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Classical conditioning of the nictitating membrane response of the rabbit

III. Connections of cerebellar lobule HVI

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Summary. We report the connections of cerebellar cortical lobule HVI in the rabbit. We have studied the anterograde and retrograde transport of wheatgerm-agglutinated horseradish peroxidase (WGA-HRP) following its injection into HVI to reveal efferent and afferent connections. All of the cases showed strong anterograde transport to the anterior interpositus nucleus (AIP) – indicating that this is the major efferent target of HVI. Retrogradely labelled cells were found in the inferior olivary, spinal trigeminal, lateral reticular, inferior vestibular and pontine nuclei. Within the olive, the medial part of the rostral dorsal accessory olive (DAO) and the adjacent medial part of the principal olive (PO) were consistently labelled in all cases. This area is known to receive somatosensory information from the face and neck. There was no projection to the hemispheral part of lobule VI from visual parts of the olive within the dorsal cap and medial parts of the medial accessory olive. Likely sources of visual and auditory information to HVI are the dorsolateral basilar pontine nuclei and nucleus reticularis tegmenti pontis, which were densely labelled in all cases. These anatomical findings are consistent whith the suggestion that, during NMR conditioning, information related to the periorbital shock unconditional stimulus (US) may be provided by climbing fibres to HVI and light and white noise conditional stimulus (CS) information may be supplied by pontine mossy fibres.

Key words: Cerebellum – Lobule HVI – Corticonuclear projection – Precerebellar nuclei – Horseradish peroxidase

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Introduction

The classically conditioned nictitating membrane response (NMR) of the rabbit is crucially dependent upon the anterior interpositus nucleus (AIP) of the cerebellum (Yeo et al. 1985a) and upon a small region of cerebellar cortex, within the hemispheral division of lobule VI (HVI) which is also known as lobulus simplex (Yeo et al. 1984, 1985b). A lesion of HVI abolishes previously established NMR conditioning to light and white noise stimuli and prevents its relearning.

Is this restricted region of cerebellar cortex the actual locus of the associative processes underlying NMR conditioning or, rather, is it an essential relay to or from such a locus? In order to begin to answer this question, we need to know the connections of cerebellar HVI. Some of these connections have been studied previously in the rabbit. Brodal and Jansen (1946) and Brodal (1939, 1940) mapped retrograde degeneration in the pons and the olive after making lesions of the cerebellar cortex. Van Rossum (1969) used fibre degeneration staining methods to analyse efferent projections from the cerebellar cortex to the cerebellar and vestibular nuclei. In none of these studies were lesions entirely confined to HVI. The horseradish peroxidase (HRP) technique has the advantage of much greater sensitivity for retrograde tracing, and allowed us to analyse orthograde as well as retrograde transport in the same case.

The purpose of this paper is to report the connections of HVI in the rabbit. We have studied the anterograde and retrograde transport of wheatgerm-agglutinated horseradish peroxidase (WGA–HRP) following its injection into cerebellar HVI to reveal efferent and afferent connections.

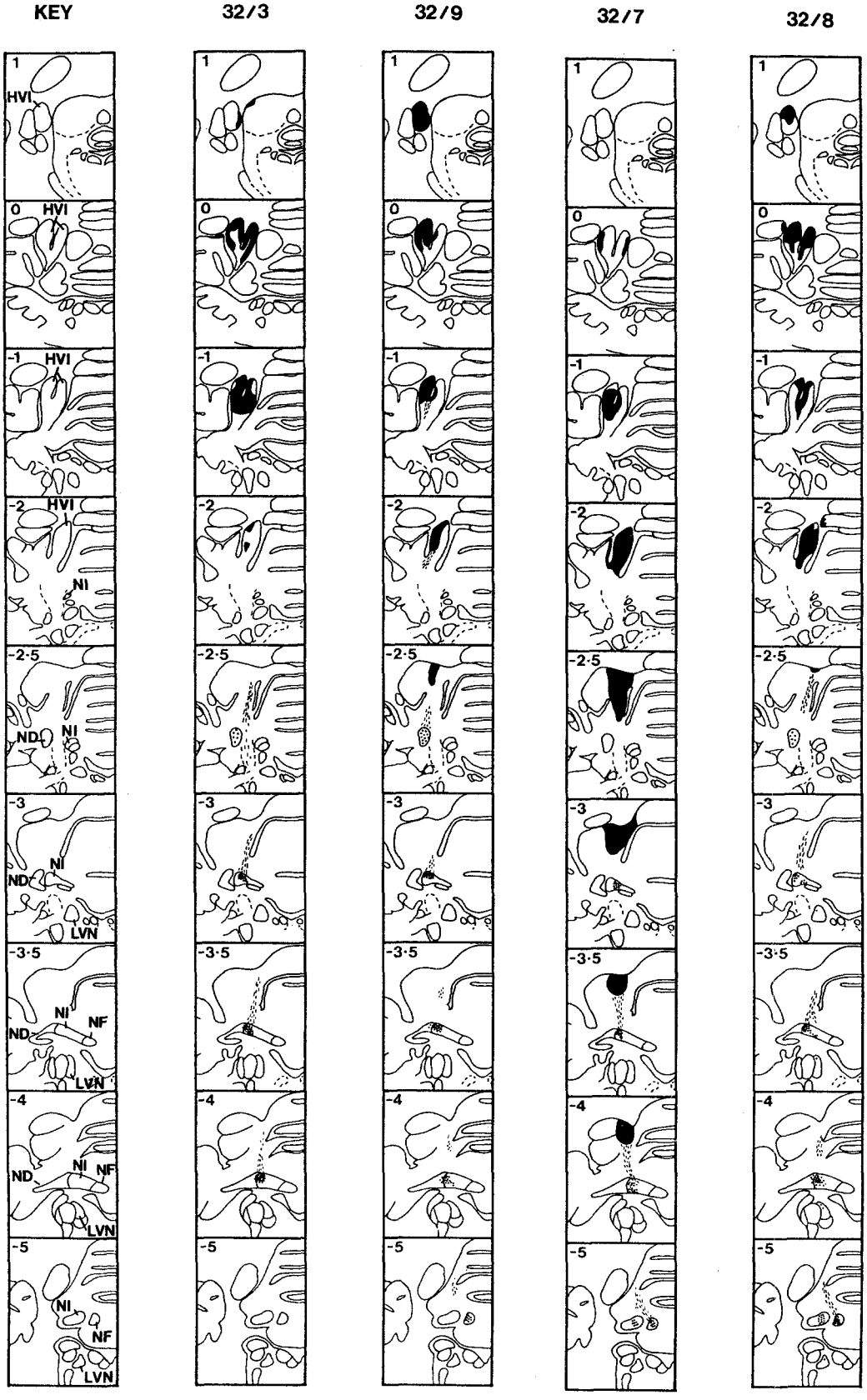

Fig. 1. Primary injection sites of HRP and its orthograde transport. The injection sites are shown in black, labelled fibres as dashed lines and terminal label as stipple. The density of stipple indicates the number of labelled terminals. The transverse sections show the right side of the brain from 1 mm anterior to lambda (1) to 5 mm posterior to lambda (–5).
Abbreviations. HVI – hemispheral division of cerebellar lobule VI; LVN – lateral vestibular nucleus; ND – dentate nucleus; NF – fastigial nucleus; NI – interpositus nucleus

