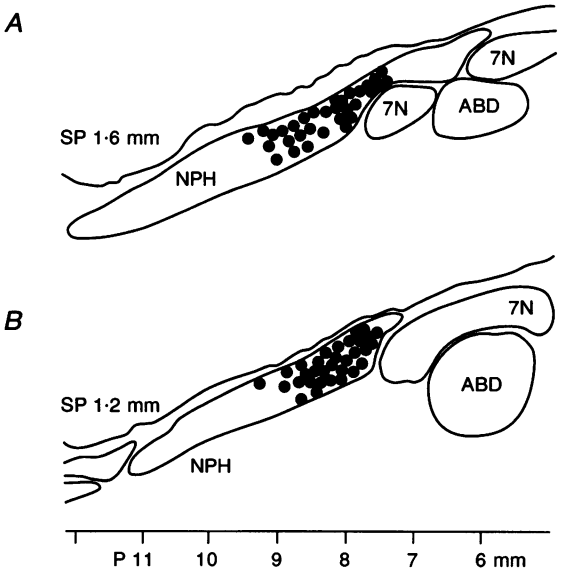

Figure 2. Parasagittal sections showing the locations of the nucleus prepositus hypoglossi (NPH) neurones

All cells located at a laterality from 1·6 to 1·4 mm and from 1·4 to 1·2 mm are plotted on parasagittal sections corresponding to a laterality of 1·6 (*A*) and 1·2 mm (*B*), respectively. Abbreviations: ABD, abducens nucleus; NPH, nucleus prepositus hypoglossi; 7N, genu of the facial nerve; SP, sagittal plane.

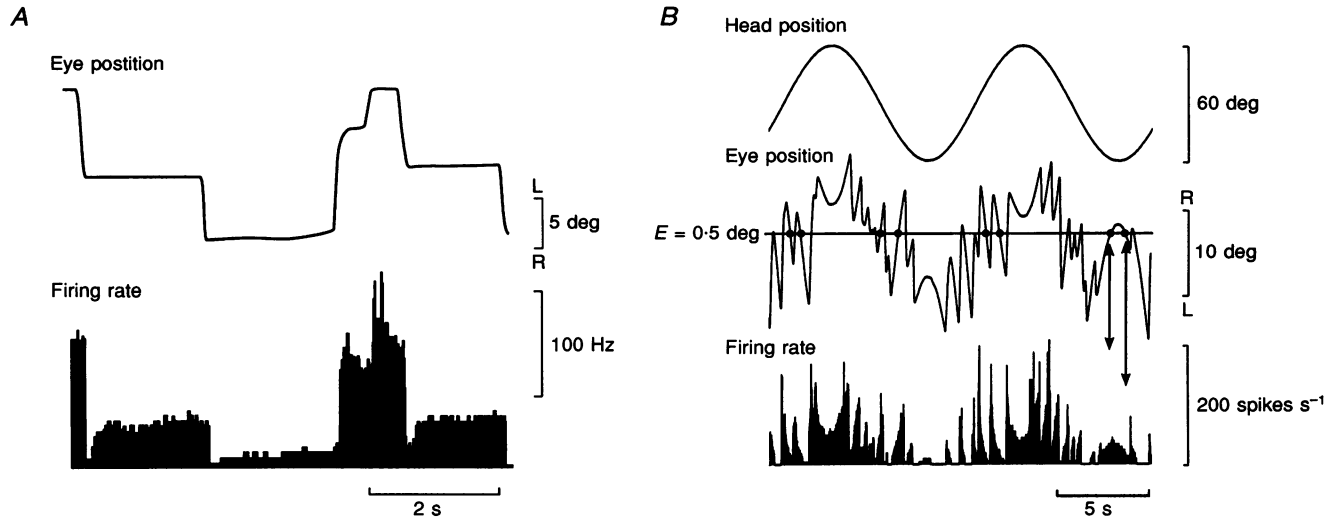

Figure 3. Activity of a burst-tonic neurone of the left NPH (unit 14 of Table 1) during spontaneous eye movements (*A*) and during the vestibulo-ocular reflex (VOR) (*B*)

A, from top to bottom: horizontal component of the eye position and firing rate. *B*, from top to bottom: horizontal head position during a sinusoidal rotation of the head (0·10 Hz, ±20 deg), horizontal eye position, and firing rate. L, leftward; R, rightward.

