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RESEARCH ARTICLE

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Cerebellar inhibition of inferior olivary transmission in the decerebrate ferret

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Abstract Stimulation around the superior cerebellar peduncle or within the deep cerebellar nuclei is known to inhibit the inferior olive with a very long latency. It has been suggested that this inhibition is mediated by the GABA-ergic nucleo-olivary pathway, but alternative explanations such as activation of an indirect excitatory pathway or a pathway via the red nucleus are possible. A long-latency inhibition via the nucleo-olivary pathway would have profound implications for cerebellar function and the present study was performed to test alternative explanations and to characterize the nucleo-olivary inhibition. Climbing fibre responses (CFRs), evoked by periorbital stimulation and recorded from the cerebellar cortex, could be inhibited by stimulation of two distinct mesencephalic areas. One was located within the superior cerebellar peduncle and the other about 1 mm further ventrally. Inhibition evoked from either area occurred in the inferior olive and was independent of a red nucleus relay. Single Purkinje cell recordings revealed that inhibition from the ventral area was not secondary to olivary activation. It is concluded that stimulation of the ventral area activated nucleo-olivary fibres. The inhibition elicited by stimulation within the peduncle probably resulted from indirect activation on the nucleo-olivary fibres via antidromic activation of the interpositus nucleus. The time courses of the inhibition from the two areas were indistinguishable. The duration of the strongest inhibition was short and had a sharp peak at about 30 ms. It is suggested that the time course of the inhibition is important for temporal regulation of learned responses.

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Introduction

A monosynaptic projection runs from the deep cerebellar nuclei just ventral to the superior cerebellar peduncle to the inferior olive (Graybiel et al. 1973; Legendre and Courville 1987; Martin et al. 1976). Several lines of experimental data suggest that this nucleo-olivary projection is inhibitory. Stimulation just ventral to the superior cerebellar peduncle in cats (Hesslow 1986) and of the cerebellar lateral nucleus in rats (Garifoli et al. 2001) inhibits the inferior olive. Blockade of the nucleo-olivary pathway eliminates oscillatory changes in climbing fibre response amplitude induced by high-frequency stimulation and increases the spontaneous complex spike frequency (Andersson et al. 1988). The cell bodies of the olive-projecting neurons are glutamate decarboxylase positive (Nelson and Mugnaini 1989) and nucleo-olivary terminals are Gamma-Amino Butyric Acid (GABA) positive (Angaut and Sotelo 1987, 1989; De Zeeuw et al. 1988, 1989). The olivo-cortico-nuclear zonal organization is preserved in the nucleo-olivary pathway (Dietrichs and Walberg 1981, 1986; Ruigrok 1997) and probably also the microzonal organization (Andersson and Hesslow 1987). Climbing fibre input controls both the background activity of Purkinje cells (Bengtsson et al. 2004; Cerminara and Rawson 2004; Colin et al. 1980; Demer et al. 1985; Montarolo et al. 1982; Rawson and Tilokskulchai 1981; Savio and Tempia 1985) and their responsiveness to parallel fibre input (Ito 1989; Linden and Connor 1995). We have previously suggested that the nucleo-olivary pathway provides a possible negative feedback mechanism for controlling background activity and learning in the cerebellar cortex (Andersson et al. 1988). Evidence for these suggestions has been presented (Bengtsson et al.

2004; Hesslow and Ivarsson 1996; Kim et al. 1998). A quite different idea that the nucleo-olivary pathway controls electrical coupling between olivary neurons has also been proposed (Lang et al. 1996; Llinas et al. 1974; Sotelo et al. 1986).

The nucleo-olivary pathway is clearly of fundamental importance, but it has turned out to be surprisingly difficult to characterize it physiologically. A previous attempt to do this by stimulation of the nucleo-olivary fibres in barbiturate-anaesthetized cats (Hesslow 1986) raised a number of questions, which have never been addressed. Most importantly, it was not clear if the inhibition was really mediated by the nucleo-olivary fibres or if it was an indirect effect of rebound inhibition after excitation, perhaps via activation of the red nucleus or an indirect inhibition via some other brain stem structure. These questions are particularly pertinent in view of the fact that previous stimulation of the superior cerebellar peduncle inhibited the olive with a latency of 30–50 ms, which is surprisingly long for a monosynaptic effect. The aim of the present study was to address these questions and to characterize the nucleo-olivary pathway in greater detail.

Materials and methods

Anaesthesia and surgery

Twenty-six ferrets, weighing 0.75–1.8 kg, were used. Each animal was anaesthetized with isoflurane (Abbot Laboratories Ltd, England; 1.5–2% in a mixture of O_2 and N_2O). When deep anaesthesia had been achieved, a tracheotomy was performed and the gas was then channelled directly into a tracheal tube. The end-expiratory CO_2 concentration, arterial blood pressure and rectal temperature were monitored continuously and kept within physiological limits. Throughout the experiment the animals were artificially ventilated and infusion (1 ml/kg/h of a mixture of equal volumes: Glucose in H_2O [50 mg/ml; Kabi Pharmacia AB, Sweden], Macrodex in 0.9% NaCl solution [60 mg/ml; Medisan Pharmaceuticals, Sweden] and isotonic Acetate Ringer [Pharmacia & Upjohn AB, Sweden]) was continuously given into the femoral vein. In some animals, Albumin (fraction V from bovine serum, 4 mg/l; from bovine serum; Merck, Darmstadt, Germany) was added to the infusion. The level of anaesthesia was regularly monitored by testing the withdrawal reflexes. This study has been reviewed and approved by the local Swedish Ethical Committee.

The animal's head was fixed in a stereotaxic frame. The skull was then opened on the left side and the caudal half of cerebral hemispheres, together with a substantial part of the thalamus on the left side, were removed by aspiration. The aspiration exposed the cerebellum and the superior and inferior colliculi. The animals were decerebrated by sectioning the brain stem with a blunt spatula 1–2 mm rostral to the superior colliculus. In order to disconnect the red nucleus from the cerebellum, a few animals were decerebrated at the border between the superior and inferior colliculus. Bleeding was controlled with gelfoam (Johnson & Johnson Medical Ltd, England).

After decerebration the anaesthesia was terminated and a mixture of O_2 and air replaced the mixture of O_2 and N_2O. A cotton reinforced agar pool was built around the opening of the skull and was filled with warm mineral oil or a high density insulating carbonfluor liquid (FC 40, 3M Company).

Periorbital stimulation

The peripheral electrical stimulation was applied through two stainless steel electrodes (insulated insect pins, de-insulated tip$\approx$ 100 μm), the tips of which were inserted about 5 mm apart into the skin of the medial part of the periorbital area. One square pulse of 0.5 ms duration and a stimulus intensity of 3 mA were used. This stimulus intensity reliably elicited CFRs in the c3 zone of the cerebellar cortex. In experiments where single Purkinje cells were recorded, the intensity was 1.5 times the threshold for eliciting a CFR.

Mesencephalic stimulation

The mesencephalon was stimulated with a cathodal tungsten needle electrode (de-insulated tip diameter $\approx$ 20 μm and length $\approx$ 75 μm). The anodal reference electrode was a silver-ball electrode (Ø $\approx$ 200 μm) placed on the surface of the inferior colliculus. The mesencephalon was tracked and stimulated around the superior cerebellar peduncle in a transversal plane close to caudal border of the inferior colliculus. During tracking, the electrode tip was moved in steps of 250 μm (depth and laterality). At each stimulation site, the threshold for eliciting an eyelid EMG with a stimulus train, consisting of three square pulses (0.5 ms; 333 Hz), was determined. Also, the ability to depress periorbitally evoked CFRs was tested at each site by applying a stimulus train consisting of five square pulses (0.5 ms; 200 Hz). The mesencephalic stimulus intensity during the depression was around 50 μA. The interval between the mesencephalic stimulus and the periorbital stimulus was usually 40 ms or 45 ms. The interval between each trial was 5 sec or 10 sec. Other stimulus parameters are described in the Result section. Figure 1a, b illustrates the experimental set-up and an example of a stimulus sequence and recorded cortical potential. The stimulation sites were verified histologically (see below). Before fixation of the tissue, the stimulation electrode was left in the best track (see Fig. 3a).

