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Classical conditioning of the nictitating membrane response of the rabbit

II. Lesions of the cerebellar cortex

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Summary. The nictitating membrane response (NMR) of 20 rabbits was conditioned to light and white noise conditional stimuli (CSs) using a periorbital shock unconditional stimulus (US). Unilateral lesions of the cerebellar cortex, sparing the underlying deep nuclei, were then made. Small lesions of cerebellar cortical lobule HVI abolished conditioning on the side of the lesion to both CSs leaving unconditional responses to the US intact. Larger lesions of the posterior lobe which spared HVI did not impair NMR conditioning. We conclude that cerebellar lobule HVI is essential for NMR conditioning in the rabbit. Degeneration following critical lesions of HVI was seen in a restricted region of the inferior olive – the medial part of the dorsal accessory olive and the adjoining medial part of the dorsal leaf of the principal olive. This region of the olive provides somatosensory information from the face to HVI. We suggest that HVI receives information related to the US via climbing fibres from the olive and CS information via mossy fibres from the pontine nuclei. The critical changes underlying NMR conditioning may be the association of these two inputs at the Purkinje cells of cortical lobule HVI.

Key words: Nictitating membrane response – Classical conditioning – Cerebellar cortex – Lobule HVI

Introduction

The conditioned nictitating membrane response (NMR) of the rabbit is a promising preparation for analyses of the essential neural circuitry and mechanisms of a simple form of mammalian motor learning. NMR conditioning is crucially dependent upon the cerebellum. Destruction of the cerebellum abolishes nictitating membrane responses conditioned to stimuli such as light or sound but leaves intact the membrane's reflex response to corneal airpuff or mild periorbital electrical shock. Unilateral cerebellar lesions disrupt NMR conditioning only on the ipsilateral side (Clark et al. 1982; McCormick et al. 1981, 1982; Lincoln et al. 1982).

The interpositus nucleus is a critical structure for NMR conditioning. Lesions of the interpositus nucleus (Glickstein et al. 1983; Yeo et al. 1982, 1985a) or the dentate-interpositus region (Clark et al. 1982, 1984; McCormick and Thompson 1984) abolish conditioned responses as effectively as much larger lesions which include the cerebellar cortex and its nuclei. It is the anterior division of the interpositus nucleus which is crucial. Lesions confined to the posterior interpositus nucleus do not abolish the conditioned NMR (Yeo et al. 1985a).

This paper addresses the question of whether the cerebellar cortical input to the nuclei is necessary for NMR conditioning. By far the largest input to the deep nuclei is from the Purkinje cells of the cerebellar cortex but the nuclei also receive climbing fibre collaterals from the inferior olive (Matsushita and Ikeda 1970) and they have reciprocal connections with the red nucleus (Courville and Brodal 1966).

The corticonuclear projection is known to be strictly ordered in longitudinal zones (Jansen and Brodal 1940, 1942; Voogd 1969). The medial cerebellar cortex, or vermis, projects to the fastigial nucleus and the lateral cerebellar cortex projects to the dentate nucleus. An intermediate zone (zone C of Voogd) projects to the interpositus nucleus.

We have studied the effects upon NMR conditioning of lesions restricted to the cerebellar cortex.

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Small lesions of cortical HVI[1], within Voogd's division C, abolish the conditioned NMR. In contrast, much larger lesions of the cerebellar cortex which do not include the critical HVI area, fail to disrupt NMR conditioning.

Methods

Animals

The subjects were 20 male, Dutch belted rabbits of weights between 2.0 kg and 2.7 kg. They were housed individually with ad libitum food and water and maintained on a 12 h light-dark cycle.

Apparatus and procedures

The conditioning apparatus and the conditioning procedures were fully described in the accompanying paper (Yeo et al. 1985a). Briefly, the subjects were placed in sound-attenuating chambers and the NMR was conditioned to both light and white noise conditional stimuli (CSs) using a periorbital AC electrical unconditional stimulus (US) of 2.5 mA intensity. The CS-US interval was 500 ms and the US was 50 ms coterminous with the CS. Blocks of 5 trials of each CS type were alternately presented to a total of 200 trials per day (100 light, 100 white noise) with an intertrial interval of 15 s.

Movement of the nictitating membrane was transduced using standard procedures employing a low-torque potentiometer. Five, daily training sessions were given with the US applied to the right side. 2–5 days after finishing this training, lesions of the cerebellar cortex were made on the right side (see below). After 3–6 days of postoperative recovery, the animals were retrained for 5 sessions with the US again to the right side. In all cases, this retraining began 7 days after the preoperative training finished. After another interval of 2 days, two sessions of training were given with the US to the left side, contralateral to the cerebellar lesion.

Surgery

The animals were anaesthetised with fentanyl/fluanisone (Hypnorm, Janssen; 0.1/5.0 mg/kg, i.m.) with a supplement of benzodiazepam (Valium, Roche; 0.75 mg/kg, i.v.). They were then placed in the headholder of a stereotaxic device and a midline incision of the scalp was made, with a different surgical approach for each. In all cases the lesions were unilateral and on the right side.

(i) Large lesions of posterior lobe. For this type of lesion, the skull directly above the vermis and ansiform and paramedian lobes was carefully removed using a dental drill. Cerebellar cortex was removed by aspiration through a fine, glass pipette. Following this, the surface of the brain was covered with sterile, absorbable gelatin foam and the scalp was sutured.

(ii) Small lesions of hemispheral lobule VI were made using a different approach in order to minimise damage to other cerebellar cortical zones. Mannitol (20% w/v, 30–40 ml per subject) was administered intravenously to shrink and harden the brin. The skull was opened above the right occipital and parietal cortex using a dental drill and fine rongeurs. The dura above occipital cortex was cut and reflected, and the cortex was carefully retracted to expose the tentorium. With the aid of an operating microscope, the tentorium was visualised and a vertical incision was made in it to expose the rostral face of the cerebellum. Lesions of lobule HVI were then made by aspiration of cerebellar cortical tissue through a fine, glass pipette. After the lesion, the exposed cerebral cortical surface was covered with sterile, absorbable gelatin foam and the scalp was sutured.

Histological methods

After behavioural training, the subjects were deeply anaesthetised with pentobarbitone sodium and perfused through the aorta with 0.9% saline followed by 10% formalin. The brains were removed from the skulls, stored in neutral buffered formalin, dehydrated and embedded in paraffin. They were serially sectioned at 15 μ in the frontal stereotaxic plane described in the previous paper (Yeo et al. 1985a) and stained with cresyl violet and luxol fast blue.

The primary cerebellar cortical lesions were reconstructed onto our own standard series of sections in the transverse stereotaxic plane. The lesions of those brains sectioned in the parasaggittal plane were reconstructed onto a series of standard parasaggittal sections based on those of Van Rossum (1969). In some of the transversely sectioned brains, secondary degeneration of the inferior olive was assessed using the criteria described by Brodal (1939, 1940) and was reconstructed on the standard inferior olive sections of that author (Brodal 1940).

Data analysis and presentation

The behavioural data were analysed as described in the previous paper (Yeo et al. 1985a). The average peak conditional response (CR) and unconditional response (UR) amplitudes on light and on white noise trials are presented for each session. Session 1–5 are preoperative training to the right side, sessions 6–10 are postoperative training to the right (lesioned) side and sessions 11 and 12 are training sessions to the left (non-lesioned) side.

Results

We found a small region of cerebellar cortex in the hemispheral part of lobule VI to be crucial for NMR conditioning. Conditioning was abolished ipsilateral to small lesions in this area. Much larger lesions had no effects upon conditioning if they spared HVI. Small lesions of HVI alone were fully effective in abolishing the conditioned NMR.

