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CEREBRO-CEREBELLAR PROJECTIONS FROM THE VENTRAL BANK OF THE ANTERIOR ECTOSYLVIAN SULCUS IN THE CAT

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SUMMARY

1. Stimulation of the ventral bank of the anterior ectosylvian sulcus (AESv) induced marked mossy fibre (MF) and climbing fibre (CF) responses in the cerebellar posterior vermis (lobules VI–VII) and moderate sized ones in the paraflocculus, paramedian lobules and crus I and II of the cat. The relay stations for these responses to the posterior vermis were investigated morphologically and electrophysiologically.

2. It can be considered that the MF responses were relayed at least in part via the dorsolateral, peduncular and paramedian pontine nuclei, since in these nuclei there were units orthodromically responsive to AESv stimulation and antidromically responsive to stimulation of the posterior vermis. The MF responses are thought to be relayed monosynaptically, since the distribution of axon terminals labelled after injection of wheatgerm agglutinin-conjugated peroxidase (WGA–HRP) into the AESv overlapped in these pontine nuclei with that of neurons labelled after injection of WGA–HRP into the posterior vermis.

3. It is thought that the CF responses are relayed in the caudomedial part of the medial accessory olive (MAOcm), because neurons in the MAOcm were orthodromically responsive to AESv stimulation and antidromically responsive to stimulation of the posterior vermis.

4. It is suggested that the cerebro-olivary projection which transmits the orthodromic responses in the MAOcm is indirect, via the superior colliculus (SC), because injection of WGA–HRP into the AESv labelled axon terminals not in the MAOcm but in the SC, and injection of WGA–HRP into the MAOcm gave rise to retrograde labelling of cells in the SC. Synaptic connections between the axon terminals of the cerebrotectal projection and the tecto-olivary neurons were demonstrated by extracellular unit studies in the SC.

5. The hypothesis that the CF responses were transmitted via the SC was supported by the finding that the CF responses disappeared transiently after muscimol or lidocaine was injected into the SC.

6. These findings provide evidence that the MF responses are transmitted at least in part via the cerebro-ponto-cerebellar projection, while the CF responses are relayed via the cerebro-tecto-olivo-cerebellar projection. These cerebro-cerebellar pathways from the AESv are suggested to participate in conducting visual information to the posterior vermis.

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INTRODUCTION

The ventral bank of the anterior ectosylvian sulcus (AESv) is classified as a visual cortex named 'anterior ectosylvian visual area' (Mucke, Norita, Benedek & Creutzfeld, 1982). Neurons in the AESv are markedly responsive to objects moving in the field of view (Mucke *et al.* 1982; Hicks, Benedek & Thurlow, 1988). It is known that stimulation of the AESv evokes saccadic eye movements (Tamai, Miyashita & Nakai, 1989), and that neurons in the AESv discharge before saccades (Tamai, Miyashita & Kimura. 1990). The properties of the cerebellar posterior vermis are similar to those of the AESv, that is, the posterior vermis responds to visual stimulation (Snider & Stowell, 1944) and stimulation of the posterior vermis induces saccadic eye movements (Ron & Robinson, 1973; Noda & Fujikado, 1987). The posterior vermis projects to the caudal part of the fastigial nucleus (Courville & Diakiw, 1976), which in turn projects to the AESv (Noda & Oka, 1985; Kyuhou & Kawaguchi, 1987). Because reciprocal connections exist between some parts of the cerebrum and cerebellum (Sasaki, Matsuda, Kawaguchi & Mizuno, 1972; Sasaki, Oka, Matsuda, Shimono & Mizuno, 1975; Kawaguchi, Miyata & Kato, 1983; Kato, Kawaguchi & Miyata, 1987, 1988), the existence may be predicted of pathways from the AESv to the posterior vermis. The present study sought to test this prediction and to locate the anatomical connections underlying the predicted pathways.

METHODS

Electrophysiological study

The electrophysiological experiments were performed on fifteen cats. In two cats, the middle ectosylvian gyrus was ablated aseptically under pentobarbitone anaesthesia (38 mg kg^{-1}, I.P.) 2 weeks before the experiments. Whenever necessary, the anaesthesia was supplemented by intraperitoneal injection (3·0–6·0 mg kg^{-1}). A bone flap over the temporal cortex was removed and the underlying dura was incised and reflected. The grey matter of the middle ectosylvian gyrus was aspirated. The white matter revealed was covered with the dura, the bone flap was replaced and the skin was sutured. The animals were kept warm with supplemented heating until they recovered from anaesthesia. Antibiotics were injected intramuscularly and applied to the scalp incision. The animals were fed after full recovery from anaesthesia.

During the experiments, the head was fixed in a stereotaxic apparatus and a wide craniotomy was made to expose the cerebrum and cerebellum under pentobarbitone anaesthesia (38 mg kg^{-1}, I.P.). The level of anaesthesia was maintained adequate by continuous intravenous injection of the anaesthetic (2·5–5·0 mg kg^{-1} h^{-1}) via the radial vein which was cannulated. In cats used to record field potentials in the cerebellum, respiration was natural. The other cats were immobilized by continuous injection of pancuronium bromide (Mioblock, Organon Co.; initial dose 0·2 mg kg^{-1}, continuous dose 0·05–0·1 mg kg^{-1} h^{-1}) via the cephalic vein which was cannulated and they were artificially ventilated. Whenever the electroencephalogram or pupil size indicated arousal, the rate of anaesthetic infusion was increased to maintain adequate anaesthesia at all times. The rectal temperature was maintained within the range 36–38 °C. Several pairs of bipolar stimulation electrodes were placed on the AESv and the cerebellar posterior vermis. Concentric electrodes with tapered tips were placed in the intermediate layers of the superior colliculus (SC) or the caudomedial part of the medial accessory olive (MAOcm). A concentric electrode with a flat tip (LOC) was put on the surface of the cerebellum to excite parallel fibres. Triple pulses (0·3 ms duration, 0·3–0·8 mA intensity) were used to stimulate the cerebral cortex and the SC and single pulses (0·3 ms duration, 0·5 ms interval, 0·1–0·2 mA intensity) to stimulate the parallel fibres. For stimulation of the MAOcm, weak single-pulse stimulation (0·3 ms duration, under 50 μA intensity) was used. In order to place the concentric stimulating electrode exactly in the MAOcm, the field potentials evoked by stimulation of the AESv were recorded from the inner electrode and the