Olivary stimulation

In two animals the peripheral stimulus was replaced by a direct cathodal stimulus (0.5 ms) of the inferior olive

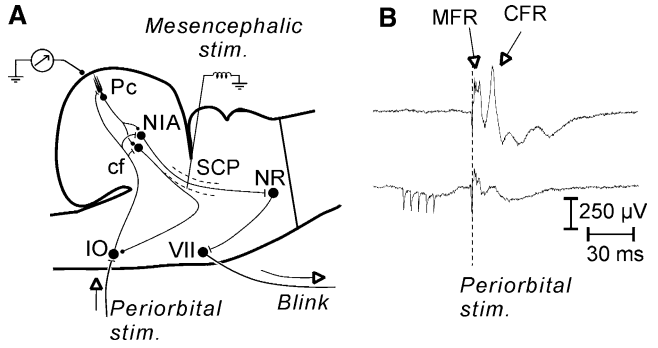

Fig. 1 Wiring diagram and experimental set-up. **a** The periorbital stimulus generates a climbing fibre response in the Purkinje cell (*Pc*) via the inferior olive (*IO*). The mesencephalic stimulation is hypothesized to activate two pathways; firstly, the nucleo-olivary pathway from the nucleus interpositus anterior (*NIA*) to the *IO* and, secondly, the nucleo-rubral pathway from the *NIA* to the red nucleus (*NR*), which via the facial nucleus (*VII*) elicits a blink. **b** Example of the depression of a climbing fibre response (*CFR*). In the *upper trace*, the periorbital stimulus pulse (*vertical line*) elicits a characteristic mossy fibre response (*MFR*) and a *CFR*. In the *lower trace*, a mesencephalic stimulus train (5 shock artefacts) is applied just before the periorbital stimulus. Average of ten consecutive trials

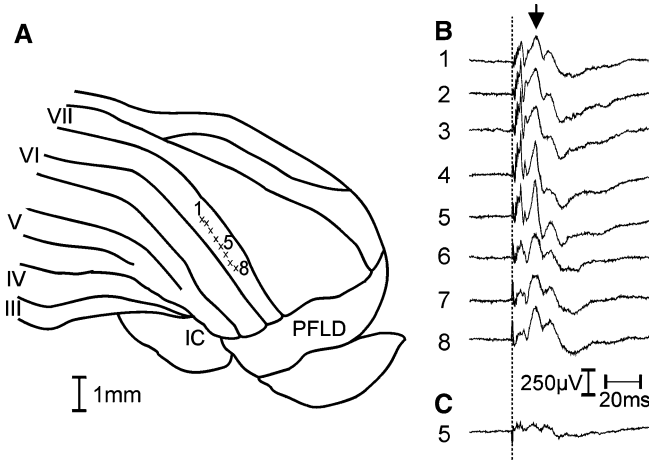

Fig. 2 Blink-controlling area in the c3 zone of the cerebellar cortex. **a** Rostro-ventral view of the left cerebellar cortex. Recording sites 1–8 are indicated in *lobule VI*. **b** and **c** Cerebellar surface recordings from site 1–8 (average of ten consecutive trials) during stimulation of the left (**b**) and right periorbital area (**c**). The *arrow* indicates the time of the second peak, which corresponds to the analysed climbing fibre response in the c3 zone. The climbing fibre response with the largest amplitude was recorded from area five. *PFLD*: dorsal paraflocculus; *IC*: inferior colliculus

contralateral to the cerebellar surface recording. We aimed for the rostro-medial part of the dorsal accessory olive, since this part is known to transmit periorbitally evoked climbing fibre signals to the cortical c3 zone in lobule VI (Gellman et al. 1983; Hesslow 1994; Oscarsson 1980).

A metal electrode (insect pin with deinsulated tip ≈ 75 μm) was positioned on the surface of the medulla close to the caudal edge of the cerebellum about 1 mm lateral to the midline. The electrode was then lowered in a rostro-caudal angle of 30° about 7 mm to the approximate location of the dorsal accessory olive. The exact position of the electrode was adjusted in three dimensions so that a stimulus intensity of ∼100 μA elicited CFRs in the blink-controlling area of the c3 zone in lobule VI. The localization of the stimulation site was verified in histological sections.

EMG recording

Electromyographic (EMG) activity in the orbicularis oculi muscle was recorded through two stainless steel electrodes (insulated insect needles, de-insulated tip≈100 μm). The electrodes were inserted through the skin at 2–3 mm dorso-caudal to the lateral margin.

Surface and single unit recordings

Evoked potentials were recorded from the blink-controlling area of the c3 zone in the hemispheral parts of lobule V and VI. Surface potentials were recorded with a silver-ball electrode (Ø≈250 μm) and single Purkinje cell activity with a glass microelectrode (filled with 0.09% NaCl) or a glass-coated metal microelectrode (platinum 80%, iridium 20%; FHC Inc, ME, USA). The blink-controlling area of the c3-zone was identified by well-established physiological criteria (See Fig. 2; (Armstrong 1990; Garwicz 1997; Hesslow 1994; Oscarsson 1980). The animals were immobilized by Norcuron (Organon Teknika bv, Boxtel, NL) (0.2 mg/ml; 1–2 ml/kg/h. Electrophysiological sampling and analysis was made with computer software from Cambridge Electronics Design (CED, Cambridge, England). Single units were recorded at the sampling rate of 10 kHz and band-pass filtered at 50–150 kHz. Sample traces of CFRs with and without preceding mesencephalic stimulation are shown in Fig. 1b.

Red nucleus inactivation and lesion

To test the contribution of the red nucleus in nucleo-olivary inhibition, the red nucleus was either inactivated by lignocaine or mechanically lesioned by local aspiration or decerebration. Inactivation by lignocaine was performed in two animals. Lignocaine (10 μl; 40 mg/ml; Xylocain; Astra, Sweden) was aspirated into a Hamilton syringe (Hamilton Bonaduz AG, Switzerland). The tip of the syringe was positioned at the dorsal surface of the brainstem 1 mm rostral to the contralateral superior colliculus and was then lowered to the red nucleus. In the first animal 1-μl lignocaine was applied at a depth of 5.3 mm and in the second animal 1-μl lignocaine was injected at three sites along the injection track at the depths 4.3 mm, 5.3 mm and 6.3 mm. The red nucleus was considered blocked when stimulation of the superior