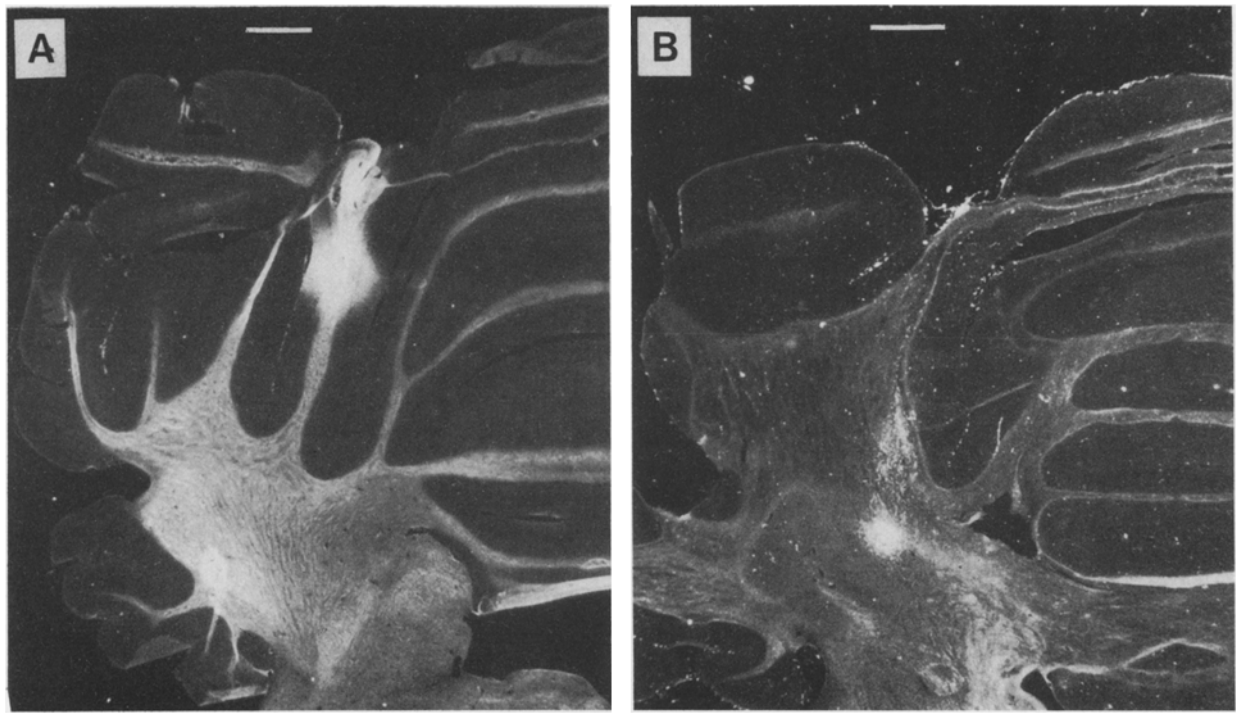

Fig. 2A and B. Dark field photomicrographs of transverse sections through the cerebellum of subject 32/3 at **A** – 1 mm and **B** –3 mm. **A** shows the primary injection site which is restricted to HVI. **B** shows the course of labelled fibres down to the anterior interpositus nucleus and terminals in this nucleus. Calibration bars – 1 mm

Methods

The subjects were 4 male, Dutch-belted rabbits of weight between 2.0 kg and 2.8 kg. They were housed individually with ad libitum food and water and maintained on a 12 h light-dark cycle.

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.). A catheter was placed in the marginal ear vein and mannitol solution (20% w/v in water, 30 ml per subject) was slowly infused to shrink and harden the brain. The scalp was cut and reflected along the midline. The cranium overlying occipital and parietal cortex on the right side was removed. The occipital cortex was carefully retracted and a vertical incision was made in the tentorium to expose cerebellar HVI.

WGA-HRP (Sigma, 4% aqueous solution) was pressure injected through a glass micropipette. The micropipette was introduced through the incision in the tentorium under direct vision until its tip penetrated the rostral bank of HVI to a depth of approximately 0.5 mm. All animals received 0.03 µl of WGA-HRP, injected over a period of 5 min. The pipette was left in situ after the injection for a further 10 min and then withdrawn. The cerebral cortical surface was then covered with sterile absorbable gelatin foam and the scalp was sutured.

After 48 h survival, each animal was given an overdose of pentobarbitone sodium and perfused through the aorta with 1.5 l of 0.09% saline, followed by 1.5 l of mixed aldehydes fixative in phosphate buffer and then postperfused with 1.5 l buffer-sucrose (Mesulam 1982). The brain was removed and stored for 1 or 2 days in sucrose-buffer at 4° C. Before sectioning, the brain was rapidly frozen and thickly coated with 5% carboxymethylcellulose solution to support the tissue. This frozen block was then serially sectioned at 20 µ using a cryostat. Every 10th section (subjects 32/3 and 32/9) or every 7th section (subjects 32/7 and 32/8) was collected on a gelatinised slide and allowed to air dry overnight. Alternate sections were processed using the tetramethylbenzidine method, following the method of Mesulam (1982) but omitting the stabilisation step. The remaining sections were stained for Nissl substance with cresyl fast violet.

Results

Primary injection site and orthograde transport

The primary injection site and orthograde transport to the deep cerebellar and vestibular nuclei are shown for each subject in Fig. 1. Representative sections were plotted on a series of nine standard transverse sections through the cerebellum. The primary injection sites are shown in black and the labelled fibres as dashed lines. Terminal label is shown stippled; the density of stipple indicates the density of label.

The boundaries between hemispheral and vermian parts of lobule VI are not obvious at caudal levels of the lobule, corresponding to levels –2.5 mm to –3.5 mm in Fig. 1. So, only in subject 32/3 was the primary injection site clearly confined to hemispheral parts of lobule VI, with little or no HRP in this