electrode was moved to the point where substantial field potentials were induced by AESv stimulation. Glass microelectrodes filled with 3 M-NaCl (DC resistance 1·2 MΩ) or Pontamine Sky Blue in 0·75 M-sodium acetate (DC resistance 5–15 MΩ) were used respectively to record field potentials in the cerebellar cortex and extracellular units in the MAOcm and SC. A glass-coated Elgiloy microelectrode was used to record from the pontine nucleus. Locations of recorded units were marked by passing DC current (10 μA for 10 min for glass microelectrodes; 5 μA for 1 min for Elgiloy microelectrodes). Lidocaine (10%) or muscimol dissolved in saline (3 mg μl^{-1}) was injected into the SC using a Hamilton syringe with a glass micropipette on the tip. These drugs were injected slowly for 10 min. After the experiments, under deep pentobarbitone anaesthesia (70 mg kg^{-1}, I.P.), the brain was perfused with a 10% formalin solution via the aorta. In the cases using the Elgiloy electrodes, the brain was perfused with a 10% formalin containing 3% potassium ferrocyanide to detect iron deposits. The brain was cut on a freezing microtome and stained with Neutral Red. Subsequently, the marked recording sites and tracks of the stimulating electrodes were examined histologically.

Morphological study

Seven cats were used for morphological study. Wheatgerm agglutinin-conjugated horseradish peroxidase (WGA–HRP, Toyobo) was used as the tracer. The animals were anaesthetized with pentobarbitone (38 mg kg^{-1}, I.P.), placed in a stereotaxic apparatus, and craniotomies were made aseptically to expose the temporal part of the cerebral hemisphere and/or the most caudal part of the cerebellum. In four cats, the efferents of the AESv and the afferents of vermal lobules VI–VII were investigated. WGA–HRP (0·2–0·6 μl of a 5% solution of WGA–HRP dissolved in saline) was injected into one or two sites in the AESv and one site in vermal lobule VI or lobule VII using a Hamilton syringe with a glass micropipette. The tip of the glass micropipette, filled with a 5% solution of WGA–HRP dissolved in saline, was placed in the MAOcm by monitoring the field potentials evoked by stimulation of the AESv. After identifying the location of the MAOcm, WGA–HRP was injected electrophoretically through the micropipette by passing 1 μA of anodal current for 30 min. After a survival period of 24–40 h the animals were anaesthetized deeply (70 mg kg^{-1}, I.P.) and perfused through the aorta with 200 ml of 7% formalin in 0·1 M-phosphate buffer (pH 7·6) containing 10% sucrose, and kept 2–4 days in a refrigerator. The brain was then cut serially at 60 μm on a freezing microtome. The sections were reacted with benzidine dihydrochloride according to the method of DeOlmos & Heimer (1977), washed in 0·9% saline, mounted on glass slides, and stained with Neutral Red. Some sections were initially unstained to avoid fading of the anterogradely labelled axon terminals. They were stained later if necessary.

RESULTS

Field potentials evoked in the cerebellum by AESv stimulation

Stimulation of AESv induced two distinct negative potentials in the molecular layer of cerebellar vermal lobule VII (Fig. 1*A*). In order to determine whether the potentials were evoked via mossy fibres (MF) or climbing fibres (CF), parallel fibre and double-shock stimulations were performed. The parallel fibre stimulation was given 20 ms before AESv stimulation. In the superficial part of the molecular layer (Ms), parallel fibre stimulation depressed the early negative potential (called the N3 potential by Eccles, Llinàs & Sasaki, 1966) but slightly enhanced the late negative potential. In the deep part of the molecular layer (Md) and the granular layer, the early potential was unchanged and the later potential was slightly enhanced. The depression of the early negative potential by parallel fibre stimulation in the Ms is thought to result from Golgi cell inhibition which blocks mossy fibre–granule cell transmission (Eccles *et al.* 1966).

After double-shock stimulation of the AESv, the early potential evoked by the second stimulus was decreased only when the interval between the stimulations was less than 20 ms, while the late negative potential was markedly decreased when the

interval between the stimulations was 10–100 ms (Fig. 1*B*). The prolonged double-shock depression of the late negative potential is characteristic of responses evoked via the CF (Eccles, Provini, Strata & Toborikova, 1968). It is thought that the depression of CF responses by double-shock stimulation is due to a powerful

