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INHIBITORY CONNECTIONS OF IPSILATERAL SEMICIRCULAR CANAL AFFERENTS ONTO RENSHAW CELLS IN THE LUMBAR SPINAL CORD OF THE CAT

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SUMMARY

1. In intercollicularly decerebrate cats, the excitability of lumbar spinal Renshaw cells (tested by single shocks to ventral roots and deafferented muscle nerves) decreased for 600–1000 ms after conditioning electrical stimulation of ipsilateral semicircular canal nerves.

2. Conditioning stimulation of posterior canal afferents and combined stimulation of anterior and lateral canal afferents were equally effective in causing inhibition of Renshaw cells. No significant differences were observed for Renshaw cells excitable from hind-limb flexor or extensor nerves.

3. Inhibition appeared when one to five stimuli were applied to the canal afferents and arrived at the spinal segmental level 11–15 ms after the onset of conditioning stimulation.

4. Evidence is adduced to suggest that the inhibitory effects on Renshaw cells following stimulation of semicircular canal afferents were mediated directly, i.e. they were not caused by alterations of motoneurone activity.

5. Excitation of Renshaw cells due to stimulation of the canal afferents was rarely observed; it could not be excluded that it was secondary to motoneurone discharges.

6. It is suggested that vestibular inhibition of Renshaw cells ensure a high gain of hind-limb α-motoneurones during postural adjustments following a massive disturbance of body equilibrium.

INTRODUCTION

It has been repeatedly demonstrated that individual Renshaw cells and recurrent inhibition of α-motoneurones can be influenced by conditioning electrical stimulation of supraspinal structures (for references see Haase, Cleveland & Ross, 1975; Baldissera, Hultborn & Illert, 1981). Both enhancement and reduction of Renshaw cell activity have been observed which was shown to be either due to direct effects on the interneurones themselves or secondary to changes in the discharge pattern of α-motoneurones converging on them. The existence of direct descending pathways to Renshaw cells would be consistent with the hypothesis that the recurrent inhibitory pathway would primarily function as a variable gain control governing the input–output relation of α-motoneurones (Hultborn, Lindström & Wigström, 1979).

Unfortunately, the supraspinal structures investigated thus far (cerebral cortex, capsula interna, red nucleus, cerebellum, reticular formation and thalamus) participate in a variety of motor acts which makes it difficult to assess the functional significance of the results obtained.

A more clear-cut situation is encountered when signals originating in vestibular afferents descend to the spinal segments, because they produce vestibulo-spinal reflexes which basically serve a well-defined purpose, namely to maintain and restore body equilibrium.

In recent studies applying paired Hoffmann's reflexes, recurrent inhibition has been estimated in humans during voluntary contractions of the triceps surae muscle. The experimental results indicate that recurrent inhibition of triceps surae motoneurones is weak during strong movements and strong during weak movements (Pierrot-Deseilligny, Morin, Katz & Bussel, 1977; Hultborn & Pierrot-Deseilligny, 1979*a*; Katz & Pierrot-Deseilligny, 1984).

If this reflects a general rule describing the behaviour of the recurrent control of motor neurones one would expect that during massive disturbances of body equilibrium, which would call for rapid and vigorous compensatory muscle activity, Renshaw cells ought to be inhibited by vestibular afferent input. We present evidence that Renshaw cells can indeed be suppressed for up to at least 600 ms after electrical stimulation of semicircular canal afferents, and that these effects are mediated through a separate channel, i.e. without prior alterations of α-motoneurone activity.

A preliminary report of some of these results has been presented (Ross, Thewissen, Cleveland & Purrmann, 1982).

METHODS

Experiments were carried out on twenty-three intercollicularly decerebrate cats. Blood pressure was monitored continuously; measurements were discontinued if it dropped below 80 mmHg.

Preparation was done under fluothane–N_2O–O_2 anaesthesia. After decerebration, anaesthesia was removed, and the animals were immobilized by intravenous injection of alcuronium (Alloferin, Hoffmann–La Roche, 0·25 mg/kg) and artificially ventilated. Body temperature was kept at approximately 37 °C.

Measurements were begun no earlier than 2 h after decerebration and removal of anaesthesia.

Preparation and recording

The right hind limb was completely denervated; after laminectomy, the left dorsal roots L6–S2 were cut intradurally. The following nerves of the left hind limb were prepared and mounted on bipolar platinum wire electrodes for electrical stimulation: gastrocnemius–soleus, anterior tibial, deep peroneal, anterior biceps–semimembranosus, and posterior biceps–semitendinosus. In a second series of experiments, the left ventral roots L6–S2 were cut and the central ends of the ventral roots L7 or S1 were used for stimulation.

The exposed spinal cord and the nerves prepared for stimulation were covered with liquid paraffin at body temperature.

Renshaw cells were recorded extracellularly in spinal segments L7 or S1 by means of glass micro-electrodes filled with 3 M-KCl, and identified according to common criteria (Renshaw, 1946; Eccles, Fatt & Koketsu, 1954; Frank & Fuortes, 1956).

For vestibular conditioning, steel needle electrodes were implanted ipsilaterally close to individual semicircular canal nerves according to the technique published by Suzuki, Goto, Tokumasu & Cohen (1969). Briefly, the surgical intervention consisted of the following steps, which were all carried out under deep anaesthesia. Following incision of the skin, the temporal muscle

and underlying tissue were retracted to give access to the tympanic bulla. After opening the bulla and atticotomy, the ear drum, the tensor tympani muscle and the auditory ossicles were removed under a dissection microscope; only the foot-plate of the stapes was preserved. The fallopian canal was opened as far as the lateral genu and the exposed portion of the facial nerve removed. Close to the two dark areas at the bottom of the fallopian canal which indicate the site of the ampullae of the anterior and lateral canals, small holes were drilled through which the bipolar stimulus electrodes were inserted. A third pair of electrodes (for stimulation of the posterior canal afferents) was inserted through a small opening drilled just posterior to the round window (compare Suzuki *et al.* Fig. 1). The electrodes were fixated with acrylic cement. As a modification we also employed monopolar stimulation, the cathode being implanted near a canal nerve, while the anode (an Ag–AgCl plate) was either placed under the tongue or buried in the musculature of the neck.

