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# CONTRIBUTION OF CEREBELLAR INTRACORTICAL INHIBITION TO PURKINJE CELL RESPONSES DURING VESTIBULO-OCULAR REFLEX OF ALERT RABBITS

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SUMMARY

1. Ionophoretic application of bicuculline, an antagonist of $\gamma$-aminobutyric acid (GABA), was used to examine the contribution of intracortical inhibition to vestibular responses of Purkinje cells in the cerebellar flocculus of alert rabbits.

2. Purkinje cells were sampled extracellularly (with triple-barrelled micropipettes) from the floccular area where electrical stimulation through the micro-electrode evoked abduction of the ipsilateral eye, indicating its close functional relationship to the horizontal vestibulo-ocular reflex. These cells exhibited frequency modulation of *simple* spike discharges in-phase or out-phase with sinusoidal head rotation (0·5 cycles/s, 5° peak-to-peak) in the horizontal plane.

3. Bicuculline was ejected ionophoretically through one barrel with a 20–60 nA current. The pharmacological effectiveness of the ejected bicuculline was confirmed for each Purkinje cell by its blocking action upon the depressant action of GABA applied ionophoretically through another barrel.

4. Bicuculline usually shifted the *simple* spike modulation in the in-phase direction: it reduced the amplitude of out-phase modulation in three cells, converted out-phase modulation to the in-phase type in four cells, and increased in-phase modulation in five cells. In three other cells, however, bicuculline shifted the modulation in the out-phase direction.

5. Because bicuculline application usually increased the resting discharge level of a Purkinje cell, ionophoretic application of DL-homocysteate was used in ten Purkinje cells to control for the effect of a generalized increase in excitability. In contrast to bicuculline, DL-homocysteate generally induced a slight increase of the *simple* spike modulation regardless of the phase relationship.

6. Since frequency modulation of the *simple* spike discharges of flocculus Purkinje cells is presumed to contribute to the control of vestibulo-ocular reflexes, these results point to an important functional role of intracortical post-synaptic inhibition in the cerebellar cortex.

## INTRODUCTION

The major pathway for signal flow through the cerebellar cortex is formed from serial, excitatory connexions from mossy fibres to granule cells to Purkinje cells. However, three types of inhibitory neurones can affect this basic circuit (Eccles, Ito & Szentágothai, 1967). Basket cells and superficial stellate cells are activated by granule cell impulses and in turn inhibit Purkinje cells; Golgi cells are activated by impulses of both mossy fibres and granule cells and in turn inhibit granule cells. Purkinje cell axon collaterals add another inhibitory element to the cerebellar circuitry. It is natural to suppose that these inhibitory neurones play an important role in determining the response patterns of Purkinje cells. Up to now, however, this supposition has not been substantiated experimentally in alert animals under natural, behavioural conditions.

In the cerebellar flocculus, Purkinje cells respond to sinusoidal head rotation in the horizontal plane with a frequency modulation of *simple* spike discharges at the same frequency as the head rotation (Ghelarducci, Ito & Yagi, 1975; Lisberger & Fuchs, 1978). The phase relationship between the modulation and the head velocity varies widely. However, when Purkinje cells are sampled selectively from a region of rabbit flocculus where local stimulation evokes horizontal eye movements, the major response type is out-phase, and a minor response type is in-phase (Dufossé, Ito, Jastreboff & Miyashita, 1978). These response patterns of flocculus Purkinje cells are presumed to be formed during passage of vestibular mossy fibre impulses through the cerebellar cortical circuitry.

This paper reports the effect of bicuculline, a specific antagonist of $\gamma$-aminobutyric acid (GABA) (Curtis, Duggan, Felix, Johnston & McLennan, 1971), on the response patterns of flocculus Purkinje cells in alert rabbits. Since bicuculline blocks the basket cell inhibition of Purkinje cells (Curtis & Felix, 1971), effects arising from ionophoretic application of bicuculline to Purkinje cells would be indicative of a possible contribution of the basket cell inhibition to behaviour of Purkinje cells. Flocculus Purkinje cells are connected with relay cells of the vestibulo-ocular reflex (v.o.r.) in the vestibular nuclei (Fukuda, Highstein & Ito, 1972; Baker, Precht & Llinás, 1972; Dufossé, Ito & Miyashita, 1977) and are presumed to be involved in an adaptive control of the v.o.r. (Ito, 1972; Robinson, 1976; Ito, Jastreboff & Miyashita, 1982). Thus, the present results point to a role played by the cerebellar intracortical inhibition in the control of eye movements.

A preliminary report has been published (Miyashita & Nagao, 1982).