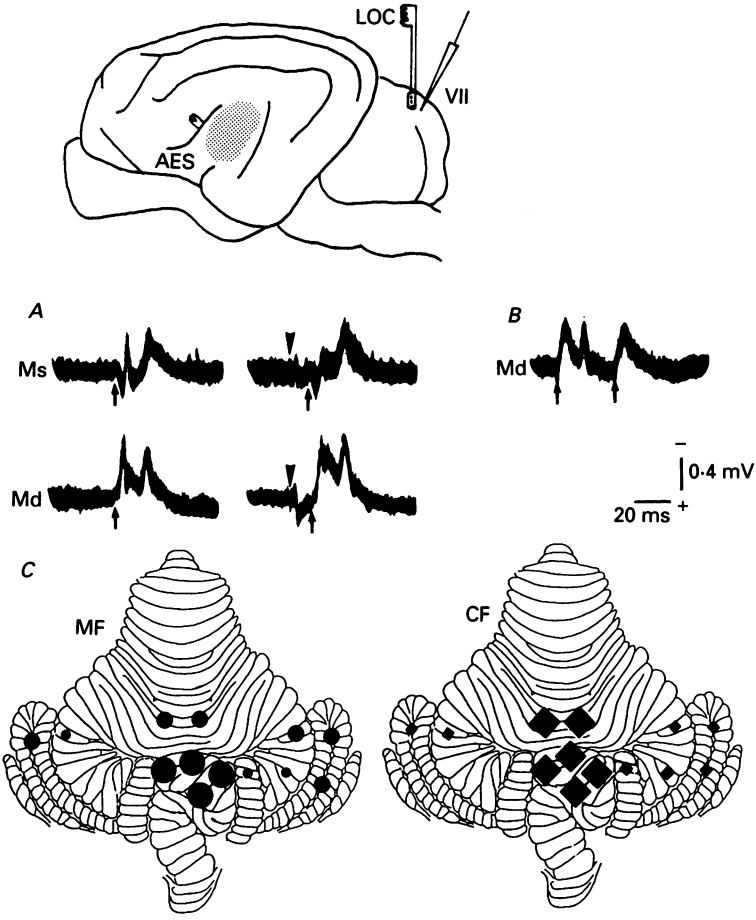

Fig. 1. Responses to AESv stimulation in the cerebellar cortex. Upper diagram shows experimental arrangements. The middle ectosylvian gyrus had been ablated 2 weeks before recording (stippled area). *A*, field potentials evoked by stimulation of AESv. AES, anterior ectosylvian sulcus; VII, cerebellar vermal lobule VII, LOC, parallel fibre stimulation (arrowhead) on the field potentials evoked by AESv stimulation. *B*, responses to double-shock AESv stimulation. Polarity is upward negative. *C*, distribution of the MF (left) and CF (right) responses evoked by AESv stimulation. The magnitude of the responses is classified into three groups according to the amplitudes of the field potentials, which were averaged from five animals, and were shown as ● or ◆ of three different sizes. Large sizes indicate larger responses. Left side is the side ipsilateral to the AESv stimulation.

inhibitory mechanism in the inferior olive (Armstrong & Harvey, 1966). These findings indicate that the early and late negative potentials are MF and CF responses, respectively.

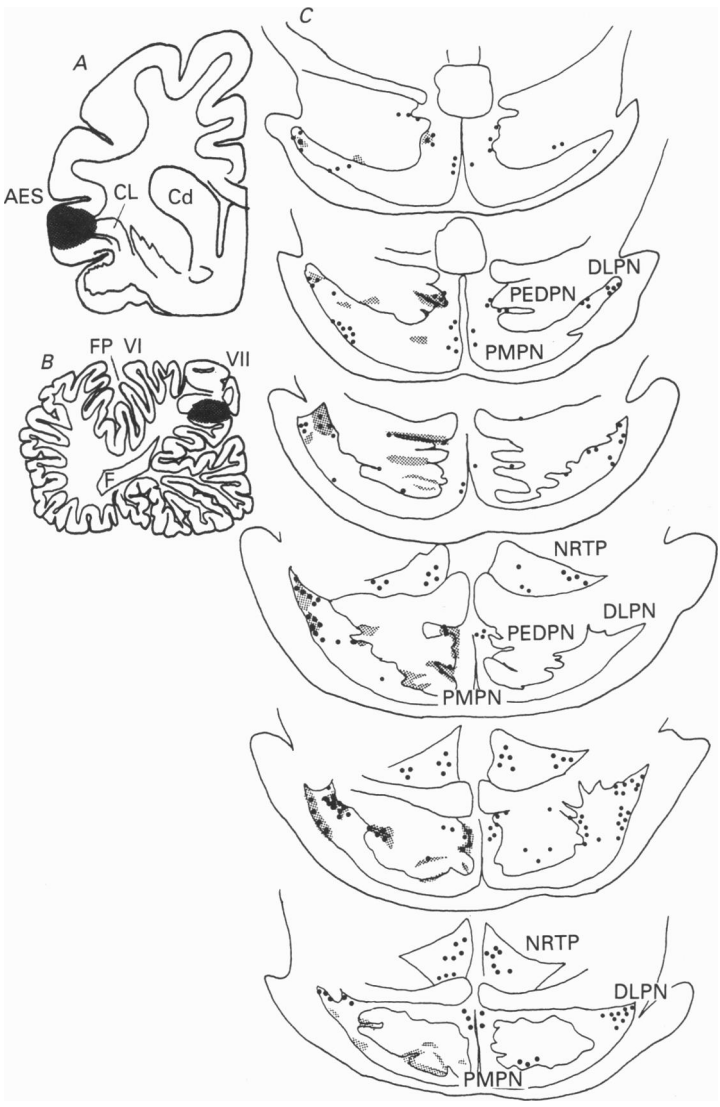

Fig. 2. Morphological investigation of the relay stations that transmit the MF responses to the posterior vermis. Diffusion of the WGA–HRP injected in the AESv (*A*) and cerebellar vermal lobule VII (*B*). *C*, anterogradely labelled axon terminals (stippled) and retrogradely labelled neurons (dots) are plotted on a rostral (top) to caudal (bottom) series of frontal sections of the pons. One dot indicates three labelled neurons. Note the overlapping of the distribution of the labelled axon terminals and neurons. CL, claustrum; Cd, caudate nucleus; F, fastigial nucleus; FP, fissure prima; PEDPN, peduncular pontine nucleus; DLPN, dorsolateral pontine nucleus; PMPN, paramedian pontine nucleus; NRTP, nucleus reticularis tegmenti pontis.