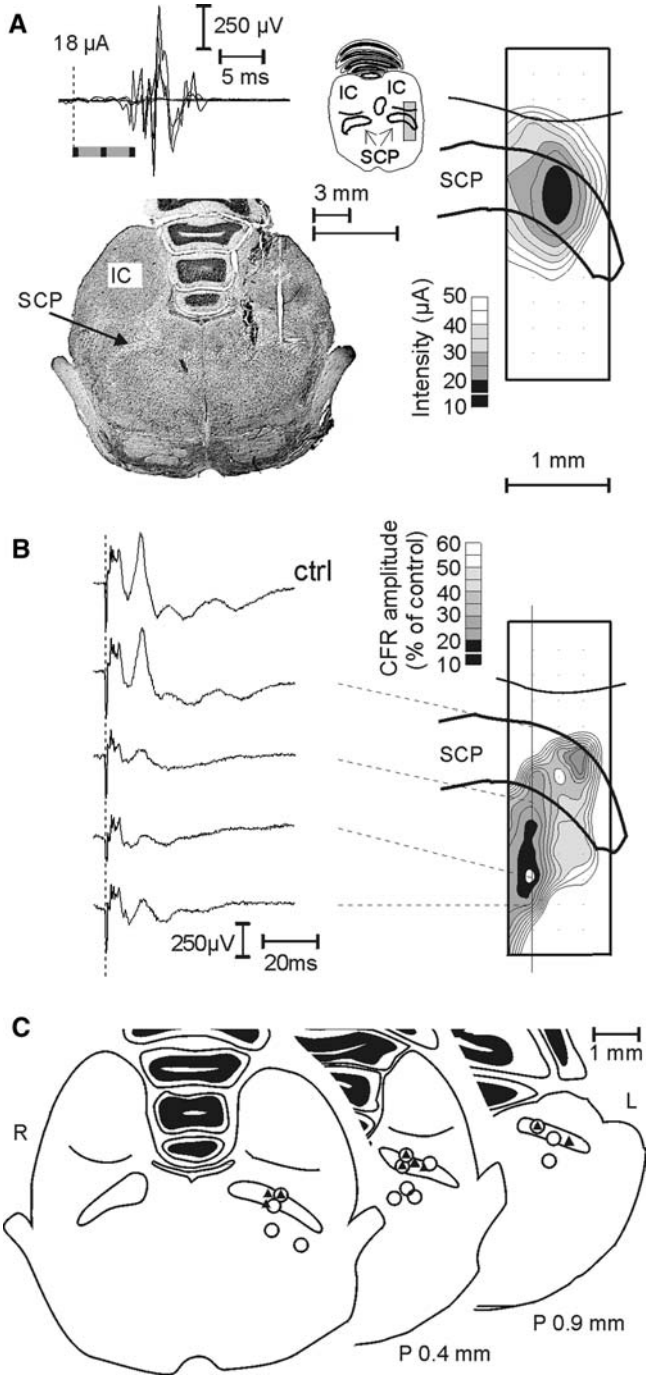

Fig. 3 Localization of blink-eliciting and *CFR*-depressing areas. **a** *Upper left* five superimposed consecutive eyelid *EMG* records ($n = 5$) during stimulation of the superior cerebellar peduncle (*vertical line*). **a** *Lower left* transverse mesencephalic section containing an electrode track on the animals' left side. *SCP* superior cerebellar peduncle, *IC* inferior colliculus. **a** *Right* isocurrent map of eyelid *EMG* thresholds in the area is indicated in the small reconstructed mesencephalic section. Each stimulation site is illustrated with a *small black dot*. **b** *Left* cerebellar surface recordings (average of ten consecutive trials). The periorbital stimulus (*vertical line*) is preceded by mesencephalic stimulation at different depths along the track indicated in the *right panel*. **b** *Right* Isopotential map of peripherally elicited *CFR* amplitudes from the same area as in **a** *right*. **c** Summary of optimal stimulation sites in the animals. The stimulation site with the lowest threshold for eliciting an ipsilateral short-latency blink response (*filled triangles*) and the site from which the *CFR* was maximally depressed (*circles*) for each animal is indicated. *R* right; *L* left; *P* posterior distance from the first section

cerebellar peduncle could no longer elicit any eyelid EMG. Mechanical lesions were performed in six animals. In two animals the red nucleus was locally aspirated by a blunt syringe needle attached to a vacuum pump. In four other animals the decerebration was performed at the border between the inferior and superior colliculus. After local aspiration as well as after intracollicular decerebration, stimulation of the superior cerebellar peduncle could not evoke any short-latency eyelid EMG. When the eyelid EMG was abolished, the CFR inhibition was tested by applying a mesencephalic stimulus (pulse duration 0.5 ms; 5 pulses; 200 Hz) before the periorbital stimulation. An interval of 40 ms or 45 ms was applied since these intervals usually gave a reliable CFR inhibition. The injection sites and the extent of lesions were verified in histological sections (Fig. 3).

Analysis

The ability of an electrical stimulus to elicit short-latency eyelid EMG (latency 5–8 ms) from the mesencephalic area was analysed for each animal by calculating isocurrent maps (Kriging algorithm; Surfer Mapping System; Golden Software Inc, Colorado, USA) from the blink-eliciting current thresholds. This threshold was defined as the current intensity that elicited EMG responses in 50% of the trials. Depression of CFRs was analysed by calculating isopotential maps with the same Kriging algorithm but from the amplitudes of periorbitally elicited CFR (% of control; mean of ten consecutive trials). The maps were then superimposed on tissue-section photos. The site with the lowest threshold for eliciting an eyelid movement and the site with the strongest CFR inhibition were transferred on to mesencephalic frontal-section templates. The amplitude of each CFR was always the amplitude of the second positive potential. The amplitude was calculated as the difference between the mean baseline level, 1–10 ms before the superior cerebellar peduncle stimulation, and the peak amplitude 7.5–12.5 ms after the periorbital stimulus. The amplitude of the climbing fibre responses elicited by direct olivary stimulation was calculated as the difference between mean baseline (see previous sentence) and the peak amplitude between 2.5 and 7.5 ms after the olivary stimulus.

Histology

The results of lesions and placement of electrodes were examined in histological sections. After the experiment

the animals were perfused with sodium chloride followed by 10% (w/v) formaldehyde (Merck, Germany) in phosphate buffer (0.2 M, pH 7.7) solution. The cerebellum and brainstem were removed from the skulls, stored in 10% formaldehyde for at least 3 weeks, frozen and sectioned transversally in 60-μm slices in a cryostat. The slices were mounted and stained with Cresyl Violet and then examined under a light microscope.

Results

Identification of the blink-controlling area in the c3 zone

Climbing fibre responses were recorded either as surface field potentials or as single-cell complex spikes from the blink-controlling area in the cortical c3 zone of lobule VI. This area was localized by recording characteristic surface potentials from the cerebellar cortex during electrical stimulation of the periorbital area as previously described (Hesslow 1994). Figure 2 illustrates characteristic potentials recorded from different areas in lobule VI. The first positive potential shows mossy fibre response activity while the second positive peak reflects CFRs in the c3 zone. The third positive peak with considerably smaller amplitude was not typical and only seen in a few animals. This third peak corresponds to CFRs found in the c3 zone, which may indicate not only the sub-zonal organization of the c3 zone, but also the CFRs found in neighbouring c2 or d1 zones. In addition, the amplitude of the third peak is smaller in site four and five where the second peak has the largest amplitude. The CFRs were selectively depressed by a high-frequency stimulation of the periorbital area (4 Hz). In the animal illustrated in Fig. 2, the largest CFR amplitude and the sharpest peak were observed in recording site five. This information, together with the laterality of the recording site, led us to conclude that this site was located within the blink-controlling area of the c3 zone. The almost complete lack of contralateral input to this area further supported this conclusion (Fig. 2c). Stimulation of an area with the same characteristics in the cat has previously been shown to elicit EMG responses in the eyelids (Hesslow 1994).

Stimulation both in and below the superior cerebellar peduncle can depress CFRs

The blink-eliciting nucleo-rubral fibres run within the superior cerebellar peduncle (for ref. see: (Ito 1984)) and the nucleo-olivary fibres, at least in cat, run ventral to the peduncle (Legendre and Courville 1987) but see (Tolbert et al. 1976). To outline the exact anatomical relation between these two fibre bundles in the ferret, the mesencephalon was stimulated in and around the superior cerebellar peduncle. Tracks were made in a transversal plane at the caudal border of the superior colliculus. The nucleo-rubral fibres were localized by determining the lowest current threshold for eliciting ipsilateral eyelid EMG activity with an onset latency of 5–8 ms. Eyelid EMG activity could be evoked from a relatively large area of the mesencephalon. However, when the current intensity was below 60 μA, short-latency EMG activity with an onset latency of 5–8 ms could only be evoked from an area in and near the superior cerebellar peduncle (Fig. 3a). The different current thresholds for eliciting short-latency eyelid EMG were combined into an isocurrent map of the area around the ipsilateral superior cerebellar peduncle. In the animal illustrated in Fig. 3a, a stimulus intensity of 15 μA elicited short-latency EMG only from a restricted area within the superior cerebellar peduncle. As illustrated in Fig. 3c (*filled triangles*), the lowest threshold was found within the superior cerebellar peduncle in all animals ($n = 9$), mostly in the middle part of the peduncle. The lowest current threshold was on average 28 μA (median 28 μA; range 15–45 μA). When the current intensity was increased, a contralateral eyelid EMG with somewhat longer latency was elicited (not illustrated; (Ivarsson and Hesslow 1993)). When the intensity was increased even further, contraction of other muscles was observed. The ability of the stimulation to depress periorbitally elicited CFRs was also tested from each mesencephalic stimulation site. A stimulus train consisting of five pulses (200 Hz) and with an intensity of 50 μA was applied 40 ms before the periorbital stimulation. In the animal illustrated in Fig. 3b, the CFR amplitude was maximally depressed to 12% of control when stimulation was applied at a depth of 4.25 mm along the illustrated track (Fig. 3b).

More surprisingly, it was found that the CFRs were also strongly depressed by stimulation in a second area located *within* the middle cerebellar peduncle (Fig. 3b). In six out of seven animals, two areas with distinct maxima of depression were found. One area was found in the superior cerebellar peduncle, closely corresponding to the area from which eye blinks could be elicited with low-current strengths (Fig. 3c, *open circles*). The second area was found about 1 mm further ventrally (Fig. 3c, *open circles*). In the seventh animal, the CFRs could only be inhibited by stimulation in the superior cerebellar peduncle. The inhibitory area within the superior cerebellar peduncle will hereafter be referred to as the dorsal inhibitory area and the ventral one as the ventral inhibitory area.

In some cases we observed a depression of the mossy fibre response as well as of the CFR. Examples are shown in Figs. 1 and 3. This was not seen in all experiments and is not the focus of the present paper but it does raise the question whether the depression of the CFRs results from inhibition of the IO or if it is secondary to an inhibition of a pre-olivary relay.