There were thus twenty-one type I and forty-four type II neurones.

Method for determining position sensitivity during intersaccadic fixation

The sensitivity of a neurone to the eye position achieved during intersaccadic fixation was obtained by plotting the firing rate as a function of the horizontal or the vertical components of the eye position. To determine these relations, a computer program first searched for the fixation intervals where both horizontal and vertical eye positions remained stable (eye velocity less than 2 deg s^{-1}). In order to avoid interference from some postsaccadic slide and presaccadic change in the firing rate of the neurone, the initial 300 ms and the last 50 ms portions of each fixation interval were discarded. The firing rate of the studied neurone was determined for different eye positions by averaging the instantaneous firing rates occurring during each stable intersaccadic period. For each neurone, the correlations between its firing rate and each of the two components (horizontal and vertical) of the eye position were determined. By considering that a correlation coefficient was significant when it exceeded 0·6, all the studied neurones were found to encode horizontal position. The corresponding correlation coefficients ranged from 0·75 to 0·98. For each unit, firing rate was plotted against the horizontal component of eye position during fixation (Fig. 4). A regression line was then calculated. Its slope, termed K_f (spikes s^{-1} deg^{-1}), corresponded to the sensitivity of the neurone to horizontal eye position measured during intersaccadic fixation in the light.

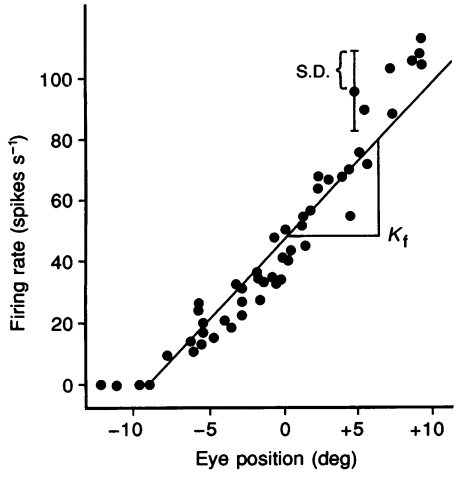

Figure 4. Relationship between firing rate and the horizontal component of eye position during intersaccadic fixation for a burst-tonic neurone of the left NPH (unit 14 of Table 1)

The slope of the regression line is 5·68 spikes s^{-1} deg^{-1} ($r = 0·95$). The standard deviation associated with each point is the mean of the standard deviations of the firing rate measured for the different analysed intersaccadic fixation periods.

Table 1. Sensitivity to eye position of the neurones of the nucleus prepositus hypoglossi

