Bilateral disruption of conditioned responses after unilateral blockade of cerebellar output in the decerebrate ferret

M. Ivarsson, P. Svensson and G. Hesslow

Department of Physiology and Neuroscience, Lund University, Sölvegatan 19, S-223 62 Lund, Sweden

1. Lesions of the cerebellar cortex can abolish classically conditioned eyeblink responses, but some recovery with retraining has been observed. It has been suggested that the recovered responses are generated by the intact contralateral cerebellar hemisphere. In order to investigate this suggestion, bilaterally acquired conditioned responses were studied after the unilateral blockade of cerebellar output.

2. Decerebrate ferrets were trained with ipsilateral electrical forelimb stimulation (300 ms, 50 Hz, 1 mA) as the conditioned stimulus and bilaterally applied peri-orbital stimulation (40 ms, 50 Hz, 3 mA) as the unconditioned stimulus. The conditioned and unconditioned eyeblink responses were monitored by EMG recordings from the orbicularis oculi muscle. The output from one cerebellar hemisphere was blocked either by injecting small amounts of lignocaine (lidocaine; 0·5–1·0 μl) into the brachium conjunctivum, or by a restricted mechanical lesion of the brainstem rostral to the cerebellum.

3. As described by previous investigators, the unilateral blockade of cerebellar output abolished ipsilateral conditioned responses.

4. More importantly, such blockade also abolished or strongly depressed contralateral conditioned responses. When mechanical lesions of the brachium conjunctivum were made, contralateral responses, in contrast to ipsilateral responses, recovered within 1–2·5 h.

5. When the unconditioned stimulus was removed on one side, causing extinction of conditioned responses on this side, conditioned responses were temporarily depressed on the trained side as well.

6. Unilateral interruption of cerebellar output had no clear effect on contralateral unconditioned reflex responses.

7. The results demonstrate that one cerebellar hemisphere in ferrets exerts a marked control of contralateral conditioned eyeblink responses, probably via premotor neurones involved specifically in conditioned, and not in unconditioned, responses.

Since the early observations by McCormick & Thompson, (1984) that cerebellar lesions abolish classically conditioned eyeblink responses, a considerable amount of data has been accumulated supporting the hypothesis that the cerebellum is necessary for both the learning and performance of such responses. Lesions and reversible inactivations of the ipsilateral deep cerebellar nuclei abolish conditioned responses (CRs) while having a smaller effect on unconditioned responses (URs). CRs are also abolished by aspirations of the hemispheral lobule VI of the cerebellar cortex which leaves the nuclei intact. Lesions and pharmacological inactivation of the output from the cerebellum via the brachium conjunctivum (BC) and the red nucleus have the same effect (see Thompson, 1990; Yeo, 1991 for reviews and further references). A reversible inactivation of the lateral cerebellum has been shown to prevent learning of a CR, while blockade of the red nucleus only prevented the expression of a learned response (Krupa, Thompson & Thompson, 1993). Brief electrical stimulation of specific areas in the cerebellar cortex that control eyeblink can inhibit a CR without affecting the unconditioned response (Hesslow, 1994*b*). Purkinje cells in these areas respond with climbing fibre responses to tapping around the eye and show a powerful suppression of their simple spike firing in eyeblink-conditioned animals (Hesslow & Ivarsson, 1994). Although there is some disagreement concerning the relative importance of the cerebellar nuclei and cortex (Lavond, Steinmetz, Yokaitis & Thompson, 1987; Lavond & Steinmetz, 1989; Yeo & Hardiman, 1992) almost all studies agree that interference with the cerebellum impairs conditioning.

One interpretation of these findings is that the cerebellum is involved in some essential way in the learning and generation of CRs (Thompson, 1990). This interpretation has been challenged by others who claim that the effects observed after interference with the cerebellum are due to a deficit only in the performance of the CR, not in the learning (Welsh & Harvey, 1989; Kelly, Zuo & Bloedel, 1990). These investigators have suggested that the cerebellum provides a tonic background facilitation of the motor pathways involved in the generation of an eyeblink and that the loss of this facilitation induced by interference with the cerebellum is what abolishes CRs.

This alternative explanation of the lesion and inactivation data has been supported, for instance, by the observations that cerebellar lesions and inactivations of the red nucleus, not only affect the CRs, but also the URs (Welsh & Harvey, 1989; Bracha, Stewart & Bloedel, 1993). This effect had been missed in previous studies, possibly because supramaximal stimuli (in most studies an air puff directed towards the cornea) had been used as unconditioned stimuli (USs), but when weaker air puffs were tested, it was found that the UR frequency was depressed and the latency increased after cerebellar lesions. It was concluded that the lesions caused a non-specific depression of the performance of eyeblinks. Although other groups have obtained different results (e.g. Steinmetz, Lavond, Ivkovich, Logan & Thompson, 1992; Ivkovich, Lockard & Thompson, 1993), the Welsh & Harvey report has been widely cited (e.g. Llinás & Welsh, 1993) as evidence against a cerebellar locus of conditioning. It should be noted that this evidence is not, strictly speaking, incompatible with a cerebellar involvement in learning. It merely shows that an alternative explanation is possible.

More direct evidence against a critical role for the cerebellum in classical conditioning is supplied from observations which indicate that cerebellar lesions in decerebrates are not as effective in abolishing CRs as the early experiments in intact animals suggested. For instance, the report by Kelly *et al.* (1990) suggests that the cerebellum may not be important at all in the learning of a CR. They trained decerebrate rabbits to emit stable CRs and then removed the ipsilateral cerebellum by aspiration. The rabbits could still give CRs but with a more variable frequency than the non-decerebellate animals. Although it has been questioned whether the CRs in this study were authentic CRs (Nordholm, Lavond & Thompson, 1991), the paper by Kelly *et al.* (1990) has also been cited as evidence against a cerebellar locus of conditioning.

A similar difficulty is the fact that many lesions of the cerebellar cortex have failed to abolish conditioning (McCormick & Thompson, 1984; Lavond *et al.* 1987). These results have been interpreted as evidence that the critical site of learning is in the cerebellar nuclei rather than in the cortex. Others have found that even when conditioning has been abolished after cerebellar cortical lesions, continued post-operative training often causes some recovery of CRs, although always with changes in topography and onset latency (Lavond *et al.* 1987; Harvey, Welsh, Yeo & Romano, 1993). Recovery of CRs could also mean that conditioning does not involve the cerebellum at all, except in the sense that it facilitates an extracerebellar CR pathway.

An alternative explanation for the reoccurrence of ipsilateral CRs after ipsilateral cortical lesions and extensive training, which would be consistent with the above observations, is the suggestion by Yeo & Hardiman (1992) that CRs can be generated by the contralateral cerebellar hemisphere. There are two observations which suggest that this is a possible mechanism. Firstly, there is direct physiological evidence of a bilateral pathway from one cerebellar hemisphere to the orbicularis oculi muscles (Ivarsson & Hesslow, 1993). Electrical stimulation of the BC evoked both ipsi- and contralateral EMG activity in the orbicularis oculi muscles. Secondly, there is evidence that training on one side causes some contralateral learning. For instance, when an animal has learned to emit stable CRs on one side and the training is then shifted to the other side, it takes fewer acquisition trials to reach the learning criterion than it did originally. But if the contralateral side has learned and there is a crossed motor output from the cerebellum, it should not be surprising that some small CRs can occur after an ipsilateral lesion. This suggestion has recently received some confirmation in work by Gruart & Yeo (1995). They compared the effect of unilateral and bilateral cortical lesions, respectively, in well-trained rabbits and found that while unilateral lesions did not cause a permanent loss of CRs, bilateral lesions did.

In this report we will present direct evidence demonstrating that one cerebellar hemisphere exerts a marked control over contralaterally generated conditioned eyeblink responses.

Some of the results have been presented in a preliminary form (Ivarsson, Svensson & Hesslow, 1995).