The MF responses were large in the posterior vermis (lobules VI–VII) but moderate sized or small in the lateral part of the crus I and crus II, the paramedian lobules and the dorsal paraflocculus (Fig. 1*C*, left). the CF responses were large and

stable in the posterior vermis but weak and variable from trial to trial in the lateral part of crus I and crus II, the paramedian lobule and dorsal paraflocculus (Fig. 1*C*, right). Latencies of the MF and CF responses in the posterior vermis (measured at the inset of the negative potential in the Md) were 2–3 and 14–17 ms, respectively.

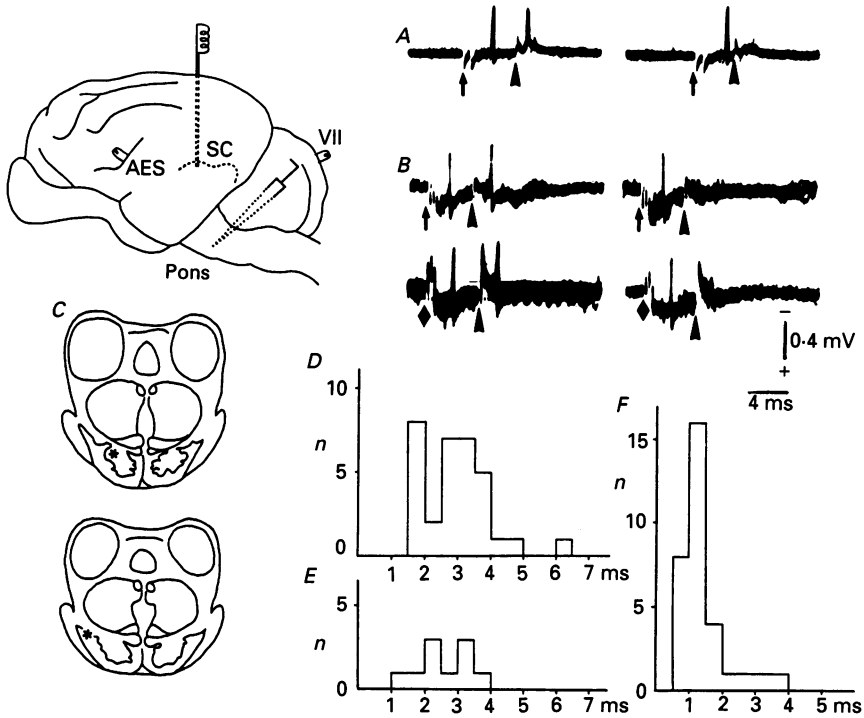

Fig. 3. Electrophysiological investigation of the relay stations that transmit the MF responses in the posterior vermis. Upper left diagram shows the arrangement of the experiment. *A*, a unit in the peduncular pontine nucleus responded orthodromically to AESv (arrow) stimulation and antidromically to stimulation of the posterior vermis (arrowhead). Collisions occlude when the interval between stimulation was less than 4 ms (right). *B*, a unit in the dorsolateral pontine nucleus was orthodromically responsive to both AESv (arrow) and SC (diamond) stimulation, and was antidromically responsive to stimulation of the posterior vermis. *C*, the recording sites (asterisk) were plotted on frontal sections of the pons. Upper and lower diagrams correspond to the sites of the recordings shown in *A* and *B*, respectively. Latency histograms of the responses evoked by stimulation of the AESv (*D*), the SC (*E*) and the posterior vermis (*F*), respectively. Vertical axis in each of *D*, *E*, and *F* shows number of responsive units.

MF and CF responses were also evoked by stimulation of the middle ectosylvian gyrus (probably corresponding to the secondary auditory area, Aii). The MF responses evoked by Aii stimulation were larger than those evoked by AESv stimulation, while the CF responses evoked by Aii and AESv stimulation were of almost the same size. The spatial distributions on the cerebellar surface of the MF and CF responses evoked by Aii and AESv stimulation were the same. Since the AESv is next to the Aii, when the AESv is stimulated stimulating current might well

spread and stimulate the efferent fibres of the Aii. To check this possibility, the Aii had been ablated 2 weeks before AESv stimulation in two cats. In the cats whose Aii was ablated, the MF and CF responses evoked by AESv stimulation were as large as those in normal cats. Thus, the responses evoked by AESv stimulation do not result from a current spread to the Aii.

As the MF and CF responses after stimulation of the AESv were largest and most stable in the posterior vermis, they were chosen for further investigations aimed at identifying the relay stations mediating the responses.

Investigation of the MF response relay station

In order to locate the relay station that transmits the MF responses to the posterior vermis, WGA–HRP was injected into the AESv and the posterior vermis and the distribution of labelled axon terminals and neurons was investigated. Injection of WGA–HRP into the AESv labelled axon terminals anterogradely in the dorsolateral, peduncular and paramedian pontine nuclei (Fig. 2*C*, stippled areas). Injection of WGA–HRP into the cerebellar posterior vermis labelled neurons retrogradely in the dorsolateral, peduncular and paramedian pontine nuclei and the nucleus reticularis tegmenti pontis (Fig. 2*C*, dots). These two distributions overlapped extensively. This finding suggests that the MF responses in the posterior vermis are relayed at least partly in the dorsolateral, peduncular and paramedian pontine nuclei. To confirm the existence of synaptic connections between the axon terminals of the cerebro-pontine projections and the ponto-cerebellar neurons, extracellular unit recordings were performed in the pons. Synaptic connections were identified by the impulse collision test (Fig. 3*A* and *B*, upper trace). Thirty-two relay units, which responded orthodromically to AESv stimulation and antidromically to stimulation of the posterior vermis, were observed in the pontine nuclei. The latencies of the orthodromic and antidromic responses were $2{\cdot}9 \pm 1{\cdot}0$ and $1{\cdot}3 \pm 0{\cdot}7$ ms (mean $\pm$ S.D.), respectively (Fig. 3*D* and *F*). The sum of the orthodromic and antidromic latencies corresponds to the latencies of vermal MF responses, confirming that the pontine units contribute to transmitting the MF responses. The relay units were located in the dorsolateral pontine nucleus ($n = 18$), the peduncular pontine nucleus ($n = 9$) and the paramedian pontine nucleus ($n = 5$).