Unit	Type	K_f	K_v	T	Unit	Type	K_f	K_v	T
1	II	14·66 ± 0·71	14·74 ± 0·53	0·08	34	II	3·30 ± 0·32	4·02 ± 0·31	1·59
2	II	12·25 ± 0·48	12·38 ± 0·47	0·24	35	II	3·30 ± 0·70	3·17 ± 2·07	0·05
3	II	10·73 ± 1·31	8·10 ± 0·23	1·96	36	II	3·22 ± 0·42	3·14 ± 0·61	0·11
4	II	9·62 ± 0·82	9·55 ± 0·52	0·07	37	II	3·02 ± 0·25	3·26 ± 0·58	0·55
5	I	9·07 ± 0·29	9·02 ± 0·29	0·05	38	I	2·91 ± 0·81	2·88 ± 0·70	0·03
6	II	8·36 ± 2·56	5·03 ± 0·86	1·24	39	II	2·68 ± 0·43	2·89 ± 0·11	0·45
7	I	7·61 ± 1·20	7·34 ± 1·10	0·15	40	I	2·44 ± 0·37	3·02 ± 1·76	0·31
8	II	7·43 ± 0·77	7·54 ± 0·17	0·12	41	II	2·39 ± 0·38	3·97 ± 1·83	0·84
9	I	6·29 ± 1·39	6·40 ± 0·35	0·07	42	II	2·37 ± 0·29	2·54 ± 0·35	0·50
10	II	6·10 ± 0·46	5·05 ± 0·77	1·38	43	II	2·27 ± 0·71	2·06 ± 0·23	0·27
11	II	5·98 ± 0·49	5·13 ± 0·16	1·61	44	I	2·02 ± 0·30	2·33 ± 0·88	0·90
12	II	5·78 ± 0·35	5·80 ± 0·27	0·03	45	II	1·41 ± 0·42	1·83 ± 0·91	0·45
13	II	5·73 ± 0·29	5·75 ± 0·15	0·07	46	I	1·39 ± 0·44	2·77 ± 0·57	1·88
14	II	5·68 ± 0·57	4·94 ± 0·47	1·25	47	II	1·29 ± 0·33	1·06 ± 0·94	0·23
15	II	5·61 ± 0·49	6·08 ± 0·36	0·76	48	I	1·03 ± 1·20	0·77 ± 0·55	0·18
16	II	5·59 ± 0·76	6·04 ± 0·76	0·37	49	II	0·86 ± 0·13	0·87 ± 0·51	0·01
17	II	5·25 ± 0·59	4·50 ± 0·60	1·07	50	I	0·77 ± 0·36	0·51 ± 0·18	0·62
18	I	5·23 ± 1·20	4·02 ± 0·90	0·76	51	I	0·77 ± 0·88	0·19 ± 0·18	0·64
19	II	5·23 ± 0·84	5·04 ± 0·63	0·18	52	I	0·76 ± 0·54	0·64 ± 0·22	0·18
20	II	4·80 ± 0·71	4·97 ± 0·68	0·11	53	II	0·64 ± 0·44	1·01 ± 0·40	0·60
21	II	4·77 ± 1·08	3·90 ± 1·20	0·52	54	II	0·64 ± 0·22	0·54 ± 0·12	0·41
22	II	4·75 ± 1·01	4·29 ± 0·15	0·45	55	I	0·56 ± 0·21	0·99 ± 0·35	1·72
23	II	4·66 ± 0·40	4·62 ± 0·42	0·08	56	I	0·50 ± 0·14	0·24 ± 0·51	0·47
24	II	4·45 ± 0·46	4·53 ± 0·66	0·37	57	I	0·37 ± 0·30	0·27 ± 0·13	0·28
25	II	4·42 ± 0·64	4·97 ± 0·24	0·85	58	II	0·34 ± 0·45	0·21 ± 0·74	0·18
26	II	4·13 ± 0·95	4·01 ± 0·23	0·12	59	I	0·34 ± 0·41	0·68 ± 0·44	0·73
27	II	4·03 ± 0·68	5·07 ± 0·66	1·08	60	II	0·30 ± 0·50	0·51 ± 0·24	1·43
28	II	3·93 ± 1·37	2·69 ± 0·96	0·74	61	II	0·28 ± 0·37	0·52 ± 0·65	0·31
29	II	3·58 ± 0·71	3·54 ± 0·37	0·04	62	I	0·24 ± 0·27	0·22 ± 0·95	0·02
30	II	3·56 ± 0·28	3·56 ± 0·45	0·009	63	I	0·21 ± 0·56	0·74 ± 0·27	1·50
31	II	3·48 ± 0·44	3·17 ± 0·16	0·65	64	II	0·18 ± 0·28	0·19 ± 0·36	0·80
32	I	3·46 ± 0·50	4·96 ± 0·26	0·97	65	I	0·14 ± 0·48	0·24 ± 0·08	0·19
33	I	3·32 ± 1·10	2·46 ± 0·91	0·58					

K_f is the sensitivity to eye position measured during intersaccadic fixation (in the light). K_v is the sensitivity to eye position measured during the VOR (in complete darkness). Values for K_f and K_v (spikes s^{-1} deg^{-1}) are means ± S.D. T is a statistical variable used to establish whether K_f and K_v are statistically different with a confidence level of 0·01. T is defined by the ratio $|K_f - K_v|$ divided by $\sqrt{(\sigma^2(K_f) + \sigma^2(K_v))}$. K_f is different from K_v if $T > 2{\cdot}6$. This is the case in none of the 65 units. The NPH neurones are arranged in increasing values of K_f. Following Duensing & Schaefer (1958), units are referred to as type I units when they increase their discharge during rotation toward the recording side and as type II units when they increase their discharge during rotation in the opposite direction.

