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ORGANIZATION OF THE CORTICO-PONTO-CEREBELLAR PATHWAY TO THE DORSAL PARAFLOCCULUS.

An experimental study with anterograde and retrograde transport of WGA-HRP in the cat

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In 1946 Alf Brodal together with Jan Jansen published the first comprehensive experimental study of the pontocerebellar projection using the modified Gudden method (6). They were able to show a certain topographical order, and their main conclusions have stood the test of time. It was obvious from their study, however, that the pontocerebellar system did not show the very clear-cut and precisely distributed retrograde changes and areas of cell loss that were so striking in the olivocerebellar projection studied some years earlier with the same technique (5). Contemporary physiological studies with the evoked potential technique and surface electrodes, also gave the impression that cerebrocerebellar connections, mainly relayed throught the pontine nuclei, only showed a rough topographical order (21, 34, 49). The introduction of intra-axonally transported markers made it possible to understand more of the organization the cerebrocerebellar pathway. It then became obvious that one reason for the lack of evidence of clear-cut topography in the experiments of Brodal and Jansen (6) was the extremely convergent (and to a lesser degree divergent) nature of the pontocerebellar projection. Thus, neurons projecting to one small part of the cerebellum are concentrated in groups that may be widely dispersed and intermingled with (although not necessarily overlapping) cell groups projecting to other parts of the cerebellum (for a review, see (13)). More sensitive methods allowing precise anterograde tracing of fiber connections disclosed that the first link in the cortico-ponto-cerebellar pathway, the corticopontine projection, is highly ordered with a high degree of divergence (to a lesser extent convergence). When recent data from the corticopontine and pontocerebellar projections are put together, the picture emerges of a highly ordered but extremely complicated system distributing information from each specific cell group in the cerebral cortex to presumably many specific parts of the cerebellar cortex. Also, modern electrophysiological micromapping studies of afferent pathways to the cerebellar cortex bear witness of an extreme degree of order in several mossy fiber projections to the cerebellar cortex (for reviews, see (53, 54)).

Yet, we still lack precise information and a basic understanding of the organization of cerebrocortical inputs to major parts of the cerebellum. In order to learn more on this point, we have studied systematically with a double labelling technique which parts of the cortex that project to a particular lobule of the cerebellar hemisphere via the pontine nuclei (1, 12, 14, 23). Injections of WGA-HRP (Wheat Germ Agglutinin-Horseradish Peroxidase conjugate) were made in specific parts of the cerebral cortex in conjunction with injections of the same tracer in one lobule of the cerebellar hemisphere. The degree of overlap between anterogradely labelled terminal fibres and groups of retrogradely labelled cells most likely gives an approximate indication of the amount of synaptic contacts between these two elements.

This paper presents data on the dorsal paraflocculus that constitutes a substantial part of the cerebellar hemispheres in most mammals (38) notably also in the cat. Its functional role is, however, largely unknown. Electrophysiological investigations in the cat by Jansen (34) indicated that short latency potentials (transmitted by mossy fibers) were evoked in the dorsal paraflocculus only after stimulation of the parietal association cortex and parts of the orbital gyrus. On the other hand, Robinson and co-workers (45) emphasized the importance of inputs from the visual cortex via the pontine nuclei on the basis of double anterograde-retrograde labelling experiments in the cat. They did not, however, explore whether other parts of the cortex also contribute to the cortico-ponto-paraflocculus pathway. That this may be the case is indicated by findings of Rosina and Provini (46). They mention briefly in a paper adressing another question that pontine neurons retrogradely labelled from the "paraflocculus lobes" overlapped corticopontine fibres from visual and parietal cortex (anterogradely labelled with Fast Blue). From studies of the pontocerebellar projection in the cat with retrograde transport of HRP (26, 29) conclusions as to cortical inputs to the dorsal paraflocculus are discordant.

By studying systematically all the main functional regions of the cerebral cortex, we hoped to get a clearer picture than what is presently available of the total cerebral input to the dorsal paraflocculus. We also wanted to see if the organizational principles found for the cortico-ponto-cerebellar inputs to the ansiform lobule (Crus I and II) and the paramedian lobule are applicable to the dorsal paraflocculus.

METHODS

Altogether 17 cats received pressure injections of 1% or 2% WGA-HRP (Sigma) under pentobarbital anesthesia (Mebumal, Rikshospitalets apotek). In all, three injections of 0.05 µl each were placed among the six medialmost folia of the left dorsal paraflocculus. The same animals received cerebrocortical injections in the right hemisphere, systematically varying the region injected from case to case. A total of 0.2-1.5 µl was injected through 1-10 injections, to cover most of the cortical area in question, without encroachment upon functionally different neighboring areas. Since the corticopontine projection from most cortical regions is strictly unilateral, we added in some cases injections in the left cerebral hemisphere, to compare the location of terminal areas on the two sides.