(i) Large lesions of the posterior lobe. Six animals received lesions of the posterior lobe which were intended to include as much as possible of the likely

1 There are several different terminologies describing subdivisions of the cerebellar cortex. Bolk's original cerebellar nomenclature (1906) is still widely used, particularly for the hemispheral subdivisions. Larsell's system (Larsell 1952, 1953) numbers vermian lobules from I to X (Bolk's lingula to nodulus) and hemispheral parts of the lobule are prefixed with H. Thus HVI corresponds to the hemispheral part of Bolk's lobulus simplex. Throughout this report we use the Larsell numbering of vermian lobules and in those instances in which we wish to clearly distinguish between the hemispheral and vermian parts of a lobule. We use the more customary terminology of Bolk, however, for the hemispheral ansiform and paramedian lobes

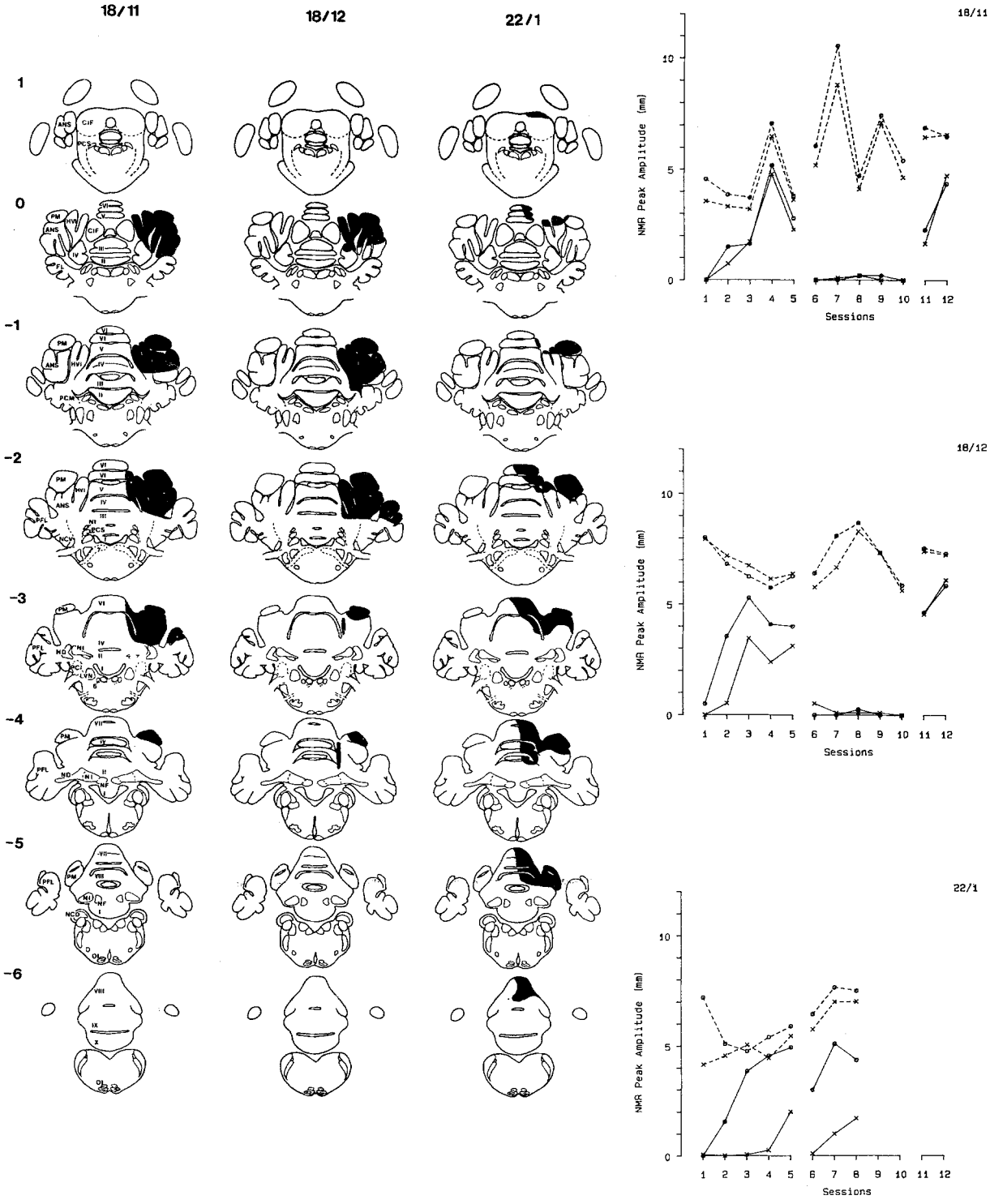


Fig. 1. Reconstructions of the cerebellar cortical lesions of subjects 18/11, 18/12 and 22/1, and their pre- and postoperative NMR conditioning. The transverse sections through the cerebellum are in the stereotaxic plane of Matricali (1961) and are from 1 mm anterior to lambda (1) to 6 mm posterior to lambda (–6). The lesions are on the right side and are indicated in black.
Abbreviations. ANS – ansiform lobe; CIF – inferior colliculus; FL – flocculus; NCD – dorsal cochlear nucleus; NCV – ventral cochlear nucleus; ND – dentate nucleus; NF – fastigial nucleus; NI – interpositus nucleus; OI – inferior olivary nucleus; PCI – inferior cerebellar peduncle; PCM – middle cerebellar peduncle; PCS – superior cerebellar peduncle; PFL – paraflocculus; PM – paramedian lobe; I–X cerebellar vermian lobules 1 to 10; HVI – hemispheral lobule six; 6 – sixth nerve; 7 – seventh nerve. The NMR conditioning data are the session means of peak CR and UR amplitudes on light and white noise trials. Sessions 1–5 are postoperative training to the right (lesioned) side and sessions 11 and 12 are training sessions to the left (non-lesioned) side. Key. Solid lines – CR amplitudes; broken lines – UR amplitudes; circles – responses on white noise trials; crosses – responses on light trials