However, for statistical comparison, it was necessary to assess not only the slopes of the linear regressions, K_f, but also the associated errors in the slopes. K_f and its standard deviation were obtained by the following procedure. For any intersaccadic fixation period (excluding the first 300 ms and the last 50 ms), the firing frequency was measured each 10 ms and the mean and standard deviation of these measurements were calculated. Then the mean of the standard deviations associated with the different intersaccadic fixation periods was calculated. The result of this computation was estimated to be the mean error associated with each mean firing rate. K_f and its standard deviation, $\sigma(K_f)$, were then calculated using a linear regression analysis adapted to the case where both the following conditions are fulfilled (see Meyer, 1975, pp. 365–367). (i) For each value of the independent variable (eye position in this case), there are several measurements of the dependent variable (firing rate in this case), whose mean and standard deviation are calculated by classical formulae. (ii) For all values of the independent variable, the corresponding standard deviations of the dependent variable are equal. This procedure is displayed in Fig. 4 for NPH neurone 14. Thus, for all NPH neurones, we determined $K_f \pm \sigma(K_f)$ (Table 1). Because the on-direction was always considered as positive, all the K_f values were positive.

Method for determining position sensitivity during the VOR

During sinusoidal vestibular stimulation, the eye movements do not consist of a single slow and continuous movement, but instead, of a number of discrete continuous movements in the compensatory direction separated by rapid resetting movements in the opposite direction (Fig. 3*B*). Those anti-compensatory movements occur repeatedly and are independent of eye position, thereby causing the sinusoidal profile of the eye position recording to be shifted along the eye-position axis at recurring intervals. As a consequence, eye velocity does not covary in a fixed manner with position during the VOR: the eye may pass through any given position at different velocities. Furthermore, the utilization of four different maximal velocities of the sinusoidal vestibular stimuli promotes the occurrence of a variety of velocities at the same position. Because eye velocity and eye position change simultaneously during the VOR, and because it is known that brainstem neurones may encode either of these movement parameters, it is important to separate the component of the neural activity related to eye velocity from that related to eye position (E). This was accomplished according to a method described in detail in a previous paper (Godaux & Cheron, 1993). Briefly, an algorithm scanned all the crossing points of the slow phases through a given position (see Fig. 3*B*; points on the line $E = 0{\cdot}5$ deg). For each crossing point, the eye velocity and the corresponding instantaneous firing rate were calculated. The firing frequency was then plotted against the eye velocity (Fig. 5*A*) and the corresponding regression line was calculated excluding points where the firing rate was zero. Its slope, termed R_v (spikes s^{-1} (deg s^{-1})$^{-1}$) corresponded to the sensitivity of the neurone to eye velocity when the eye crossed a given eye position. The firing rate at zero velocity, termed $F(0)$, could be obtained by interpolation (Fig. 5*A*, ●). Similar rate–velocity regressions were computed for different eye positions over the whole range of positions achieved by the eye, at 0·5 deg intervals (Fig. 5*B*). Each rate–velocity regression was shifted along the firing-rate axis by a value depending on the eye position at which it was established. For each given position, $F(0)$ could be obtained by interpolation (Fig. 5*B*). Finally, $F(0)$ for each rate–velocity relationship was plotted against its eye position (Fig. 6). The regression line was calculated and its slope K_v (spikes s^{-1} deg^{-1}) gave the sensitivity of the neurone to eye position for movements induced by vestibular stimuli.