Inhibition occurs at the inferior olive

The inferior olive has been reported to be inhibited via a rubro-spinal pathway (Weiss et al. 1990). The red nu-

cleus can also inhibit the trigeminal nucleus (Davis and Dostrovsky 1986). It is therefore conceivable that the depression of the CFRs in the present study was caused by an effect on the periorbitally evoked neural signal before reaching the inferior olive. If this were the case, CFRs elicited by direct stimulation of the inferior olive would not be depressed. This possibility was tested in two animals by replacing the periorbital stimulus with direct stimulation of the contralateral dorsal accessory olive (50 μA or 60 μA). The climbing fibre responses were depressed by stimulation in the dorsal inhibitory area to 58 ± 2% and 45 ± 1% of control (Mean ± SEM of ten consecutive trails; *t* test, $P < 0.001$), respectively, and from the ventral area to 43 ± 2% and 36 ± 1% of control (Mean ± SEM of ten consecutive trails; *t* test, $P < 0.001$), respectively. Results from one animal are illustrated in Fig. 4.

Inhibition is not dependent on a red nucleus relay

Stimulation of the red nucleus has previously been reported to inhibit the inferior olive (Horn et al. 1998). It is therefore conceivable that the mesencephalic stimulation activated a red nucleus-dependent mechanism. This possibility was tested by inactivating the red nucleus contralateral to the cortical recording with lignocaine in two animals (1 μl and 3×1 μl, respectively) and by mechanical lesions in six animals.

The red nucleus was considered blocked by the lignocaine injection when stimulation of the superior cerebellar peduncle (three pulses, 100 μA) could no longer elicit any eyelid EMG (Fig. 5a). This occurred at 6 and 8 min after the injection, respectively. The red nucleus inactivation did not change the amplitude of the control CFR. The CFR depression was then tested by stimulating either the dorsal or the ventral inhibitory area. In neither case was the depression caused by stimulation significantly different after lignocaine injections (*t* test, $P > 0.1$; Fig. 5b). Thirty minutes after the lignocaine injection, stimulation of the superior cerebellar peduncle again elicited normal eyelid EMG. Histological examination of the injection areas revealed that the lignocaine had been injected about 1 mm rostral to the red nucleus in both animals. A histological section from the same animal as in Fig. 5b is shown in Fig. 5d. The lignocaine injection did not immediately abolish the eyelid EMG. It is therefore possible that the EMG abolishment was caused by a non-specific inactivation of other structures than the red nucleus. To further investigate the possible role of the red nucleus, it was mechanically removed by a localized aspiration contralateral to the cortical recording ($n = 2$) or disconnected by an intracollicular decerebration ($n = 4$). After the localized aspiration, the eyelid EMG, evoked from the superior cerebellar peduncle, was abolished.

Both the control CFR amplitude and the CFR depression elicited from the dorsal or the ventral inhibitory areas were the same as before the aspiration. The CFR amplitudes were depressed to below 30% of control in both animals. The histological inspection showed that a relatively large area around the red nucleus had been removed. After the inter-collicular decerebration, no eyelid EMG could be evoked by direct activation of the superior cerebellar peduncle. Here, the ventral and the dorsal areas were crudely localized according to coordinates and finely localized by testing for maximal CFR depression. Histological examination revealed that the intra-collicular decerebration only partly removed the red nucleus in two animals. In these two animals, the CFR amplitude was normal and stimulation of the ventral and dorsal area depressed the CFR to below 25% of control. In the two animals with complete removal of the red nucleus, the CFR amplitude was depressed to 23 ± 3% and 18 ± 4% of control (mean ± SD) by stimulation of the ventral area and to 16 ± 3% and 7 ± 3% of control (mean ± SD) by stimulation of the dorsal area (Fig. 5c, e). It should be noted that the intra-collicular level of decerebration also must have removed all of the so-called mesodiencephalic junction since this area is located rostral to the red nucleus. Several parts of this area were most likely also removed in the animals with pre-collicular decerebration.

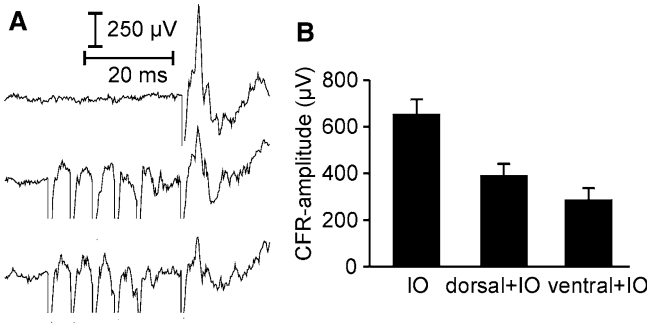

Fig. 4 Inhibition at the IO. **a** Cerebellar surface records show *CFRs* evoked by direct stimulation of the dorsal accessory olive (*top trace*), preceded by stimulation of the dorsal (*middle trace*, *hock artefacts*) or the ventral inhibitory areas (*bottom trace*; *hock artefacts*). **b** Amplitude of *CFRs* (mean ± SD, ten consecutive trials) evoked by direct olivary stimulation (*IO*) and preceded by stimulation of the dorsal (*dorsal + IO*) and the ventral inhibitory areas (*ventral + IO*), respectively

Depression is not caused by olivary activation

Activation of the inferior olive is known to cause a refractory period that lasts about 100 ms (Armstrong et al. 1968; Armstrong and Harvey 1968). It is therefore conceivable that the CFR depression was secondary to olivary activation. To test this hypothesis, we recorded periorbitally elicited CFRs in single Purkinje cells. The all or none character of the CFR during stimulation at the threshold for eliciting a response (stimulus intensity: 150–850 μA) confirmed the unitary nature of the recordings (Fig. 6a, b). When the periorbital stimulus was increased to 1.5 times this

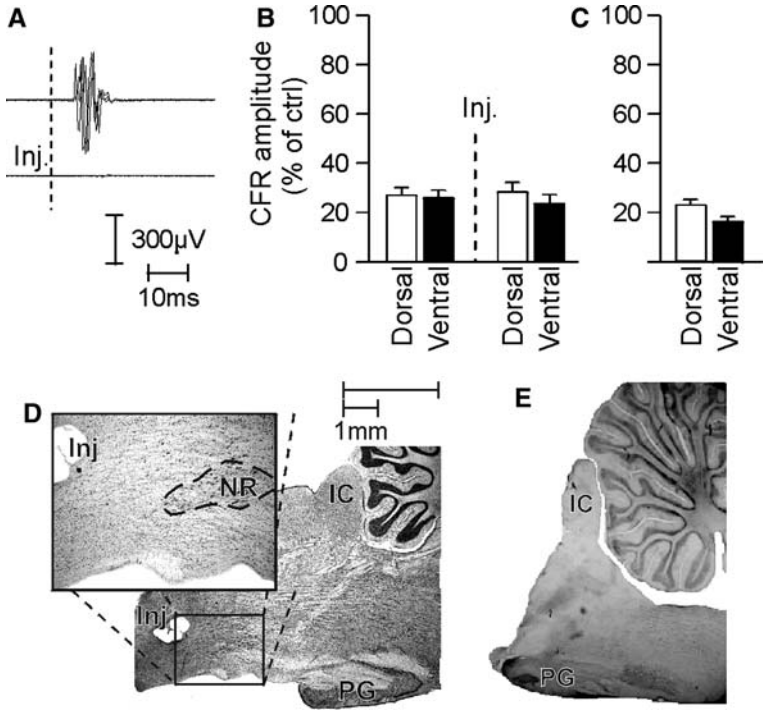

Fig. 5 Pharmacological and mechanical inactivation of the red nucleus. **a** Three superimposed consecutive eyelid *EMG* records before (*upper traces*) and 6 min after (*lower traces*) injection of lignocaine (1 μl). The *vertical line* indicates the onset of the superior cerebellar peduncle stimulus. **b** Amplitude of periorbitally elicited *CFRs* (mean ± SEM; ten consecutive trials) preceded by stimulation of the dorsal and the ventral area (50 μA) before and after lignocaine injection (*Inj.*). **c** Amplitude of periorbitally elicited *CFRs* after intra collicular decerebration (mean ± SEM; ten consecutive trials) preceded by stimulation of the dorsal and the ventral inhibitory areas (60 μA). **d** Sagittal section showing the lignocaine injection site for **a** and **b** (Inj). **e** Sagittal section showing the level of intracollicular decerebration for **b**. *NR*: Red nucleus; *IC*: Inferior colliculus; *PG*: Pontine grey

threshold, CFRs were reliably elicited (Fig. 6d, *upper trace*, e, *upper trace*). A mesencephalic stimulus train (five pulses; 200 Hz) was then applied to the ventral stimulation site at 45 ms before the periorbital stimulation. When the mesencephalic stimulus train elicited a CFR (present in 1.8 ± 1.2 of 20 trials; mean ± SD; $n = 7$), the periorbitally evoked CFR was abolished (Fig. 6c). Periorbitally elicited CFRs were also abolished when stimulation of the ventral area did not elicit any CFR (Fig. 6d, *lower trace*, e, *lower trace*). All seven tested cells displayed a powerful depression in the absence of CFRs elicited by the mesencephalic stimulus (Fig. 6f).