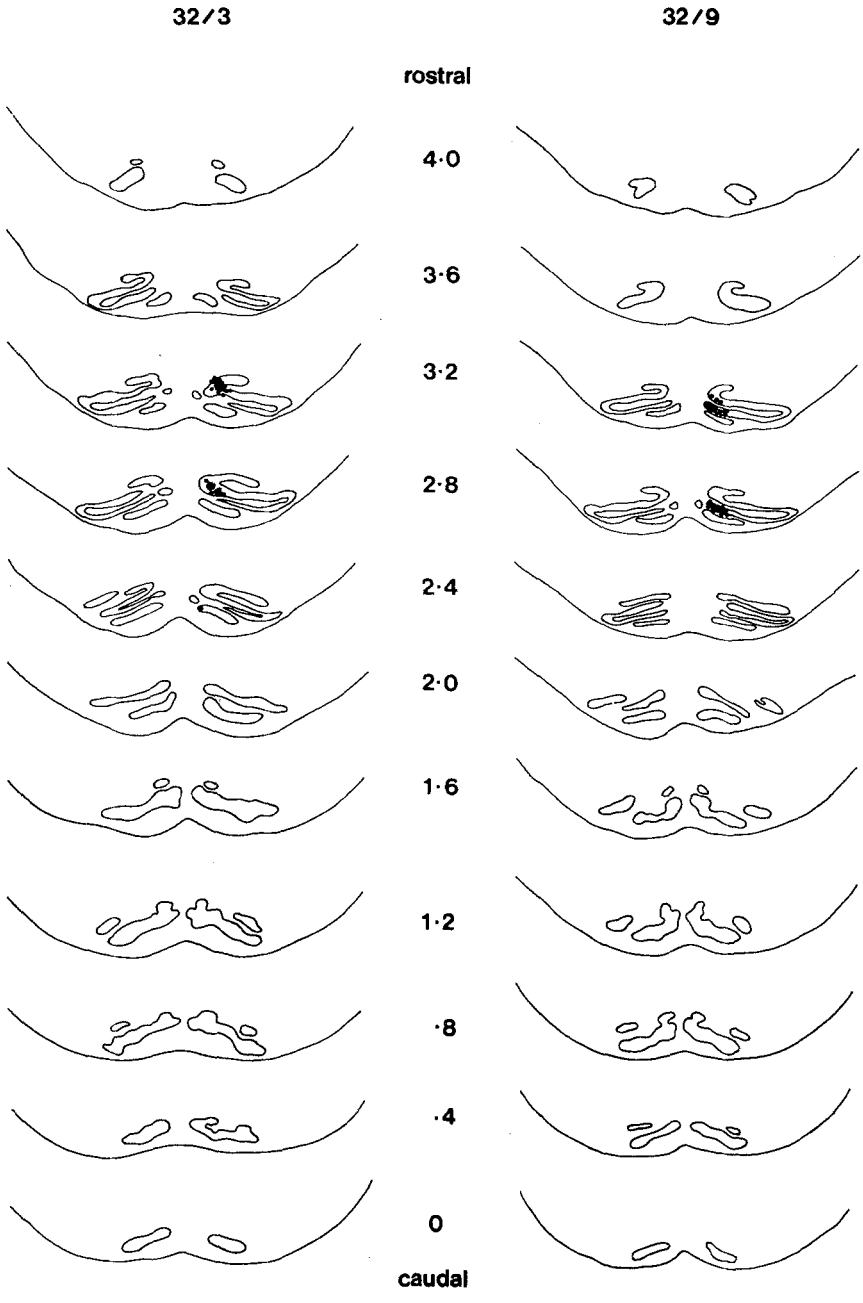

Fig. 3. Series of evenly spaced (0.4 mm) transverse sections through the inferior olives of subjects 32/3 and 32/9. The distances are in mm from the most caudal olivary section. Individual labelled cells are indicated

caudal part of the lobule. Rostral parts of the lobule were also not labelled.

In 32/9, the injection site extended a little more caudally to level –2.5 mm where no parasagittal fissure divides HVI from vermian lobule VI medially. the injection site of 32/8 was similar, but there was a clear region of primary label in lobule VI of vermis at –2 mm.

32/7 had the largest spread of primary label, to the depths of HVI at level –2 mm and at more caudal levels into lateral parts of vermian VI and slightly into paramedian lobe.

All subjects had a heavy fibre projection to the anterior interpositus nucleus (AIP) and terminals within it. The terminals extended medially from the dentate-interpositus borders and in some instances extended slightly into the dentate nucleus. In addition to this consistently prominent projection to AIP, those subjects which had primary label in HVI at level +1 mm and substantial amounts at 0 mm (32/3, 32/8 and 32/9) had sparse terminal label throughout the most rostral level of the dentate nucleus at level –2.5 mm. The terminal label extended into the posterior interpositus nucleus in those two cases in

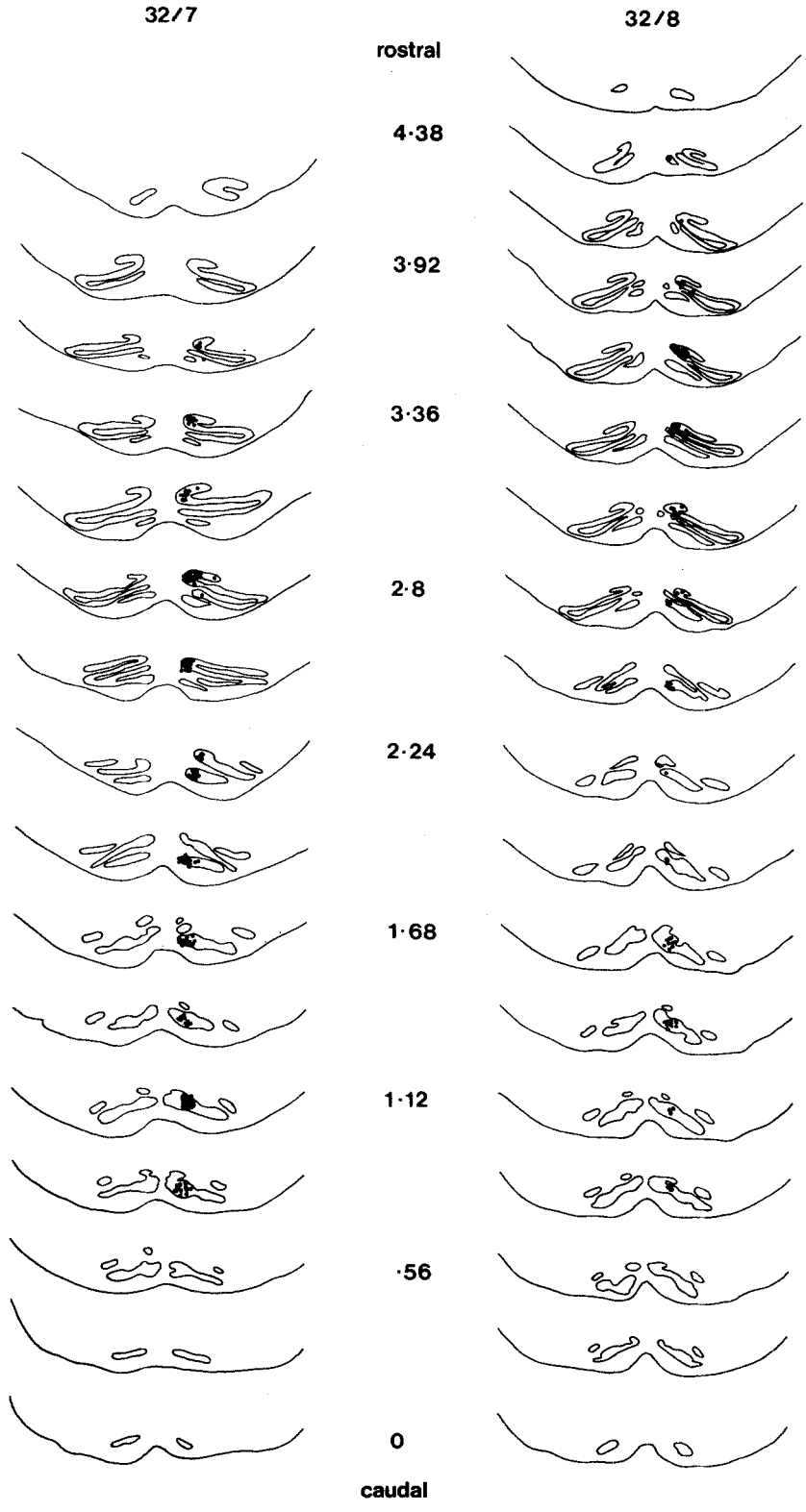

Fig. 4. Series of evenly spaced (0.28 mm) transverse sections through the inferior olives of subjects 32/7 and 32/8. Conventions as in Fig. 2

which there was heavy primary label more caudally, at –2 mm (32/8) and beyond (32/7).

In addition, three subjects (32/7, 32/8, 32/9) had a clearly separate track of fibres which coursed medially at more caudal levels to terminate in the most posterior part of the fastigial nucleus. It was these subjects (32/7, 32/8 and 32/9) which had primary label extending medially, at more caudal levels, into

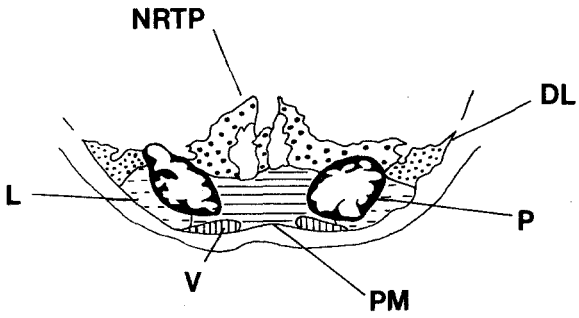

Fig. 5. Diagram of a transverse section through the pons of subject 32/7 at 1.12 mm anterior to the caudal end. The divisions of the pontine nuclei are those of Brodal and Jansen (1946). The medial division is not present at this caudal level.
Abbreviations. DL – dorsolateral nucleus; L – lateral nucleus; NRTP – nucleus reticularis tegmenti pontis; P – peduncular nucleus; PM – paramedian nucleus; V – ventral nucleus

vermian VI. In one of these cases (32/8) there was a very small amount of terminal label in the dorsal part of the lateral vestibular nucleus.