The cerebrocortical injections were placed using gyri and sulci on the surface of the brain as landmarks, in order to restrict the cortical staining to the areas mapped electrophysiologically (17, 32, 44, 52, 55, 56) and cytoarchitectonically (27, 43). After 24 to 48 hours the cats were perfused intracardially under deep pentobarbital anesthesia. Perfusion started with 1 l of physiological saline followed by 2 l of a fixative consisting of a mixture of 1% paraformaldehyde and 1.25% glutaraldehyde, both at body temperature. One l cooled 10% sucrose in phosphate buffer terminated the perfusion. The brain was dissected out immediately. To facilitate identification of the injection sites the cerebrum and cerebellum were photographed.

After storage in 30% sucrose by 4 C overnight, 50 μm sections were cut on a freezing microtome. The sections were collected in groups of five. The cerebral cortex was cut frontally, the cerebellum horizontally and the pons transversely. Two sections out of each group of five were incubated with Tetramethylbenzidine (TMB) according to Mesulam (39). One section of each pair was weakly counterstained with neutral red, the other was left unstained.

The section through both the cerebrocortical and cerebellar injection sites were drawn in a Camera lucida, and the extent and exact localization of staining due to TMB reaction product were marked and checked microscopically. The findings were then transferred to standard diagrams of the cerebral and the cerebellar hemispheres (Figs. 4-10), using the photographs and drawings of sections as guides.

The unstained sections through the pontine nuclei were drawn with an X-Y plotter and the retrograde and anterograde labelling was carefully entered in the correct positions. About 15 sections through the pons were plotted for each case. In ten cats labelled cells were counted in ten equally spaced sections through the pons. To facilitated comparison between cases the distribution of labelling was transferred to standard diagrams (Fig. 4). In one experiment (Fig. 5) we combined a cerebrocortical WGA-HRP injection with an adjacent lesion made by transdural thermocoagulation. The lesion was made 5 days prior to the WGA-HRP injection. Neighboring sections were used for silver impregnation (24) and TMB processing, respectively. After plotting of the two series, size difference of the sections due to different shrinkage was determined and adjusted for photographically. The neighbouring silver- and TMB-sections were subsequently superimposed using vessels as reference points and transferred to one standard diagram of the pontine nuclei (Fig. 5).

RESULTS

The majority of the *cerebellar injections* involve the medial third, fourth and fifth folium of the left dorsal paraflocculus (Fig. 1c). They usually cover the molecular and granular layers and some white matter. Only staining of the granular layer, however, is considered as the injection site since ponto-cerebellar axons end as mossy fibres (36). Staining due to the injections is limited to the dorsal paraflocculus, except in two cases with slight staining of the neighboring paramedian lobule and crus II, respectively (see ccol 337 in Fig. 4 and ccol 363 in Fig. 10). There is no spread to the intracerebellar nuclei in any of the cases.

The *cerebrocortical injections* mostly cover all cortical laminae and the white matter immediately beneath (Fig. 1*a* and *b*). Only the region with staining involving lamina 5 is considered effective injection site, since apparently all cortical neurons projecting to the pontine nuclei are situated in this lamina (2, 35). There is no staining of subcortical nuclei in any of our cases.

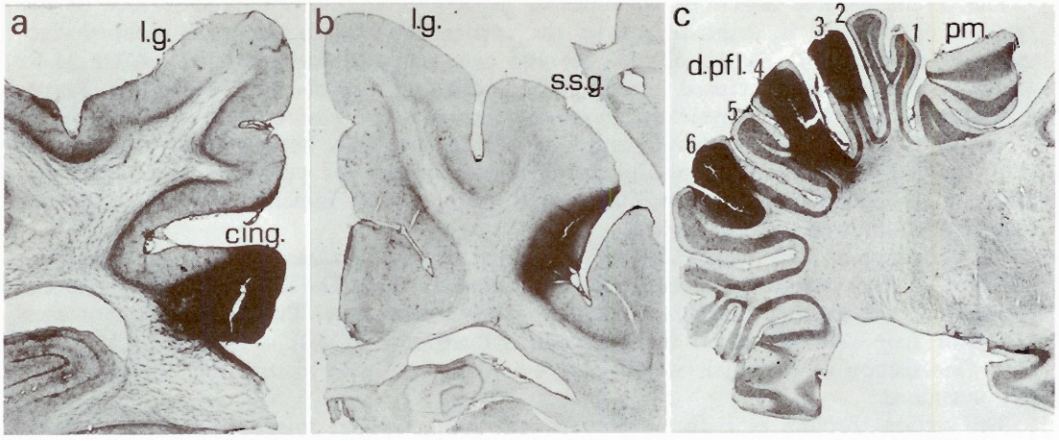


Fig. 1. – *Photomicrographs of typical cerebrocortical and cerebellar WGA-HRP injection sites.*

A: Injection in the cingulate gyrus, frontal section (cat ccol. 338). B: Injection restricted to the visual area PMLS in cat ccol 354 (see also Fig. 7). C: Three injections in the medial part of the dorsal paraflocculus (cat ccol 327, see also Fig. 8).