For statistical comparison, it was necessary to assess both the slopes of the linear regressions, K_v, and the associated errors in the slopes. As explained above, R_v gave the sensitivity of the neurone to eye velocity at a given eye position. R_v, the firing rate at zero velocity, $F(0)$, and the

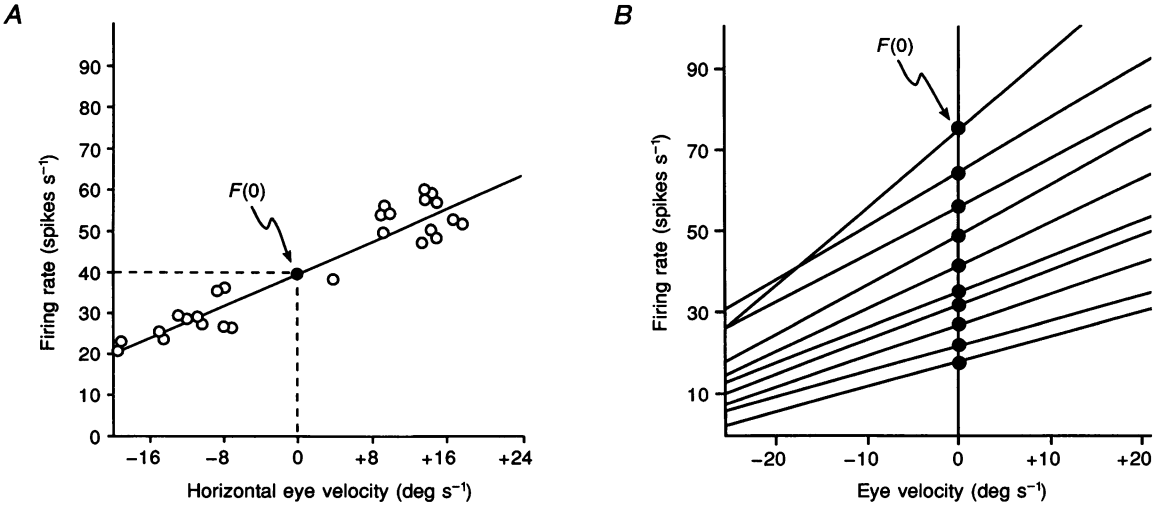

Figure 5. Illustration of the method used to establish the relation between firing rate and eye velocity when the eye passed through selected positions during the slow phases of the VOR

A, relationship between firing rate and eye velocity during the slow phases of the VOR and for a given position of the eye (0·5 deg to the right). A computer algorithm automatically selected the slow phases and scanned all the crossings of the slow phases through a chosen position (see points on the line $E = 0{\cdot}5$ deg to the right in Fig. 3*B*). For each crossing point, the instantaneous eye velocity and the related firing rate were determined. Firing rate was then plotted against eye velocity. The slope of the corresponding regression (R_v) line corresponds to the sensitivity of the neurone to horizontal head velocity (expressed in spikes s^{-1} (deg s^{-1})$^{-1}$). The y-intercept, termed $F(0)$, gives the firing rate when velocity is zero for the chosen eye position (0·5 deg to the right in this case). *B*, the linear regressions between firing rate and horizontal eye velocity were established for a set of eye positions according to the procedure displayed in *A* for one eye position. For each of these lines, $F(0)$ was calculated by interpolation.

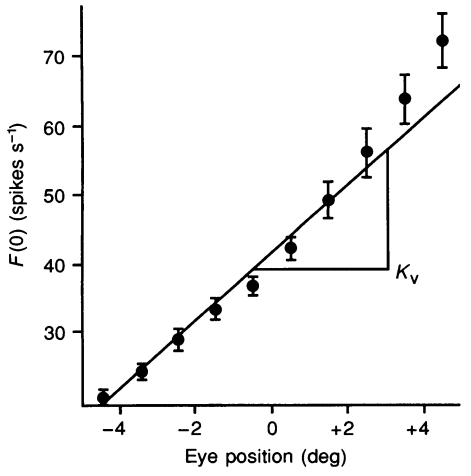

Figure 6. Method used to determine the sensitivity of a NPH neurone (unit 14 of Table 1) to eye position during the slow phases of the VOR