Test of stimulation parameters

Optimal stimulus parameters for CFR depression were determined by testing three different aspects of the mesencephalic stimulation. Since no systematic differences were observed between stimulation of the dorsal ($n = 2$) and the ventral area ($n = 5$), data were treated as one group. The standard stimulus parameters were 60 μA, five pulses, 200 Hz (20 ms duration) with a fixed interval between the mesencephalic stimulus onset and the periorbital stimulus of 45 ms. One parameter was altered while the other parameters were kept constant.

First, current intensities from 10 to 70 μA were tested in six animals (Fig. 7a). The CFR amplitude decreased gradually as the stimulus intensity was increased up to 40 μA. Higher intensities did not further depress the amplitude. When the stimulus intensity was 40 μA, the average CFR was 38% of control.

Second, mesencephalic stimulus trains with different durations from one to seven pulses (200 Hz; train duration from 0.5 to 30 ms) were tested in eight animals (Fig. 7b). The CFR amplitude decreased gradually as the number of pulses was increased from one to five. An increase to six and seven pulses did not further decrease the amplitude. When the mesencephalic stimulus train consisted of five pulses, the average amplitude was 38% of control.

When the interval between the offset of the mesencephalic stimulus train and the periorbital stimulus was kept constant at 25 ms ($n = 3$), the CFR amplitude gradually decreased to 11% of control as the number of pulses was increased from one to five. An increase to six or seven pulses did not further decrease the amplitude. This suggests that the depression was not connected to the offset of the mesencephalic stimulation.

Third, frequencies from 50 to 200 Hz of a 20 ms mesencephalic stimulus train were tested in four animals (Fig. 7d). The CFR amplitude decreased gradually from 91 to 55% of control as the frequency was increased from 50 to 200 Hz.

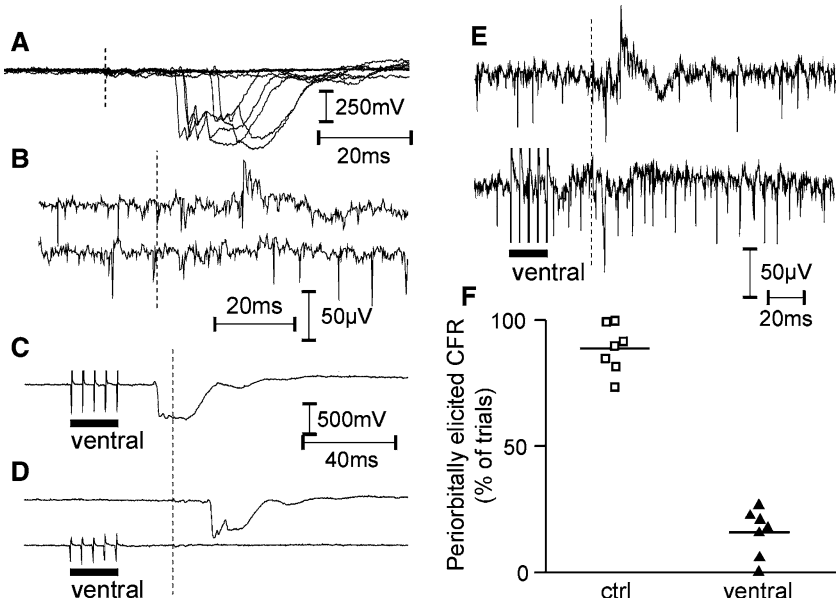

Fig. 6 Inhibition of single olivary neurons. **a** and **b**, Two different *Pc* recordings during stimulation of the periorbital area at threshold intensities for eliciting the *CFR* (**a**, 550 μA, B, 900 μA). **c** *Pc* activity during stimulation of the ventral inhibitory area (*shock artefacts*, 65 μA). **d** and **e** Activity of single *Pcs* during supra-threshold periorbital stimulus (*top traces*) preceded by stimulation of the ventral inhibitory area (*shock artefacts*, 55 μA; *bottom traces*). *Vertical lines* indicate the periorbital stimulation. **f** Number of periorbitally elicited *CFRs* (% of 20 trials) in 7 *Pcs*, without (*ctrl, open squares*) and with preceding stimulation of the ventral inhibitory area (60 μA, *filled triangles*). *Horizontal bar* illustrate mean value

Time course of nucleo-olivary inhibition

In order to determine the onset latency of the olivary inhibition, a stimulus train consisting of one to five pulses (200 Hz; 1–20 ms) was applied to the ventral inhibitory area at 0–50 ms before the periorbital stimulus (60 μA) in three animals (Fig. 8a). During the first intervals of 0–15 ms between the ventral stimulus onset and the periorbital stimulus, the CFR amplitude was never depressed by more than 1 SD of control, that is never below 89% of control. At the interval of 20 ms, the CFR in the three tested animals was depressed to an average 80 ± 4% (mean ± SD; $P < 0.005$) of control with one pulse. The CFR depression caused by two to four pulses was almost identical (61 ± 3% of control; mean ± SD) and the depression caused by five pulses was similar to the one pulse depression (78 ± 3% of control; mean ± SD). This suggests that the stimulus pulses that came after about 20 ms were too late to contribute to the inhibition. As the interval was further increased, the CFR depression gradually increased. The magnitude of depression increased with the duration of the mesencephalic train, consistent with the test of different durations described in the section above. The maximal depression by one pulse occurred at 25, 30 and 40 ms. The average of the maximal depression to one pulse was 72 ± 2% of control (mean ± SD). With the other train durations, the interval for maximal depression was constant in each animal at 30 ms in two animals and 35 ms in one animal. Results of one animal are illustrated in Fig. 8a.

To determine the entire time course of the inhibition, different intervals between the mesencephalic and the

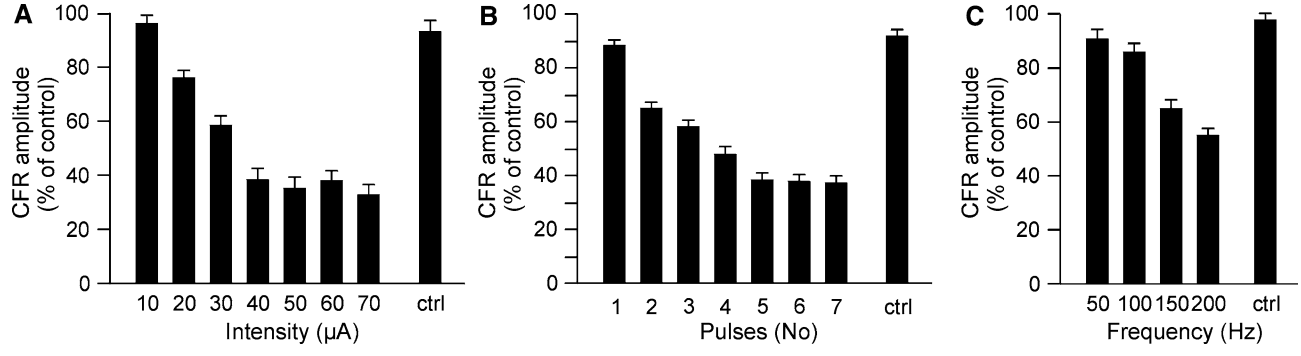

Fig. 7 Amplitudes of peripherally elicited *CFRs* during stimulation of the inhibitory areas with different parameters (Mean and SEM; ten consecutive trials from each animal). Separate tests of the current intensity (**a**; $n = 6$), the number of pulses (**b**; $n = 8$) and the frequency of pulses (**c**; $n = 4$) while the other parameters (60 μA, five pulses, 200 Hz) were kept constant