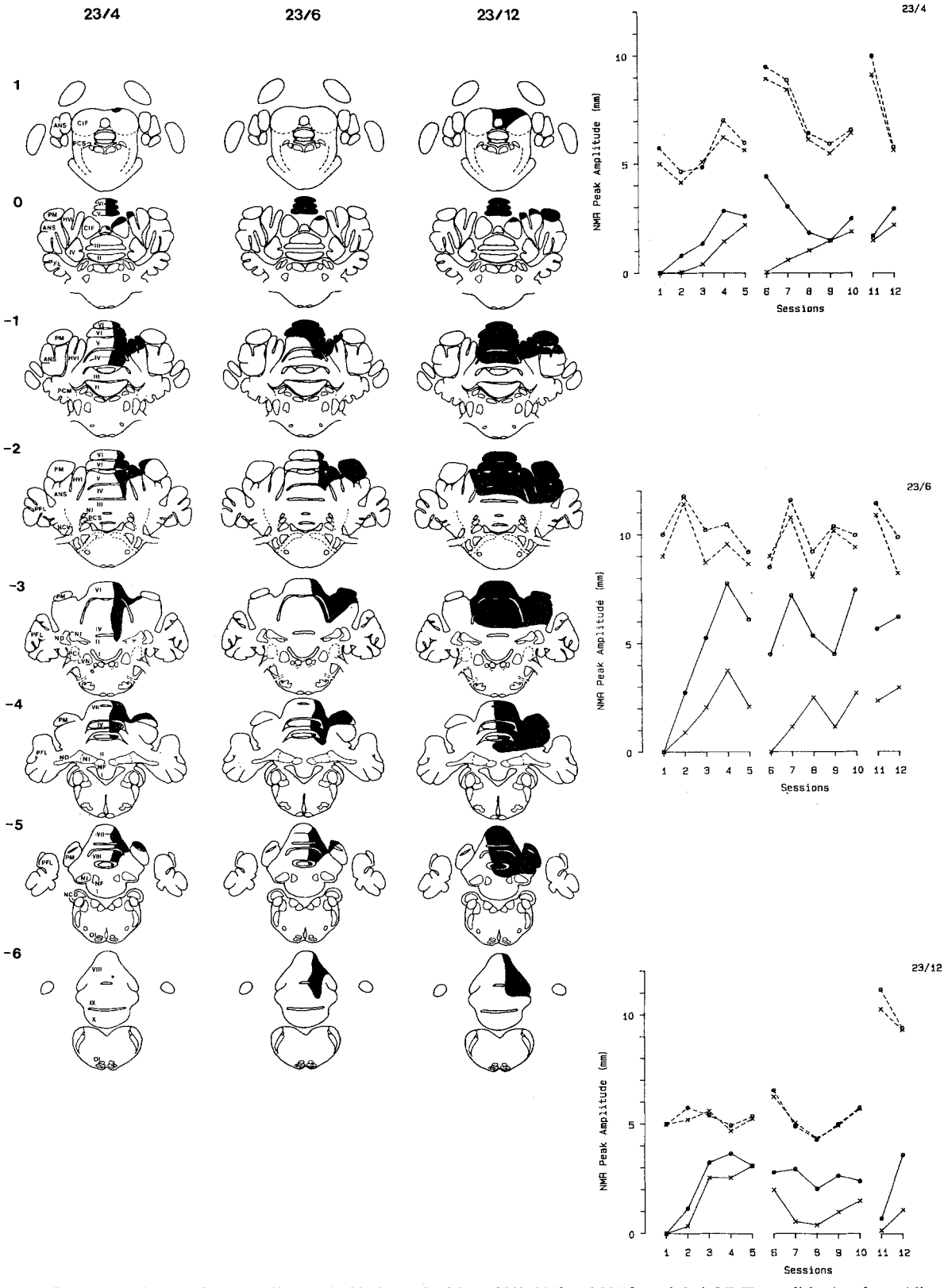


Fig. 2. Reconstructions of the cerebellar cortical lesions of subjects 23/4, 23/6 and 23/12, and their NMR conditioning data. All conventions as in Fig. 1

Fig. 3. A–H Photomicrographs of Nissl stained sections at four levels (0, 1, 2 and 3 mm posterior to lambda) through the brains of subjects 23/12 and 34/5. 23/12 had a large cerebellar cortical lesion but which spared rostral HVI and did not abolish NMR conditioning. The lesion of 34/5 was much smaller and was restricted to HVI. NMR conditioning was abolished in this subject. Calibration bar – 2 mm