Some of the relay units in the dorsolateral pontine nucleus (10 out of 14 units responsive to AESv stimulation) responded to both SC and AESv stimulation (Fig. 3*B*). The latencies of the responses to SC stimulation were $2{\cdot}5 \pm 0{\cdot}7$ ms (mean $\pm$ S.D.; Fig. 3*E*).

Investigation of the CF response relay station

In order to identify the CF response relay station, extracellular recordings were made from the inferior olivary nucleus. Stimulation of the posterior vermis (Fig. 4*A*, arrowhead) induced action potentials which were regarded as antidromic since latency was fixed and there was clearly an inflexion in the initial downstroke of the spike (Fig. 4*A*, left trace) indicating initial segment–somadendrite (IS–SD) delay. With double-shock stimulation of the posterior vermis (Fig. 4*A*, right trace), the antidromic spike only partially invaded after the second stimulation. Units in the

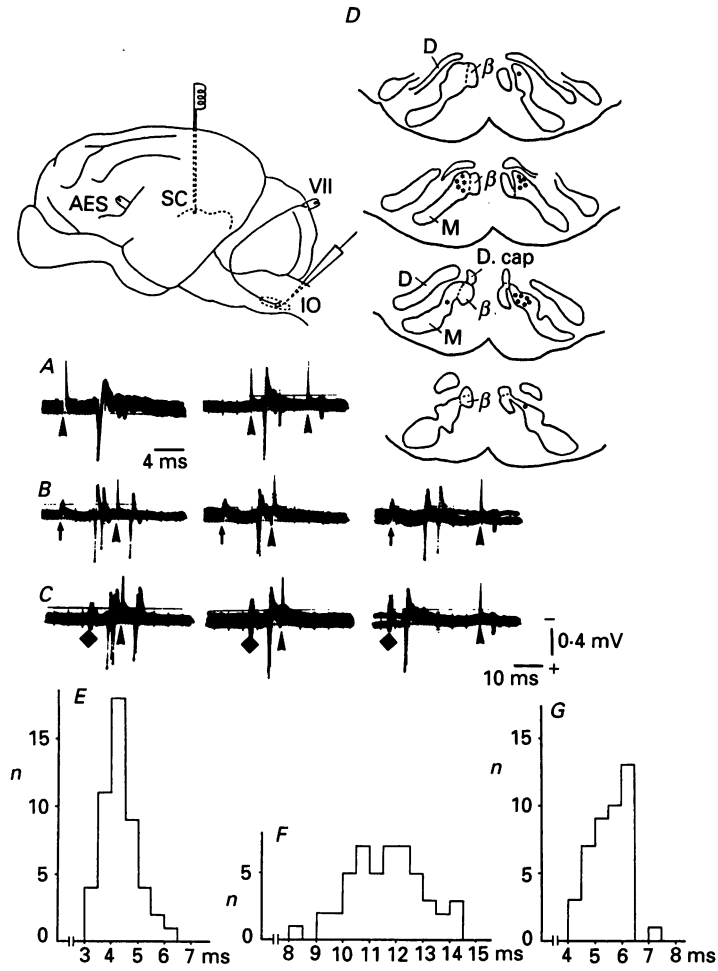

Fig. 4. Electrophysiological investigation of the relay station that transmits the CF responses in the posterior vermis. Upper diagram shows the experimental arrangement. *A*, left record shows antidromic responses of an olivary neuron to stimulation of the posterior vermis (arrowhead). In a slower swept recording (right trace), after double-shock stimulation, only a partial spike was recorded after the second stimulation. *B*, orthodromic responses to AESv stimulation (arrow) and antidromic responses to stimulation of the posterior vermis (arrowhead). *C*, orthodromic responses to SC stimulation (diamond) and antidromic responses to stimulation of the posterior vermis (arrowhead). The traces in *A–C* were recorded from the same unit. Note that the time calibration below the left-hand trace in *A* applies only to the trace while the calibration for other traces is given below the right-hand record in *C*. *D*, location where unit activities or field potentials were evoked by AESv stimulation. The locations where large field potentials with spike discharge (●) or weak field potentials (✱) were recorded are plotted on the nearest of four frontal sections of the brainstem including the caudal half of the medial accessory olive, that are aligned from rostral to caudal in descending order. The left side is ipsilateral to the AESv stimulation. *β*, nucleus of beta; M, medial accessory olive; D, dorsal accessory olive; D. cap, dorsal cap of Kooy; IO, inferior olive. *E*, *F* and *G* show respectively the frequency distribution for the responses evoked by stimulation of the posterior vermis, AESv and SC.

MAOcm were also orthodromically responsive to AESv stimulation (Fig. 4*B*, arrow). Responses were markedly evoked on the side contralateral to the stimulation but weaker on the side ipsilateral to the stimulation. When the interval between stimulation of the AESv and the posterior vermis was less than 15 ms, the antidromic spikes disappeared by collision (Fig. 4*B*, middle trace). When the AESv was stimulated at a near-threshold strength, the antidromic spikes appeared only when the orthodromic spikes failed to occur (Fig. 4*B*, left trace). When the AESv was stimulated above the threshold, the antidromic spikes only partially invaded (Fig. 4*B*, right trace) if the interval between stimulation of the AESv and the posterior vermis was 15–100 ms. The antidromic spikes fully invaded when the interval between the stimulations was over 100 ms. The failure of the full spikes may be due to the powerful and prolonged IPSP (inhibitory postsynaptic potential) which follows orthodromic discharges in the inferior olive (Llinàs, Baker & Sotelo, 1974). These findings indicate that neurons in the MAOcm receive input from the AESv and provide output to the posterior vermis.