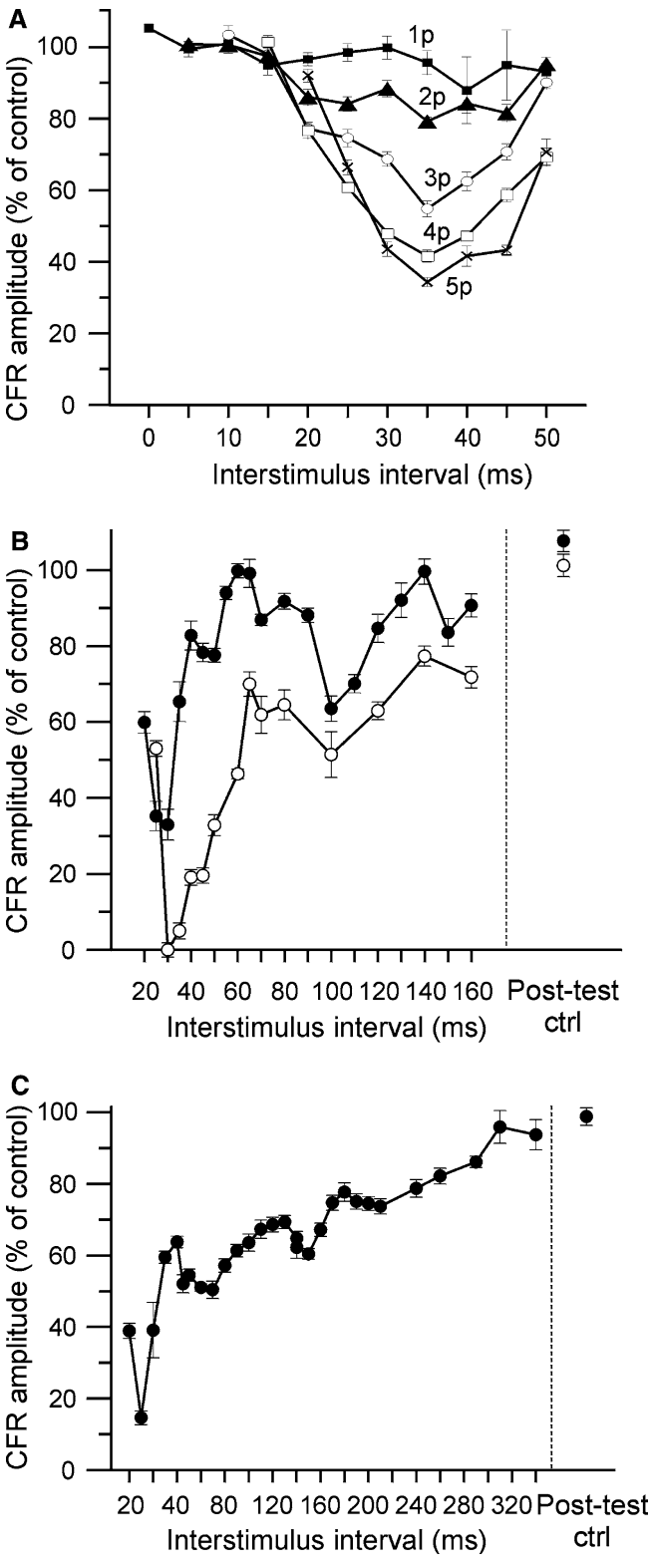

Fig. 8 Time course of the *CFR* inhibition. **a** Amplitude of *CFRs* elicited by periorbital stimulation at different times after the ventral stimulation onset. The duration of the stimulus train applied to the ventral area was one to five pulses (1–20 ms; 200 Hz) as indicated. **b** *CFR* depression from the ventral area in two animals, now with a stimulus duration of five pulses (20 ms, 200 Hz). The animal with the most depressed *CFRs* (*open circles*) and the animal with the least depressed *CFRs* (*filled circles*) are illustrated. **c** *CFR* depression up to 340 ms after the stimulation of the ventral site (five pulses, 20 ms). Note the different time scales. Mean ± SEM of ten consecutive trials

periorbital stimulus onsets were tested in six animals. Here, the duration of the mesencephalic train was kept at 20 ms (five pulses; 200 Hz) since this induced a close to maximal inhibition. The mesencephalic stimulus intensity of 50 μA (30 and 60 μA in two animals, respectively) remained constant in each animal. At the interval of 20 ms, i.e. when the end of the mesencephalic stimulus train coincides with the periorbital stimulus, the CFR amplitude in the six animals was 45 ± 14% of control (mean ± SD). This was the shortest interval tested with the five-pulse train since the stimulus artefacts from the mesencephalic stimulus train made the cortical surface recordings difficult to interpret. When the interval was increased to 30 ms, the depression of the CFRs became stronger. The depression was maximal at 30 ms in five tests in four animals and at 25 and 45 ms in two other animals, respectively. When maximally depressed, the CFR amplitude was 15 ± 15% of control (mean ± SD). The animal with the weakest (filled circles) and that with the strongest depressions (open circles) are illustrated in Fig. 8b. In the study by Hesslow (1986), a small but consistent increase in CFR amplitude was observed during the shortest intervals. Also, stimulation of deep cerebellar nuclei caused an initial excitation followed by a depression in about 30% of recorded olivary cells (Garifoli et al. 2001). In the present study, no such increase was observed in any animal.

Following maximal depression, the CFR amplitude gradually increased. The amplitude increased relatively fast as the interval was increased up to about 70 ms. After this relatively sharp peak of depression, the amplitude increased somewhat slower as the interval was further increased (Fig. 8b, c). The CFR amplitudes remained depressed below 50% of control during 25 ± 16 ms (mean ± SD; range: 5ms–60 ms) and had recovered to above 95% of control after 141 ± 51 ms (mean ± SD; range: 40–310 ms). It is noteworthy that the CFR amplitudes in the animal with the weakest maximal depression (Fig. 8b; filled circles) were least depressed in all the other tests. When the location of the stimulation was compared with the isopotential map after the experiment, it was noticed that the stimulation in this particular animal had been applied outside the area of optimal inhibition.

The peak and duration of the depression was characterized by stimulation of the ventral inhibitory area in five tests in four animals and by stimulation of the dorsal inhibitory area in two animals. The only difference between the effects of dorsal and ventral stimulation was that the latency to the maximal depression was slightly longer (30 and 45 ms) in the two experiments with dorsal

site stimulation. Otherwise, the time courses of inhibition elicited from the two stimulation areas did not differ systematically.

As the olivary inhibition gradually decreased with longer interstimulus intervals, two short-lasting periods (duration: 15–40 ms) of brief depression was observed in all animals as can be seen in Fig. 8c.

Discussion

Mechanism of olivary inhibition

Olivary responses to periorbital stimulation were strongly inhibited by stimulation of either of the two distinct mesencephalic areas. One of these was located within the superior cerebellar peduncle and the other about 1 mm ventraly to the first. Inhibition from both areas was independent of the red nucleus. Since the ventral inhibitory area corresponded perfectly to the location of the nucleo-olivary projection in cat (Legendre and Courville 1987), the most plausible explanation for the inhibition of the olive from the ventral site is that the stimulation activated these fibres.

An alternative interpretation is that stimulation of the ventral site activated output from the red nucleus, which can inhibit the trigeminal relay to the olive (Davis and Dostrovsky 1986) and the olive itself (Horn et al. 1998). However, the CFR depression in the present study remained unchanged after removal of the red nucleus.

The observation in some animals that mossy fibres were depressed by the mesencephalic stimulus raises the question whether CFR depression could have been caused by inhibition at a pre-olivary relay. However, mossy fibres were not always depressed. Furthermore, CFRs elicited by direct stimulation of the inferior olive were also depressed, demonstrating the presence of an inhibitory mechanism acting on the olive itself.

Another possibility is that the stimulation caused a short latency *excitation* of the olive and that the inhibition was due to some kind recurrent inhibition (Armstrong et al. 1968; Armstrong and Harvey 1968). This would explain the long latency of the effect. Such a mechanism is suggested by the observation that CFRs are slightly increased in amplitude just before inhibition sets in after stimulation close to the nucleo-olivary pathway (Hesslow 1986). An indirect excitatory pathway to the olive via the mesodiencephalic junction has also been reported (Ruigrok and Voogd 1995). Indeed, when the mesencephalic stimulation elicited a CFR in a single Purkinje cell, the periorbital stimulation could not evoke any CFR in the same Purkinje cell. However, in the present study we found that when the intensity of the mesencephalic stimulus was reduced below threshold for eliciting CFRs, the periorbitally elicited CFRs were still abolished. This supports the conclusion that a true inhibitory pathway was activated.