METHODS

Anaesthesia and surgery

The experiments were performed on fifteen male ferrets (1·4–1·9 kg). The animals were deeply anaesthetized with halothane or isoflurane (ISC Chemicals Ltd, UK, or Abbott Laboratories Ltd, UK, respectively; 1·2–1·5% in a mixture of $O_2 > 30\%$ and $N_2O < 70\%$). They were initially placed in a box into which anaesthetic gas was directed. When deep anaesthesia had been achieved, a tracheotomy was performed and the gas was led directly into a tracheal tube. The animal was put on artificial ventilation. The level of anaesthesia was monitored by testing withdrawal reflexes. In order to prevent oedema of the cerebellum and disseminated coagulation, 0·5 ml betamethasone (Betapred, I.V. 4 mg ml^{-1}; Glaxo Operations Ltd, Greenford, UK) and 0·5 ml doktacillin (I.M. 250 mg ml^{-1}; Astra, Södertälje, Sweden) were given at the start of the experiments.

After the head of the animal had been fixed to a stereotaxic frame and the skull had been opened, a decerebration was performed. In

previous experiments in which all of the forebrain was removed, it was very difficult to control bleeding from the middle cerebral artery. For this reason, the most rostrolateral part of the cerebral cortex and the head of the caudate nucleus were spared. About two-thirds of the forebrain, all of the thalamus, most hypothalamic structures rostral to the superior colliculus and large parts of the basal ganglia were removed by aspiration. This exposed the cerebellum and the superior and inferior colliculi of the brainstem. In order to eliminate any possibility of ascending input to the remaining parts of hypothalamus and forebrain, the brainstem was sectioned at the rostral border of the superior colliculus and the red nucleus with a blunt spatula. In two animals, which were used in a different study but prepared in a similar manner, bilateral EEG recordings from several sites on the remaining cortex several hours after the decerebration, showed no activity. The extent of the aspiration and the completeness of the brainstem section was always checked by post-mortem examination. Bleeding was controlled with Gelfoam (Ferrosan, Søborg, Denmark). A pool was built around the cerebellum with cotton-reinforced agar and filled with warm (37 °C) mineral oil. A small hole in the cerebellar dura mater was made so that the border between the inferior colliculus and the cerebellum was exposed.

After decerebration the anaesthesia was terminated. The arterial blood pressure was measured continuously through a catheter inserted into the right femoral artery. The end-expiratory CO_2 concentration and the rectal temperature were also monitored throughout the experiment and kept within physiological limits. At the end of the experiment the decerebrate animals were killed by exsanguination through the femoral catheter.

The experimental procedures used were approved in advance by the local ethics committee.

Stimulation

The conditioned stimulus (CS) was a 300 ms, 50 Hz train of electrical stimuli (0·1 ms, square-wave pulse) applied through two needle electrodes inserted subcutaneously 15 mm apart on the proximal part of the left forelimb. The strength was 1 mA, which was usually sufficient to elicit weak reflex movements of the forelimb.

The unconditioned stimulus (US) was a 50 Hz train of three electrical stimuli (0·5 ms duration, negative square-wave pulse) applied bilaterally starting 300 ms after CS onset. The animal received the US through two needle electrodes inserted 5 mm apart in the lower eyelid, close to the infraorbital nerve. The stimulus strength on both sides was 3–4 mA. These stimulus strengths were chosen to reliably evoke a maximal eyeblink reflex. For reasons given in the Results, in order to study the UR on US-alone trials we occasionally also used stimulus strengths of 0·5–2 mA.

It has been shown previously that classical conditioning training with these parameters in the ferret does not cause either sensitization or pseudoconditioning (Hesslow & Ivarsson, 1996).

The intertrial interval (ITI) was kept constant at 20 s throughout most of the experiment. In order to exclude the possibility that the responses acquired during training were due to temporal conditioning, the ITI was occasionally increased in a pseudorandom manner to 30–60 s for at least 5 min. The acquired responses always remained time-locked to the CS. This test was performed in every animal.

Surgery, mounting of the animal in a stereotaxic frame and placement of electrodes, etc., took 3–4 h. Conditioning training on both ipsi- and contralateral sides was started and continued until the animal emitted CRs on at least 95% of the trials. CRs were always easy to distinguish and there were no problems in ruling non-CRs out. The training had to be discontinued for periods of varying duration in order to localize the part of the BC related to eyeblink.

Recording

The eyeblink responses, from both sides, were monitored by EMG recordings from the orbicularis oculi muscles through two stainless-steel electrodes about 5 mm apart. They were inserted into the upper eyelid about 5 mm above the lateral margin.

EMG responses were rectified and integrated during predetermined time intervals in every CS–US trial. The EMG activity during a 100 ms interval before CS onset was used to estimate the spontaneous activity of the orbicularis oculi muscle. The CR size was calculated from EMG activity during the interval 80–298 ms after CS onset. This interval was chosen because it included all of the CR activity and excluded, as much as possible, interference from spontaneous eyeblinks. In previous studies during the last couple of years, we have studied more than a hundred animals that have been trained with the parameters described above and which have acquired responses with stable latencies and amplitudes to the CS. Only in one of those animals did the learned response have a latency under 80 ms. The high-pass filtration of the EMG signal distorted the records slightly before the shock artefact caused by the US at 300 ms. To eliminate this error, the upper limit of the integration interval was set to 298 ms.

The interval used to calculate the size of the UR was 5–19 ms after US onset. The stimulus strength of 3 mA elicited EMG activity in the orbicularis oculi muscle in our preparation that lasted from 5–18 ms after US onset and the interval chosen thus included all of the reflex activity. In order to compare EMG activity during intervals of different lengths the size of the responses was expressed per millisecond (spontaneous activity and CRs). The trials immediately before extinction training, injection of lignocaine in the BC or lesion of the BC were used as control sessions and the mean size of the CRs in these trials were estimated. The EMG activity was then expressed as a percentage of the mean size of the CRs during the control sessions. A consequence of this is that the size measures include the spontaneous background activity. When CRs were abolished, the size of the responses in the graphs may therefore be larger than zero.

EMG records were converted to digital data with an A/D converter from RC Electronics Inc. (Goleta, CA, USA) and the acquisition and analysis of the data was made by software from our own department.

Lignocaine injections

When stable CRs had been established bilaterally the conditioning training was stopped and a tungsten electrode was lowered in a series of tracks at various distances from the mid-line into the anterior part of the cerebellum close to its border with the inferior colliculus. Single-pulse stimuli or trains of stimuli were given at different depths in each track in order to locate the site with the lowest threshold for eliciting EMG activity in the ipsilateral orbicularis oculi muscle. A site where EMG activity could be elicited with a stimulus strength of 8–15 μA was usually located about 3 mm lateral to the mid-line and at a depth of 4–5 mm. If the electrode was placed above or below this site the threshold increased. The depth was in agreement with earlier findings (Ivarsson & Hesslow, 1993) and previous histological localizations of the BC in the ferret. The same co-ordinates were then used to place a micropipette (tip diameter about 50–100 μm) attached to a Hamilton syringe (Hamilton Bonaduz Ag, Bonaduz, Switzerland)

and 0·5–1·0 μl of 4% lignocaine (lidocaine) HCl (Xylocaine; Astra, Södertälje, Sweden) was injected at this site. The injection site was later verified histologically. The micropipette was left in the tissue during formalin fixation, thereby leaving a visible track in the tissue. When examined under the microscope, the end of the track was interpreted as the location of the tip of the micropipette. An example of the location of the site of injection is shown in Fig. 2*C*. In some cases, it was difficult to identify the pipette track in the sections. Because of our previous experience with histologically confirmed stimulation of the ferret BC, however, we are confident that the micropipette placements cannot have erred by more than a millimetre, which in the present context would not invalidate our conclusions.

Mechanical lesions

In three of the experiments a unilateral mechanical lesion of the BC was made. To make a complete lesion, sharp watchmaker's forceps were lowered in the rostrocaudal direction at the border between the inferior colliculus and the cerebellum with a 2–3 mm distance between the tips and to a depth of 4–5 mm. The area between the tips was completely cut off by the forceps and the extent of the lesion was histologically verified (one example is shown in Fig. 3*C*). In two other experiments a mechanical lesion of the BC was aimed for but the lesion did not involve the BC.