Figure 4*D* shows the histological locations of such relay neurons in the inferior olive. They were localized within the MAOcm. Units in which orthodromic responses were readily induced by stimulation of the contralateral AESv are shown in Fig. 4*D* by filled circles, while units responding weakly to ipsilateral stimulation are shown by asterisks.

All units responsive to AESv stimulation were also responsive to stimulation of the contralateral SC (Fig. 4*C*, diamond). Unlike the responses to AESv stimulation, significant responses were not induced by stimulation of the SC ipsilateral to the recording side.

The latency of the antidromic responses evoked by stimulation of the posterior vermis (Fig. 4*E*) was $4{\cdot}3 \pm 0{\cdot}6$ ms (mean $\pm$ S.D.). The latencies of the orthodromic spikes evoked by stimulation of the AESv (Fig. 4*F*) and the SC (Fig. 4*G*) were $11{\cdot}5 \pm 1{\cdot}4$ and $5{\cdot}5 \pm 0{\cdot}6$ ms (mean $\pm$ S.D.), respectively. Latency to SC stimulation was about half that to AESv stimulation (compare Fig. 4*B* and *C*).

Morphological investigation of cerebro-olivary projections

In order to investigate morphologically the pathway from the AESv to the inferior olive, WGA–HRP was injected into the AESv and MAOcm. After AESv injection, labelled axon terminals were observed in the intermediate and deep layers of the SC but not in the inferior olive (Fig. 5*A*). The labelled terminals were dense on the side ipsilateral to the injection and sparse on the contralateral side. After electrophoretic injection of WGA–HRP into the MAOcm, no neurons were retrogradely labelled in the AESv but many were observed in the intermediate and deep layers of the SC (Fig. 5*B*). They were small in size ($18{\cdot}5 \pm 6{\cdot}3$ μm; mean $\pm$ S.D., $n = 53$) and distributed mostly on the side contralateral to the injection. The distribution of axon terminals anterogradely labelled after AESv injection overlapped in the SC with that of the cell bodies retrogradely labelled from MAOcm. Thus, the cerebro-olivary projection from the AESv to the MAOcm is considered to be indirect via the SC.

Extracellular unit studies in the SC

To verify electrophysiologically whether such a cerebro-olivary pathway exists, extracellular unit activities were recorded in the SC. Some units in the SC responded antidromically to weak stimulation of the MAOcm (e.g. Fig. 6, arrowhead). Figure

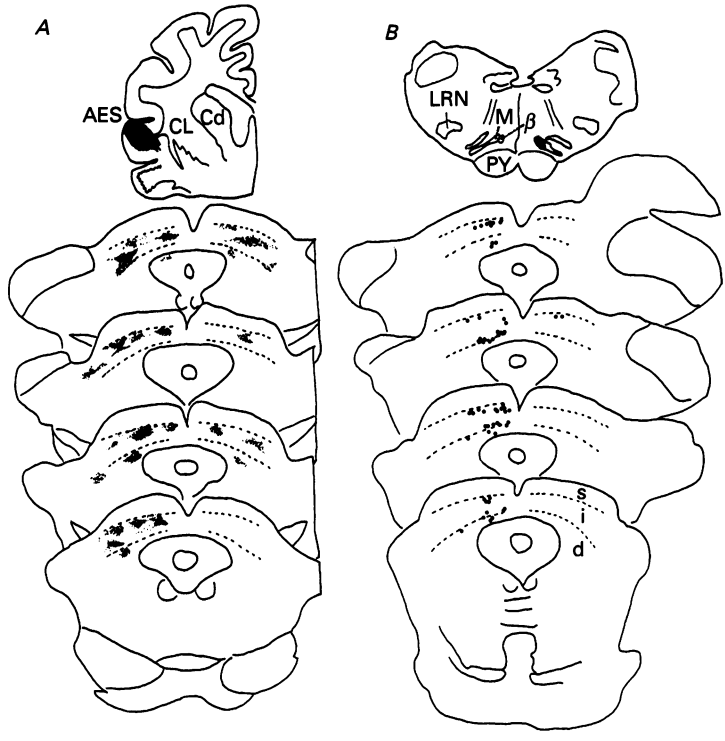

Fig. 5. Morphological investigation of the relay station of the cerebro-olivary projection from the AESv. Distribution of the anterogradely labelled axon terminals after WGA–HRP injection into the AESv (*A*) and retrogradely labelled neurons in the SC after electrophoretic injection of WGA–HRP into the MAOcm (*B*). WGA–HRP injection sites are shown in uppermost diagrams. Anterogradely labelled axon terminals and retrogradely labelled neurons were plotted in four representative midbrain frontal sections from rostral to caudal in descending order. In *B*, one dot indicates one labelled cell. PY, pyramidal tract; s, i and d, superficial, intermediate and deep layers of the SC, respectively.

6 also shows orthodromic responses of the same neuron to AESv stimulation (arrow). When the interval between the stimulations of the MAOcm and AESv was less than 8·1 ms, the antidromic spike evoked by MAOcm stimulation collided with the orthodromic spike evoked by AESv stimulation. This finding indicates that the axon terminals from the AESv have excitatory synaptic contact with the tecto-olivary neurons. Such relay units were localized in the intermediate layers of the SC (Fig. 6, photomicrograph, arrowhead). Because the somata of the tecto-olivary neurons are small in size, responses were hard to detect, and in fact only eight neurons were recorded.