Electrode positioning was checked by observing the eye movements evoked by short trains of rectangular pulses (width, 0·2 ms; frequency, 500 Hz) immediately prior to decerebration. Since both common carotid arteries had been ligated about 2 h before decerebration, nineteen animals were anaemically decorticated by that time, indicated by forelimb extensor rigidity (Andén, Jukes, Lundberg & Vyklicky, 1966). The absence of cortical blood supply was verified by direct inspection of the exposed cortical surface. In four animals in which anaemic decortication was incomplete, the cortex was removed using a spatula under deep anaesthesia before the eye movements were tested. Since electrical stimulation of the canal nerves did not produce eye movements under the deep anaesthesia we employed during surgery, a transient reduction was unavoidable during the eye-movement tests. However, care was taken to keep the level of anaesthesia sufficiently deep to leave the animals insentient, as judged by the following criteria:

(1) no aversive reactions or withdrawal reflexes were observed following noxious stimuli, such as pin-prick or manipulating of the surgically exposed areas of the back,

(2) noxious stimulation produced no changes in depth or frequency of respiration, in blood pressure, or heart frequency,

(3) during vestibular stimulation, no reactions other than eye movements and minor contractions of neck muscles occurred; blood pressure and respiration remained unaffected.

Our observation that eye-movement responses to vestibular stimulation in the cat can be obtained under adequate anaesthesia is in accordance with earlier findings (e.g. Szentágothai, 1950).

As judged by the eye movements, it was a common observation that while selective stimulation of the posterior canal nerve was always achieved, the effects due to stimulation of the anterior canal nerve were in most cases contaminated by obvious co-excitation of the lateral canal nerve and vice versa. The results obtained from these experiments were therefore taken as being due to combined stimulation of the anterior *and* lateral canal nerves.

After the eye movements had been documented anaesthesia was deepened again to a level which caused blood pressure to drop to 40–50 mmHg. The exposed brain stem was then transected with a spatula at intercollicular level under visual control. If bleeding occurred the corresponding branches of the arterial circle were clamped.

Stimulus parameters

Renshaw cell test responses were elicited by single electrical shocks to the deafferented muscle nerves or to portions of the ipsilateral ventral roots L7 or S1, delivered every 3 s; pulse width 0·2 ms, amplitude supramaximal for α-fibres. Every second test shock was preceded by repetitive conditioning stimulation of the vestibular afferents at various conditioning–testing intervals. We usually applied ten rectangular pulses (0·2 ms wide) at a frequency of 500 Hz; the stimulus amplitude was kept the same as for eliciting the eye movements (1–3 V).

In five experiments the number of vestibular stimuli was varied systematically between one and twenty-five to define the threshold and maximum of the observed effects.

In three experiments the eighth cranial nerve was exposed and either cut during cell recording or blocked by local anaesthesia.

Distinction between Renshaw cells and other interneurones

It was a common finding that action potentials appeared during conditioning vestibular stimulation. To test whether this early activity was originating from the Renshaw cells under investigation or from other interneurones, we moved the micro-electrode up and down while

monitoring the resulting changes in the spike form and amplitude; this procedure is illustrated in Figs. 1 and 7.

Motor activity

Using the same kind of preparation, motor activity following semicircular canal afferent stimulation was recorded from the muscle nerves and from filaments isolated from the ipsilateral ventral root L7.

RESULTS

Results are based on a total of eighty-four Renshaw cells. Of these, seventy-nine units exhibited an inhibition following vestibular conditioning while five showed the same kind of inhibition, but preceded by an excitation which appeared during vestibular stimulation.

From thirty-three Renshaw cells stable recordings could be obtained for at least 10 min which was sufficient to determine the maximal amount of the vestibular effects. Seventeen units were observed for up to 2 h allowing extended quantitative measurements summarized in Figs. 4 and 6.

Vestibular inhibition of Renshaw cells

The experiment depicted in Fig. 1 is a typical example of the pronounced inhibition of a Renshaw cell due to vestibular conditioning. The burst response elicited by a single electrical shock to the deafferented gastrocnemius–soleus nerve (Fig. 1*A* and *C*) consisted of 9–11 spikes/stimulus; it was reduced to 3–4 spikes/stimulus (Fig. 1*B* and *D*) when the antidromic nerve volley was preceded by conditioning stimulation of the posterior canal nerve (conditioning–testing interval, 50 ms). The reduced response was very stable, as is evident from the superimposed traces (Fig. 1*B*). The form of the field potential caused by the antidromically excited α-motoneurones and the first two spikes of the Renshaw cell remained virtually uninfluenced; minimal fluctuations of amplitude are due to superposition of base-line irregularities which appeared during vestibular afferent stimulation (Fig. 1*D*, *E* and *F*) and outlasted it for at least 600 ms. The early action potentials which appeared during canal afferent stimulation were originating from interneurones other than this Renshaw cell because both kinds of responses changed differently when the microelectrode was moved up or down (Fig. 1*E* and *F*).

No further attempt was made to classify the cells which produced the early activity. It should be noted, however, that these units could not be excited by electrical shocks to ipsilateral ventral roots or deafferented muscle nerves and hence are neither motoneurones nor Renshaw cells.

Time course of vestibular inhibition of Renshaw cells

Renshaw cell test activity evoked by an antidromic volley in α-axons showed a maximal depression when the conditioning vestibular stimulation preceded the test shock by 30–200 ms, but inhibition was still present at intervals up to at least 600 ms. Fig. 2 exemplifies this for two Renshaw cells, one driven by an extensor nerve (medial gastrocnemius) the other by a flexor nerve (deep peroneal).