## METHODS

### *Materials*

Eleven adult albino rabbits (3–4 kg body weight) were used. The method of single unit recording from alert rabbits through painless immobilization of the head has been described (Ghelarducci *et al.* 1975). In brief, before the operation, the animals were trained to be gently held in a rubbered brass cylinder; most rabbits quickly grew accustomed to the experimental environment so that a provisional eye movement test could select those which showed a relatively large gain of the horizontal v.o.r. (0·3–0·6, Ito, Jastreboff & Miyashita, 1982). Under full general anaesthesia with sodium pentobarbitone (40–60 mg/kg I.V., Mintal, Tanabe), the recording chamber for a hydraulic

micro-drive (MO-95, Narishige) was attached to the skull over the cerebellar hemisphere. A small craniotomy of about 3 mm diameter was made and plugged with a sterile paraffin wax. Three bolts were implanted on the skull with dental cement for fixation of the head on a turntable.

Not earlier than the fourth post-operative day, the rabbits were placed on the horizontal turntable with the trunk restrained loosely in a plastic cylinder, where their limbs were free to move. This system resulted in complete immobilization of the head without any painful pressure and permitted micro-electrodes to be driven stereotaxically. In recording sessions which normally lasted for 3–4 h, the animals remained calm and showed no signs of stress or struggling. After each session the animals were returned to their home cages and kept there for at least 2–4 days. Recordings were made several times from each rabbit and none showed any signs of unwillingness to enter the next recording session.

*Electrodes and drugs*

Triple-barrelled glass micropipettes with tip diameters from 2 to 6 $\mu$m were used. The recording barrel contained 2 M-NaCl solution, and the electrical resistance was 2–5 M$\Omega$. In some experiments the NaCl solution was saturated with Fast Green FCF for marking the recording sites. The other two barrels contained (1) 0·5 M-GABA (Sigma Chemical Co.) in aqueous solution (pH 3, adjusted with HCl), (2) 5 mM-bicuculline methiodide (Pierce Chemical Co.) in 165 mM-NaCl solution (pH 3, adjusted with HCl), or (3) 0·2 M-DL-homocysteic acid (Sigma Chemical Co.) in aqueous solution (pH 7·5, adjusted with NaOH). In some control experiments the GABA solution was replaced with HCl solution (pH 3). The retaining and ejecting currents used for controlling the release of the drugs were (1) $-20$ to $-50$ nA (retaining) and 0 to $+30$ nA (ejecting) for GABA, (2) $-20$ to $-50$ nA (retaining) and $+20$ to $+60$ nA (ejecting) for bicuculline, and (3) $+20$ to $+30$ nA (retaining) and 0 to $-20$ nA (ejecting) for DL-homocysteate ($+$, tip positive; $-$, tip negative).

*Stimulation and recording*

The methods used for rotating the whole body of the animal and for measuring the evoked eye movements with a television eye tracking system were described previously (Batini, Ito, Kado, Jastreboff & Miyashita, 1979). The horizontal turntable was rotated sinusoidally by 5° (peak-to-peak) at 0·5 cycles/s, in total darkness.

The micro-electrode was inserted dorso-ventrally into the flocculus through the cerebellar hemisphere. Purkinje cells were identified by the presence of both *simple* and *complex* spikes in spontaneous discharges (Thach, 1968). *Simple* and *complex* spikes were examined separately by means of an electronic slicer device.

After recording from each Purkinje cell, electrical pulse trains (frequency 500 cycles/s, pulse width 0·2 ms, duration 1 s, current intensity 5–40 $\mu$A) were applied through the micro-electrode. When this local stimulation evoked abduction of the eye ipsilateral to the stimulated flocculus, the site was defined as within the horizontal eye movement area and was assumed to be related to the horizontal vestibulo-ocular reflex (Dufossé *et al.* 1977; Ito, Orlov & Yamamoto, 1982). In some experiments the recording sites were located in the rostral area of the flocculus by marking with Fast Green FCF.

*Data processing*

A spike density histogram was constructed on-line on a minicomputer (NOVA-01, Data General) by dividing each period of rotation into 32 bins, then counting and averaging the number of spikes occurring during each bin over fifty periods. The histogram was subjected to a modified Fourier analysis and a statistical test of fitness (Jastreboff, 1979). The phase shift of the sine curve relative to the head velocity was taken as the phase angle of the modulation. As a convention, the head velocity ipsilateral to the flocculus under study was taken as positive. Positive values of the phase angle indicate a phase lag, and negative ones a phase lead. The amplitude and phase of these sine curves were plotted on a polar diagram (Fig. 1*C*; see Ghelarducci *et al.* 1975).