Histology

The animals were perfused with sodium chloride followed by 10% (w/v) formaldehyde in H_2O. After each experiment, the cerebellum was removed from the skull and stored in 10% formaldehyde in H_2O for at least 2 weeks. Before sectioning, the tissue was placed in a sucrose (30% w/v)–phosphate buffer (0·2 M, pH 7·7) solution. The brains were frozen and sectioned either sagittally or transversely in 50 or 60 μm slices. These were mounted and stained with Cresyl Violet and then examined under a microscope.

RESULTS

Conditioned responses

Training of the animal with paired CS–US stimuli began about 1 h after completion of the surgery and was continued until CRs with similar latency and size occurred on virtually every trial on both sides. This required 200–300 trials. In three of the animals the first CRs appeared after only around forty trials, but these animals needed 200–300 trials to reach a stable level of responding. The onset latency of the CRs varied little from trial to trial in the same well-trained animal, but varied between 80–140 ms after CS onset between animals. Representative samples of CRs, with recordings from both orbicularis oculi muscles, are shown in Fig. 2*B*.

The first question that we addressed was whether the two sides really could generate CRs independently after bilateral learning. Four animals were therefore presented with a reinforcing US on one side and extinction training, i.e. no US on the contralateral side, after they had learned to generate CRs bilaterally. Figure 1 shows the extinction of the CRs on the non-reinforced side in two animals. In Fig. 1*A* the reinforced side continues to emit CRs, while the CRs on the non-reinforced side gradually disappear. When

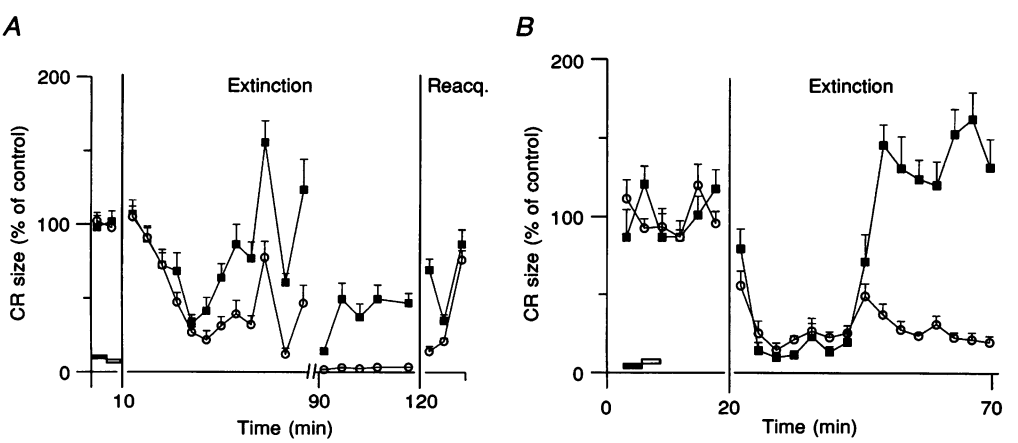

Figure 1. Unilateral extinction training

Animals were trained with a reinforcing unconditioned stimulus (US) on one side only. *A*, unilateral extinction training showing the gradual decrease in the size of the conditioned responses (CRs) on the non-reinforced side with a minor accompanying effect on the reinforced side in one animal. When paired stimulation (reacquisition, Reacq.) was resumed on the non-reinforced side, responses recovered. *B*, unilateral extinction training in another animal showing a more marked effect on the CRs generated on the reinforced side. Each symbol in both *A* and *B* represents the mean size of CRs during ten consecutive trials. The size is expressed as a percentage of the mean CR size during the control sessions. The CRs on the non-reinforced side are represented by ○ and the reinforced side by ■. Error bars show +S.E.M. CR sizes should be related to the spontaneous EMG activity, which is indicated in the plots by bars. The filled bar represents the level of spontaneous activity on the reinforced side and the open bar represents the level of activity on the non-reinforced side. The time base is broken when a pause in the sampling was necessary and otherwise consecutive trials are shown.

Table 1. The bilateral effect on CR size of unilateral extinction training, injection or lesion

	Animal	Effect	Ipsilateral side (% of control)	Contralateral side (% of control)	Number of trials
Extinction	E74	I	2 ± 2·3	−6 ± 0·6	10
		II	43 ± 9·4	−4 ± 0·1	10
	E105	I	62 ± 4·2	23 ± 3·5	10
		II	118 ± 9·1	2·5 ± 0·7	10
	E108	I	3 ± 3·4	0·5 ± 8·5	10
		II	166 ± 18	8 ± 3·8	10
	E127	I	60 ± 8·2	52 ± 9·6	10
		II	84 ± 5·6	3 ± 0·5	10
Injection	E64	—	0*	30*	10
	E66	—	−3 ± 2·4	1 ± 0·5	7
	E67	—	9 ± 1·2	61 ± 11	10
	E72	—	0·09 ± 3·1	55 ± 3	10
	E77	—	−0·3 ± 0·9	0 ± 0·4	10
	E78	—	0·2 ± 6	23 ± 13	10
	E81	—	0·9 ± 0·6	23 ± 5·4	10
	E99	—	0·5 ± 2·2	46 ± 29	10
Lesion	E71	I	−0·1 ± 0·2	−0·3 ± 0·6	5
		II	−0·8 ± 0·2	23 ± 11	5
	E80	I	−0·07 ± 0·4	7·6 ± 3	10
		II	−0·5 ± 1·4	122 ± 24	10
	E110	I	0·03 ± 0·08	−0·6 ± 0·2	10
		II	−0·05 ± 0	120 ± 6·7	10

The effects on CR size of extinction training, lignocaine injections into the BC and lesions of the BC are shown as percentages of the mean size of the CRs during the control sessions ± S.E.M. The part of the table with the extinction experiments shows the mean CR size in the session in which the bilateral effect was largest (I) and one session when the CRs were extinguished on the contralateral side and had returned to a stable level on the reinforced ipsilateral side (II). The part of the table with the injection experiments shows the session in each experiment in which the bilateral effect of unilateral lignocaine injection in the BC was most pronounced. The part of the table showing the lesion experiments shows the session in which the bilateral effect on CR size was the largest (I) and one session when the CRs on the non-lesioned side had returned to a stable level (II). In order to compensate for the possible contribution of spontaneous activity in the calculation of the size of the CRs, the average spontaneous activity was subtracted from the size of the CRs. A decrease in the spontaneous activity during the CS period explains why the CR size appears to be less than zero in a few sessions. * Data from this experiment were stored in a way that was inaccessible to the analysis software. The numbers represent a crude approximation of the effect.

studying the individual trials it was noticed that in some trials, at the time when contralateral CRs were beginning to disappear, ipsilateral CRs were also depressed. This depression was mainly the result of a complete absence of CRs on some trials, rather than a mean decrease in amplitude. After continued extinction training, the CRs on the reinforced side recovered, although not to their original level. This effect on the CRs on the reinforced side due to the extinction training on the opposite side was more pronounced in one animal and is shown in Fig. 1*B*. In this animal the CRs on the reinforced side were almost completely depressed during the initial unilateral extinction training and after continued extinction training the CRs reappeared in full size on the reinforced side without accompanying CRs on the non-reinforced side. However, when the CRs on the reinforced side recovered, there was a small transient increase in EMG activity on the non-reinforced side as well. The reinforced side can clearly continue to generate CRs even though the CRs on the contralateral side have disappeared. This was the case in every animal (see Table 1).

An initial decrease in the frequency of CRs on the reinforced side when beginning unilateral extinction training was seen in every animal ($n = 4$) undergoing extinction training, although the duration of the contralateral effect varied greatly. These findings suggest that some interaction exists between the expression of CRs on the two sides and this interaction was further studied by interrupting the cerebellar outflow.

Lignocaine injections and conditioned responses

In eight experiments, the outflow from one cerebellar hemisphere was reversibly blocked by injections of lignocaine in the BC (see Methods).

Consistent with previous reports that CRs are abolished when output from one cerebellar hemisphere is blocked, we found in all experiments that when a small amount of lignocaine was injected unilaterally in the BC the ipsilateral CRs disappeared immediately. A more surprising, but consistent finding, was that the injection also suppressed the CRs on the side contralateral to the injection.