Fig. 3 shows the time course of the inhibition of another Renshaw cell (driven by the gastrocnemius–soleus nerve) when the conditioning stimulus was applied either to the posterior or the anterior–lateral canal nerves. The cell's response is reduced

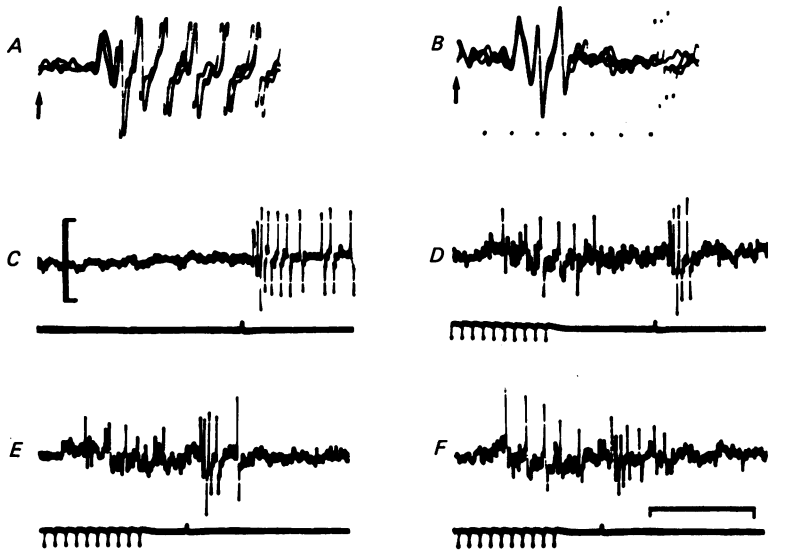

Fig. 1. Reduction of Renshaw cell burst responses and appearance of discharges of another interneurone due to vestibular stimulation. *A*, test response (superimposed traces) of a Renshaw cell in spinal segment L7 following single electrical shocks (arrow) to deafferented gastrocnemius–soleus nerve (pulse width 0·2 ms; supramaximal for α-fibres). *B*, reduction of response (superimposed traces) caused by conditioning stimulation of ipsilateral posterior canal nerve (ten impulses at 500 Hz; pulse width 0·2 ms, pulse amplitude 1·5 V). Conditioning–testing interval, 50 ms. *C*, Renshaw cell test response (upper trace) to single electrical shock (stimulus mark on lower trace, upward deflection); same cell as in *A*. *D*, reduction of Renshaw cell burst response after conditioning stimulation of ipsilateral posterior canal nerve (stimulus marks on lower trace, downward deflections). Note the additional early repetitive action potentials beginnning about 10 ms after onset of vestibular nerve stimulation. *E* and *F*, converse changes of Renshaw cell discharges and of early action potentials produced by other interneurones when the micro-electrode was moved up (*E*) or down (*F*). Conditioning–testing interval, 40 ms (*D*) and 30 ms (*E* and *F*). Time calibration, 1 ms (*A* and *B*) and 20 ms (*C*–*F*); amplitude calibration, 0·5 mV.

from about 10 spikes/stimulus (test) to about 4 spikes/stimulus after additional vestibular conditioning. This maximal depression occurred at a conditioning–testing interval of 30 ms (anterior–lateral canal nerves) and 200 ms (posterior canal nerve).

No long-term adaptation of the vestibular effects on Renshaw cells was observed during the course of the measurements. This was true for all experiments, no matter whether the conditioning–testing interval was varied systematically or at random.

There was considerable variation between individual experiments concerning both the extent of vestibular inhibition and its time course (cf. Fig. 3) but we have seen no *systematic* differences, no matter whether the Renshaw cells were driven by the extensor or flexor nerves tested or which semicircular canal afferents were used for conditioning. This was also true for the vestibular inhibition of one Renshaw cell which received excitatory convergence from extensor (gastrocnemius–soleus) and flexor (deep peroneal) nerves.

Therefore, all results are pooled together in Fig. 4. In the main graph, which includes twenty-four measurements on twelve Renshaw cells, the average reduction

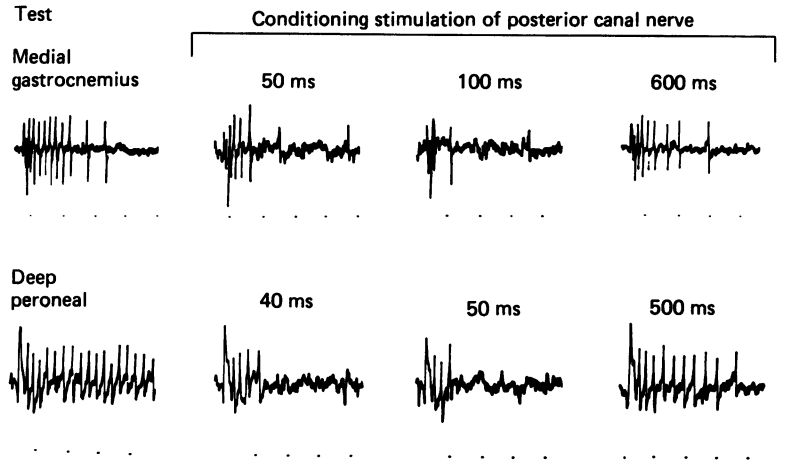

Fig. 2. Dependence of vestibular inhibition of Renshaw cells on conditioning–testing interval. Responses of two Renshaw cells from spinal segment L7, one driven by the medial gastrocnemius nerve (cell 1, upper row), the other by the deep peroneal nerve (cell 2, lower row). Left column: burst responses without vestibular conditioning (test), elicited by single electrical shocks to deafferented nerves (pulse width 0·2 ms, amplitude supramaximal for α-fibres). Other columns: responses elicited by the same nerve stimulation, but preceded by stimulation on ipsilateral posterior canal nerve (ten pulses at 500 Hz) at conditioning–testing intervals indicated above original recordings. Time marks, 10 ms.