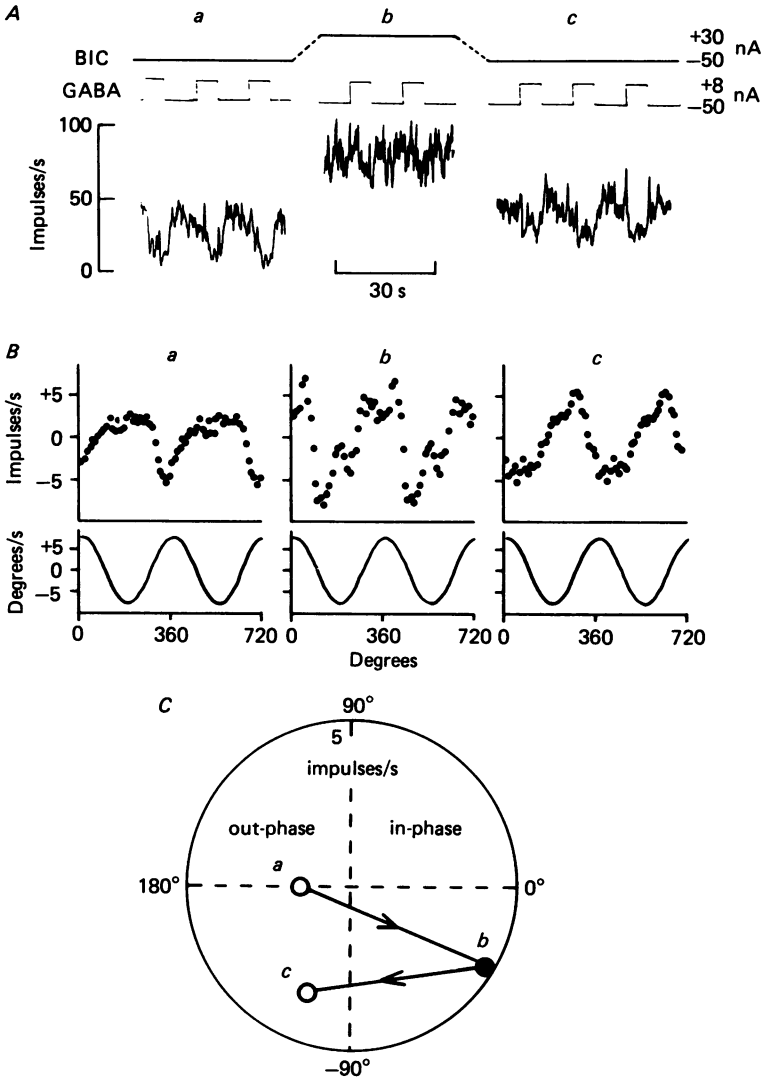


Fig. 1. Effects of ionophoretically applied bicuculline on Purkinje cell responses to GABA and head rotation. Records *a* in *A*, *B* and *C* were taken before bicuculline application with a −50 nA retaining current. Records *b* in *A*, *B* and *C* were taken 2 min after the onset of bicuculline application with a +30 nA current, and records *c* in *A*, *B* and *C* were taken 3 min after cessation of bicuculline application. The trace 'BIC' shows bicuculline application by an upward deflexion. *A*, GABA inhibition. The instantaneous discharge rate (ordinate) was recorded by integrating the number of *simple* spikes with a 1 s time constant. The trace 'GABA' indicates 6 s periods of ionophoretic GABA ejection at +8 nA, while a −50 nA retaining current was used for stopping GABA ejection. *B*, out-phase modulation of *simple* spike discharges induced by head rotation in darkness at 0·5 cycles/s with 5° (peak-to-peak) amplitude. Specimen histograms were obtained by summing over 50 cycles of rotation and taking a running average of five neighbouring bins. The ordinate shows deviations from mean discharge frequency. Sine curves at the bottom represent head velocity during two successive cycles of rotation. *C*, polar diagram plotting the *simple* spike modulation shown in *B*. The amplitude of modulation is represented by the distance from the centre of the diagram and the phase angle relative to head velocity is represented by the polar angle.