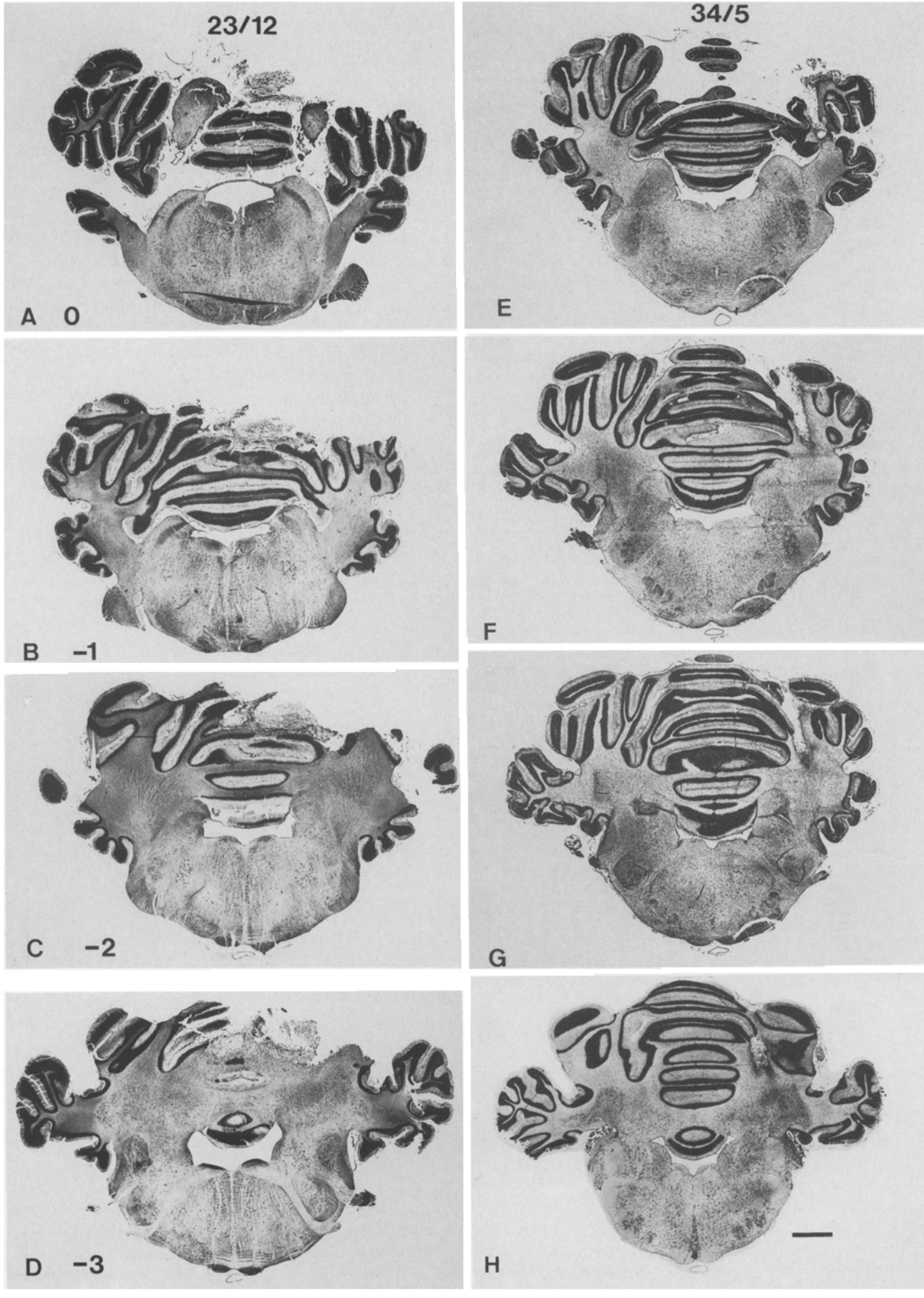


Fig. 3

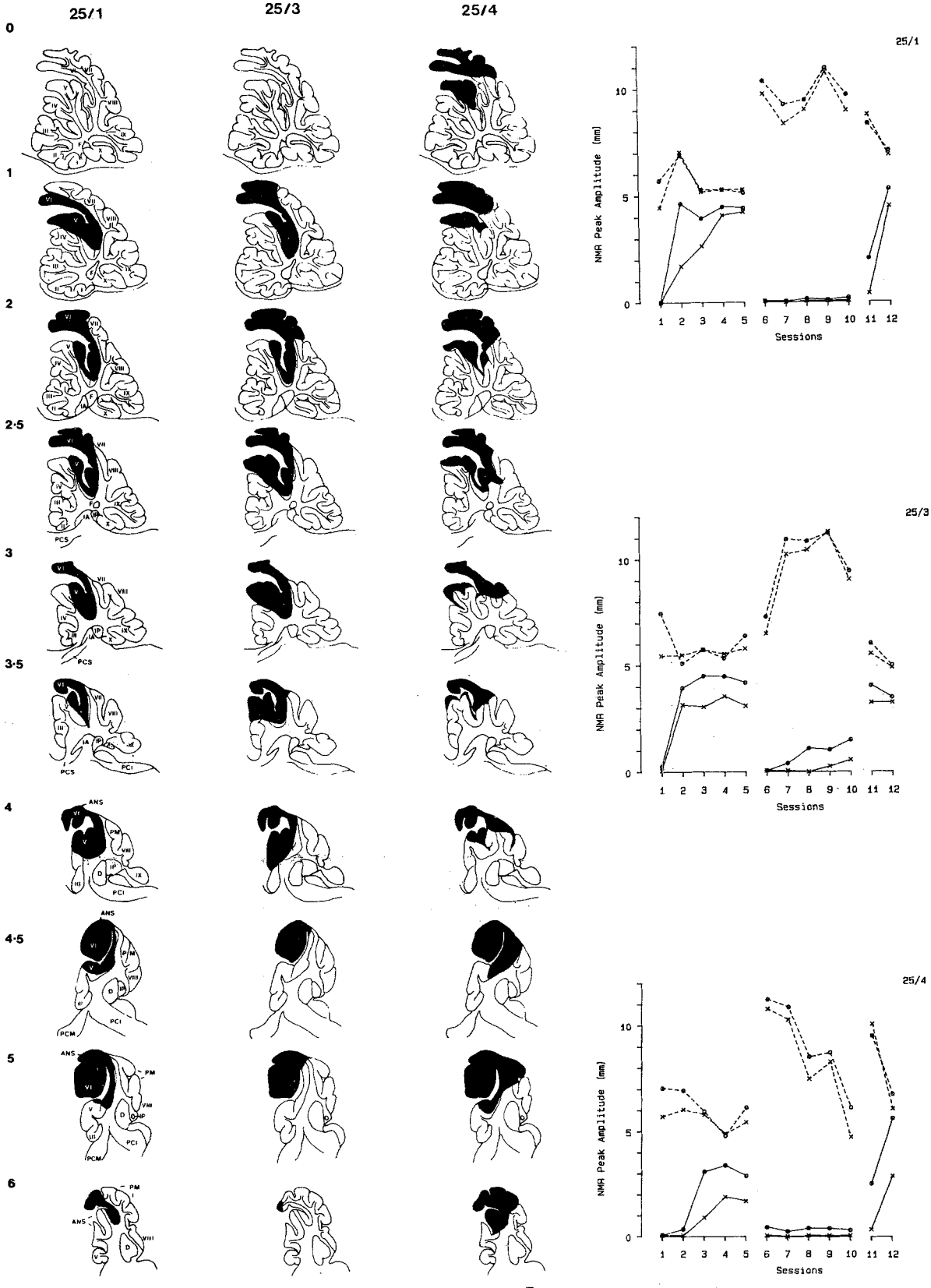


Fig. 4. Reconstructions of the HVI lesions of subjects 25/1, 25/3 and 25/4, and their NMR conditioning data. The lesions are reconstructed on a series of saggital sections through the cerebellum based on those of van Rossum (1969) and are indicated in black.
Abbreviations. D – dentate nucleus; F – fastigial nucleus; IA – anterior interpositus nucleus; IP – posterior interpositus nucleus ; PCI – inferior cerebellar peduncle; PCS – superior cerebellar peduncle; I–X – cerebellar lobules 1 to 10

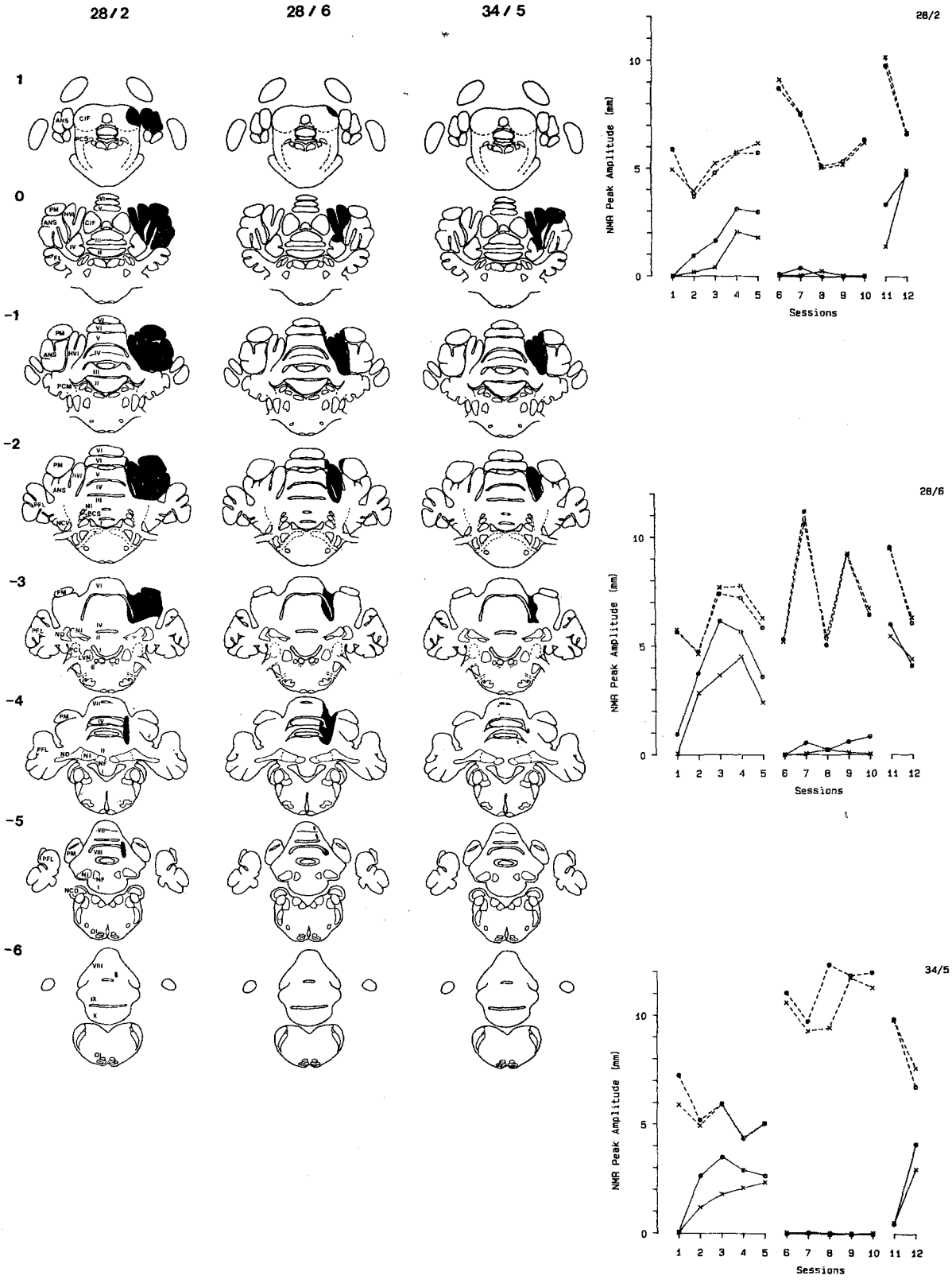


Fig. 5. Reconstructions of the HVI lesions of subjects 28/2, 28/6 and 34/5 and their NMR conditioning data. All conventions as in Fig. 1