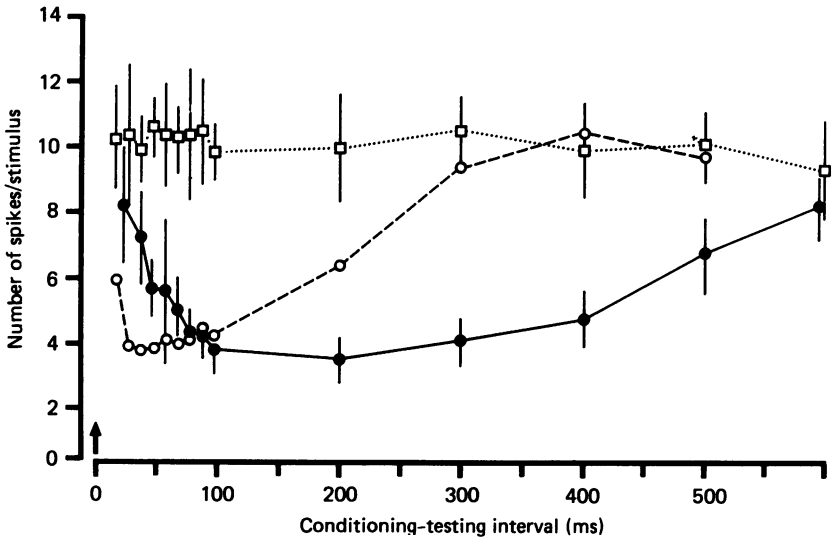

Fig. 3. Time course of vestibular inhibition of a Renshaw cell. Number of spikes/stimulus elicited by single electrical shocks to gastrocnemius–soleus nerve (pulse width 0·2 ms, supramaximal for α-fibres) *versus* conditioning–testing interval. Dotted line, squares: burst responses without vestibular stimulation. Continuous line, filled circles: Renshaw cell response when conditioning stimulation was applied to ipsilateral posterior canal nerve (ten pulses at 500 Hz; arrow on abscissa). Dashed line, open circles: same, but conditioning stimulation applied to ipsilateral anterior and lateral canal nerves. Values are averages of five measurements each $\pm$standard deviations; standard deviations for values during stimulation of anterior and lateral canal nerves omitted for clarity.

of the Renshaw cell burst response (spikes/stimulus) is plotted against the conditioning–testing interval. On average, inhibition reached its maximum between 100 and 200 ms after the onset of the conditioning stimulus and persisted for at least 600 ms. Between 700 and 900 ms, and possibly between 1200 and 1300 ms, it re-appeared. Thus the complete time course resembled that of a damped oscillation. The insets of the Figure are based on a total of sixty-five measurements on

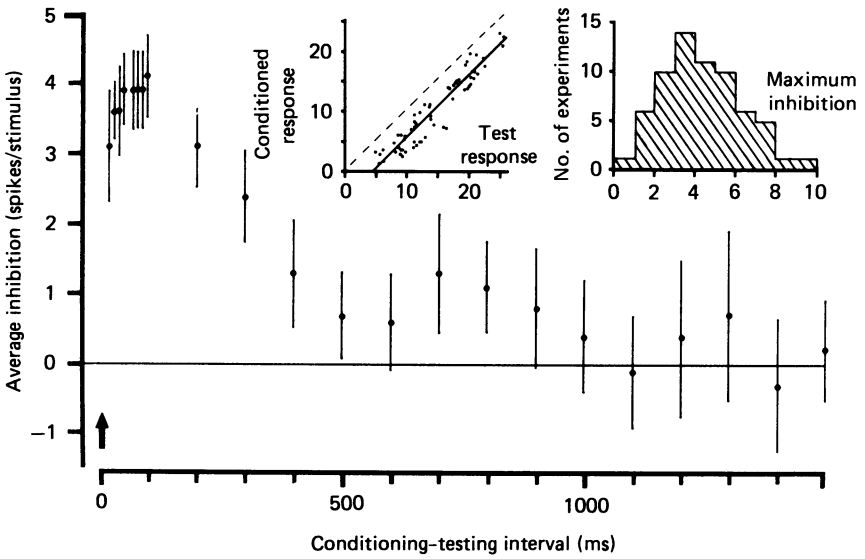

Fig. 4. Summary of vestibular inhibition of Renshaw cells. Main graph: average inhibition (reduction of antidromically elicited burst response; spikes/stimulus) *versus* conditioning–testing interval for twelve Renshaw cells. Conditioning stimulation (ten impulses at 500 Hz) indicated by arrow on abscissa. Graph includes measurements of twenty-four unitary responses ±standard error of the mean. Test responses elicited from gastrocnemius–soleus (n = 7), deep peroneal (n = 15), anterior tibial (n = 1) and anterior biceps–semimembranosus (n = 1); conditioning stimulation of anterior–lateral (n = 9) or posterior (n = 15) canal afferents. Left inset: maximum vestibular inhibition (conditioned response; spikes/stimulus.) at optimum conditioning–testing interval plotted *versus* test response (spikes/stimulus) for thirty-three Renshaw cells; sixty-five points determined by at least ten measurements each. Correlation coefficient for (continuous) regression line, 0·95; slope 1·02. Dashed line: theoretical relation without vestibular inhibition. Test responses elicited from gastrocnemius–soleus (n = 20), deep peroneal (n = 32), anterior tibial (n = 4), anterior biceps–semimembranosus (n = 2), posterior biceps–semitendinosus (n = 2) and portions of ventral root L7 (n = 5); conditioning stimulation of anterior–lateral (n = 22) or posterior (n = 43) canal afferents. Right inset: histogram of maximum vestibular inhibition (spikes/stimulus) for the same experiments (n = 65); median of the distribution, 4·02 spikes/stimulus.

thirty-three cells and include values for those units for which a complete time course could not be established because they were lost after their maximal vestibular inhibition was determined. When the conditioned responses at optimum conditioning–testing intervals are plotted *versus* the test responses (Fig. 4, left inset) it becomes obvious that the maximum amount of vestibular inhibition is independent of the size of the test response which ranged from 5·0 to 25·8 spikes/stimulus. The regression line through the points is almost perfectly parallel to the dashed line