After the unilateral injection the CRs disappeared immediately on the ipsilateral and on the contralateral side. The duration of the lignocaine effect on the CRs was shorter on the contralateral side. The contralateral CRs were abolished for at least a few trials in every injection

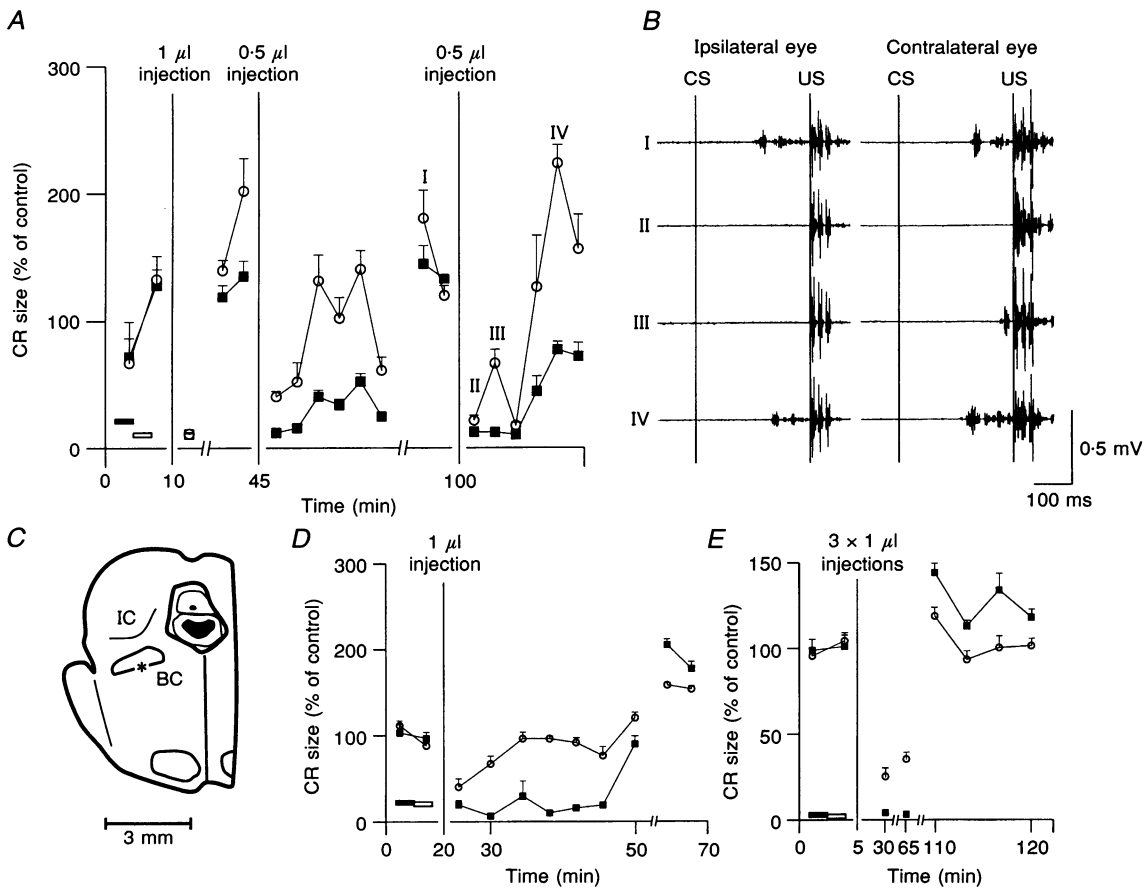

Figure 2. Effect on CR size of lignocaine injections in the brachium conjunctivum (BC) in three different animals

The effect on the size of the CRs in three different animals (*A*, *D* and *E*) of injecting (0·5–1 μl) of lignocaine in the BC. The period of integration was from 80–298 ms after the conditioned stimulus (CS) onset. The ipsilateral CRs are represented by ■ and the contralateral by ○. The size of the responses are expressed as percentages of the mean CR size during control sessions. *A*, the BC was blocked repeatedly and the CRs were abolished bilaterally after each injection. The contralateral CR regained its pre-injection size before the ipsilateral CRs did. Each data point in *A* represents the mean size of the CRs during seven consecutive conditioning trials. *B*, EMG records from both eyelids. The Roman numerals correspond to those in *A* and indicate from which periods the records are taken; US, unconditioned stimulus. *C*, a histological reconstruction of a frontal section of the brainstem, near the border between the inferior colliculus and the cerebellum, from the same animal as in *A* and *B*. The injection site is indicated by an asterisk. IC, inferior colliculus. *D* and *E*, these plots show the effect of lignocaine injections in the BC of two other animals. Each data point in the graphs represents the mean size of the CRs during ten consecutive conditioning trials. *D*, the bilateral CRs were abolished for a longer period by repeated injections of 1 μl (3 × 1 μl) and the plot shows the post-injection period when contralateral CRs reappear before the ipsilateral CRs do. Error bars show +S.E.M. CR sizes should be related to the spontaneous EMG activity, which is indicated in the plots by bars; filled bars represent the level of spontaneous activity on the ipsilateral side and open bars represent the level of activity on the contralateral side. The time base is broken when a pause in the sampling was necessary and otherwise consecutive trials are shown.

experiment. This period of contralateral suppression was then followed by a gradual recovery of the CRs until they reached their preinjection size. This bilateral effect of the unilateral blockade of the BC was observed in all experiments. In four of the experiments the bilateral effect of the unilateral lignocaine injection was repeated at least once. Table 1 shows the bilateral effect on the CR size of unilateral BC injection in every animal.

Figure 2 shows the injection effect on the size of the CRs on the ipsilateral and the contralateral side in three representative animals (Fig. 2*A*, *D* and *E*). The CRs were immediately abolished on both sides by the injection of 1 μl lignocaine. The responses on the contralateral side returned to their original size before those on the ipsilateral side did. Figure 2*A* also shows that the bilateral effect on the CRs could be repeated several times. The first injection of 1 μl abolished the CRs bilaterally. The second and third injections of 0·5 μl were as effective in abolishing the contralateral CRs initially but the responses recovered within fewer trials compared with the first injection of 1 μl. In Fig. 2*B* EMG recordings from both the ipsi- and the contralateral orbicularis oculi muscle before and after an injection of 0·5 μl of lignocaine are shown. The records clearly show that the ipsilateral and the contralateral CRs are both abolished by the injection. The CR on the contralateral side reappears before the CR on the ipsilateral side. The EMG records in Fig. 2*B* are from the same animal as shown in Fig. 2*A* and the time of each recording is indicated by the corresponding Roman numerals (I–IV). The location of the injection site in the same animal is shown in Fig. 2*C*.