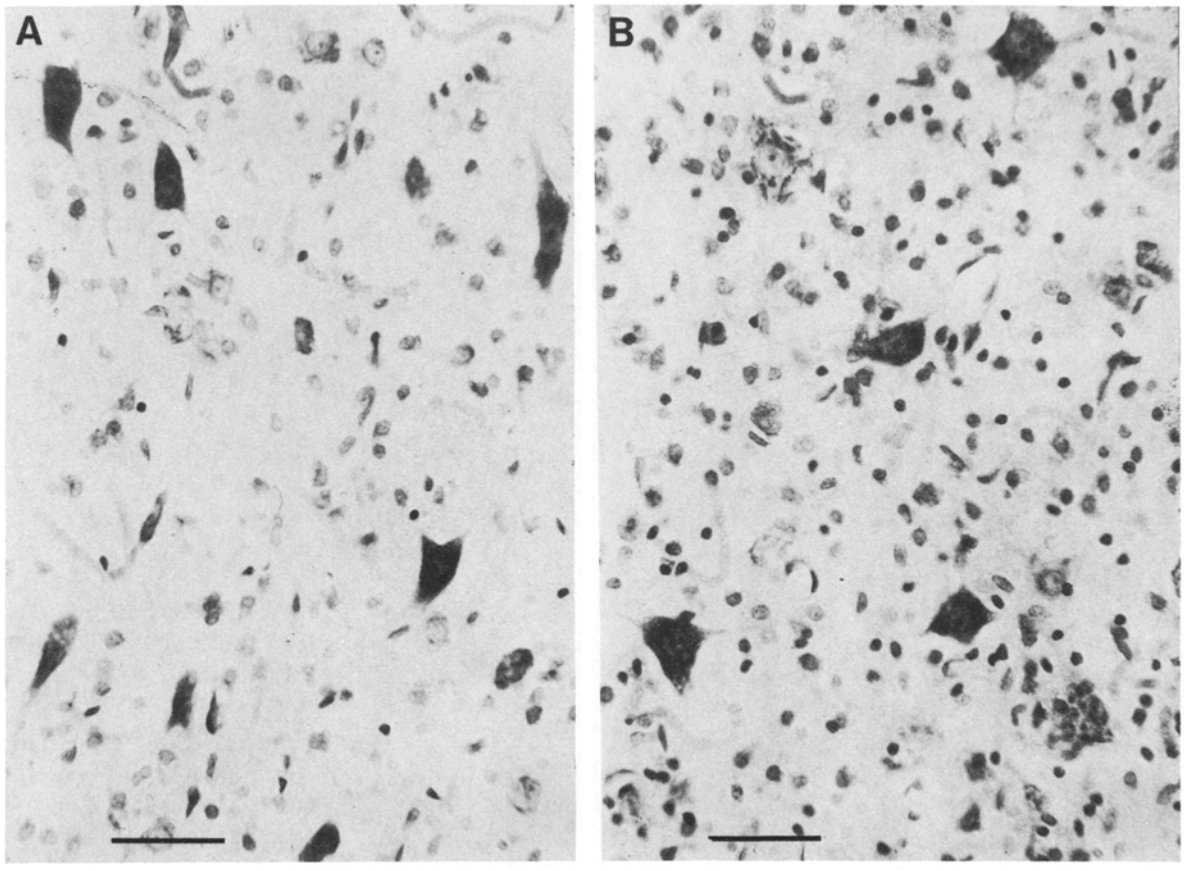


Fig. 6A and B. Photomicrographs of cells of the interpositus nuclei of subject 34/5 at 3 mm posterior to lambda. **A** shows cells from the left, non-lesioned side and **B** shows cells from the right, lesioned side. There is an increase of glia on the lesioned side, but the nuclear cells are similar in number and normal in appearance on both sides. Calibration bar – 50 μ

cortical projection to the interpositus nucleus (division C of Voogd). The behavioural data and the reconstructed cerebellar lesions of these six subjects (18/11, 18/12, 22/1, 23/4, 23/6 and 23/12) are shown in Figs. 1 and 2. Photomicrographs of a series of transverse sections through the cerebellum of 23/12 are shown in Fig. 3.

Animals 18/11 and 18/12 received similar lesions which included almost all of HVI to the depths of the lobule, much of ansiform lobe and the more rostral parts of paramedian lobe. 18/12 also had damage to dorsal paraflocculus. The cortical lesion of 22/1 included much of paramedian lobe and vermian lobules VI–VIII. There was only slight damage to the most dorsal part of HVI and to the dorsal part of the right inferior colliculus. Animals 23/4 and 23/6 received very similar lesions which included almost the entire lateral edge of the vermis and some adjacent hemispheral tissue, mainly of the paramedian lobe. Ansiform lobe and paraflocculus were spared, though there was slight damage to the most dorsal part of HVI in both cases. Animal 23/12 had a much larger lesion of the cerebellar cortex. Almost the entire paramedian lobe, posterior parts of the ansiform lobe and much of the vermis was lesioned. The vermian lesion extended over the midline to include the left side at most levels. Only vermian lobules I–III and X were completely spared. Additionally, there was some damage to the right inferior colliculus. Photomicrographs of a series of transverse sections through the cerebellum of 23/12 are shown in Fig. 3.

In no case did the lesion extend to the underlying cerebellar nuclei; the lesions were restricted to the cerebellar cortex. The cells of the cerebellar nuclei were critically examined for signs of degeneration – shrinkage, chromatolysis of loss of cells – which would have been the consequences of damage to their blood supply or cutting their efferent axons. There were no differences in the number or appearance of cells on the lesioned and unlesioned sides.

Two entirely distinct types of effect upon conditioning were seen in this group of animals. The two subjects (18/11 and 18/12) in which the lesion included

all of HVI showed virtually complete abolition of conditioned responses to light and white noise stimuli following the cortical lesion. The other four subjects (22/1, 23/4, 23/6 and 23/12) had either slight or no disruption of conditioning.

After the lesions, CRs of 18/11 and 18/12 were virtually absent, except for some very low amplitude responses by 18/12 to the light CS on sessions 6 and 10. The unconditional responses of these two subjects were of equal, or greater, amplitude than preoperative levels. Acquisition of the conditioned NMR by the non-lesioned, left side was rapid. There was evidence of immediate, positive transfer of conditioning to this side on session 11.

In the other four subjects (22/1, 23/4, 23/6 and 23/12) CRs to white noise were almost unchanged from preoperative levels. Postoperative CRs to light were, in some cases, mildly disrupted. Subject 22/1 which had shown poor preoperative conditioning to light, showed little retention with this stimulus, but did reacquire CRs over sessions 6–8. 23/4 showed a similar disruption of conditioning to the light CS over the early postoperative sessions, but reacquired CRs which equalled their preoperative levels by session 10.

Subjects 23/4 and 23/6 showed immediate positive transfer of conditioning when trained on the left (non-lesioned) side during sessions 11 and 12. 23/12 did not show this immediate transfer, but acquired CRs to both light and white noise stimuli rapidly over sessions 11 and 12. The unconditional responses of all subjects were normal and consistent through the training sessions.

In summary, moderate to extensive lesions of the ansiform and paramedian lobes, the most dorsal parts of HVI and lobules IV and V of anterior lobe did not abolish the conditioned NMR to light or to white noise; there was, in some instances, temporary disruption of CRs to light. Only in cases in which the cortical damage included most of the hemispheral part of lobule VI, was there abolition of the conditioned NMR to both light and white noise.

In order to establish whether HVI is the cerebellar cortical zone crucial for NMR conditioning, we attempted to remove this small area alone, sparing the adjacent ansiform, paramedian and anterior lobes which were variously damaged in the subjects reported above.

(ii) Small lesions of hemispheral lobule VI. 10 subjects received lesions which were directed at HVI, and all of these animals showed either abolition of the conditioned NMR or severe disruption of it. The results of these two groupings are presented separately.

(a) Six animals (25/1, 25/3, 25/4, 28/2, 28/6 and 34/5) showed virtually complete abolition of CRs to light and white noise after lesions of HVI (see Figs. 4 and 5).