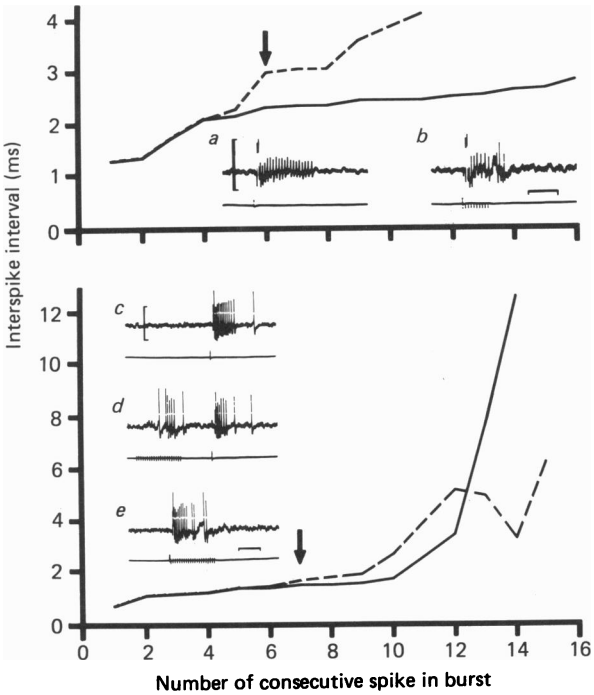

Fig. 5. Onset of vestibular inhibition of Renshaw cells as evidenced by interval trend analysis. For two Renshaw cells, interspike intervals are plotted against the number of consecutive spikes in burst when test shocks are given alone (continuous lines) or when the same stimulus is applied together with semicircular afferent conditioning (dashed lines). Values are averages of ten burst responses each; the first interval is that between the field potentials of the α-motoneurones and the first Renshaw cell spike. The upper graph pertains to a cell exhibiting pure inhibition, the lower to a unit exhibiting mixed excitatory and inhibitory effects. Interspike intervals are significantly prolonged beginning at the points indicated by arrows, corresponding to 10·1–12·2 ms (cell 1, upper graph) and 11·1–11·6 ms (cell 2, lower graph) after onset of canal afferent stimulation. Resolution for measuring the intervals was 0·05 ms. *a–e*, original recordings. *a*, Renshaw cell from spinal segment L7; test response elicited by single shock to deafferented gastrocnemius–soleus nerve (stimulus mark on lower trace, upward deflection). *b*, when stimulation of posterior canal afferents (ten impulses at 500 Hz; stimulus marks on lower trace, downward deflection) and test shock are applied together, number and density of Renshaw cell discharges are reduced. *c*, same Renshaw cell and stimulus parameters as in Fig. 7. *d*, Renshaw cell excitation during vestibular stimulation together with reduced response to nerve shock. *e*, when vestibular nerve stimulation and test shock are applied simultaneously, mixed inhibitory and excitatory effects occur. Time calibration, 20 ms; amplitude calibration, 0·5 mV.

describing the relation without vestibular effects. The histogram (Fig. 4, right inset) illustrates the distribution of the maximum amount of vestibular inhibition which ranged from 0·60 to 9·25 (median 4·02) spikes/stimulus.

Latency, threshold, and maximum of vestibular inhibition of Renshaw cells

Since no intracellular recordings were obtained from Renshaw cells, the exact onset of the vestibular inhibition (i.p.s.p.s) could not be measured. Furthermore, because

of the early action potentials from other interneurones superimposed on the Renshaw cell discharges at short conditioning–testing intervals (compare Fig. 1), it was difficult to determine the beginning of the reduction of the burst response.

From four cells, recordings were obtained in which action potentials from other interneurones were absent or few. For these units it was possible to determine the time which elapsed between the onset of vestibular conditioning and the beginning of the thinning out of the Renshaw cell burst response when the test shock and the conditioning stimulus train were applied simultaneously.

This is exemplified for two cells in Fig. 5. When the intervals between successive spikes in the control bursts are compared to those during additional conditioning in a unit exhibiting pure inhibition (Fig. 5, upper graph), a significant difference first appears for the interval between the fifth and sixth action potential (arrow) which corresponds to about 11 ms after the onset of vestibular stimulation.

The second cell in Fig. 5 (lower graph) is one of the five units which, in addition to the long-lasting inhibition, exhibited excitation during canal afferent stimulation. The interval trend analysis of this unit reveals that inhibition (increase of the interspike intervals) begins between the sixth and seventh action potential (arrow) corresponding to about 11·5 ms after the onset of canal afferent stimulation. The thinning out of the Renshaw cell burst is a transient effect, however, since vestibular excitation prevails a few milliseconds later, as can be seen from the shorter interspike intervals appearing towards the end of the response.

It is interesting to note that this sequence of inhibition and excitation can only be detected by measuring the changes in instantaneous spike density (interval trend), since both effects cancel each other almost perfectly when the number of spikes is taken as an indicator. The responses consisted of $13{\cdot}4 \pm 1{\cdot}3$ (control) and $14{\cdot}4 \pm 0{\cdot}7$ (conditioned) spikes per burst.

In the four units which could be analysed by the interval trend method the latencies for the onset of vestibular inhibition ranged from 11·0 to 15·0 ms.

To determine the threshold and maximum of the inhibitory effects, the number of conditioning vestibular stimuli was varied between one and twenty-five (frequency 500 Hz) at optimum conditioning–testing intervals in nine experimental series on five Renshaw cells. In two units, inhibition appeared when only one stimulus was applied; for the remaining three units the threshold was reached at two, four, and five impulses, respectively.

From the right graph of Fig. 6, which contains the results of ninety measurements, it can be seen that once the threshold was reached, inhibition increased along with increasing number of impulses in the conditioning stimulus train. On average, a tendency towards saturation appeared between ten and fifteen impulses.

From the data presented thus far it can be predicted that vestibular conditioning may result in a complete depression of Renshaw cells, provided their unconditioned activity is low. That this prediction is valid could be proved by the experiment depicted in the left graph of Fig. 6. We observed total suppression of this cell's discharges when a low test activity (2 or 3 spikes/stimulus) was induced by stimulation of a very thin filament isolated from the ventral root L7 (Cleveland, Kuschmierz & Ross, 1981). Activity was first reduced with four conditioning stimuli (lateral canal nerve), and completely suppressed with twenty-two or more impulses. A similar reaction was observed in another unit under the same conditions.