Effect of injection of muscimol or lidocaine into the SC

To confirm that the cerebro-olivary projection from the AESv was relayed in the SC, muscimol (0·4 μg) or lidocaine (0·4 μg) was injected into the SC. Muscimol, a GABA agonist, increases tonic inhibition of the SC cells and deafferents them

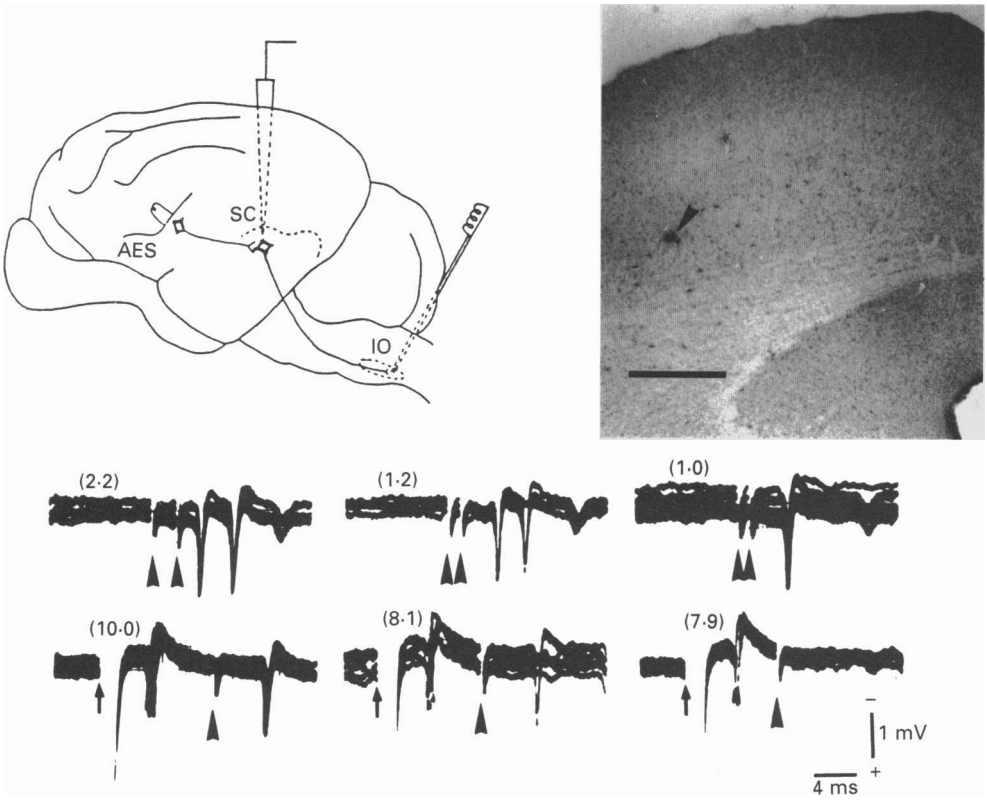

Fig. 6. Identification of SC neurons relaying the cerebro-olivary projection from the AESv. Diagram shows the experimental arrangements. Example of a unit recorded from the SC with antidromic activation by MAOcm stimulation (arrowhead and orthodromic activation by AESv stimulation (arrow). The antidromic spikes collided with the orthodromic one and disappeared when the interval between the stimulations was less than 8·1 ms. Numerals in parentheses indicate the stimulus intervals in milliseconds. The recording site is demonstrated on the photomicrograph of the frontal section of the SC. An arrowhead points at the dye spot where the units were recorded. Calibration bar indicates 1 mm.

(Hikosaka & Wurtz, 1985). Lidocaine, a local anaesthetic, blocks sodium channels and eliminates generation or conduction of action potentials (Ritchie, 1979). After injection, the CF response evoked in the posterior vermis by AESv stimulation was reduced and transiently disappeared (Fig. 7). The disappearance of the CF responses lasted 1–2 h after lidocaine injection and over 15 h after muscimol injection. The MF responses evoked by AESv stimulation showed almost no change after injection of

lidocaine or muscimol. These findings support the hypothesis that the CF responses evoked by AESv stimulation are relayed in the SC while most of the MF responses are relayed elsewhere.