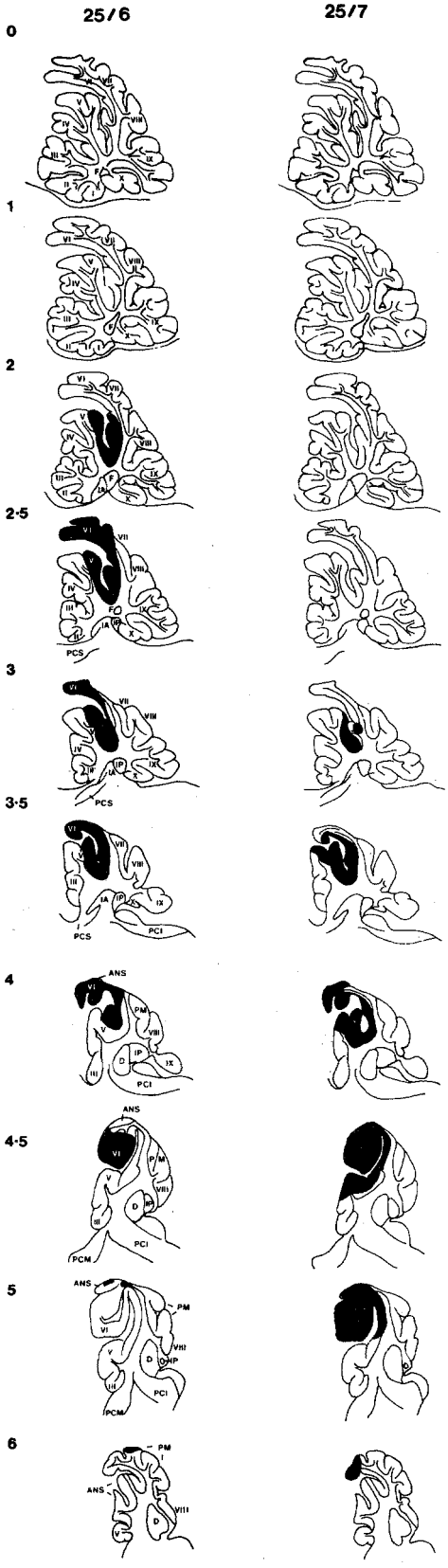
The lesions of 25/1, 25/3 and 25/4 have been reconstructed from parasaggital sections which were used to give an alternative visualisation of the cerebellar peduncles and nuclei. HVI is extensively lesioned in these three cases, together with some parts of ansiform lobe and lobule V of anterior lobe. There is some damage to paramedian lobe in 25/4. The cerebellar nuclei and all three cerebellar peduncles are completely free from damage in these subjects.

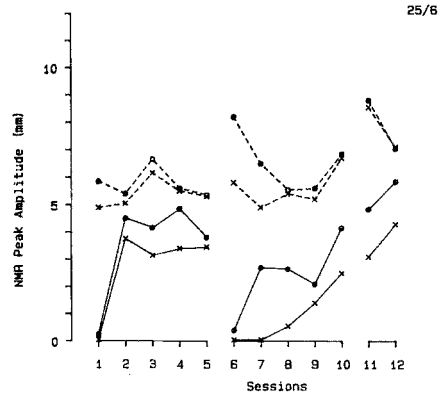
The lesion of 28/2, 28/6 and 34/5 have been reconstructed from transverse sections of the brain. All have a virtually complete lesion of HVI. The lesions extend to the depths of the lobule but, again, all three cerebellar peduncles and nuclei are completely free from damage. Photomicrographs of a series of transverse sections through the cerebellum of 34/5 are shown in Fig. 3.

The cells of the deep nuclei were critically examined for signs of degeneration. They were normal in size and appearance and no differences between the nuclei of each side were visible in the transversely sectioned brains. There was slight to moderate gliosis in the white matter between the primary lesion and the cerebellar nuclei. This we attribute to the expected degeneration of afferent and efferent fibres of the cerebellar cortex. Photomicrographs of cells of the left (non-lesioned side) and right (lesioned side) interpositus nuclei of subject 34/5 are shown in Fig. 6. Additionally, the retrograde degeneration in the inferior olive was examined according to the full and detailed descriptions of Brodal (1939, 1940). These data are presented in a following section.

HVI lesions almost totally abolished conditioning in all six subjects. Preoperative conditioning was normal in all cases, with the usual slightly smaller amplitude CRs to light than to white noise. Postoperatively, CR amplitudes to both stimuli were never more than 0.5 mm for any subject, except 28/6 which showed CR amplitudes slightly higher than this to white noise on session 10. In all cases the UR amplitudes remained at preoperative levels. All of these animals conditioned rapidly on the side contralateral to the lesion during sessions 11 and 12; they achieved CR amplitudes equal to, or greater than, those on the last preoperative session.

In summary, complete lesion of HVI which included the cortical tissue at the depths of the lobule and which spared the deep nuclei abolished NMR





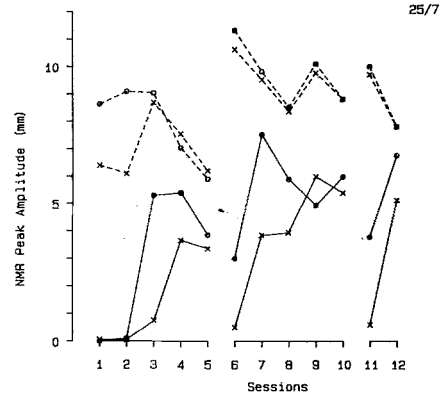


Fig. 7. Reconstructions of the subtotal HVI lesions of subjects 25/6 and 25/7, and their NMR conditioning data. All conventions as in Fig. 4