Retrograde transport to the precerebellar nuclei

The retrograde transport of HRP to cells of the inferior olive and the pontine nuclei is presented for each subject (see Figs. 3–7). Label in the remaining nuclei of the brainstem was similar in each case, so that of subject 32/7 is presented in full (Fig. 8), since it had the largest spread of primary label in HVI.

Inferior olive

The terminology of Brodal (1940) is used for the subdivisions of the inferior olive. These subdivisions are reproduced in the accompanying paper (Yeo et al. 1985b). Retrogradely labelled cells were found exclusively in the contralateral inferior olive in all cases (Figs. 3 and 4). Rostral levels of the most medial part of the dorsal accessory olive (DAO) and the adjacent part of the dorsal leaf of the principal olive (PO) were the only olivary regions which were consistently labelled in all four subjects.

Two subjects (32/8 and 32/9) with slightly greater spread of primary injection, particularly into the most rostral parts of HVI, also had labelled cells in the most medial part of the ventral leaf of rostral PO, but in no case was a projection found from the most caudal extension of PO, the dorsal cap.

Subjects 32/7 and 32/8 had labelled cells in the medial accessory olive (MAO) at its intermediate levels but none at its most rostral or caudal levels. 32/3 had only a single cell in MAO. In 32/7 and 32/8, the cells towards the more caudal end of MAO were clustered medially, but not in the nucleus beta, whose border may, therefore, beseen sharply defined at level 1.12 mm in these two subjects.

Pontine nuclei

The terminology of Brodal and Jansen (1946) is used for divisions of the pontine nuclei. These are illustrated in Fig. 5, which shows a section through the caudal part of the pons.

The labelled cells of the pontine nuclei were entirely confined to the caudal pons (Figs. 6 and 7). Almost all of the retrogradely labelled pontine cells were in the most caudal 1.2 mm of each subject. In addition to this sharp rostro-caudal demarcation, there were more labelled cells on the contralateral side in each animal.

The pattern of label within the pontine nuclei presented a consistent picture. All subjects had a prominent projection from the contralateral dorsolateral and lateral nuclei and from dorsal parts of the peduncular nucleus and a weaker projection from these areas ipsilaterally. In all cases there were a few labelled cells, mainly contralateral, in the ventral nucleus and small numbers of labelled cells bilaterally in the paramedian nucleus and in the nucleus reticularis tegmenti pontis (NRTP). This minimal projection was found in 32/3. 32/8 had rather more labelled pontine cells; their distribution was similar to that of 32/3 but with a stronger bilateral projection from NRTP. 32/9 was similar to 32/8, but with a heavier projection from the contralateral dorsolateral and lateral nuclei and more labelled cells in these divisions on the ipsilateral side. Consistent with its largest site of primary label, 32/7 had the greatest number of labelled pontine cells. These were distributed as in the other subjects but with more labelled cells in the dorsolateral and lateral pontine nuclei on both sides and greater numbers bilaterally in the paramedian and peduncular divisions.

Lateral reticular nucleus (LRN)

LRN of the rabbit may be divided into parvo- and magnocellular divisions and a smaller sub-trigeminal region more rostrally (Walberg 1952). Three subjects (32/3, 32/7 and 32/8) had labelled cells bilaterally in the LRN. The subtrigeminal resion was consistently labelled in these three animals, with similar numbers of cells on each side. In 32/7 and 32/8, labelled cells were also found more caudally, mainly in the mag-

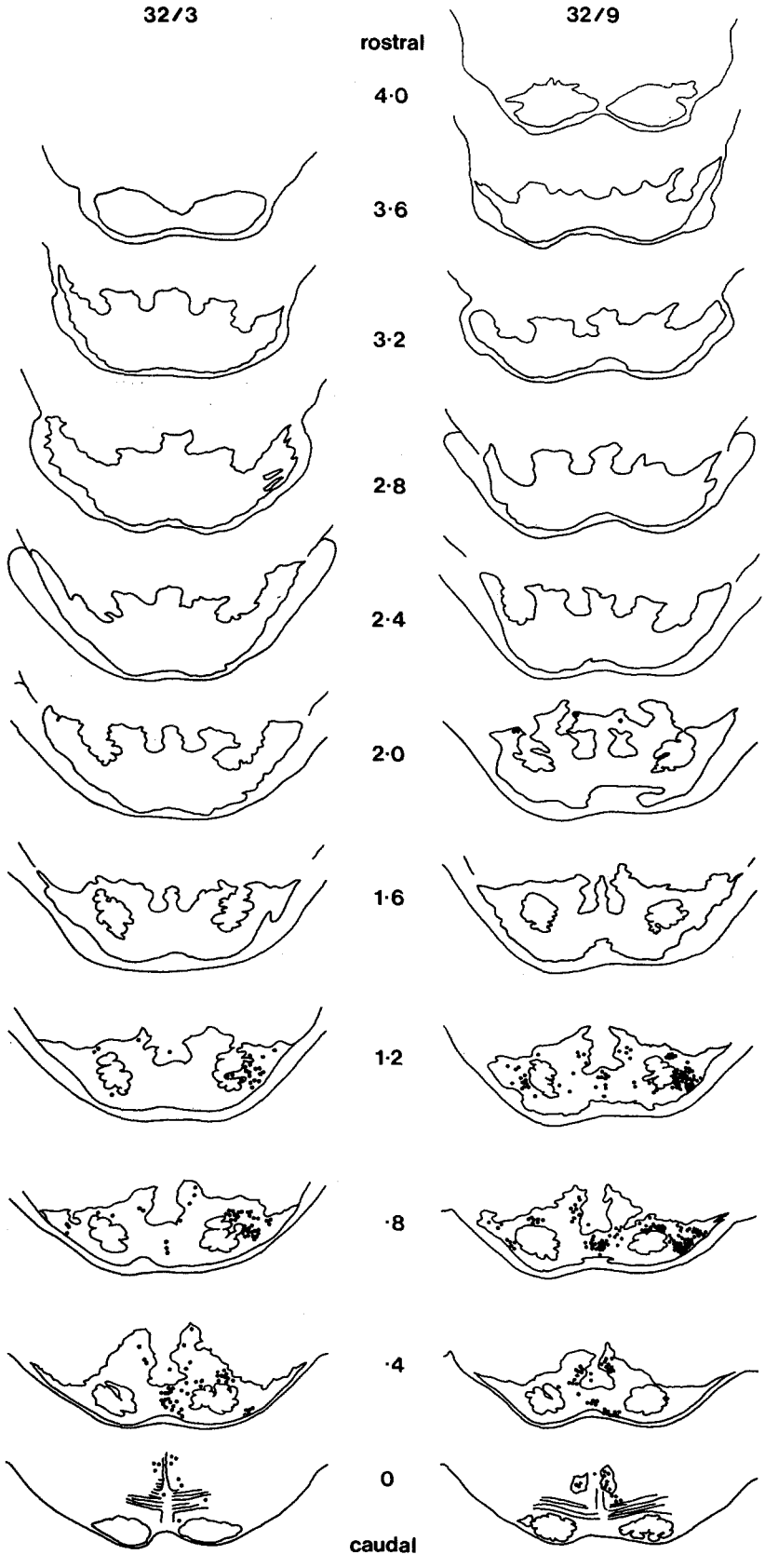

Fig. 6. Series of transverse sections through the entire pons of subjects 32/3 and 32/9 at 0.4 mm intervals from the most caudal level. Each labelled cell is indicated.