Abbreviations used in all figures: a.e.g., anterior ectosylvian gyrus; a.s.g., anterior sigmoid gyrus; br.p., brachium pontis; cing., cingulate gyrus; cor., coronal gyrus; cr. I, crus I of the ansiform lobule; cr. II, crus II of the ansiform lobule; cru.s., cruciate sulcus; d.pfl., dorsal paraflocculus; e.g., ectosylvian gyrus; l.g., lateral gyrus; p.e.g., posterior ectosylvian gyrus; p.l.g., posterolateral gyrus; p.s.g., posterior sigmoid gyrus; ped., corticospinal and corticobulbar fibres in the pons; pm., paramedian lobule; s.s.g., suprasylvian gyrus; spl.g., splenial gyrus; v.pfl., ventral paraflocculus.

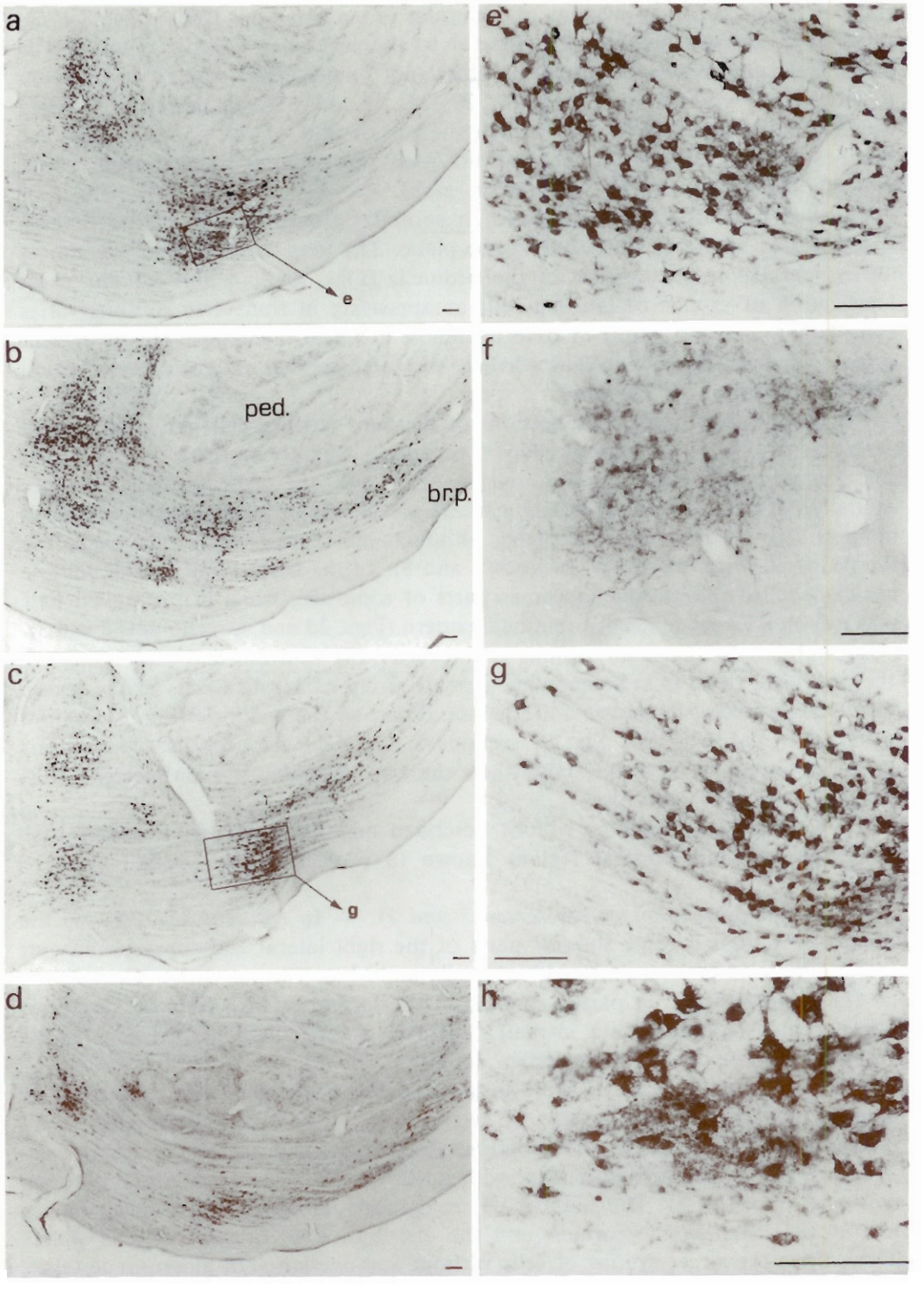
Anterograde terminal labelling is present in the pontine nuclei ipsilateral to the injections in all our cases (Figs. 2 and 3). Localization of terminal areas coincides well with previous descriptions based on various anterograde tracing methods (7-9, 11-12, 14, 18, 23, 40, 46).