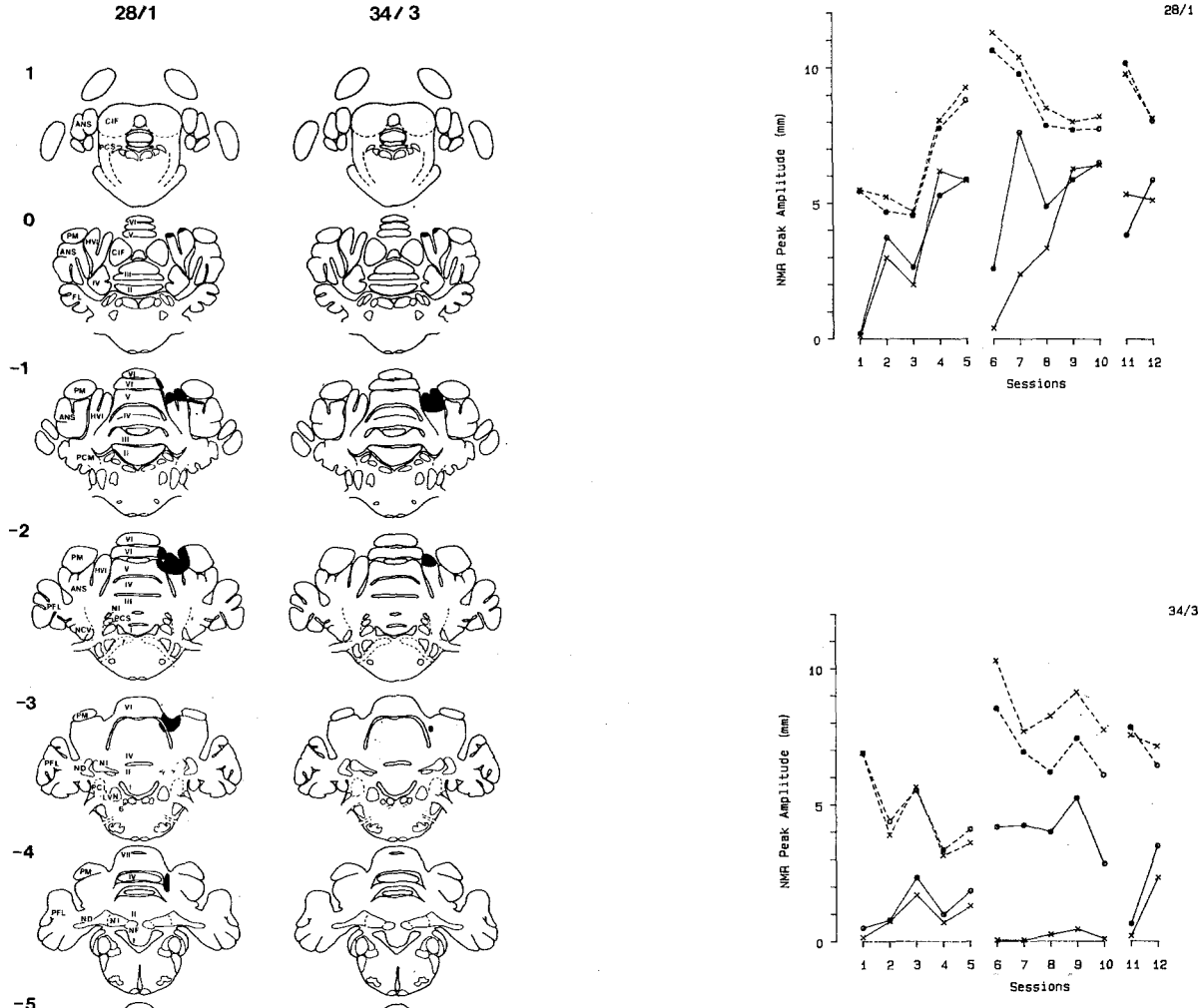


Fig. 8. Reconstructions of the subtotal HVI lesions of subjects 28/1 and 34/3, and their NMR conditioning data. All conventions as in Fig. 1

conditioning to light and white noise stimuli on the side of the lesion.

(b) A group of four animals (25/6, 25/7, 28/1 and 34/3) showed temporary or incomplete disruption of NMR conditioning after the HVI lesion (see Figs. 7 and 8).

25/6, 28/1 and 34/3 had lesions with considerable sparing of parts of HVI. 25/6 had sparing of HVI at 5 mm lateral, 28/1 and 34/3 showed damage only to the most dorsal part of HVI, the cortical tissue towards the base of the lobule was quite undamaged. 25/7 had a much larger lesion of HVI, but some of this lobule is spared alongside vermis, and at the lateral margin of the lobule (see Fig 7, 3 mm and 5 mm lateral respectively).

Three subjects (25/6, 25/7 and 28/1) showed very similar postoperative NMR conditioning. They had low CR amplitudes on the first postoperative session – session 6. These amplitudes were noticeably lower ot the light CS than to the white noise CS, even for subject 28/1, whose preoperative CR amplitudes to both stimuli had been very similar. Over the remain-

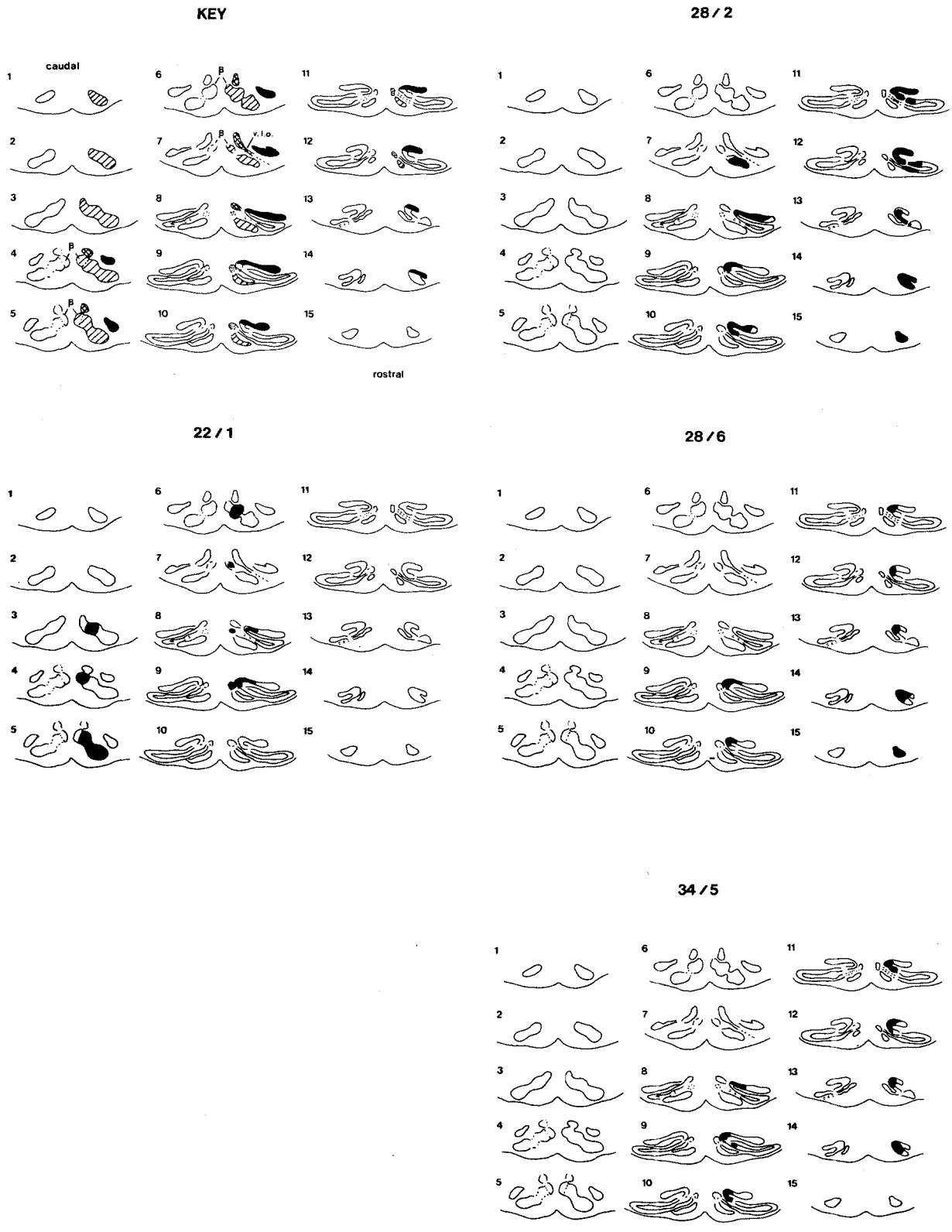


Fig. 9. Reconstructions of olivary degeneration following cerebellar cortical lesions. 22/1 had a large cortical lesion which spared HVI and did not abolish NMR conditioning. 28/2, 28/6 and 34/5 had lesions of HVI which abolished NMR conditioning. Areas of olivary cell loss or substantial shrinkage are indicated in black. The transverse sections are based on those of Brodal (1939, 1940).
Key. White areas – principal olive; black areas – dorsal accessory olive; oblique lines – medial accessory olive; cross hatching – dorsal cap; stipple – dorsomedial cell column; β – nucleus beta; v.l.o. – ventrolateral outsrowth

ing postoperative sessions, these three subjects relearned, giving CR amplitudes comparable with preoperative levels for both stimuli. In subject 34/3 there was a complete loss of CRs to the light CS, but CRs to white noise were unimpaired. All of the subjects in this group showed rapid conditioning to both stimuli on the non-lesioned side and the lesions had no effects upon UR amplitudes on either side.

(iii) Retrograde degeneration in the inferior olivary nucleus. The inferior olivary nuclei of all subjects were examined for signs of retrograde degeneration. For transversely sectioned brains, olivary cell loss and shrinkage were plotted on Brodal's standard series of transverse sections through the inferior olive of the rabbit (Brodal 1939, 1940). Because the cerebellar cortical lesions were unilateral and the olivo-cerebellar pathway decussates completely (Brodal 1940; Walberg 1980), the contralateral olive showed signs of degeneration but the ipsilateral olive did not. In Fig. 9 we present reconstructions of olivary degeneration from (a) a subject with a large posterior lobe lesion which did not abolish NMR conditioning and (b) subjects with discrete lesions of HVI which did abolish NMR conditioning.