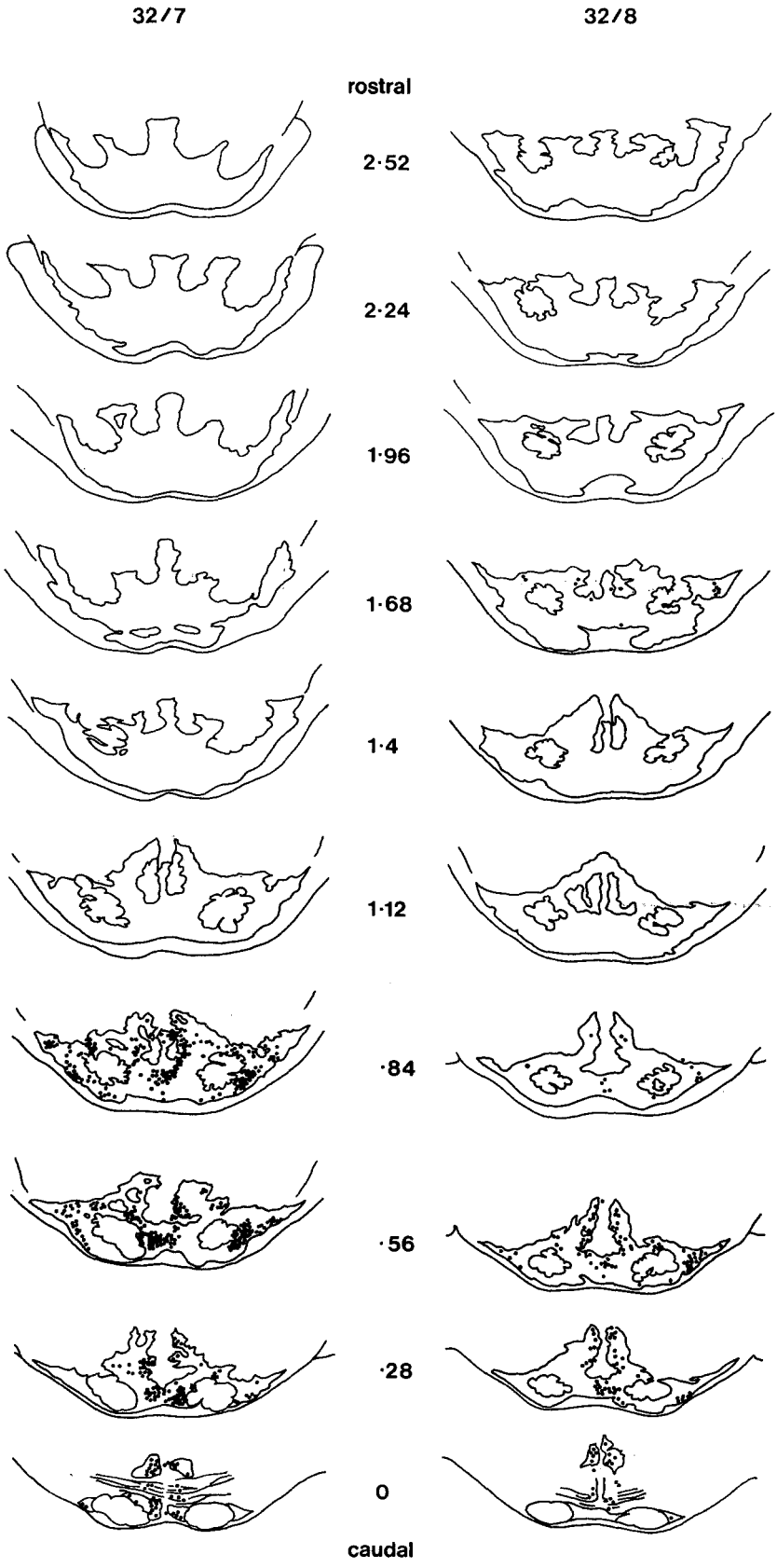

Fig. 7. Series of transverse sections through the more caudal two thirds of the pons of subjects 32/7 and 32/8. Each labelled cell is indicated. There were no labelled cells in sections rostral to those shown

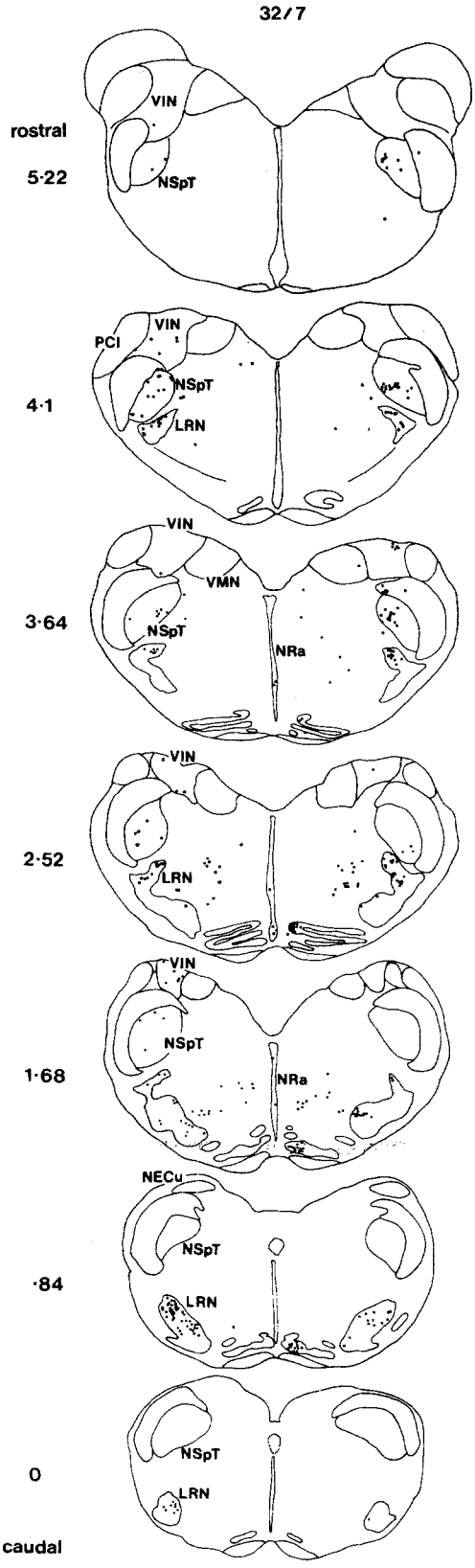

Fig. 8. A series of evenly spaced (0.84 mm) sections through the brainstem of subject 32/7 from the most caudal level of the inferior olive (see also Fig. 3 for fuller detail of olivary label) to the dorsal cochlear nucleus. Each labelled cell is indicated.
Abbreviations. LRN – lateral reticular nucleus; NECu – external cuneate nucleus; NRa – raphe nuclei; NSpT – spinal nucleus of the trigeminal nerve; PCI – inferior cerebellar peduncle; VIN – inferior vestibular nucleus; VMN – medial vestibular nucleus

nocellular division. In this region, the projection to HVI was stronger on the ipsilateral side. 32/9 had very few labelled cells in LRN.