Furthermore, two spontaneously active Renshaw cells were totally inhibited for about 300 ms following vestibular stimulation (twenty impulses, 500 Hz); the control activity was fully restored during the next 400 ms. The time course determined from averaged responses of these units resembled that presented in Fig. 4.

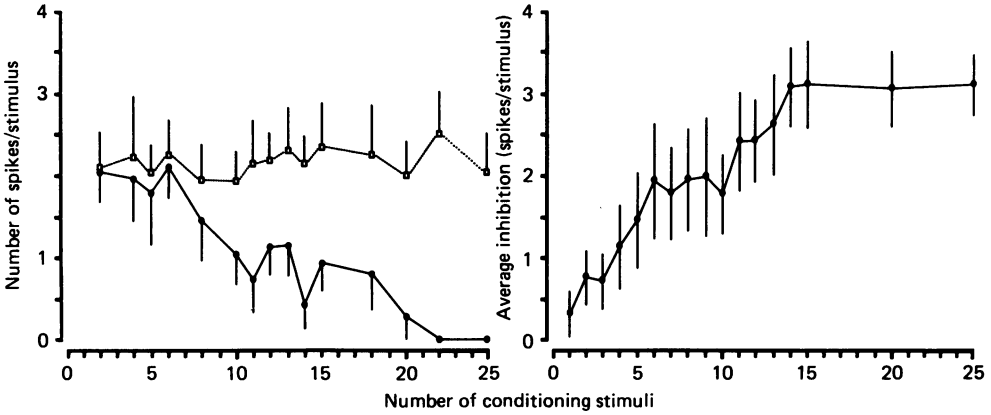

Fig. 6. Dependence of vestibular inhibition of Renshaw cells on the number of conditioning stimuli. Left graph: squares and dotted line indicate test activity (ordinate) of a Renshaw cell from spinal segment L7 responding with an average of 2 spikes/stimulus to single electrical shocks to a fine filament isolated from ventral root L7; filled circles and continuous line indicate increasing reduction of response with increasing number of conditioning stimuli to ipsilateral lateral canal nerve (abscissa). Conditioning–testing interval 50 ms throughout; points are averages of twenty responses each ±standard deviation. Right graph: average dependence of inhibition of Renshaw cells (ordinate) on the number of stimuli in the conditioning stimulus train to canal afferents (abscissa) at optimum conditioning–testing interval. Points are averages of ninety measurements each ±standard error of the mean, determined in five Renshaw cells driven by ipsilateral ventral root L7 or portions of it. Conditioning stimuli (frequency, 500 Hz) applied to afferents from lateral canal ($n = 1$), anterior–lateral canal ($n = 1$), or posterior canal ($n = 3$).

As a test for specifity, the eighth cranial nerve was cut in two experiments during cell recording; no vestibular inhibition of Renshaw cells was detectable thereafter. In one experiment the eighth nerve was blocked by injection of 0·01 ml of a 0·5% solution of lidocaine hydrochloride (Xylocain, Astra Chemicals) into the nerve trunk. A few minutes later the vestibular inhibition of the Renshaw cell recorded diminished and totally disappeared during the next 15 min. Inhibition re-appeared 165 min after administration of the drug and approached its control level after 200 min.

Vestibular excitation of Renshaw cells

In five Renshaw cells electrical stimulation of canal afferents not only evoked the long-lasting inhibition described in the previous sections but also caused additional excitation. In two of these units, early excitation appeared only when more intense conditioning stimulation (twenty stimulus impulses) was applied.

Such responses of a Renshaw cell driven by the gastrocnemius–soleus nerve and

conditioned by the ipsilateral anterior and lateral canal afferents are depicted in Figs. 5 and 7. As determined from ten averaged cycles, Renshaw cell excitation appeared $19{\cdot}3 \pm 3{\cdot}7$ ms after the semicircular afferent stimulation began and consisted of $6{\cdot}6 \pm 1{\cdot}5$ spikes. Similar values were obtained from the remaining four experiments. Of the five cells exhibiting vestibular excitation, four were driven by the gastrocnemius–soleus and one by the deep peroneal nerve. The procedure described in Methods yielded clear evidence that the early discharges originated from