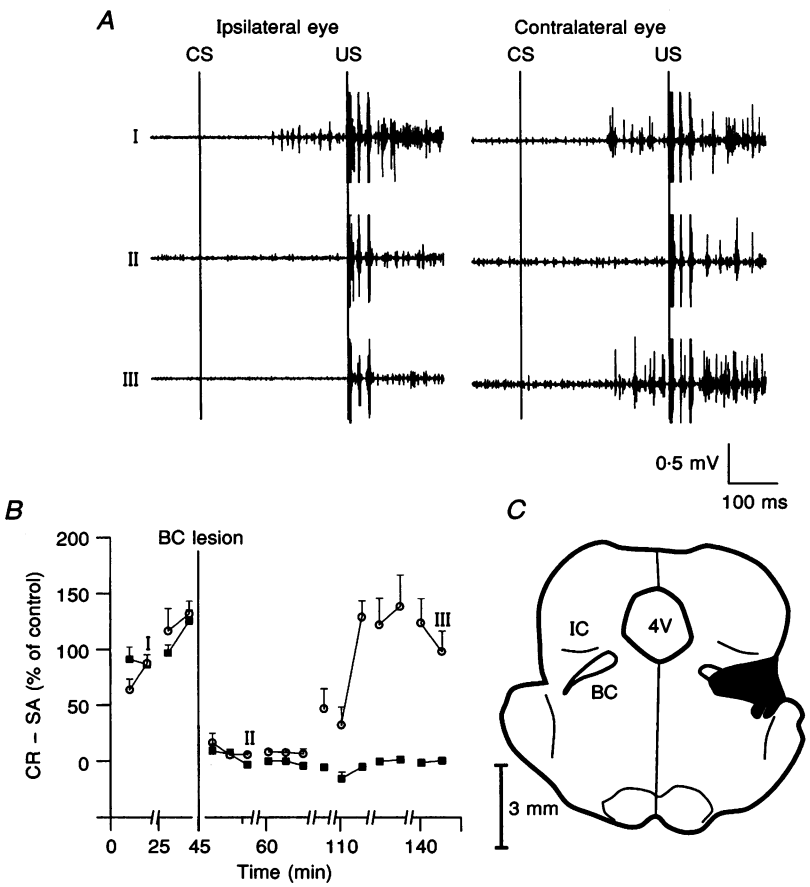

Figure 3. The effect on CRs of a lesion to the BC

A, EMG records from the orbicularis oculi muscles 28 min before (I), 10 min after (II) and 105 min after (III) a lesion to the BC. The CRs were abolished in the orbicularis oculi muscle ipsilateral to the lesion and were suppressed for a limited period on the contralateral non-lesioned side. *B*, the plot shows the mean size of the CRs on the two sides. The lesion caused an increase in the spontaneous activity (SA) in the orbicularis oculi muscles and the plot has been corrected for this by subtracting the spontaneous EMG activity. Because of fluctuations in the background activity, this subtraction could occasionally have the effect that the size of an individual CR was less than zero. The ipsilateral CRs are represented by ■ and the contralateral by ○. Each symbol represents the mean size of the CRs during ten consecutive conditioning trials; error bars show +S.E.M. The time base is broken when a pause in the sampling was necessary and otherwise consecutive trials are shown. *C*, histological reconstruction of the lesion. The grey area corresponds to the lesioned area. The lesion was made in a rostrocaudal direction.

The CRs were quantified by measuring the size of the rectified and integrated EMG activity as described in Methods. In the graphs, the sizes of the CRs are expressed as percentages of CRs during a control session. Since there is usually some spontaneous background EMG activity, the plotted CR sizes are sometimes greater than zero even when CRs are abolished. This is clearly seen in Fig. 2*B*, where the records can be directly compared with the corresponding data points in Fig. 2*A*. Bars which indicate the level of background EMG activity have been inserted in these plots.

It might be thought that the effect on the CRs after injection could be caused by a change in the spontaneous activity of the orbicularis oculi muscles, but this cannot be the case since there was no significant change in the spontaneous activity after the lignocaine injection, even though the CRs were abolished bilaterally.

Another very interesting observation made in the injection experiments was that when CRs returned they were initially larger than the control responses. This increase lasted only a short period after which the responses returned to the control size. This effect is seen in the plots of Fig. 2.

Conditioned responses after mechanical lesions

In three experiments mechanical lesions were made at the level between the inferior colliculus and the cerebellum. The lesions clearly affected the ipsilateral BC. The CRs on both the ipsi- and the contralateral sides were initially abolished by the lesion, but the contralateral CRs reappeared. After the lesion, an increase in the spontaneous activity in the orbicularis oculi muscle was noted. The upper EMG records in Fig. 3*A* show CRs on both sides before the mechanical lesion (I), 10 min after (II) and 105 min after (III) the lesion. Figure 3*B* shows the sizes of CRs during 80–298 ms after CS onset. The spontaneous activity in each trial during 100 ms before CS onset has been subtracted from the CR size. The subtraction of the spontaneous activity from the size of the CRs was done in order to compensate for the increased spontaneous activity which occurred after the lesion. The records clearly show that the CRs are abolished. The ipsilateral CRs were abolished immediately after the lesion, as were the contralateral responses, and not a single CR on the lesioned side was observed during the remaining part of the experiment (5 h) even though the CRs on the contralateral side had recovered after approximately 75 min. Even with double CS intensities, CRs could not be elicited on the lesioned side. The extent of the lesion is shown in Fig. 3*C*. The other two lesion experiments that included the BC showed the same effect as those described above. CRs reappeared on the non-lesioned side within 1–2·5 h and no CR was recorded on the lesioned side during the remaining part of the experiment. The effect of the unilateral lesion on bilateral CRs is shown in Table 1.

Two other animals received unilateral mechanical lesions that only temporarily abolished the CRs bilaterally. In both of these cases the lesions did not involve any part of the BC.

Unconditioned responses

We have previously described that a single electrical stimulus applied to the peri-orbital area of the ferret elicits an eyeblink composed of a first EMG component with an onset latency of 6–7 ms and a more variable second response at 10–15 ms (Ivarsson & Hesslow, 1993). The two EMG responses in the ipsilateral orbicularis oculi muscle in the ferret ends before 18 ms with stimulus strengths of up to 3 mA. The first early component (R1) is thought to be mediated by a direct pathway from the trigeminal nucleus

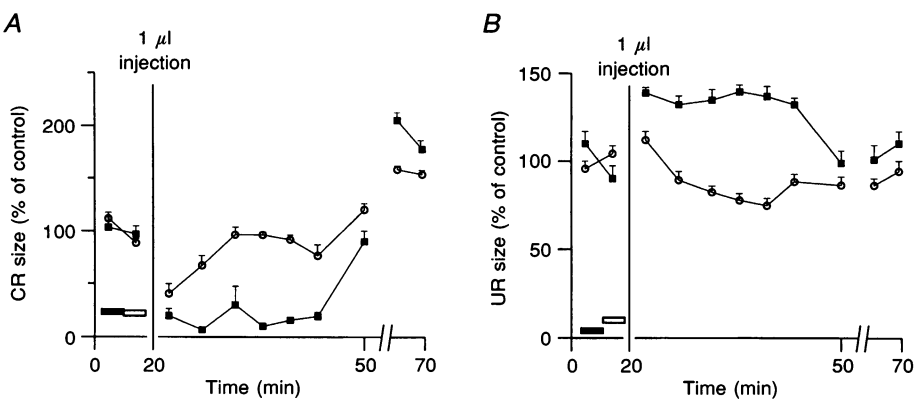

Figure 4. The UR size on CS–US trials after lignocaine injection in the BC

A, the injection of lignocaine in the BC caused an abolition of the CRs on the ipsilateral side (■) and a suppression of the CRs on the contralateral side (○). *B*, the corresponding URs elicited by the US from the same period of the same experiment. The effect of the injection is a marked increase in the size of the ipsilateral reflex responses during the period of abolished CRs. The size of the responses should be related to the spontaneous EMG activity, which is indicated in the plots by bars; filled bars represent the level of spontaneous activity on the ipsilateral side and open bars represent the level of activity on the contralateral side. Error bars show +S.E.M. The time base is broken when a pause in the sampling was necessary and otherwise consecutive trials are shown.

to the facial nucleus and is more stable in size and onset latency than the second component (R2). The R2 is thought to be mediated by a polysynaptic pathway through brainstem sites (Kugelberg, 1952; Kimura, Powers & Van Allen, 1969; Shahani & Young, 1972; Hiraoka & Shimamura, 1977). In addition to the relays in the brainstem it has recently been shown that the C1 segment of the spinal cord is critical for the generation of R2 (Pellegrini, Horn & Evinger, 1995). The motoneurones of the facial nucleus are the final common pathway and a change in the excitability on the facial nucleus would be expected to affect both of the components.

The effects of blocking the BC by lignocaine on URs was tested in five animals on paired CS–US trials. An example of such an experiment is shown in Fig. 4. When the BC was blocked and the CRs were temporarily depressed (Fig. 4*A*), there was a marked increase in the size of the ipsilateral URs, while the contralateral URs remained unaffected (Fig. 4*B*). This phenomenon was observed in all of the five animals tested.

The effects of BC lesions were tested in three animals. Figure 5*A* shows the effect of a BC lesion on UR size during CS–US trials in the same experiment as illustrated in Fig. 3. After the lesion, the URs were immediately reduced on the ipsilateral side, which is the opposite effect of that observed after lignocaine injections. The responses contralateral to the lesion were not decreased. The observation that the ipsilateral but not the contralateral URs are depressed by the BC lesions, was seen in one of the two other lesion experiments. In the third experiment, the ipsilateral URs were unaffected while the contralateral URs were increased.