## RESULTS

### *Effects of bicuculline on GABA inhibition*

The effectiveness of bicuculline as an antagonist of ionophoretically applied GABA was tested for each of the flocculus Purkinje cells, prior to each measurement of vestibular responses. All of the fifty-one Purkinje cells tested were inhibited by ionophoretic application of GABA (current: 0–30 nA), confirming the results of an earlier report (Kawamura & Provini, 1970). The tip position of the micro-electrodes was adjusted to produce maximal GABA-evoked inhibition of the Purkinje cells, and currents from 10 to 20 nA were usually sufficient to suppress almost all spontaneous discharges (Fig. 1 *A a*). Ionophoretic application of bicuculline through another barrel (current: 20–60 nA) produced a marked reduction of the inhibitory action of GABA within a few minutes (Fig. 1 *A b*). Similar observations were made for thirty-one Purkinje cells. For the other twenty cells, bicuculline induced jerky burst discharges which prevented observation of the antagonism of GABA inhibition. In twenty-seven of the former thirty-one cells, the spontaneous activity increased appreciably during application of bicuculline; the increase was not clear in the remaining four cells. After terminating bicuculline application, there was a substantial recovery of the inhibitory action of GABA and spontaneous discharge rates (Fig. 1 *A c*), as confirmed in twenty-one cells. Control ejection through the HCl-filled electrode with current up to 100 nA did not change the discharge rate of Purkinje cells, even though spike amplitudes were altered slightly in some cases.

### *Effects of bicuculline upon vestibular responses*

When a Purkinje cell was sampled from the horizontal eye movement area of the flocculus, horizontal rotation of the head regularly induced frequency modulation of *simple* spike discharges of the Purkinje cell. The majority of the cells exhibited an out-phase modulation, i.e. an increase in discharge frequency during contralateral rotation and decrease during ipsilateral rotation (Dufossé *et al.* 1978). An example of such out-phase modulation is shown in Fig. 1 *B a*. During the period when bicuculline blocked the inhibitory action of GABA (Fig. 1 *A b*), this out-phase response was altered to an in-phase modulation, i.e. an increase during ipsilateral head rotation and a decrease during contralateral rotation (Fig. 1 *B b*). This effect is represented in the polar diagram of Fig. 1 *C* by a shift of the plotted point from the left side to the right. Three minutes after terminating bicuculline application, the inhibitory action of GABA recovered (Fig. 1 *A c*), and, concomitantly, the response to sinusoidal head rotation returned to an out-phase modulation (Fig. 1 *B c*, 1 *C c*), although the recovery of the phase angle was not complete.

Some Purkinje cells in the horizontal eye movement area modulated their *simple* spike discharges in-phase with head velocity, as exemplified in Fig. 2 *B* and *C* (+). During bicuculline ejection (30 nA current), spontaneous activity gradually increased and the GABA inhibition decreased until it was blocked a few minutes later (Fig. 2 *A*). Concomitantly, the amplitude of *simple* spike modulation to head rotation increased (Fig. 2 *B*, *C*) to a level that was about five times as large as before bicuculline application. By contrast, the phase angle of the modulation changed only a little (29° in the direction of phase lead). All of these changes diminished within a few minutes after terminating bicuculline ejection (Fig. 2 *A*, *B* and *C*).

*Effects of* DL-*homocysteate*

The ionophoretic application of bicuculline often increased the resting discharge level of a Purkinje cell (Figs. 1*A* and 2*A*). The possibility that the bicuculline effect is secondary to a generalized increase in excitability was countered by testing the effect of raising excitability with an excitatory amino acid, DL-homocysteate (Sillito, 1979).