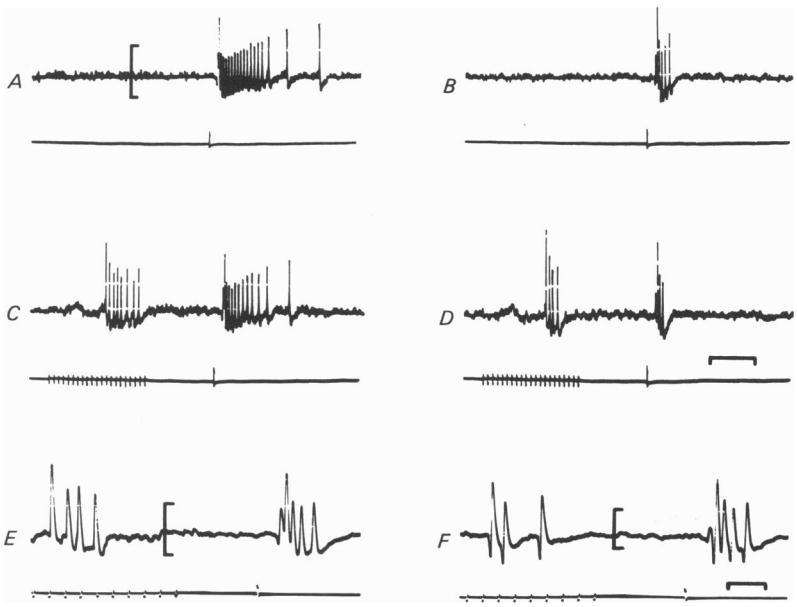

Fig. 7. Excitation of a Renshaw cell during vestibular conditioning and effect of dihydro-β-erythroidine. *A*, test response of a Renshaw cell in spinal segment S1 (upper trace) elicited by single electrical shock to gastrocnemius–soleus nerve (pulse width 0·2 ms, supramaximal for α-fibres; stimulus mark on lower trace, upward deflection). *B*, same as *A*, but 10 min after intravenous injection of dihydro-β-erythroidine (0·5 mg/kg body weight). *C*, reduction of Renshaw cell response to nerve shock after conditioning stimulation of ipsilateral anterior and lateral canal nerves (twenty impulses at 500 Hz; stimulus marks on lower trace, downward deflections) together with Renshaw cell discharges appearing during vestibular stimulation. *D*, same as *C*, but after dihydro-β-erythroidine. *E* and *F*, concomitant changes in spike form and amplitude of early action potentials and spikes following nerve shock when the micro-electrode is moved up (*E*) or down (*F*). Records also taken after dihydro-β-erythroidine; note that only the second half of the vestibular stimulus train is depicted. Time calibration, 20 ms (*A–D*), 5 ms (*E*, *F*); amplitude calibration, 0·5 mV

the same Renshaw cell which was driven by antidromic nerve excitation: both kinds of action potentials changed in the same way during displacement of the micro-electrode (Fig. 7 *E* and *F*) which is never observed when the responses stem from different units (compare Fig. 1 *D–F*).

Electrical stimulation of vestibular afferents has been reported to induce liminal α-motoneurone firing in forelimbs (Maeda, Maunz & Wilson, 1975) and hind limbs (Partridge & Kim, 1969). Since this may have contributed to the early excitation of

Renshaw cells, we measured motor activity during semicircular afferent stimulation in the deafferented muscle nerves used for eliciting the Renshaw cell test responses and in portions of the ipsilateral ventral root L7 in five animals.

Even when ten to twenty stimuli (500 Hz) were applied, motor activity was extremely low, and averages of 100–200 cycles were necessary before a clear distinction from background noise was achieved. This is in accord with the observations of Partridge & Kim (1969) who found that the tension developed in the triceps surae muscle during repetitive electrical stimulation of individual canal nerves (up to 300 Hz) was only about 1 % of its maximum force.

Correcting for the axonal conduction time, the first detectable motoneurone activity at segmental level occurred in our experiments 11–13 ms after the onset of canal afferent stimulation, i.e. 6–8 ms before Renshaw cell excitation began.

Dihydro-β-erythroidine which reduces the burst response of Renshaw cells following antidromic excitation of α-axons (Eccles *et al.* 1954) or following orthodromic activation of α-motoneurones (Haase & Vogel, 1971), was applied in two experiments (0·5 mg/kg I.V.). Both the burst response following nerve stimulation and the early excitation of the Renshaw cells during canal afferent stimulation were reduced, as depicted in Fig. 7*A–D*. The average number of spikes decreases from $15{\cdot}2 \pm 1{\cdot}3$ to $3{\cdot}4 \pm 1{\cdot}1$ (response to nerve shock) and from $6{\cdot}6 \pm 1{\cdot}5$ to $4{\cdot}2 \pm 1{\cdot}4$ (response to vestibular afferent stimulation). The latency of the response to vestibular stimulation increased from $19{\cdot}3 \pm 3{\cdot}7$ ms to $22{\cdot}0 \pm 4{\cdot}2$ ms; no such effect on the latency was detectable in the burst response following nerve excitation.

DISCUSSION

Excitation of Renshaw cells following vestibular canal nerve stimulation

Excitation of Renshaw cells was detected only in five units. The excitation had the form of a burst response, although the spike density was less than in bursts elicited by antidromic volleys in α-axons (see Figs. 5 and 7).

Since a detectable – though very low – motor activity preceded this early Renshaw cell activity, an excitatory projection from semicircular afferents to lumbar spinal Renshaw cells excluding α-motoneurones could not be established with certainty. That a low level of motor activity may well be sufficient to explain the Renshaw cell activity observed can be inferred from the observation that single α-motoneurones are capable of eliciting action potentials and even burst responses in these interneurones (Ross, Cleveland & Haase, 1975, 1976; van Keulen, 1981). The reduction of the vestibularly induced Renshaw cell responses by dihydro-β-erythroidine seen in our experiments is also in favour of the idea that motoneurones are intercalated in the pathway. On the other hand, the minimal motor activity observed in our preparations is most probably due to small motoneurones, which, according to the size principle, are the first to be recruited by low synaptic drive (e.g. Henneman, Somjen & Carpenter, 1965). Since small motoneurones have few axon collaterals and might thus contribute less to Renshaw cell excitation, it is conceivable that the activity of these interneurones observed during semicircular afferent stimulation may be partly due to direct descending cholinergic pathways (Pompeiano, Wand & Scivastava, 1985*b*).

Inhibition of Renshaw cells following vestibular canal nerve stimulation

A reduction of antidromically induced Renshaw cell bursts after vestibular conditioning was observed in all units tested.

Observations on an additional set of fifteen Renshaw cells driven by ipsilateral ventral roots L7, S1 or portions of them yielded no evidence for the existence of other patterns of ipsilateral canal afferent influence on these interneurones in our kind of preparation. Although all of these units were inhibited, measurements on them have not been included in Figs. 4 and 6 because stable recordings were not obtained from sufficiently long periods to determine either the complete time course or the maxima of the effects.

A striking fact is that this kind of inhibition outlasted the vestibular canal stimulation by about 1000 ms (Fig. 4). Thus, the information contained in a short bout of vestibular afferent activity is available at the output stage of the spinal motor system for a comparatively long period.

The mechanism which enables the system to store this information is at present unknown. It is conceivable, however, that it may be located at the spinal or even at the segmental level, e.g. in the form of reverberating interneuronal chains. Even membrane properties of interneurones intercalated in the pathway from canal afferents to Renshaw cells may be an important factor. That such a possibility does indeed exist at this site has been demonstrated for a long-lasting increase in the excitability of α-motoneurones (Hultborn & Wigström, 1980; Hounsgaard, Hultborn, Jespersen & Kiehn, 1984). Furthermore, the reciprocal inhibition of spinal interneurones intercalated in the pathway of the late long-lasting effects from flexion reflex afferents exhibits a time course similar to that which we found for the vestibular inhibition of Renshaw cells (Bergmans, Fedina & Lundberg; in Lundberg, 1969).