The inconsistency of these effects could be due to the fact, reported by several authors, that the CR can interact with the UR. For instance Canli, Detmer & Donegan (1992) observed a decreased UR amplitude on paired CS–US trials as compared with US-alone trials. Baker, Hardiman & Yeo (1991) found a decrease only in the R1 component of the UR when this response had been preceded by a CR. Hence, the changes in UR size, when the CR pathway was blocked, could be due to the disappearance of CRs and need not be an effect of interrupting cerebellar outflow *per se*.

For this reason, we studied UR sizes after blocking the ipsilateral cerebellar output on US-alone trials in four animals. We used single peri-orbital stimuli of different strengths (0·5–3 mA). Figure 5*B* shows the results from one animal.

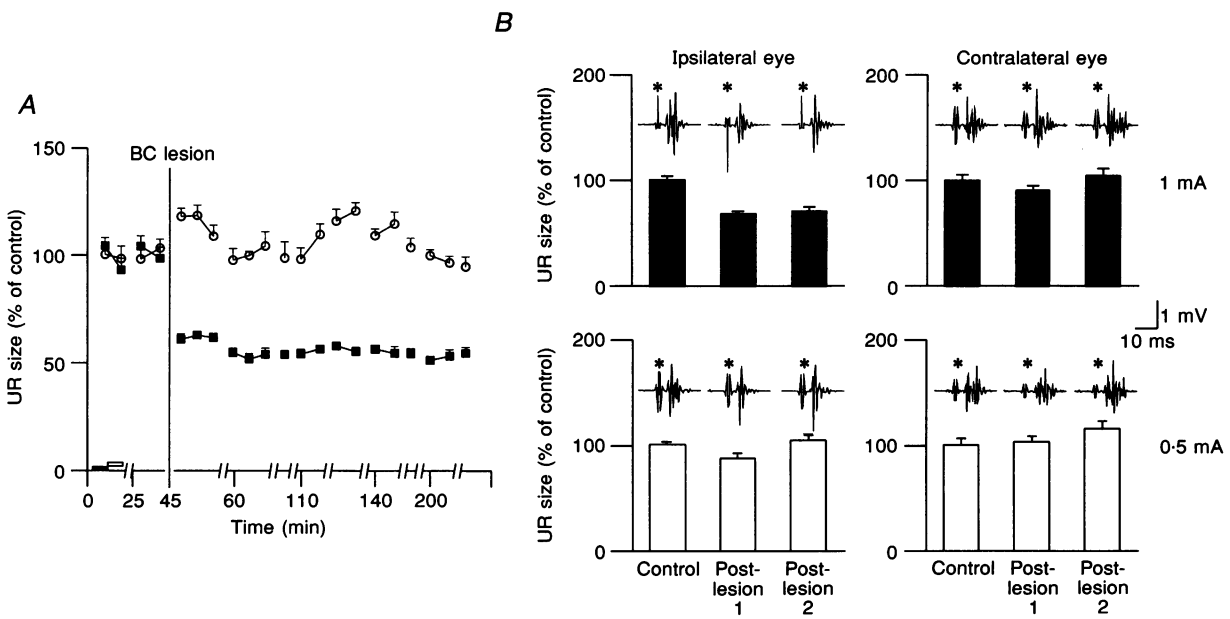

Figure 5. The effect on URs of a lesion to the BC in CS–US trials and on US-alone trials

A, each data point represents the mean size of the URs during ten consecutive conditioning trials. The ipsilateral URs are represented by ■ and the contralateral by ○. The size of the contralateral URs are not decreased by the lesion. UR sizes should be related to the spontaneous EMG activity, which is indicated in the plot by bars; the filled bar represents the level of spontaneous activity on the ipsilateral side and the open bar represents the level of activity on the contralateral side. Error bars show +S.E.M. The time base is broken when a pause in the sampling was necessary and otherwise consecutive trials are shown. *B*, bar charts showing the mean UR size on US-alone trials with weak stimulus strengths. Every bar represents the mean size of ten trials and are expressed as percentages of the mean size of the control session. A representative EMG record is shown above each corresponding bar and the stimulus artefact is marked by an asterisk. Some of the shock artefacts have been retouched for clarity. The top two bar charts represent the size of the UR on sessions with a stimulus strength of 1 mA and the two lower charts show the response size with a stimulus strength of 0·5 mA. Post-lesion 1 represents trials immediately after the lesion. Post-lesion 2 represents trials after recovery of the contralateral CRs.

Bars represent mean sizes of URs on US-alone trials with stimulus strengths of 1 and 0·5 mA. The sizes of the URs are expressed as percentages of the mean UR size during a preceding control session and an EMG record from each session is shown above the corresponding bar. The first post-lesion session represents the time when the CRs were abolished bilaterally and the second post-lesion session corresponds to the time when the CRs had reappeared on the contralateral side. Although there was a clear depression of the contralateral URs at 1 mA, the effect on 0·5 mA as well as the effects on the contraleral side were small and went in opposite directions. Such inconsistencies in the effect on the URs were also found in the other two lesion experiments in which this was tested.

In one animal, the interruption of the cerebellar outflow caused an 11% decrease in the size of the ipsilateral UR at 0·5 mA and a 32% decrease at 1 mA. In the second animal there was an 11% increase at 0·5 mA and a 1% decrease at 1 mA of the ipsilateral UR. In the other two animals, in which only 2 and 3 mA stimuli were tested, the effects were also in opposite directions.

The effects on the size of contralateral URs were similarly inconsistent and also unrelated to the ipsilateral effects. For instance, in the animal in which there was an 11% decrease in the ipsilateral UR size at 0·5 mA, there was a 3% increase in the contralateral UR size. In the animal in which the ipsilateral URs were increased by 11% the contralateral URs were decreased by 11%. To summarize, no consistent effects on the URs of blocking cerebellar outflow were found.

Although the effects of blocking the BC on URs were highly variable, these data suggest that a non-specific decrease in the excitability of the motoneurones is not likely to be the cause of the disappearance of the contralateral CRs.

DISCUSSION

Effect on ipsi- and contralateral CRs after ipsilateral blockade of the BC

The results presented in this study show that injections of lignocaine as well as mechanical lesions of the area involving BC abolish CRs on the ipsilateral side. This effect is probably due to an interruption of cerebellar outflow which passes through the BC. A plausible alternative would be an interference with the rubrobulbar pathway. The projection from the red nucleus to the facial nucleus is not known in detail in the ferret but presumably the fibres cross over to the contralateral side soon after leaving the red nucleus and then descend caudally, ventral to the BC. The mechanical lesions may have damaged both the BC and the rubrobulbar pathway. Although it cannot be excluded, it is less likely that the lignocaine injections affected the rubrobulbar pathway. The BC was tracked with a stimulation electrode before the injection and the fibres related to eyeblink were localized. The injection pipette was then inserted according to the same co-ordinates and the placement was later verified histologically. The CRs disappeared immediately after the injection. If the lignocaine had blocked the rubrobulbar pathway, one would expect a temporal delay to allow for diffusion of the lignocaine.

This result confirms a large number of reports from several laboratories which show that interference with ipsilateral cerebellar function or outflow abolishes CRs. This has been shown for lesions of the cerebellar nuclei (McCormick & Thompson, 1984; Clark, McCormick, Lavond & Thompson, 1984; Yeo, Hardiman & Glickstein, 1985*a*; Welsh & Harvey, 1989; Lavond, Logan, Sohn, Garner & Kanzawa, 1990; Steinmetz, Lavond, Ivkovich, Logan & Thompson, 1992), lesions of cerebellar cortical areas (Yeo, Hardiman & Glickstein, 1985*b*), pharmacological inactivation of the interpositus nucleus (Haley, Thompson & Madden, 1988; Chapman, Steinmetz, Sears & Thompson, 1990; Welsh & Harvey, 1991; Krupa *et al.* 1993) and physiological inactivation of the interpositus nucleus (Hesslow, 1994*b*).