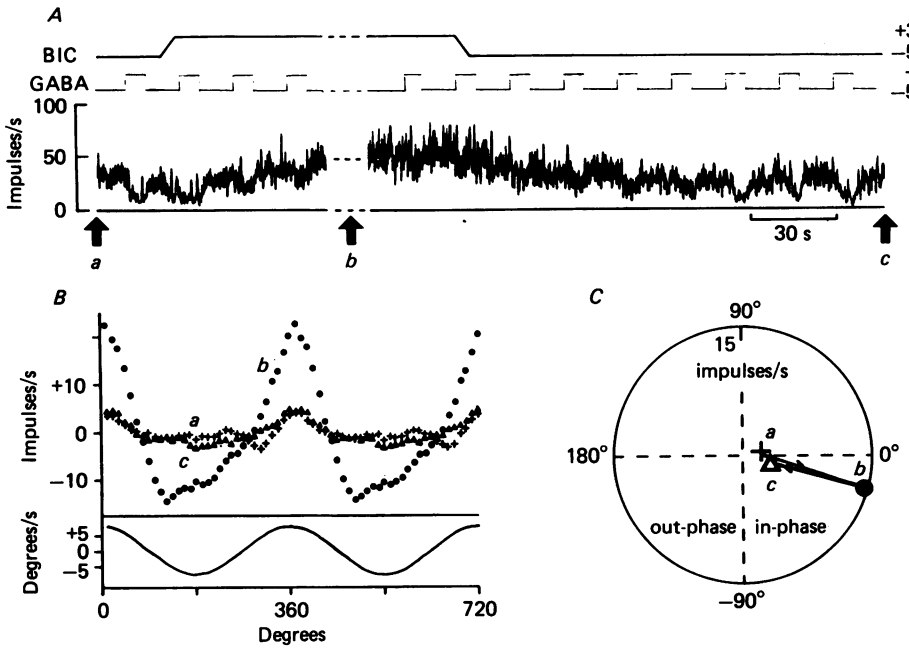


Fig. 2. Effects of bicuculline on responses of a Purkinje cell with in-phase modulation. *A*, bicuculline-induced blockage and recovery of the GABA-induced inhibition of spontaneous discharges. Ordinate, instantaneous discharge rate as in Fig. 1*A*. Arrows indicate test periods when *simple* spike modulation to head rotation was examined as *a*, *b*, *c* in *B* and *C*. *B*, *simple* spike modulation induced by head rotation in darkness. Spike density histograms were recorded *a* before (+), *b* during (●), *c* after (△) bicuculline application. The ordinate shows deviations from mean discharge frequency. The sine curve at the bottom represents head velocity. *C*, polar diagram plotting the *simple* spike modulations shown in *B*.

In Fig. 3*A*, DL-homocysteate was applied ionophoretically to a Purkinje cell which exhibited an out-phase modulation of *simple* spike discharge to head rotation. The out-phase response increased slightly while the resting discharge level increased appreciably. This effect of DL-homocysteate is represented in the polar diagram of Fig. 3*C* by a relatively small shift in the left direction. An in-phase Purkinje cell was tested similarly in Fig. 3*B* and showed a slight increase of the amplitude of modulation during ejection of DL-homocysteate (Fig. 3*C*). Thus, DL-homocysteate application tended slightly to increase the amplitude of modulation, irrespective of the phase angle of the modulation.

*Statistical analysis of responses during bicuculline and* DL*-homocysteate application*

Fifteen of the fifty-one tested Purkinje cells satisfied four criteria: (1) they were sampled from the horizontal eye movement area of the flocculus, (2) head rotation produced significant modulation of *simple* spike discharges, (3) bicuculline blocked the GABA inhibition, and (4) the effect of bicuculline was reversible. The amplitude and phase angle of their response to sinusoidal head rotation were plotted on polar diagrams (a) before, (b) during, and (c) after bicuculline application (Fig. 4*A*, *B*).

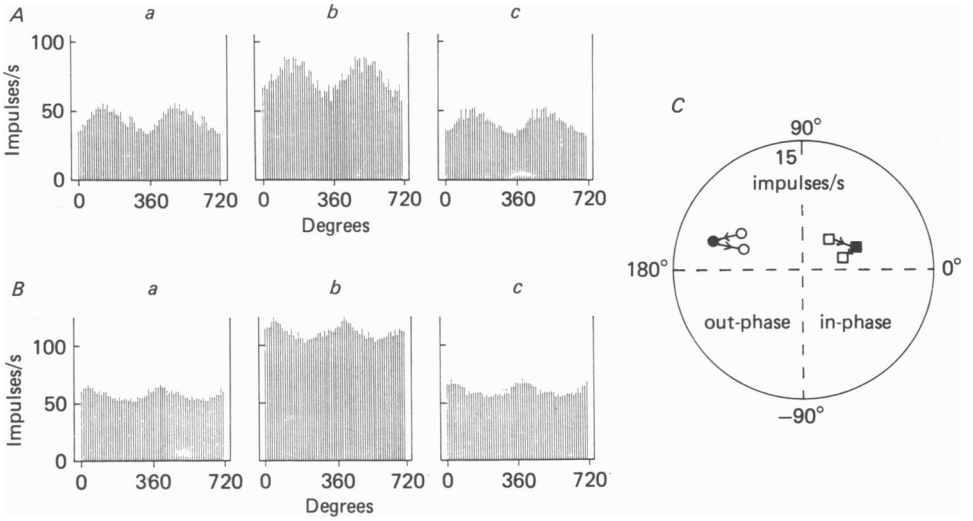


Fig. 3. Effects of DL-homocysteate on two Purkinje cells with in-phase or out-phase modulation. Records *a* in *A* and *B* were taken before DL-homocysteate application with a +30 nA retaining current. Records *b* in *A* and *B* were taken 30 s after the onset of DL-homocysteate application with a −10 nA current, and records *c* were taken 3 min after cessation of DL-homocysteate application. *A*, spike density histograms obtained from an out-phase-modulating Purkinje cell during head rotation in the dark. *B*, same as *A*, but for an in-phase-modulating Purkinje cell. *C*, polar diagram plotting the modulations shown in *A* and *B*.