The most important point is, however, to rule out the possibility that the vestibular inhibitory effects on Renshaw cells are in any way a secondary consequence of alterations in α-motoneurone activity.

Since the control activity of the Renshaw cells was evoked by antidromic excitation of α-axons, any subthreshold potentials in α-motoneurones following vestibular stimulation could not have influenced the pathway to these interneurones. The remaining alternative would be a refractory state of α-axons. This would imply that α-motoneurones converging on the Renshaw cells under investigation were discharged by vestibular conditioning and hence unexcitable by the test shock. This can be excluded for two reasons.

(1) The maximum duration of motor activity observed in our experiments was 40–50 ms while the depression of the Renshaw cell excitability lasted for at least 600 ms.

(2) None of the Renshaw cells included in Figs. 4 and 6 was fired by vestibular afferent excitation, as would have been expected if a significant number of α-motoneurones converging on them had discharged following the conditioning stimulus.

There is of course the remote possibility that a small fraction of α-motoneurones converging on the Renshaw cells under investigation (and not detectable in the averaged motor response) was indeed activated when the response of the interneurones was reduced and their influence remained subthreshold. From the experiments

of Hultborn & Pierrot-Deseilligny (1979*b*) it can be inferred, however, that under these conditions a facilitation rather than a depression would have occurred.

A final, though even more remote possibility would be that the effects observed are due to presynaptic inhibition acting on the recurrent collaterals. Although primary afferent depolarization of Ia afferents has been reported following stimulation of vestibular nuclei (Cook, Cangiano & Pompeiano, 1969) there is no evidence for descending fibers terminating on recurrent collaterals of α-axons.

We thus conclude that the vestibular inhibition of Renshaw cells is mediated through a direct channel without prior influence on α-motoneurones.

The latency of vestibular inhibition of Renshaw cells could only be estimated indirectly, i.e. by the onset of reduction of the spike density of antidromically evoked burst responses in a few units. From the interval trend graphs (Fig. 5) it was determined that the antidromically elicited Renshaw cell burst response was reduced about 11·0–15·0 ms after the vestibular stimulation began.

This closely coincides with the onset of motoneuronal activity (11–13 ms) observable under the same conditions. Thus, α-motoneurone excitation and Renshaw cell inhibition occur at practically the same time at the segmental level when both are induced by electrical stimulation of vestibular afferents.

Functional considerations

Since our primary interest was focused on the search for a direct link between semicircular canal afferents and Renshaw cells, the only appropriate method was electrical stimulation. Since this will hardly simulate natural patterns, our method implies some restrictions on the functional interpretations of our results.

It should be recalled, however, that both increase and decrease of Renshaw cell activity have also been observed with natural stimulation of vestibular afferents (Pompeiano, Wand & Scrivastava, 1985*a*, *b*). Their study was, however, primarily focused on the effects of macular input and they have ascribed the reduction of Renshaw cell activity to disfacilitation rather than to inhibition.

Our discussion will be limited to some general comments on the possible role of the inhibitory pathway from semicircular canal afferents to Renshaw cells, although we are aware of the fact that the excitatory influence rarely observed in our preparations may be much more prominent in intact motor systems.

Renshaw cells associated with both extensor and flexor nerve activity were inhibited by afferents from any ipsilateral semicircular canal. Therefore, our current hypothesis is that semicircular canal excitation causes a general inhibition of all Renshaw cells connected to motoneurones of the lower limbs.

At least one of the peculiarities of the recurrent inhibitory system does indeed suggest a general rather that a differential supraspinal control, namely that its effects are widely distributed among motoneurones serving fairly different functions.

On the input side, this is reflected in the fact that individual Renshaw cells are excited by motor axons contained in different flexor and extensor nerves from the same limb (Eccles *et al.* 1954; Longo, Martin & Unna, 1960; Eccles, Eccles, Iggo & Lundberg, 1961; Deliagina & Feldman, 1981). On the output side, antidromic inhibition following electrical stimulation of a given muscle nerve is in turn detectable in a number of motor nuclei. Although it is most potent between motoneurones linked

by Ia afferent excitation, it can also be strong between antagonists in a wider sense, i.e. between flexors and extensors of the same limb (Thomas & Wilson, 1967; Hultborn *et al.* 1971*a*). The only exception is the absence of antidromic inhibition between strict antagonists acting at the same joint, where the antidromic effects consist of a reciprocal facilitation via a depression of Ia inhibitory interneurones (Hultborn, Jankowska & Lindström, 1971*b*; Cleveland, Haase, Ross & Wand, 1972). Thus, if Renshaw cells act as variable gain regulators they do this in at least two ways, one being focally organized (recurrent control within closely synergistic motor nuclei), the other following a more extended pattern. Hultborn *et al.* (1971*b*) suggested that this extended pattern may be important during synergies not reflected in Ia input. One may speculate that certain movements would require a general depression or facilitation of Renshaw cells rather than localized variations. One such situation, which we tried to mimic by strong stimulation of the canal afferents, may be a short-lasting massive disturbance of body equilibrium, which would certainly call for rapid motor acts. In this case motoneurones having a high gain (due to inhibition of Renshaw cells) would probably be advantageous. Since a high gain ensures a prompter reaction to excitation as well as to inhibition, any descending command to motor nuclei would be executed more effectively than through motoneurones with high recurrent inhibition. The long duration of semicircular afferent inhibition of Renshaw cells could help to maintain this response characteristic of the motoneurones for a complete sequence of postural adjustments.

Since successful postural adjustments depend on co-ordinated motor activity on both sides of the body, our hypothesis would require that Renshaw cells contralateral to the semicircular canals activated are inhibited as well. This we have indeed observed in two experiments.

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