The firing rate at zero velocity, $F(0)$, is plotted against horizontal eye position. The data points are fitted by a linear regression line. The slope of this line, termed K_v (expressed in spikes s^{-1} deg^{-1}), corresponds to the sensitivity of the studied neurone to horizontal eye position.

associated error in $F(0)$, $\sigma(F(0))$, were obtained by using the same linear regression analysis as was used to calculate K_f and its variation. K_v was not calculated in the same way as K_f. K_v and its variation, $\sigma(K_v)$, were calculated using a linear regression analysis adapted to the case where the standard deviations of the dependent variable corresponding to the different values of the independent variable are not equal (see Meyer, 1975, pp. 365–367). This procedure is displayed in Fig. 6 for NPH neurone 14. Thus, for all neurones, we determined $K_v \pm \sigma(K_v)$ (Table 1). The direction along which firing rate increased was considered as positive. Hence, all the values of K_v were positive.

Comparison of the position sensitivities of the NPH neurones

Values of K_f and K_v are listed in Table 1. The eye-position sensitivities during intersaccadic fixation (K_f) ranged from 0·14 to 14·66 spikes s^{-1} deg^{-1}. Their mean (± S.D.) was 3·73 ± 3·11 spikes s^{-1} deg^{-1}. The eye-position sensitivities during the VOR (K_v) ranged from 0·19 to 14·74 spikes s^{-1} deg^{-1}. Their mean (± S.D.) was 3·65 ± 2·95 spikes s^{-1} deg^{-1}.

Figure 7 plots K_v values against the corresponding K_f values for the sixty-five NPH neurones. If the sensitivity to eye position for these neurones was the same during the VOR and intersaccadic fixation, K_v and K_f would be equal and the corresponding slope of the plot of K_v *versus* K_f would be 1·0. This was found to be roughly the case. Indeed, the calculated regression line was $y = 0{\cdot}91x + 0{\cdot}22$ ($r = 0{\cdot}97$), the 95% confidence interval for the slope of that regression line being 0·91 ± 0·06.

In order to determine further whether there was a difference between the sensitivity of NPH neurones to eye position during intersaccadic fixation and during the VOR, the values of K_f and K_v were compared at the individual level. For each neurone, the K_f value and its associated error ($K_f \pm \sigma(K_f)$) was compared with the K_v value and its associated error ($K_v \pm \sigma(K_v)$) by a test of comparison of two Gaussian populations. Since the worst risk in the statistical decision was to decide that a K_f value was different from the related K_v value, the chosen confidence level was 0·01. The statistical variable used to establish whether K_f and K_v were statistically different was the T

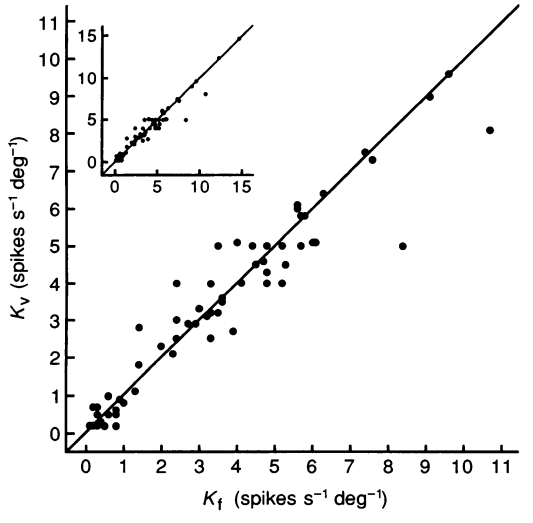

Figure 7. Relationship between K_v and K_f

Measurements were made on 65 neurones of the nucleus prepositus hypoglossi (NPH). The inset plots the same data but at a larger scale to show the two neurones that had K_f values larger than 11 spikes s^{-1} deg^{-1}.

variable defined by the ratio $|K_f - K_v|$ divided by $\sqrt{(\sigma^2(K_f) + \sigma^2(K_v))}$. K_f is different from K_v at the confidence level of 0·01 if $T > 2{\cdot}6$. The individual values of K_f and K_v were not statistically different for any of the sixty-five studied neurones ($P > 0{\cdot}01$). Thus for the NPH neurones, the sensitivity to eye position measured during intersaccadic fixation in the light is equal to the sensitivity to eye position during the VOR elicited in complete darkness.