Spinal nucleus of the trigeminal (NSpT)

Cells of the NSpT were consistently labelled in all subjects. This projection to HVI was bilateral and from the region corresponding to NSpT pars oralis of Meesen and Olzewski (1949). The densest projection was from the more rostral part of the nucleus.

Vestibular, raphe and reticular nuclei

A few labelled cells were found bilaterally in the inferior vestibular nucleus (VIN) and the raphe nuclei (NRa) and there was a sparse projection to HVI bilaterally from reticular areas medial to LRN.

Discussion

The major efferent projection of cerebellar HVI is to the interpositus nucleus. This is in agreement with the sagittal zonation of the cerebellum identified by Voogd (1969) and demonstrated in the corticonuclear projection of the rabbit by van Rossum (1969). Using the Nauta-Gygax technique to identify degenerating terminals following cortical lesions, van Rossum found a paravermal strip C projecting to the interpositus nucleus. These lesions were rather large, so it was impossible to subdivide further zone C. In cats, zone C is recognised as having three longitudinal subdivisions – C1, C2 and C3 which lie medial to lateral respectively. C1 and C3 project to the anterior interpositus nucleus (AIP) and C2 projects to the posterior interpositus nucleus (PIP) (Voogd and Bigaré 1980). In two of our cases (32/7 and 32/8) there were labelled terminals in both AIP and PIP but in the other two (32/3 and 32/9) label was confined to AIP. This finding is consistent with the suggestion that, in the rabbit, there were zones corresponding to C1, C2 and C3 of the cat and that C2 escaped primary label in two of our cases.

The most medial longitudinal zone A (Voogd 1969) projects to the fastigial nucleus in the rabbit (van Rossum 1969) and in the cat (Voogd and Bigaré 1980). Lateral to A, a narrow zone B projects to the lateral vestibular nucleus (Voogd 1969, van Rossum 1969). Recently, Ekerot and Larson (1979), using electrophysiological techniques, found an additional zone X between A and B in the anterior vermis of the cat. The presence of this zone has been confirmed

anatomically (Voogd 1983). Both A and X project to the fastigial nucleus but they differ in their connections from the olive.

The presence of terminal label in the posterior part of the fastigial nucleus in subjects 32/7 and 32/8 is consistent with the observed spread of primary label into the vermis, at level –2 mm in 32/8 and at levels –2.5 mm and –3 mm in 32/9. Although there was no spread of primary label into vermis at the more rostral levels in subject 32/9, surface features do not accurately define a vermis/hemisphere border more caudally, at levels –2.5 mm and beyond. It is likely that the primary distribution of HRP has invaded a vermian zone at this level in 32/9.

Only subject 32/8 had labelled terminals in the lateral vestibular nucleus (VLN). These were at levels –3 mm to –5 mm and were extremely sparse. Our finding that label was either sparse or absent in VLN is in agreement with the suggestion that zone B is very narrow in lobule VI of the rabbit (van Rossum 1969).

The border between AIP and the dentate nucleus (ND) of the rabbit is not readily apparent in normal material (Ono and Kato 1938; van Rossum 1969) so the labelled terminals which we observed in this region may not be definitely ascribed to either nucleus. However, at its most rostral levels (see Fig. 1, level –2.5 mm) ND is distinct and it contained sparse terminal label in three subjects (32/3, 32/8 and 32/9) with primary injection sites which included rostral HVI. This is consistent with the presence of Voogd's zone D in these more rostral levels, where HVI extends most laterally.

The major projection of HVI is to AIP, hence our lesions abolished NMR conditioning by destroying either of two stages in a necessary circuit (Yeo et al. 1985a, b). The location of an effective lesion site in AIP and the location of the densest terminal label in the present study are very similar. So, a lesion or cortical HVI is as effective in disrupting NMR conditioning as is a lesion of AIP because the latter is the major efferent target of HVI.

The sagittal zonation of the cerebellum is related not only to corticonuclear connections but also to the olivocerebellar projection (Armstrong et al. 1974; Campbell and Armstrong 1983; Courville 1975; Groenewegen and Voogd 1977; Groenewegen et al. 1979). Following the classical degeneration studies by Brodal (1939, 1940) this olivocerebellar projection to HVI has not been widely studied using the HRP technique. Studies in the cat (Brodal and Kawamura 1980; Gould 1980; Kotchabhakdi et al. 1978; Rosina and Provini 1982) report the retrograde transport of HRP following injections which spread into lobulus simplex (HVI) from crus 1 (HVII). Examples of the olivary projection to HVI in the rat (Bernard et al. 1984; Furber and Watson 1983) and in the opossum (Linauts and Martin 1978) have also been reported. The olivary projection in the rabbit is similar to that of the rat, but there are not sufficiently distinct data on lobulus simplex in the cat to make close comparisons.

The olivocerebellar projection to HVI in the rabbit arises mainly from medial DAO, but also from the medial parts of the rostral DAO and from the adjacent medial part of the dorsal leaf of the PO. This projection was particularly clearly seen in 32/3, which had a purely hemispheral injection. It is consistent with the olivocorticonuclear connections int he cat, in which DAO projects to C1 and C3 cortical zones, which themselves project to AIP (Groenewegen et al. 1979).

In the rabbit, the medial part of DAO is continuous with the dorsal leaf of PO (Brodal 1940). Labelled cells in this medial part of PO were found in 32/3 and 32/8 and in the medial part of the ventral leaf of PO in 32/8 and 32/9. It was these three subjects which had terminal label in the dentate nucleus. So, our findings are in agreement with those in the cat, in which PO projects to the cortical D zone and this projects to the dentate nucleus (Groenewegen et al. 1979). The distribution of primary label indicates that the D zone is present at the more rostral levels of HVI in the rabbit, where the lobule extends most laterally (see Fig. 1).

Subjects 32/7 and 32/8, which had spread of primary label into vermian VI, had an olivary projection from medial parts of the MAO. This projection from MAO to zone A and thence to the fastigial nucleus is consistent with that found in the cat. But subject 32/9, which had a clear projection to the fastigial nucleus, had no labelled cells in MAO. It did, however, like all of the subjects in this study, have a projection from DAO. This result is evidence for the presence of cortical zone X in the rabbit since in the cat, this zone receives an olivary input from rostral DAO (Voogd 1983).

We found no projections from the dorsal cap of PO, which is known to have visual connections (see Ito 1984). But the medial part of MAO, where we found labelled cells in 32/7 and 32/8 has an input from deep layers of the superior colliculus in the cat (Saint-Cyr and Courville 1982; Weber et al. 1978) and visual responses have been recorded here (Gellman et al. 1983). Because we did not find this projection in subject 32/3, with a purely hemispheral injection, it is likely that this part of MAO contributes the climbing fibre responses to visual stimulation which have been recorded in the vermian visual area of lobule VI in the cat (Buchtel et al. 1972). We may conclude that

the olive does not supply visual information to HVI. The distribution of auditorily responsive cells in the olive may be rather more widespread (Gellman et al. 1983), so it remains to be determined if auditory information may reach HVI via the olive.

In the light of our lesion experiments with NMR conditioning (Yeo et al. 1985a, b), the projection to HVI from medial DAO is of particular interest. In the cat this region of the olive responds to somatosensory stimulation of the face (Gellman et al. 1984) and receives input from the spinal nucleus of the trigeminal nerve (Berkley and Hand 1978). It is, therefore, most likely that the climbing fibre responses recorded in HVI of the cat to tactile stimulation of the face or direct stimulation of the trigeminal nerve (Miles and Wiesendanger 1975a, b) arise from this region of the olive. In our conditioning rexperiments, the unconditional NM response is elicited by stimulation of the periorbital region of the face with a brief electrical shock. If the medial part of the DAO contributes climbing fibres to HVI essential for the conditioning, then it is most likely that the information it contributes is related to this shock unconditional stimulus (US).