Points obtained from the same neurones were connected with continuous or dashed lines.

In Fig. 4*A*, the original modulation of seven cells was out of phase with head velocity. These points are plotted on the left half of the circle. Bicuculline reduced the amplitude of modulation and reduced the phase delay in three of these cells. In the other four of the seven cells, bicuculline reversed the response to the in-phase type; as a result, the points are shifted to the right half of the circle. In another five cells (Fig. 4*A*), the modulation was originally in-phase with head velocity. Bicuculline increased the amplitude of modulation with only a slight change in the phase angle. Thus, the responses of all twelve cells plotted in Fig. 4*A* shifted on the polar diagram in the in-phase direction during bicuculline application.

Fig. 4*B* plots other three cells which exhibited a shift in the opposite direction. Two cells with in-phase responses shifted to out-phase direction and one cell with an

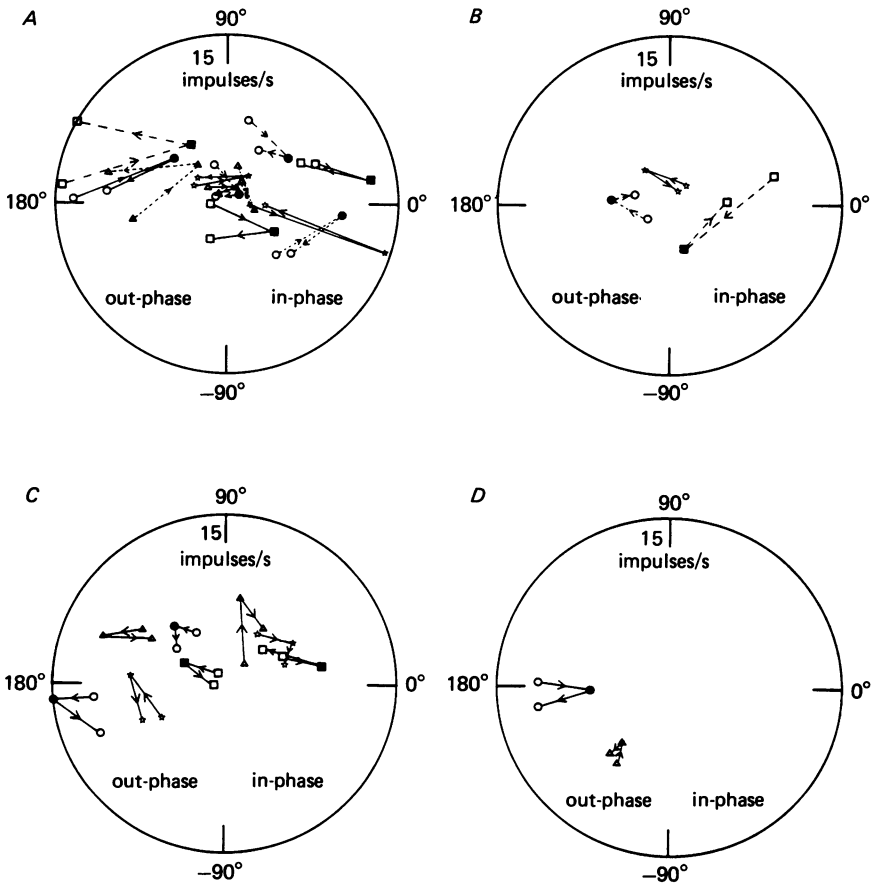


Fig. 4. Polar diagrams showing effects of ionophoretically applied bicuculline (*A*, *B*) or DL-homocysteate (*C*, *D*) on *simple* spike modulation of Purkinje cells induced by head rotation in the dark. Filled symbols represent responses taken during drug application, and open symbols represent the responses before, and after drug application. *A*, Purkinje cells in which the modulation shifted in the in-phase direction during bicuculline application. *B*, Purkinje cells in which the modulation shifted in the out-phase direction during bicuculline ejection. *C*, Purkinje cells in which the amplitude of modulation increased during DL-homocysteate application. *D*, Purkinje cells in which the amplitude of modulation decreased during DL-homocysteate ejection.

out-phase response increased its amplitude of modulation. It was noticed that two of these cells were at the margin of the horizontal eye movement area, that is, where the local stimulation evoked only a small abduction of the ipsilateral eye by currents at the upper extreme (40 $\mu$A), or the threshold for evoking the abduction was only slightly smaller than that for evoking a downward eye movement. The twelve cells plotted in Fig. 4*A* also contained three marginal cells. If these marginal cells are discarded, bicuculline application shifted the responses on nine cells in the in-phase and one cell in the out-phase direction.