DISCUSSION

Eye movements are triggered by various neurones that encode only velocity signals (Robinson, 1968). Saccadic burst cells of the paramedian pontine reticular formation discharge at rates that reflect saccadic eye velocity (Van Gisbergen *et al.* 1981). Vestibular afferents carry information on head velocity (Fernandez & Goldberg, 1971; Blanks *et al.* 1975). Cells within the visual cortex and brainstem nuclei encode retinal-error velocity signals (Collewijn, 1975; Hoffmann & Schoppmann, 1975; Komatsu & Wurtz, 1988). Alone, these signals would move the eye but would not allow the eye to hold the achieved position. For this reason, Robinson proposed that a mathematical integration was necessary to convert velocity signals into position signals (Robinson, 1968; Skavinski & Robinson, 1973). The question posed in this paper was whether integration from separate inputs is performed by separate integrators (Fig. 1*A*) or by a single, common integrator (Fig. 1*B*).

The chosen target structure

In the present paper, we focused on the NPH. This nucleus was first suggested by Baker, Evinger & McCrea (1981) to be the locus of the oculomotor integrator(s). Lesion experiments have demonstrated that the main 'processor' of the neural integrator was indeed in the NPH, although the latter needed the collaboration of the flocculus in order to work properly (Robinson, 1974; Godaux & Vanderkelen, 1984). Following lesions experimentally induced in the NPH, the cat was no longer able to maintain an eccentric position in its gaze. After each saccade, a centripetal exponential drift with a time constant of about 0·16 s was observed (Cheron *et al.* 1986*b*; Cheron & Godaux, 1987; Cheron *et al.* 1992; Mettens *et al.* 1994). After cerebellectomy, the cat could not maintain its eyes in an eccentric position either. However, the eyes drifted back toward the primary position with a longer time constant (about 1·4 s) than after a NPH lesion (Robinson, 1974; Godaux & Vanderkelen, 1984). In addition to a gaze-holding failure, lesions of the NPH (Cheron *et al.* 1986*b*), and its inactivation by muscimol microinjections (Mettens *et al.* 1994), also induced VOR abnormalities consistent with a loss of the eye-position signal. This favours the hypothesis of a common neural integrator, since a brain lesion inducing abnormality of one ocular subsystem while sparing another subsystem would have ruled out the existence of such a common integrator. However, the converse is not true. Even if NPH lesions caused total horizontal integrator loss in all versional oculomotor subsystems, there might still be separate, independent integrators for the different systems, all located within the NPH. The neurones making up these integrators would not even need to be segregated, but might be thoroughly intermingled. Conventional lesion studies are thus not sufficient to test the hypothesis of a common integrator. Studies using cell recordings are needed. In this study, we focused on the neuronal discharges of the NPH. However, recent evidence has suggested that the central part of the medial vestibular nucleus also plays a important role in the integration process (McFarland & Fuchs, 1992; Mettens *et al.* 1994). Therefore, this structure should be explored in the same way as the NPH in the future.

The used methods and strategy

In order to assess both the sensitivity to eye position and the sensitivity to eye velocity of a neurone in circumstances where eye velocity and eye position covary, the multivariate regression analysis (with two variables) is generally used. Actually, the method used here is basically similar to that multivariate regression analysis. The only difference between these two methods stems from the fact that the eye-position and the eye-velocity sensitivities are calculated simultaneously with the multiple regression analysis while they are calculated sequentially with our method. In reality, both methods assume that the system is linear and do not take into account either hysteresis or sensitivities to variables other than eye position and eye velocity. Nevertheless, disagreements between the hypothesized linear behaviour of the system and its actual behaviour can be detected by a bad fit in the multivariate regression and by the lack of parallelism between the firing rate–eye velocity relationships in our method. In fact, the method used here has been specifically developed in order to test the uniqueness of the oculomotor neural integrator (see Godaux & Cheron, 1993). Indeed, during postsaccadic fixation, the sensitivity to eye position is measured while eye velocity is null. For comparison purposes, it is thus more suitable to measure the sensitivity of the same neurone to eye position during the VOR only in cases where eye velocity is null. Our method allows us to measure K_v at any chosen eye velocity and hence at zero velocity. This is the major advantage of the method and is crucial for our purpose.