There are two likely sources of mossy fibre visual information to HVI – caudal parts of the dorsolateral and lateral pontine nuclei and nucleus reticularis tegmenti pontis (NRTP). Both were labelled bilaterally with a contralateral preponderance. The dorsolateral nucleus receives afferents from the superior colliculus (Holstege and Collewijn 1984). In cats, only the caudal pons receives input from the superior and inferior colliculi (Kawamura 1975; Kawamura and Brodal 1973); the rostral pons receives input from visual areas of the cerebral cortex (Robinson et al. 1984). This caudal pontine projection to HVI is, therefore, consistent with findings that lesions of the superior colliculus and/or pretectum abolish NMR conditioning to a light CS (Skelton et al. 1984) but decortication leaves NMR conditioning intact (Oakley and Russell 1972, 1977). NRTP may also provide visual or auditory input to HVI. It receives connections from the pretectum (Terasawa et al. 1979), the superior colliculus (Kawamura et al. 1974) and the inferior colliculus (Altman and Carpenter 1961).

The remaining projections to HVI may not be crucial for NMR conditioning but this remains to be determined. The strongly bilateral projection from the spinal trigeminal nucleus – mainly from pars oralis (Meesen and Olzewski 1949) – differs from the strictly ipsilateral projection in the cat (Ikeda 1979; Matsushita et al. 1982; Somana et al. 1980). But this projection is clearly coincident with the face representation on HVI via olivary climbing fibres.

These findings are consistent with the suggestion that, during NMR conditioning, US-related information is supplied to the cerebellum via climbing fibres from the DAO and CS-related information via mossy fibres, either from the basilar pontinenuclei or NRTP. Other evidence provides additional support for this idea. During NMR conditioning with a bilaterally presented CS and a unilateral US, in addition to the conditioning usually recorded ipsilateral to the US, there is also some weak, contralateral conditioning (Disterhoft et al. 1977). It follows that the US pathway must be strongly ipsilateral, but with a weak contralateral component. Just such a condition exists for the olivary climbing fibre input to HVI. Miles and Wiesendanger (1975a, b) recorded climbing fibre responses in HVI of the cat, ipsilateral to tactile stimulation of the face. In addition to the ipsilateral climbing fibre response, they also found responses in contralateral HVI when the stimulation was stronger, using direct electrical stimulation of the trigeminal nerve. Although the olive itself is strictly contralateral in its input to cerebellum, the trigemino-olivary projection is mainly contralateral, but with a small ipsilateral component (Berkley and Hand 1978). These findings further support the suggestion that the olive supplies US information to HVI. In principle, the US information could be relayed direct to HVI via the mossy fibre afferents from the spinal nucleus of the trigeminal nerve. But this projection was seen to be strongly bilateral, and so is a less likely source of the necessary US information.

In our lesion experiments, conditioning was established on the right side. After a cerebellar lesion on the right side, further conditioning sessions revealed that learning was abolished on the side of the lesion and that there was no reacquisition. This is in keeping with the known anatomy and physiology of the cerebellum: each side of the cerebellum influences the ipsilateral side of the body. However in our experiments, subsequent conditioning on the other, non-lesioned side, was more rapid than conditioning in a completely naive animal. It is most unlikely that this represents some sort of "transfer" process, since the critical cerebellar region was now damaged, and the cerebellum is, in any case, essentially non-commissural. It is more likely that conditioning proceeded rapidly because of the preexisting weak trace, which had developed during earlier training sessions on the contralateral side.

Our findings are consistent with the models of cerebellar cortical function in motor learning proposed by Marr (1969) and Albus (1971). They suggested that mossy fibre information (CS) is modified, at the level of the cortical Purkinje cell, under the

influence of the olivary climbing fibres (US). Other interpretations are possible. It has been suggested that the critical locus of conditioning is at the level of the cerebellar nuclei (McCormick and Thompson 1984, Clark et al. 1984). It is known that climbing fibres give collaterals to the nuclei (Eccles et al. 1967; Matsushita and Ikeda 1970; Ikeda and Matsushita 1974; Courville et al. 1977; Groenewegen and Voogd 1977). Our lesions of HVI (Yeo et al. 1985b) produced degeneration in DAO and may have deprived the interpositus nucleus of collaterals supplying possible US information. It is doubtful, however, whether there is a direct, non-cortical route for CS information to the nuclei. The pontocerebellar projection does not have a collateral to the nuclei (Dietrichs et al. 1983), and though there is electrophysiological evidence for a collateral from NRTP (Tsukahara et al. 1983) this projection has not been found with anatomical methods (McCrea et al. 1977).

Our findings support the Marr-Albus model of conditioning at the cerebellar cortex. Further studies will test these models and complete the characterisation of the essential circuitry for NMR conditioning.

References

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

Altman J, Carpenter MB (1961) Fiber connections of the superior colliculus of the cat. J Comp Neurol 116: 157–178

Armstrong DM, Harvey RJ, Schild RF (1974) Topographical localization in the olivocerebellar projection: an electrophysiological study in the cat. J Comp Neurol 154: 287–302

Berkley KJ, Hand PJ (1978) Projections to the inferior olive of the cat. II. Comparisons of input from the gracile, cuneate and spinal trigeminal nuclei. J Comp Neurol 180: 253–264

Bernard JF, Buisseret-Delmas C, Compoint C, Laplante S (1984) Harmaline induced tremor. III. A combined simple units, horseradish peroxidase, and 2-deoxyglucose study of the olivocerebellar system in the rat. Exp Brain Res 57: 128–137

Brodal A (1939) Experimentelle Untersuchungen über retrograde Zellveränderungen in der unteren Olive nach Läsionen des Kleinhirns. Z Ges Neurol Psychiat 166: 622–704

Brodal A (1940) Experimentelle Untersuchungen über die olivocerebellare Lokalisation. Z Ges Neurol Psychiat 169: 1–153

Brodal A, Jansen J (1946) The ponto-cerebellar projection in the rabbit and cat. Experimental investigations. J Comp Neurol 84: 31–118

Brodal A, Kawamura K (1980) Olivocerebelar projection: a review. Adv Anat Embryol Cell biol 64: 1–140

Buchtel HA, Iosif G, Marchesi GF, Provini L, Strata P (1972) Analysis of actuvity evoked in cerebellar cortex by stimulation of the visual pathways. Exp Brain Res 15: 278–288

Campbell NC, Armstrong DM (1983) Topographic localization in the olivocerebellar projection in the rat: an autoradiographic study. Brain Res 275: 235–249

Clark GA, McCormick DA, Lavond DG, Thompson RF (1984) Effects of lesions of cerebellar nuclei on conditioned behavioural and hippocampal neuronal responses. Brain Res 291: 125–136

Courville J (1975) Distribution of olivocerebellar fibers demonstrated by a radioautographic tracing method. Brain Res 95: 253–263

Courville J, Augustine JR, Martel P (1977) Projections from the inferior olive to the cerebellar nuclei in the cat demonstrated by retrograde transport of horseradish peroxidase. Brain Res 130: 405–419

Dietrichs E, Bjaalie JG, Brodal P (1983) Do pontocerebellar fibers send collaterals to the cerebellar nuclei? Brain Res 259: 127–131

Disterhoft JF, Kwan HH, Lo WD (1977) Nictitating membrane conditioning to tone in the immobilized albino rabbit. Brain Res 137: 127–143