DL-homocysteate application also altered *simple* spike modulation, but in a manner different from that of bicuculline. An increase of the *simple* spike modulation was

common irrespective of the phase angles in eight of ten Purkinje cells tested (Fig. 4*C*), and a decrease was seen only in the remaining two (Fig. 4*D*).

Table 1 summarizes the effects of bicuculline and DL-homocysteate. Since the base-line values of each parameter shown in the left column varied from cell to cell, data for each cell are expressed as the change from the base-line value both during ($D_1$) and after ($D_2$) bicuculline application. The spontaneous discharge frequency was 58·3 impulses/s (S.D. 37·8; $n = 15$) before bicuculline application and 68·6 impulses/s (S.D.

TABLE 1. The effects of bicuculline and DL-homocysteate

| | Bicuculline | | DL-homocysteate | |
|---|---|---|---|---|
| | $D_1$ | $D_2$ | $D_1$ | $D_2$ |
| Spontaneous discharge (impulses/s) | 27·0 ± 5·8** | −7·2 ± 5·9 | 41·1 ± 7·5** | −3·0 ± 3·4 |
| Phase angle (degrees) | −33·7 ± 13·5* | −6·7 ± 7·3 | 1·7 ± 7·4 | 9·6 ± 8·3 |
| Amplitude × cos [phase angle] (impulses/s) | 4·52 ± 1·47** | 0·05 ± 0·45 | −0·93 ± 1·27 | −0·09 ± 0·48 |

$D_1$: values during, minus those before drug application. $D_2$: values after, minus those before drug application. Numerical figures are: mean ± S.E. of mean. Number of cells are fifteen (bicuculline) and ten (DL-homocysteate). * $P < 0·05$; ** $P < 0·01$.

33·8; $n = 10$) before DL-homocysteate application, which are close to the value of 66·2 impulses/s (S.D. 27·8; $n = 293$) reported previously (Dufossé *et al.* 1978). Bicuculline and DL-homocysteate significantly increased spontaneous discharges and they recovered after termination of drug ejections. The phase angles before bicuculline (or DL-homocysteate) application were between 135° and 225° in seven (five) cells, between −45° and 45° in four (two) cells, and between 45° and 135° or −135° and −45° in another four (three) cells. A similar distribution of phase angles was obtained previously (Dufossé *et al.* 1978). Bicuculline reversibly advanced the phase angles by an average of 33·7°, while DL-homocysteate did not change the phase angles significantly.

In Fig. 4, the main effect of bicuculline is represented by a shift of the points along the abscissa. This shift was evaluated by calculating a modulation index '$M$' for each point on the diagram as: $M = a \times \cos\theta$, where $a$ is the amplitude of modulation and $\theta$ is the phase angle. The mean value of this index $M$ was −2·23 impulses/s (S.D. 7·23; $n = 15$) before bicuculline application, and −3·29 impulses/s (S.D. 5·69; $n = 10$) before DL-homocysteate application. As shown in Table 1, this index increased significantly by 4·52 impulses/s during bicuculline application, reflecting a shift of the points from the left to the right in a polar plot (Fig. 4*A*, *B*). This shift diminished after termination of bicuculline application. On the contrary, DL-homocysteate did not change the index $M$ significantly. This is because the modulation change of each Purkinje cell was relatively small and occurred in all directions (Fig. 4*C*, *D*).

## DISCUSSION

The present observation that ionophoretically applied bicuculline blocks the inhibitory action of ionophoretically applied GABA is consistent with the previous

reports of Curtis *et al.* (1971) and Curtis & Felix (1971). In the vast majority of horizontal eye-movement related Purkinje cells tested, bicuculline affected their vestibular responses and shifted the *simple* spike modulation to in-phase direction (Table 1). Although bicuculline also increased the spontaneous discharges of Purkinje cells, the control experiment with DL-homocysteate excluded the possibility that the effects on the vestibular responses were due to increased 'tonic' excitability. Application of DL-homocysteate increased the excitability more effectively than that of bicuculline, but did not produce a uni-directional shift of *simple* spike modulation such as caused by bicuculline (Table 1).