Versional ocular movements can be triggered by the four following command signals, individually or in partnership: (1) a signal from the saccade generator (Van Gisbergen *et al.* 1981), (2) a vestibular signal (Fernandez & Goldberg, 1971), (3) an optokinetic signal (Collewijn, 1975; Hoffmann & Schopmann, 1975) and (4) a pursuit signal (Lisberger, Lisberger & Westbrook, 1985; Morris & Tychsen, 1987; Komatsu & Wurtz, 1988). In the cat, only the first three command signals appear to act effectively (Evinger & Fuchs, 1978). Any test of the common integrator hypothesis

requires the comparison of two ocular movements that are not triggered, even in part, by a common signal. For instance, comparison of the behaviour of a neurone during saccades made in the light and during vestibulo-ocular reflex elicited in the light, would be inappropriate. In our study, we compared the behaviour of the NPH neurones when integration was elicited either by saccades in the light or by the vestibulo-ocular reflex in darkness. We recorded saccades in the light and not in darkness because alertness, which strongly influences the sensitivity of neurones to eye position (Delgado *et al.* 1986), was difficult to maintain in the cat held still in complete darkness.

Criterion for proving that the integrator is common

In the Introduction, we have stated that the existence of a common integrator would be proved if the relation between firing rate and eye position in the neurones of the integration system did not depend on the type of velocity signal that carried the eye to that position. In our protocol, that condition would be fulfilled if the K_f and K_v values of the neurones were equal. However, it remains to be decided whether the proof of the existence of a common integrator requires this criterion to be fulfilled necessarily by all the neurones involved in the integration process.

The integrator might be a non-homogeneous pool of neurones, from which the total output could be an eye position command that is indifferent to the source of the velocity input. In this case, the hypothesis of the common integrator would be proved if the K_f and K_v values were equal for the neurones sending the output signal of the integrator.

With this view in mind, we previously focused our attention on one of the main targets of the horizontal neural integrator: the abducens motoneurones (Godaux & Cheron, 1993). We found that, for each of the abducens motoneurones, the sensitivity to eye position measured during intersaccadic fixation in the light (K_f) was equal to the sensitivity to eye position, measured during the vestibulo-ocular reflex elicited in complete darkness (K_v). This result was consistent with the hypothesis of a common oculomotor integrator, but did not prove it since the abducens motoneurones could receive by coincidence equal inputs from separate integrators. However, it is worth emphasizing that the converse result would have ruled out the hypothesis of the common oculomotor integrator since the abducens motoneurones receive the output signal(s) of the integrator(s) (Escudero, De la Cruz & Delgardo-Garcia, 1992).

The major finding from the present study is that the eye-position sensitivity of the NPH neurones is the same during intersaccadic fixation (in the light) and during the slow phase of vestibular nystagmus (in complete darkness). As pointed out above, if the integrator were a non-homogeneous pool, cell recordings could reveal individual neurones with unequal K_f and K_v values. Actually, the proof of the existence of a common integrator only required that K_v and K_f values of the output neurones of the NPH were equal. Since all the NPH neurones studied had equal K_f and K_v values, the subpopulation of the output neurones also had equal K_f and K_v values.

We conclude that our results favour the hypothesis of a unique horizontal oculomotor integrator for all versional movements. Moreover, the present finding that all the individual cells in the NPH have equal K_f and K_v values actually supports a more extreme thesis: not only is there a common integrator, but the common processing extends down to all the single cells involved. The neural network of the oculomotor integrator is wired in such a way that, once a neurone integrates an input signal for a particular oculomotor subsystem, the resulting eye-position signal is immediately available for all the oculomotor subsystems.

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