The main result of the present study, was that after blocking the cerebellar output from one hemisphere, the CRs generated on the contralateral side were also suppressed. This is more surprising, especially in view of the fact that it has been assumed in virtually all previous studies of the role of the cerebellum in conditioning, that only interference with the ipsilateral cerebellar hemisphere needs to be considered. However, as described above, unilateral lesions as well as unilateral injections of lignocaine immediately suppressed CRs on both the ipsi- and the contralateral sides. However, the effect on the contralateral side was less pronounced than on the ipsilateral side. Unilateral lesions of the BC abolished the CRs on both sides but the CRs on the contralateral side recovered after 1–2·5 h, whereas the ipsilateral responses never recovered during the remaining 5 h of the experiments.

A plausible explanation for the marked effect on contralateral CRs after unilateral disruption of the cerebellar output is that one cerebellar hemisphere via some crossed output facilitates neurones in a contralateral CR pathway. It is possible, for instance, that the output from one cerebellar hemisphere terminates on brainstem neurones which in turn project to both facial nuclei so that contralateral CRs are facilitated. In a training protocol where the US is given bilaterally, so that learning occurs on both sides, some of the neurones in the CR pathway on each side may adapt to, and become dependent on, facilitatory input from the other side. When this input is interrupted, as in the present experiments, the CRs would be depressed until these neurones had adapted to the loss of facilitatory input.

There are no identified pathways that could mediate a contralateral facilitation, but the possibility is not far-fetched. Many investigators have reported that some late components of the unconditioned eyeblink reflex are bilateral (Kugelberg,

1952; Kimura *et al.* 1969; Ivarsson & Hesslow, 1993) and it is conceivable that the conditioned eyeblink utilizes some of the same central circuits. There is also direct physiological evidence for the existence of a crossed cerebellar output pathway in the ferret. We have previously shown that stimulation of the BC on one side can generate EMG activity in the contralateral eyelid (Ivarsson & Hesslow, 1993).

This interpretation also fits with the observed contralateral effects of extinction. In the present experiments, the US was applied to both peri-orbital areas and learning occurred on both sides. Four animals which had been reinforced bilaterally and gave stable bilateral CRs, were shifted to a protocol with reinforcing stimuli on only one side. One would expect this to cause extinction on the non-reinforced side, but if the reinforced side has become dependent on contralateral facilitatory input, a depression of CRs might occur on this side as well. This prediction was confirmed although the effect varied between animals. The CRs on the non-reinforced side underwent extinction, as expected, but there was also a depression of contralateral CRs of varying degrees.

How can we explain the fact that extinction of CRs on one side has a smaller effect on contralateral CRs than lesioning or pharmacological blockade? A contralateral facilitation may have two components. The interpositus neurones are presumably tonically active and may therefore generate a continuous facilitation of the contralateral CR pathway. When a CR is generated, a phasic activity in the anterior interpositus nucleus is added to the tonic activity and a corresponding phasic facilitation will be added to the tonic one. Whereas lesions and lignocaine injections would remove all input from one side, both tonic and phasic, unilateral extinction would only affect the phasic component.

Assuming that there exists a bilateral projection such that activity in one cerebellar hemisphere facilitates the CR-generating neurones on both sides, what could be the target of this projection? It is unlikely that it is the facial nucleus motoneurones themselves, since the unilateral blocks did not cause a consistent change in the contralateral URs even when URs were elicited by weak stimuli. Only the CRs on the contralateral side were affected. Neither did the injections consistently depress the URs on the ipsilateral side. In fact, when the CRs were depressed, the URs were sometimes increased in size. This might be due to the interaction between CRs and URs mentioned above in the Results section.

Thus, CRs facilitate contralateral CRs and can have varying effects on URs. These observations indicate that there is a fairly complex neuronal circuitry controlling the eyeblink and that important parts of the CR and UR pathways are distinct. This would entail the presence of relay neurones in the CR pathway located between the cerebellum and the facial nucleus. There is no anatomical evidence to suggest where such a relay might be located. An obvious possibility is the red nucleus but there are also many others, such as those brainstem areas or the C1 segment of the spinal cord which mediate the late components of the unconditioned eyeblink.

The importance of the cerebellum in conditioning

The results of this study are relevant in several ways to the hypothesis that the cerebellum plays an essential role in the learning and generation of CRs.

As was explained in the Introduction, the observation that CRs may recover after unilateral cerebellar lesions may be taken as evidence for an extracerebellar source of CRs, but it is also possible that the recovered CRs were actually generated by the contralateral cerebellar hemisphere.

The observations reported here strongly support this possibility. Our results show that each cerebellar hemisphere in the ferret exerts a marked control over contralateral CRs, such that under certain conditions the CR pathway on one side depends on facilitatory CR input from the contralateral side. It is surely reasonable to propose that the crossed cerebellar output pathway could be utilized by the cerebellar hemisphere remaining after a lesion to generate contralateral CRs, especially when it is considered that the recovery of CRs, which was observed after unilateral lesions, was only partial and required extensive overtraining (Lavond *et al.* 1987; Yeo & Hardiman, 1992).

Several authors have suggested that cerebellar lesions abolish CRs by causing a loss of tonic cerebellar facilitation of the motor pathways (see Introduction). Our results could be taken to support this suggestion. It is highly unlikely, and has never been suggested, that the main site of plasticity in eyeblink conditioning is the contralateral cerebellar hemisphere. Yet, interrupting outflow from one cerebellar hemisphere abolishes contralateral CRs without any corresponding effect on URs. Thus, it seems that loss of background facilitation does abolish CRs under some circumstances and the absence of a corresponding effect on URs is insufficient to rule this out. Therefore, the suggestion made by those who are sceptical of a cerebellar locus of conditioning, that lesion effects are mediated by loss of facilitation, has to be taken seriously.

It should be noted, however, that the loss of contralateral CRs was transient. The neurones in the CR pathway, which had become dependent on facilitatory input, adapted to the loss of this input within 1–2·5 h. In contrast, the animals in virtually all other studies have had several days or weeks of recovery after the lesions and the effects of ipsilateral lesions therefore seem relatively permanent.

Our results are relevant to the observation of Kelly *et al.* (1990), that cerebellar lesions had no effect on CRs in rabbits which had previously been decerebrated (see Introduction). They suggested that the loss of CRs seen after cerebellar lesions is due to a loss of tonic facilitation from the cerebellum and that the decerebration counteracted this

effect. Our results clearly show that this does not hold for the ferret. Both reversible and irreversible interruption of the cerebellar output always abolished ipsilateral CRs in all our decerebrate ferrets. Similar findings have also recently been obtained in the rabbit. When conditioning was established in decerebrate rabbits cerebellar lesions as well as lignocaine inactivations of the interpositus nucleus abolished the CRs (Hesslow, Hardiman & Yeo, 1990; Yeo, Hardiman, Lobo & Hesslow, 1996).

On balance, we think that the results are consistent with a critical cerebellar involvement in conditioning. They can help explain seemingly contradictory evidence and they reject other contradictory evidence.

Increase in CR size after lignocaine injection

Another interesting observation that was made in most of the animals was that when the CRs recovered after the lignocaine block, the CR amplitude was markedly increased. This suggests the operation of some kind of feedback system, such that the inability of the animal to express the CRs is signalled to a central circuit which increases the 'strength' of the CR output. At present, we cannot say if the feedback signal arises in the periphery, say from the eyelids, or if it is the block of the cerebellar output that is crucial. The latter possibility is intriguing, since we have previously suggested that the inhibitory pathway from the interpositus nucleus to the inferior olive has such a feedback function (Andersson, Garwicz & Hesslow, 1988; Hesslow & Ivarsson, 1996). It was proposed that when the animal has acquired the CR (i.e. when interpositus generates a sufficiently strong activity), the olive would be inhibited and further learning prevented. The present results are consistent with this suggestion.

Conclusions

In conclusion, our findings suggest that each cerebellar hemisphere facilitates neurones involved in the generation of contralateral CRs, probably via premotor neurones involved specifically in conditioned, and not in unconditioned, responses.

Andersson, G., Garwicz, M. & Hesslow, G. (1988). Evidence for a GABA-mediated inhibition of the inferior olive in the cat. *Experimental Brain Research* **72**, 450–456.