The post-synaptic inhibition of Purkinje cells arises from the basket cells, stellate cells, and Purkinje axon collaterals (Eccles *et al.* 1967). Basket cells contain glutamate decarboxylase (McLaughlin, Wood, Saito, Barber, Vaughn, Roberts & Wu, 1974), take up exogenously applied GABA (Sotelo, Privat & Drian, 1972), and the inhibition of Purkinje cells via parallel fibre–basket cell pathway is blocked by ionophoretically applied bicuculline (Curtis & Felix, 1971). Bicuculline also antagonizes action of the taurine (Okamoto & Quastel, 1976), which has been proposed as a transmitter of stellate cells (McBride, Nadi, Altman & Aprison, 1976; Frederickson, Neuss, Morzorati & McBride, 1978). However, both the axon terminals of stellate cells and the taurine-sensitive sites are distributed fairly distally on Purkinje cell dendrites (Eccles *et al.* 1967; Okamoto & Sakai, 1980). Since the tips of the micro-electrodes in the present experiments were positioned so that ionophoretic ejection of GABA evoked maximal inhibition of each Purkinje cell, it is likely that they were near the somatic area of the Purkinje cells, where GABA sensitivity is highest (Okamoto & Sakai, 1980). Therefore, ionophoretically applied bicuculline might act on the Purkinje cells by blocking the inhibitory action of the basket cells that is exerted at the Purkinje cell soma (Eccles *et al.* 1967) rather than by blocking the action of the stellate cells. Although Purkinje cell axon collaterals presumably utilize GABA as neurotransmitter and terminate proximally on Purkinje cells, their inhibitory action is much weaker than basket cell inhibition (Eccles *et al.* 1967). Thus, the available evidence suggests that the effects of bicuculline observed here were mainly due to antagonism of the basket cell inhibition of Purkinje cells.

The modulation of the *simple* spike discharges of flocculus Purkinje cells in response to head rotation is presumably induced by vestibular mossy fibre inputs to the flocculus. Eye velocity signals may also contribute to the modulation in the flocculus of monkeys (Lisberger & Fuchs, 1978), but this is not a major input to the rabbit's flocculus, where eye movements in darkness influence only a minority of Purkinje cells (Neverov, Sterc & Bures, 1980). In the vast majority of flocculus Purkinje cells sampled in this study, bicuculline reduced out-phase modulation (or converted it to in-phase modulation), or enhanced in-phase modulation. This situation can be explained by assuming that Purkinje cell responses are determined by an algebraic sum of an out-phase modulation arising from basket cell inhibition and an in-phase modulation from another bicuculline-insensitive source. Since ipsilateral primary vestibular neurones generate in-phase-modulating signals (Fernandez & Goldberg, 1971), a simple and likely explanation is that ipsilateral vestibular signals provide both in-phase and out-phase signals to Purkinje cells via different intracortical circuits. In-phase signals may arise via a direct granule cell–Purkinje cell pathway,

while out-phase signals may arise simultaneously via a granule cell–basket cell–Purkinje cell pathway. Since basket cells would be driven in-phase through granule cells, their subsequent action on Purkinje cells via inhibitory synapses would be an out-phase modulation. In a minority of flocculus Purkinje cells, their modulations were shifted in the out-phase direction during bicuculline application. These cells might receive vestibular signals from the contralateral labyrinth, which would provide an out-phase drive through granule cells and an in-phase drive through the granule cell–basket cell pathway. The differential roles for the major in-phase-shifting and the minor out-phase-shifting Purkinje cell groups in controlling the horizontal v.o.r. are not clear at the present stage of investigation. Since two of the three out-phase-shifting cells were found along the margin of the horizontal eye movement area, it is possible that they are related to a function other than horizontal eye movement.

Inhibitory signals from the flocculus Purkinje cells and excitatory signals from the horizontal canal converge onto relay cells of the horizontal v.o.r. (Highstein, 1973; Ito, Nisimaru & Yamamoto, 1977). Since the canal afferents are modulated in-phase with head velocity (Fernandez & Goldberg, 1971), in-phase discharges from the inhibitory Purkinje cells should depress the horizontal v.o.r. (Lisberger & Fuchs, 1978), while out-phase Purkinje cells should enhance the reflex (Dufossé *et al.* 1978). That the out-phase Purkinje cells make the predominant contribution to the horizontal v.o.r. is apparent because (1) they are the major population in the horizontal eye movement area (Dufossé *et al.* 1978), (2) destruction of the flocculus reduces the gain of the horizontal v.o.r. (Keller & Precht, 1979; Ito, Jastreboff & Miyashita, 1982), and (3) changes correlated with visually guided adaptive modification of the horizontal v.o.r. occur in the out-phase modulation (Dufossé *et al.* 1978). The present results suggest that contribution of the flocculus Purkinje cells to the horizontal v.o.r. through out-phase modulation is mainly due to basket cell inhibition, which converts in-phase signals in the ipsilateral vestibular fibres to out-phase signals in the Purkinje cells. This points to an important role of intracortical post-synaptic inhibition in motor control functions of the cerebellum.

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