(a) Olivary degeneration following a large posterior lobe lesion sparing HVI. Subject 22/1 received a large cerebellar cortical lesion which spared much of HVI, but included ansiform and paramedian lobes. The cortical lesion did not abolish NMR conditioning (see above). The pattern of retrograde degeneration was of cell shrinkage in several regions of the inferior olive contralateral to the cortical lesion. This shrinkage was evident in the more caudal parts of the dorsal accessory olive (DAO), in the dorsal cell column and in the medial accessory olive (MAO). There was no evidence of degeneration in the ipsilateral olive.

(b) Olivary degeneration following discrete lesions of HVI. We studied the olivary degeneration in three subjects (28/2, 28/6 and 34/5) after discrete HVI lesions which had abolished the conditioned NMR. They showed a very consistent pattern of degeneration. All had cell shrinkage or loss in the medial part of rostral DAO and the adjoining medial part of rostral principal olive (PO). In addition, 28/2, which had received primary damage to parts of ansiform and paramedian lobes, had cell shrinkage in more lateral parts of DAO and in central parts of PO.

Discussion

The experiments described demonstrate that discrete lesions of cerebellar lobule HVI abolish conditioning of the NMR but much larger lesions of the cerebellar cortex, sparing HVI, do not. Because lesions of the anterior interpositus nucleus also abolish this conditioning (Yeo et al. 1985a), it is important to be sure that the losses following cortical lesions were not spuriously due to incidental damage of the cerebellar nuclei. The integrity of the nuclear cells was taken as clear evidence that such damage did not occur in our study. Had such injury been produced by either vascular embarrassment, interruption of the superior cerebellar peduncle or direct damage to the nuclei, then there would have been degeneration of the nuclear cells. There was none. The moderate gliosis observed between the ventral part of the HVI lesions and the dorsal part of the interpositus nuclei is a necessary consequence of a cerebellar cortical lesion. It is produced by degenerating mossy and climbing fibres which projected to the lesioned part of cortex, and by degenerating axons of Purkinje cells. We may conclude that the abolition of NMR conditioning was due to the cortical lesion and not damage to the nuclei.

Undoubtedly, the cerebellum is important for many aspects of motor control. That the cerebellar cortex is crucial for a simple form of associative learning is in agreement with theories, advanced by several authors, that the cerebellar cortex is a locus of learning (Albus 1971; Eccles et al. 1967; Marr 1969). The general principle of these models is that the unique, dual input to cerebellar cortex, from mossy and climbing fibres, provides the type of stimulus convergence necessary for associative learning. Information arriving at the cerebellar cortex through the mossy fibre system activates Purkinje cells via granule cells and their parallel fibres. The models propose that these connections are modifiable under the control of climbing fibres from the inferior olive. Our finding that NMR conditioning depends upon HVI, and our studies of its connections, support those theories.

If the dual input to the cerebellar cortex is an important factor in motor learning, then we can learn more about the mechanisms of such learning by considering afferent connections of HVI from the inferior olive. The critical olivary region is indicated by the retrograde degeneration in the olive following our HVI lesions. The locus of olivary degeneration following HVI lesions is consistent with the basic plan of olivo-cerebellar projections in the rabbit first demonstrated by A. Brodal, who plotted the loci of degeneration in the olive following circumscribed cerebellar cortical lesions. As Walberg (1980) has pointed out, Brodal's original schema had to be modified in the light of recent discoveries based on more sensitive anatomical techniques. Study of olivary degeneration in our lesioned cases gave a consis-

tent picture of the source within the olive of the climbing fibre projection to HVI. We have now confirmed and extended these observations using horseradish peroxidase (HRP) techniques (see accompanying paper, Yeo et al. 1985b).

Degeneration following unilateral HVI lesions was seen in the contralateral inferior olive and was restricted to the medial part of the DAO and the adjacent medial part of the dorsal leaf of the PO. In cats, this olivary region receives an input from the spinal nucleus of the trigeminal nerve (Berkley and Hand 1978) and responds to ipsilateral tactile stimulation of the face (Gellman et al. 1983). Moreover, climbing fibre responses have been recorded in HVI during tactile stimulation of the face and to direct stimulation of the trigeminal nerve (Miles and Wiesendanger 1975a, b). If these connections are comparable in the cat and rabbit, then this evidence is consistent with the suggestion that the periorbital shock (or corneal airpuff in other studies) US used in NMR conditioning is represented at HVI of the cerebellar cortex via climbing fibres from the inferior olive.

It is unlikely that the inferior olive is a source of CS information to HVI. Visually responsive olivary cells are found only in the MAO and in the dorsal cap of the PO and auditorily responsive cells are also restricted to the MAO (Gellman et al. 1983). In no case did we see degeneration in the MAO or dorsal cap after HVI lesions which abolished or impaired conditioning.

If the olivary climbing fibres do not provide auditory and visual information to HVI, there is evidence that the mossy fibres do. Mossy fibre responses have been recorded in HVI of the cat during stimulation of the visual pathways (Buchtel et al. 1972) and in HVI of the monkey to auditory and visual stimulation (Mortimer 1975). The likely sources of this mossy fibre projection are visual and auditory region of the pontine nuclei which will be discussed, in more detail, in the accompanying paper (Yeo et al. 1985b).

These several lines of evidence provide experimental support for the Marr-Albus model. But there are, at first sight, some inconsistencies. When a unilateral HVI lesion abolishes conditioning on the same side, why does the contralateral side go on to condition so rapidly? Is the cerebellum merely an output stage for the CR and does a unilateral lesion of HVI destroy just the ipsilateral expression of the CR? Probably not. There is evidence that unilateral application of the US produces both ipsilateral and contralateral URs and CRs, though the contralateral CRs are weaker and of longer latency (Disterhoft et al. 1977). So, after the unilateral HVI lesions in our study, there was a preexisting weak, contralateral trace which was then potentiated by further training resulting in rapid conditioning. This conclusion is supported by electrophysiological evidence of weak climbing fibre responses in HVI contralateral to stimulation of the trigeminal nerve, in addition to the powerful ipsilateral input (Miles and Wiesendanger 1975b).

We have previously reported that large lesions of the posterior lobe did not abolish NMR conditioning, but these lesions did not include the critical region of HVI (Glickstein et al. 1983). Others have found no disruption of conditioning following extensive cerebellar cortical lesions of anterior and posterior lobes which may have included parts of HVI (Clark et al. 1984; McCormick and Thompson 1984). It may be that these were not sufficiently complete lesions of HVI. We have found that if the lesion includes only the more accessible, dorsal part of HVI, then conditioning is not abolished. The critical zone is at the base of the lobule, which may have been spared in these other studies.

It has been suggested that the locus of conditioning is the nuclei rather than the cerebellar cortex (Clark et al. 1984; McCormick and Thompson 1984). In our studies, lesions of HVI produced degeneration in a circumscribed region of the inferior olive and, presumably, may have removed the climbing fibre collaterals to the nuclei from this region of olive. So, in principle, the loss of conditioning could have been due to this removal of climbing fibres to the nuclei. However, if the neural basis of NMR conditioning were an association between mossy fibre CS information and climbing fibre US information at the cerebellar nuclei, then there would have to be a mossy fibre projection to the nuclei. The pontine nuclei are the only probably source of a visual and auditory mossy fibre projection. There is some physiological evidence for collaterals to the nuclei of mossy fibres destined for the cerebellar cortex (Ito et al. 1970; McCrea et al. 1967; Tsukahara et al. 1968) but the existence of such collaterals from the pontine nuclei is in dispute (Dietrichs et al. 1983; Robinson et al. 1984).

Our results are consistent with the Marr-Albus model of conditioning in the cerebellar cortex. Further support for this conclusion is given by a more detailed analysis of the neural connections of the critical HVI region which we report in the accompanying paper (Yeo et al. 1985b).

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