It could also be suggested that the olivary neurons were inhibited by other olivary neurons projecting to a neighbouring micro-zone (Andersson 1984). But such an inhibitory mechanism seems unlikely. Stimulation of the mesencephalon must have activated nucleo-olivary fibres indiscriminately, which in turn would inhibit several parts of the olive including the neurons projecting to the micro-zone from which we recorded as well as those projecting to neighbouring microzones.

A puzzling finding in the present study was that the olive could be inhibited by stimulation not only below, but also within the superior cerebellar peduncle. All characteristics of this inhibition were identical to that elicited from the ventral area, strongly suggesting that stimulation of both areas activated the same underlying mechanism. If we assume that the inhibition is caused by the nucleo-olivary pathway, there is a very simple explanation, namely collateral activation of interpositus neurones. It seems plausible that the excitatory output neurons of the interpositus nucleus drive the olive projecting neurons via collaterals. Antidromic activation of the superior cerebellar peduncle would then be expected to have much the same effect as direct stimulation of the nucleo-olivary pathway. Conversely, it is difficult to think of any other structure that might be activated by stimulation of the two sites and that could plausibly cause an inhibition of the inferior olive.

Time course of nucleo-olivary inhibition

The onset latency of the inhibition was determined by stimulating the presumed nucleo-olivary pathway with a train consisting of one to five pulses. Inhibition was usually observed 20 ms after the onset of the stimulation. A backward extrapolation of the inhibition time course to a five-pulse train also indicates such an onset. By adding 5 ms, which is the approximate conduction time from the periorbital area to the olive, the onset latency of the nucleo-olivary inhibition was estimated to be around 25 ms. The latency could also be estimated from another set of data. When testing different durations of the mesencephalic stimulus train, an increased number of pulses, up to five pulses, gradually increased the inhibition while pulses' number six and seven did not further increase the inhibition. The reason for the saturation could be that pulse six and seven reached the dorsal accessory olive too late. The latency of the nucleo-olivary inhibition could therefore be estimated by adding 5 ms, the conduction time from the periphery to the olive, to the time interval from just before the sixth pulse to the periorbital stimulus. This gives an onset latency of about 20–25 ms. These estimates are shorter than the previously reported latency of a putative nucleo-olivary inhibition in cat (Hesslow 1986; Ruigrok and Voogd 1995). The discrepancies may be due to differences in species or anaesthesia. Also, Ruigrok and Voogd (1995) recorded from the principal and the medial accessory olive.

The long onset latency of the nucleo-olivary inhibition is difficult to understand. The diameter of the nucleo-olivary fibres is small but cannot explain such a long delay. Furthermore, the latency of antidromically evoked nuclear responses on olivary stimulation is only around 1 ms (Ban and Ohno 1977; Tolbert et al. 1978). The long latency of nucleo-olivary inhibition might be caused by a polysynaptic nucleo-olivary pathway. However, the vast majority of inhibitory olivary afferents originates in the deep cerebellar nuclei. Furthermore, even a di- or trisynaptic pathway would be insufficient to explain the latency.

A more likely possibility is that the long latency is due to slow receptor mechanisms. In vitro work in the hippocampus has revealed hyperpolarising responses to synaptic stimulation with latencies of 20–50 ms mediated by metabotropic $GABA_B$ receptors (Thalmann and Ayala 1982; Mott et al. 1999). $GABA_B$ receptors are present in the olive (Turgeon and Albin 1994). There is also evidence that the α_3 subunit can slow activation of $GABA_A$ receptors (Gingrich et al. 1995). The α_3 subunit is present in $GABA_A$ receptors on the somata of olivary cells, which show delayed responses to GABA (Devor et al. 2001). In the latter experiments, GABA was applied by pressure injection so the exact timing of the responses is less clear.

Nucleo-olivary inhibition and olivary oscillation

Olivary neurons have the capacity to oscillate at about 10 Hz and it has been suggested that the nucleo-olivary pathway regulates the gap junction coupling between oscillating neurons (Devor and Yarom 2000; Lang et al. 1996; Llinas et al. 1974; Llinas and Yarom 1981, 1986). In this context it is of interest that two periods of brief inhibition occurred at regular intervals after the maximal inhibition. However, these two periods may be an artefact caused by an abnormally strong and synchronous activation of the nucleo-olivary fibres, but it is also possible that they reflect a real biological phenomenon where the nucleo-olivary pathway sets the start, and thereby the phase, of olivary oscillations.

Nucleo-olivary inhibition in classical conditioning

The association between the conditioned and the unconditioned stimuli in classical eyeblink conditioning is known to occur within the cerebellum (Christian and Thompson 2003; Hesslow and Yeo 2002). The conditioned stimulus, which elicits the conditioned response, is transmitted by the cerebellar mossy fibre afferents (Hesslow et al. 1999). The pathway for the unconditioned stimulus is generally assumed to be the climbing fibres (Albus 1971; Thompson and Krupa 1994; Yeo and Hesslow 1998). It has been suggested that the neural activity in deep cerebellar nuclei that generates the conditioned response also inhibits the olive at the time of the unconditioned stimulus (Andersson et al. 1988). In support of this, it has been reported (Hesslow and Ivarsson 1996; Sears and Steinmetz 1991) that the conditioned eyeblink responses are associated with an inhibition of the olive at the time of the unconditioned stimulus. The fact that the peak of the depression in the present study was sharp (below 50% for only 25 ms, cf. Fig. 5) adds a new dimension to the hypothesis about unconditioned stimulus inhibition. The conditioned stimulus not only has to be present to inhibit the unconditioned stimulus but also has to be correctly timed. This could mean that the nucleo-olivary pathway is important for fine-tuning the timing of the conditioned response so that the response becomes and remains time-locked to the onset of the unconditioned stimulus. For instance, when a correctly timed conditioned response inhibits the olivary-transmitted unconditioned stimulus signal, an extinction process would begin.

During this extinction the conditioned response not only becomes less frequent but also more erroneously timed. This, in turn, would lead to less olivary inhibition at the time of the unconditioned stimulus and a greater probability for the unconditioned stimulus signal to reach the cerebellum. Re-acquisition would therefore begin. In this way, the reinforcement by the unconditioned stimulus is under continuous control by the timing of the conditioned response via the nucleo-olivary pathway. The fine timing of the conditioned response is thereby self-sustaining.

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References

Albus J (1971) A theory of cerebellar function. Math Biosci 10:25–61

Andersson G (1984) Mutual inhibition between olivary cell groups projecting to different cerebellar microzones in the cat. Exp Brain Res 54:293–303

Andersson G, Hesslow G (1987) Activity of Purkinje cells and interpositus neurones during and after periods of high frequency climbing fibre activation in the cat. Exp Brain Res 67:533–542

Andersson G, Garwicz M, Hesslow G (1988) Evidence for a GABA-mediated cerebellar inhibition of the inferior olive in the cat. Exp Brain Res 72:450–456

Angaut P, Sotelo C (1987) The dentato-olivary projection in the rat as a presumptive GABAergic link in the olivo-cerebello-olivary loop. An ultrastructural study. Neurosci Lett 83:227–231

Angaut P, Sotelo C (1989) Synaptology of the cerebello-olivary pathway. Double labelling with anterograde axonal tracing and GABA immunocytochemistry in the rat. Brain Res 479:361–365

Armstrong DM (1990) Topographical localisation in the projections from the inferior olive to the paravermal cortex of the anterior lobe and paramedian lobule in the cerebellum of the cat. A brief review. Arch Ital Biol 128:183–207

Armstrong DM, Harvey RJ (1968) Responses to a spino-olivocerebellar pathway in the cat. J Physiol (Lond) 194:147–168

Armstrong DM, Eccles JC, Harvey RJ, Matthews PB (1968) Responses in the dorsal accessory olive of the cat to stimulation of hind limb afferents. J Physiol (Lond) 194:125–145