Baker, S., Hardiman, M. J. & Yeo, C. H. (1991). Conditioned response modulation of the R1 and R2 components of the blink reflex. *European Journal of Neuroscience* suppl. 4, 309, 4303.

Bracha, V., Stewart, S. L. & Bloedel, J. R. (1993). The temporary inactivation of the red nucleus affects performance of both conditioned and unconditioned nictitating membrane responses in the rabbit. *Experimental Brain Research* **94**, 225–236.

Canli, T., Detmer, W. M. & Donegan, N. H. (1992). Potentiation or diminution of discrete motor unconditioned responses (rabbit eyeblink) to an aversive Pavlovian unconditioned stimulus by two associative processes: Conditioned fear and a conditioned diminution of unconditioned stimulus processing. *Behavioral Neuroscience* **106**, 498–508.

Chapman, P. F., Steinmetz, J. E., Sears, L. L. & Thompson, R. F. (1990). Effects of lidocaine injection in the interpositus nucleus and the red nucleus on conditioned behavioral and neuronal responses. *Brain Research* **537**, 149–156.

Clark, G. A., McCormick, D. A., Lavond, D. G. & Thompson, R. F. (1984). Effects of lesions of cerebellar nuclei on conditioned behavioral and hippocampal neuronal responses. *Brain Research* **291**, 125–136.

Gruart, A. & Yeo, C. H. (1995). Cerebellar cortex and eyeblink conditioning – Bilateral regulation of conditioned responses. *Experimental Brain Research* **104**, 431–448.

Haley, D. A., Thompson, R. F. & Madden, J. IV (1988). Pharmacological analysis of the magnocellular red nucleus during classical conditioning of the rabbit nictitating membrane response. *Brain Research* **454**, 131–139.

Harvey, J. A., Welsh, J. P., Yeo, C. H. & Romano, A. G. (1993). Recoverable and non-recoverable deficits in conditioned responses after cerebellar cortical lesions. *Journal of Neuroscience* **13**, 1624–1635.

Hesslow, G. (1994). Inhibition of classically conditioned eyeblink responses by stimulation of the cerebellar cortex in the cat. *Journal of Physiology* **476**, 245–256.

Hesslow, G., Hardiman, M. & Yeo, C. H. (1990). Cerebellar lesions abolish eyeblink conditioning in the decerebrate rabbit. *European Journal of Neuroscience* suppl. 3, 301, 4278.

Hesslow, G. & Ivarsson, M. (1994). Suppression of cerebellar Purkinje cells during conditioned responses in ferrets. *NeuroReport* **5**, 649–652.

Hesslow, G. & Ivarsson, M. (1996). Inhibition of the inferior olive during conditioned responses in the decerebrate ferret. *Experimental Brain Research* **110**, 36–46.

Hiraoka, M. & Shimamura, M. (1977). Neural mechanisms of the corneal blinking reflex in cats. *Brain Research* **125**, 265–275.

Ivarsson, M. & Hesslow, G. (1993). Bilateral control of the orbicularis oculi muscle from one cerebellar hemisphere in the ferret. *NeuroReport* **4**, 1127–1130.

Ivarsson, M., Svensson, P. & Hesslow, G. (1995). Bilateral suppression of conditioned eyeblink responses after unilateral blockade of the brachium conjunctivum. In *The Cerebellum: from Structure to Control*, p. 75 (abstract). *Satellite Symposium of the Meeting of European Neuroscience, Rotterdam.*

Ivkovich, D., Lockard, J. M. & Thompson, R. F. (1993). Interpositus lesion abolition of the eyeblink conditioned response is not due to effects on performance. *Behavioral Neuroscience* **107**, 530–532.

Kelly, T. M., Zuo, C.-C. & Bloedel, J. R. (1990). Classical conditioning of the eyeblink reflex in the decerebrate–decerebellate rabbit. *Behavioural Brain Research* **38**, 7–18.

Kimura, J., Powers, M. & Van Allen, M. W. (1969). Reflex response of the orbicularis oculi muscle to supraorbital nerve stimulation. *Archives of Neurology* **21**, 193–199.

Krupa, D. J., Thompson, J. K. & Thompson, R. F. (1993). Localization of a memory trace in the mammalian brain. *Science* **260**, 989–991.

Kugelberg, E. (1952). Facial reflexes. *Brain* **75**, 385–396.

Lavond, D. G., Logan, C. G., Sohn, J. H., Garner, W. D. A. & Kanzawa, S. A. (1990). Lesions of the cerebellar interpositus nucleus abolish both nictitating membrane and eyelid EMG conditioned responses. *Brain Research* **514**, 238–248.

Lavond, D. G. & Steinmetz, J. E. (1989). Acquisition of classical conditioning without cerebellar cortex. *Behavioral Brain Research* **33**, 113–164.

LAVOND, D. G., STEINMETZ, J. E., YOKAITIS, M. H. & THOMPSON, R. F. (1987). Reacquisition of classical conditioning after removal of cerebellar cortex. *Experimental Brain Research* **67**, 569–593.

LLINÁS, R. & WELSH, J. P. (1993). On the cerebellum and motor learning. *Current Opinion in Neurobiology* **3**, 958–965.

MCCORMICK, D. A. & THOMPSON, R. F. (1984). Cerebellum: essential involvement in the classically conditioned eyelid response. *Science* **223**, 296–299.

NORDHOLM, A. F., LAVOND, D. G. & THOMPSON, R. F. (1991). Are eyeblink responses to tone in the decerebrate, decerebellate rabbit conditioned responses? *Behavioural Brain Research* **44**, 27–34.

PELLEGRINI, J. P., HORN, A. K. E. & EVINGER, C. (1995). The trigeminally evoked blink reflex I. Neuronal circuits. *Experimental Brain Research* **107**, 166–180.

SHAHANI, B. T. & YOUNG, R. R. (1972). Human orbicularis oculi reflexes. *Neurology* **22**, 149–154.

STEINMETZ, J. E., LAVOND, D. G., IVKOVICH, D., LOGAN, C. G. & THOMPSON, R. F. (1992). Disruption of classical eyelid conditioning after cerebellar lesions: Damage to a memory trace or a simple performance deficit? *Journal of Neuroscience* **12**, 4403–4426.

THOMPSON, R. F. (1990). Neural mechanisms of classical conditioning in mammals. *Philosophical Transactions of the Royal Society* B **329**, 161–170.

WELSH, J. P. & HARVEY, J. A. (1989). Cerebellar lesions and the nictitating membrane reflex: performance deficits of the conditioned and unconditioned response. *Journal of Neuroscience* **9**, 299–311.

WELSH, J. P. & HARVEY, J. A. (1991). Pavlovian conditioning in the rabbit during inactivation of the interpositus nucleus. *Journal of Physiology* **444**, 459–480.

YEO, C. H. (1991). Cerebellum and classical conditioning of motor responses. *Annals of the New York Academy of Sciences* **627**, 292–304.

YEO, C. H. & HARDIMAN, M. J. (1992). Cerebellar cortex and eyeblink conditioning: a reexamination. *Experimental Brain Research* **88**, 623–638.

YEO, C. H., HARDIMAN, M. J. & GLICKSTEIN, M. (1985*a*). Classical conditioning of the nictitating membrane response of the rabbit. I. Lesions of the cerebellar nuclei. *Experimental Brain Research* **60**, 87–98.

YEO, C. H., HARDIMAN, M. J. & GLICKSTEIN, M. (1985*b*). Classical conditioning of the nictitating membrane response of the rabbit. II. Lesions of the cerebellar cortex. *Experimental Brain Research* **60**, 99–113.

YEO, C. H., HARDIMAN, M. J., LOBO, D. H. & HESSLOW, G. (1996). Conditioned nictitating membrane response in decerebrate rabbits are abolished by cerebellar inactivation. *Brain Research Association, Newcastle* (abstract) 37.14.

Acknowledgements

This study was supported by grants from the Swedish Medical Research Council (project no. 09899), the Knut and Alice Wallenberg Foundation and the Segerfalk Foundation. We thank Henrik Jörntell for writing the software used in acquisition and analysis of the data.

Author's email address

M. Ivarsson: Magnus.Ivarsson@mphy.lu.se

Received 29 July 1996; accepted 25 March 1997.