Eccles JC, Ito M, Szentágothai J (1967) The cerebellum as a neuronal machine. Springer, Berlin

Ekerot CF, Larson B (1979) The dorsal spino-olivocerebellar system in the cat. I. Functional organisation and termination in the anterior lobe. Exp Brain Res 36: 201–217

Furber SE, Watson CR (1983) Organization of the olivocerebellar projection in the rat. Brain Behav Evol 22: 132–152

Gellman R, Houk JC, Gibson AR (1983) Somatosensory properties of the inferior olive of the cat. J Comp Neurol 215: 228–243

Gould BB (1980) Organisation of afferents from the brain stem nuclei to the cerebellar cortex in the cat. Adv Anat Embryol Cell Biol 62: 1–90

Groenewegen HJ, Voogd J (1977) The parasagittal zonation within the olivocerebellar projection. I. Climbing fiber distribution in the vermis of cat cerebellum. J Comp Neurol 174: 417–488

Groenewegen HJ, Voogd J, Freedman SL (1979) The parasagittal zonal organisation within the olivocerebellar projection. II. Climbing fiber distribution in the intermediate and hemispheric parts of cat cerebellum. J Comp Neurol 183: 551–602

Holstege G, Collewijn H (1984) The efferent connections of the nucleus of the optic tract and the superior colliculus in the rabbit. J Comp Neurol 209: 139–175

Ikeda M (1979) Projections from the spinal and the principal sensory nuclei of the trigeminal nerve to the cerebellar cortex in the cat, as studied by retrograde transport of horseradish peroxidase. J Comp Neurol 184: 567–586

Ikeda M, Matsushita M (1974) Electronmicroscopic observations on the olivary projections to the cerebellar nuclei in the cat. Experientia 30: 536–538

Ito M (1984) The cerebellum and neural control. Raven Press, New York, pp 268–271

Kawamura K (1975) The pontine projection from the inferior colliculus in the cat. An experimental anatomical study. Brain Res 95: 309–322

Kawamura K, Brodal A (1973) The tectopontine projection in the cat: an experimental anatomical study with comments on the pathways for teleceptive impulses to the cerebellum. J Comp Neurol 149: 371–390

Kawamura K, Brodal A, Hoddevik G (1974) The projection of the superior colliculus onto the reticular formation of the brainstem: an experimental anatomical study in the cat. Exp Brain Res 19: 1–19

Kotchabhakdi N, Walberg F, Brodal A (1978) The olivocerebellar projection in the cat studied with the method of retrograde transport of horseradish peroxidase. VII. The projection to lobulus simplex, crus I and crus II. J Comp Neurol 182: 293–314

Linauts M, Martin GF (1978) The organisation of olivo-cerebellar projections in the opossum, Didelphis virginiana, as revealed by the retrograde transport of horseradish peroxidase. J Comp Neurol 179: 355–382

Marr D (1969) A theory of cerebellar cortex. J Physiol (Lond) 202: 437–470

Matsushita M, Ikeda M (1970) Olivary projections to the cerebellar nuclei in the cat. Exp Brain Res 10: 488–500

Matsushita M, Ikeda M, Okado N (1982) The cells of origin of the trigeminothalamic, trigeminospinal and trigeminocerebellar projections in the cat. Neuroscience 7: 1439–1454

McCormick DA, Thompson RF (1984) Cerebellum: essential involvement in the classically conditioned eyelid response. Science 223: 296–299

McCrea RA, Bishop GA, Kitai ST (1977) Electrophysiological and horseradish peroxidase studies of precerebellar afferents to the nucleus interpositus anterior. II. Mossy fiber system. Brain Res 122: 215–228

Meesen H, Olzewski J (1949) A cytoarchitectonic atlas of the rhombencephalon of the rabbit. S Karger, Basel

Mesulam M-M (1982) Principles of horseradish peroxidase neurohistochemistry and their applications for tracing neural pathways – axonal transport, enzyme histochemistry and light microscopic analysis. In: Mesulam M-M (ed) Tracing neural connections with horseradish peroxidase. Wiley, Chichester, pp 1–151

Miles TS, Wiesendanger M (1975a) Organisation of climbing fibre projections to the cerebellar cortex from trigeminal cutaneous afferents and from the SI face area of the cat. J Physiol (Lond) 245: 409–424

Miles TS, Wiesendanger M (1975b) Climbing fibre inputs to cerebellar Purkinje cells from trigeminal cutaneous afferents and from the SI face area of the cerebral cortex in the cat. J Physiol (Lond) 245: 425–445

Ono M, Kato H (1938) Zur Kenntnis von den Kleinhirnkernen des Kaninchens. Anat Anz 86: 245–259

Oakley DA, Russell IS (1972) Neocortical lesions and Pavlovian conditioning in the rabbit. Physiol Behav 8: 915–926

Oakley DA, Russell IS (1977) Subcortical storage of Pavlovian conditioning in the rabbit. Physiol Behav 18: 931–937

Robinson FR, Cohen JL, May J, Sestokas AK, Glickstein M (1984) Cerebellar targets of visual pontine cells in the cat. J Comp Neurol 223: 471–482

Rosina A, Provini L (1982) Longitudinal and topographical organisation of the olivary projection in the cat ansiform lobule. Neuroscience 7: 2657–2676

Saint-Cyr JA, Courville J (1982) Descending projections to the inferior olive from the mesencephalon and superior colliculus in the cat. Exp Brain Res 45: 333–348

Skelton RW, Donegan NH, THompson RF (1984) Superior colliculus lesions disrupt classical conditioning to visual but not auditory stimuli. Soc Neurosci Abstr 10: 132

Somana R, Kotchabhakdi N, Walberg F (1980) Cerebellar afferents from the trigeminal sensory nuclei in the cat. Exp Brain Res 38: 57–64

Terasawa K, Otani K, Yamada J (1979) Descending pathways of the nucleus of the optic tract in the rat. Brain Res 173: 405–417

Tsukahara N, Bando T, Murakami F, Oda Y (1983) Properties of cerebello-precerebellar reverberating circuits. Brain Res 274: 249–259

van Rossum J (1969) Corticonuclear and corticovestibular projections of the cerebellum. Ph. D. thesis, University of Leiden

Voogd J (1969) The importance of fiber connections in the comparative anatomy of the mammalian cerebellum. In: Llinás R (ed) Neurobiology of cerebellar evolution and development. Am Med Assoc, Chicago, pp 493–514

Voogd J (1983) Anatomical evidence for a cortical "X" zone in the cerebellum of the cat. Soc Neurosci Abstr 9: 1091

Voogd J, Bigaré F (1980) Topographical distribution of olivary and corticonuclear fibers in the cerebellum: a review. In: Courville J, de Montigny C, Lamarre Y (eds) The inferior olivary nucleus. Raven Press, New York, pp 207–234

Weber JT, Partlow GD, Harting JK (1978) The projection of the superior colliculus upon the inferior olivary complex of the cat: an autoradiographic and horseradish peroxidase study. Brain Res 144: 369–377

Yeo CH, Hardiman MJ, Glickstein M (1984) Discrete lesions of the cerebellar cortex abolish the classically conditioned nictitating membrane response of the rabbit. Behav Brain Res 13: 261–266

Yeo CH, Hardiman MJ, Glickstein M (1985a) Classical conditioning of the nictitating membrane response of the rabbit. I. Lesions of the cerebellar nuclei. Exp Brain Res 60: 87–98

Yeo CH, Hardiman MJ, Glickstein M (1985b) Classical conditioning of the nictitating membrane response of the rabbit. II. Lesions of the cerebellar cortex. Exp Brain Res 60: 99–113

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