Ban M, Ohno T (1977) Projection of cerebellar nuclear neurones to the inferior olive by descending collaterals of ascending fibres. Brain Res 133:156–161
Bengtsson F, Svensson P, Hesslow G (2004) Feedback control of purkinje cell activity by the cerebello-olivary pathway. Eur J Neurosci 20(11):2999–3005
Cerminara NL, Rawson JA (2004) Evidence that climbing fibers control an intrinsic spike generator in cerebellar Purkinje cells. J Neurosci 24:4510–4517
Christian KM, Thompson RF (2003) Neural substrates of eyeblink conditioning: acquisition and retention. Learn Mem 10:427–455
Colin F, Manil J, Desclin JC (1980) The olivocerebellar system. I. Delayed and slow inhibitory effects: an overlooked salient feature of cerebellar climbing fibers. Brain Res 187:3–27
Davis KD, Dostrovsky JO (1986) Modulatory influences of red nucleus stimulation on the somatosensory responses of cat trigeminal subnucleus oralis neurons. Exp Neurol 91:80–101
De Zeeuw CI, Holstege JC, Calkoen F, Ruigrok TJ, Voogd J (1988) A new combination of WGA-HRP anterograde tracing and GABA immunocytochemistry applied to afferents of the cat inferior olive at the ultrastructural level. Brain Res 447:369–375
De Zeeuw CI, Holstege JC, Ruigrok TJ, Voogd J (1989) Ultrastructural study of the GABAergic, cerebellar, and mesodiencephalic innervation of the cat medial accessory olive: anterograde tracing combined with immunocytochemistry. J Comp Neurol 284:12–35
Demer JL, Echelman DA, Robinson DA (1985) Effects of electrical stimulation and reversible lesions of the olivocerebellar pathway on Purkinje cell activity in the flocculus of the cat. Brain Res 346:22–31
Devor A, Yarom Y (2000) GABAergic modulation of olivary oscillations. Prog Brain Res 124:213–220
Devor A, Fritschy JM, Yarom Y (2001) Spatial distribution and subunit composition of GABA(A) receptors in the inferior olivary nucleus. J Neurophysiol 85:1686–1696
Dietrichs E, Walberg F (1981) The cerebellar nucleo-olivary projection in the cat. Anat Embryol (Berl) 162:51–67
Dietrichs E, Walberg F (1986) The cerebellar nucleo-olivary and olivocerebellar nuclear projections in the cat as studied with anterograde and retrograde transport in the same animal after implantation of crystalline WGA-HRP. III. The interposed nuclei. Brain Res 373:373–383
Garifoli A, Scardilli G, Perciavalle V (2001) Effects of cerebellar dentate nucleus GABAergic cells on rat inferior olivary neurons. Neuroreport 12:3709–3713
Garwicz M (1997) Sagittal zonal organization of climbing fibre input to the cerebellar anterior lobe of the ferret. Exp Brain Res 117:389–398
Gellman R, Houk JC, Gibson AR (1983) Somatosensory properties of the inferior olive of the cat. J Comp Neurol 215:228–243
Gingrich KJ, Roberts WA, Kass RS (1995) Dependence of the GABAA receptor gating kinetics on the alpha-subunit isoform: implications for structure-function relations and synaptic transmission. J Physiol 489:529–543
Graybiel AM, Nauta HJ, Lasek RJ, Nauta WJ (1973) A cerebello-olivary pathway in the cat: an experimental study using autoradiographic tracing technics. Brain Res 58:205–211
Hesslow G (1986) Inhibition of inferior olivary transmission by mesencephalic stimulation in the cat. Neurosci Lett 63:76–80
Hesslow G (1994) Correspondence between climbing fibre input and motor output in eyeblink-related areas in cat cerebellar cortex. J Physiol (Lond) 476:229–244
Hesslow G, Ivarsson M (1996) Inhibition of the inferior olive during conditioned responses in the decerebrate ferret. Exp Brain Res 110:36–46
Hesslow G, Yeo CH (2002) The functional anatomy of skeletal conditioning. In: Moore JW (ed) A neuroscientist's guide to classical conditioning. Springer, New York, pp 86–146
Hesslow G, Svensson P, Ivarsson M (1999) Learned movements elicited by direct stimulation of cerebellar mossy fiber afferents. Neuron 24:179–185
Horn KM, Hamm TM, Gibson AR (1998) Red nucleus stimulation inhibits within the inferior olive. J Neurophysiol 80:3127–3136
Ito M (1984) The cerebellum and neuronal control. Raven, New York
Ito M (1989) Long-term depression. Annu Rev Neurosci 12:85–102
Ivarsson M, Hesslow G (1993) Bilateral control of the orbicularis oculi muscle by one cerebellar hemisphere in the ferret. Neuroreport 4:1127–1130
Kim JJ, Krupa DJ, Thompson RF (1998) Inhibitory cerebello-olivary projections and blocking effect in classical conditioning. Science 279:570–573
Lang EJ, Sugihara I, Llinas R (1996) GABAergic modulation of complex spike activity by the cerebellar nucleoolivary pathway in rat. J Neurophysiol 76:255–275
Legendre A, Courville J (1987) Origin and trajectory of the cerebello-olivary projection: an experimental study with radioactive and fluorescent tracers in the cat. Neuroscience 21:877–891
Linden DJ, Connor JA (1995) Long-term synaptic depression. Annu Rev Neurosci 18:319–357
Llinas R, Yarom Y (1981) Properties and distribution of ionic conductances generating electroresponsiveness of mammalian inferior olivary neurones in vitro. J Physiol (Lond) 315:569–584
Llinas R, Yarom Y (1986) Oscillatory properties of guinea-pig inferior olivary neurones and their pharmacological modulation: an in vitro study. J Physiol 376:163–182
Llinas R, Baker R, Sotelo C (1974) Electrotonic coupling between neurons in cat inferior olive. J Neurophysiol 37:560–571
Martin GF, Henkel CK, King JS (1976) Cerebello-olivary fibers: their origin, course and distribution in the North American opossum. Exp Brain Res 24:219–236
Montarolo PG, Palestini M, Strata P (1982) The inhibitory effect of the olivocerebellar input on the cerebellar Purkinje cells in the rat. J Physiol (Lond) 332:187–202
Mott DD, Li Q, Okazaki MM, Turner DA, Lewis DV (1999) GABA(B)-receptor-mediated currents in interneurons of the dentate-hilus border. J Neurophysiol 82:1438–1450
Nelson B, Mugnaini E (1989) Origins of GABA-ergic inputs to the inferior olive. In: Strata P (eds) The olivocerebellar system in motor control. Springer Berlin Heidelberg New York pp 86–107
Oscarsson O (1980) Functional organisation of olivary projection to the cerebellar anterior lobe. In: Courville J, de Montigny C, Lamarre Y (eds) The inferior olivary nucleus: anatomy and physiology. Raven, New York, pp 279–289
Rawson JA, Tilokskulchai K (1981) Suppression of simple spike discharges of cerebellar Purkinje cells by impulses in climbing fibre afferents. Neurosci Lett 25:125–130
Ruigrok TJ (1997) Cerebellar nuclei: the olivary connection. Prog Brain Res 114:167–192
Ruigrok TJ, Voogd J (1995) Cerebellar influence on olivary excitability in the cat. Eur J Neurosci 7:679–693
Savio T, Tempia F (1985) On the Purkinje cell activity increase induced by suppression of inferior olive activity. Exp Brain Res 57:456–463
Sears LL, Steinmetz JE (1991) Dorsal accessory inferior olive activity diminishes during acquisition of the rabbit classically conditioned eyelid response. Brain Res 545:114–122
Sotelo C, Gotow T, Wassef M (1986) Localization of glutamic-acid-decarboxylase-immunoreactive axon terminals in the inferior olive of the rat, with special emphasis on anatomical relations between GABAergic synapses and dendrodendritic gap junctions. J Comp Neurol 252:32–50
Thalmann RH, Ayala GF (1982) A late increase in potassium conductance follows synaptic stimulation of granule neurons of the dentate gyrus. Neurosci Lett 29:243
Thompson RF, Krupa DJ (1994) Organization of memory traces in the mammalian brain. Annu Rev Neurosci 17:519–549
Tolbert DL, Massopust LC, Murphy MG, Young PA (1976) The anatomical organization of the cerebello-olivary projection in the cat. J Comp Neurol 170:525–544
Tolbert DL, Bantli H, Bloedel JR (1978) Multiple branching of cerebellar efferent projections in cats. Exp Brain Res 31:305–316

Turgeon SM, Albin RL (1994) Postnatal ontogeny of gaba(b) binding in rat-brain. Neuroscience 62:601–613
Weiss C, Houk JC, Gibson AR (1990) Inhibition of sensory responses of cat inferior olive neurons produced by stimulation of red nucleus. J Neurophysiol 64:1170–1185
Yeo CH, Hesslow G (1998) Cerebellum and conditioned reflexes. Trends Cogn Sci 2:322–330