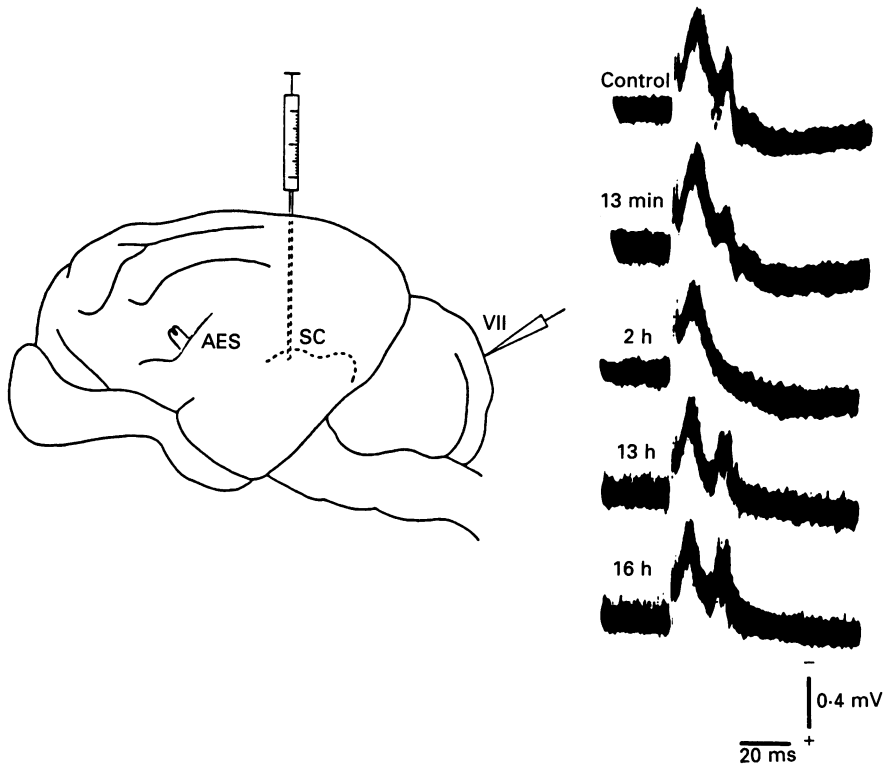

Fig. 7. Modulation of responses to AESv stimulation after injection of muscimol into the SC. Diagram shows the experimental arrangements. The recordings show the responses to AESv stimulation before (Control) and at four time intervals after injection of muscimol (lower four traces).

DISCUSSION

The present study demonstrates projections from the AESv to the posterior vermis, and provides information regarding the underlying pathways. Since the AESv receives inputs from the caudal part of the cerebellar fastigial nucleus which receives Purkinje cell inputs from the posterior vermis (Noda & Oka, 1985; Kyuhou & Kawaguchi, 1987), there is a reciprocal connection between the AESv and the posterior vermis.

Pathway that transmits the MF responses

The present data indicate that the MF responses evoked by AESv stimulation are transmitted at least in part through the cerebro-ponto-cerebellar projection via the dorsolateral, peduncular and paramedian pontine nuclei. There is a possibility that

cerebro-tecto-pontine projections to the dorsolateral pontine nucleus might exist and be related to the induction of the MF responses, because there are massive projections from the AESv to the SC and from the SC to the dorsolateral pontine nucleus (Kawamura & Brodal, 1973; Mower, Gibson & Glickstein, 1979). Nevertheless, the MF responses were not depressed by injection of lidocaine or muscimol into the SC. The latencies of the orthodromic responses in the pontine units after AESv stimulation were almost the same as those after SC stimulation. These results indicate that a cerebro-tecto-pontine projection would not play any major role on exciting the pontine neurons that evoke the MF responses in the posterior vermis after stimulation of the AESv. The fact that some of the units in the dorsolateral pontine nucleus responded to both the AESv and the SC means that inputs from the AESv and the SC converge in the dorsolateral pontine nucleus.

Indirect cerebro-olivary projection

Both direct and indirect cerebro-olivary projections have been reported (Bishop, McCrea & Kitai, 1976; Nakumura, Kitao & Okoyama, 1983). It is generally accepted that the direct cerebro-olivary projection originates only from the frontal cortex (Bishop *et al.* 1976). Although cerebral areas other than the frontal cortex are thought to project to the inferior olive indirectly via ventral midbrain structures, the pretectum and the SC, there have been few studies on the indirect cerebro-olivary projection (Oka & Jinnai, 1978; Oka, Jinnai & Yamamoto 1979; Nakamura *et al.* 1983). Oka *et al.* (1979) reported that some of the indirect cerebro-olivary projections from the parietal cortex are relayed in the parvocellular part of the red nucleus. The present data indicate that the CF responses in the posterior vermis to AESv stimulation are transmitted through another indirect cerebro-olivary projection, that is, the cerebro-tecto-olivary projection.

Since the indirect cerebro-olivary projections from the parietal cortex and AESv contain one more synapse than does the direct cerebro-olivary projection from the frontal cortex, the transmission time must be longer. The latencies of the CF responses evoked by stimulation of the frontal cortex, parietal cortex and AESv were 12–16, 17–19 and 14–17 ms, respectively (Sasaki *et al.* 1975; Oka *et al.* 1979). The CF responses to AESv stimulation were as stable from trial to trial as those evoked by stimulation of the frontal cortex. This stability contrasts with the unstable CF responses evoked by parietal stimulation, and the difference is probably due to a difference in the intensity of the input from cerebral cortex to the SC and the parvocellular part of the red nucleus. Anatomical studies indicate that the cerebro-tectal projection from the AESv is heavy, while the projection from the parietal cortex to the parvocellular part of the red nucleus does not seem to be heavy (Mizuno, Mochizuki, Akimoto, Matsushima & Sasaki, 1973).

Functional significance of the projection

It is well known that the cerebellar posterior vermis responds to visual stimuli (Snider & Stowell, 1944). It was reported that KCl applied bilaterally to the anterior ectosylvian gyrus depressed not only the click-evoked responses but also the flash-evoked responses in the cerebellar posterior vermis. The visual responses were reduced much later than the auditory responses. The auditory responses began to

diminish 1 min after the application of KCl, while the visual responses began to diminish 3 min after the application (Munson, 1968). This delay may be explained by the time required for diffusion of KCl into the ventral bank of the anterior ectosylvian sulcus. It was recently reported that neurons in the AESv respond to visual stimuli (Mucke *et al.* 1983). Thus, the cerebro-cerebellar projection from the AESv may participate in generating the visual evoked response in the cerebellar posterior vermis.

Both the AESv and the posterior vermis are related to saccadic eye movements. Sasaki and his colleagues showed that there is a reciprocal connection between the parietal cortex and the lateral part of the cerebellum. Specifically, they found that the cerebellar lateral nucleus projects to the parietal cortex (Sasaki *et al.* 1972) and that the parietal cortex projects to the lateral part of the cerebellar cortex (Sasaki *et al.* 1975). These reciprocal connections are considered to be indispensable for performing skilful and co-ordinated movements (Sasaki, 1979). Thus, by analogy, the reciprocal connections between the posterior vermis and the AESv may play an important role in the highly co-ordinated saccadic eye movements.

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