1. *General description of the ponto-paraflocculus projection.* — Pontine neurons retrogradely labelled from the paraflocculus are found in all cases and at all rostrocaudal levels of the pons (Figs. 2a-d and 4). Intensity of staining and number of labelled cells vary considerably from case to case in spite of about equal amounts of tracer injected and equal survival times, however. Also within any one section, the intensity of labelling covers a wide range from some cells that are barely visible to some that have an almost "Golgi-like" appearance (Fig. 2e).

Labelled cells are distributed bilaterally with a clear contralateral predominance

Fig. 2. – *Photomicrographs of retrograde and anterograde labelling in the pontine nuclei after cerebrocortical (parietal association areas) and cerebellar injections of WGA-HRP in the same animal.*

TMB-incubation. A-D: Low power photomicrographs of transverse sections from various levels of the pons in cat ccol 316 (see also Fig. 4). A is rostral and D caudal. E-H: Examples with higher magnification of partial overlap between anterograde and retrograde labelling in cat ccol 316 (E, G, H) and in cat ccol 322 (F) also with an injection of the parietal association cortex. Bars represent 100 μm. For abbreviations see legend to Fig. 1.


a
e
b
ped.
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h

(Figs. 4, 7 and 9). In an average of countings in ten cats only 12% (range 8-17%) of labelled neurons are found ipsilateral to the cerebellar injection site. This is in general agreement with results of other (not quantitative) studies on the pontocerebellar projection in the cat (6, 26, 29, 45, 46). The majority of labelled cells (71%, range 67-81%) is found in the rostral half of the pontine grey. The number of labelled cells in the ten cats used for counting ranged from about 2000 to 8000 (ten sections counted in each).

Labelled cells tend to be collected in several "groups", with shapes ranging from oval to band-like in the transverse plane. The long axes of these are almost always parallel to the surface of the peduncle (Figs. 2a-d, 4 and 8). The exact localization of groups of labelled cells as appearing in transverse sections varies considerably between cases, in spite of almost identical injections. This is evident when comparing Figs. 4-10. Nevertheless, the same general pattern of distribution can be recognized in all cases.

Typically, in the rostralmost sections of the pons labelled cells are concentrated ventromedially (see, for example, Fig. 4, section 9). Slightly more caudally, labelled cells occur in two major groups, one medially and one ventromedially. Further caudally but still in the rostral half, clusters and bands of labelled cells are spread more widely in the transverse plane, although most are found ventromedial to the peduncle (Figs. 2b and 4, sections 7 and 8). In the rostral half of the pontine nuclei, labelled cell groups appear as parts of a mosaic, while in the caudal half they exhibit a less complex distributional pattern (Figs. 2d and 4, sections 1, 2 and 3).

In addition to the main regions of labelled cells ventromedial and ventral to the peduncle, there is in some cases a small group of labelled cells in the corner between the medial lemniscus and the lateral part of the peduncle (Fig. 4, sections 4 and 5 in cat ccol 316; Fig. 9, sections 3 and 4 in cat ccol 357). It appears that this group is clear-cut only when the two medialmost parafloccular folia have been heavily stained.

In the following, findings will be described and illustrated for representative cases for the main cortical regions known to project to the pontine nuclei.

2. *Parietal association cortex (areas 5 and 7).* — In *cat ccol 337* (Fig. 4) the cortical injections involve anterior parts of the right lateral and suprasylvian gyri (parts of areas 5 and 7 as defined by Hassler and Muhs-Clement (27)). The cerebellar injections have led to partial staining of the second to fifth folia of the dorsal paraflocculus (counted from medial). There is a very slight involvement of an adjoining folium of the paramedian lobule.

In the right pontine nuclei there is quite extensive *overlap* between patches of anterograde labelling and groups of retrogradely labelled cells, occurring at all rostrocaudal levels. Closely corresponding observations with regard to sites of overlap are made in another experiment with a very similar combination of injections (*cat ccol 316*, Fig. 4). The number of retrogradely labelled cells is much higher in this case than in the former, and the distribution of labelled cells is wider. Thus, there are larger groups of cells without any overlap with anterograde label-

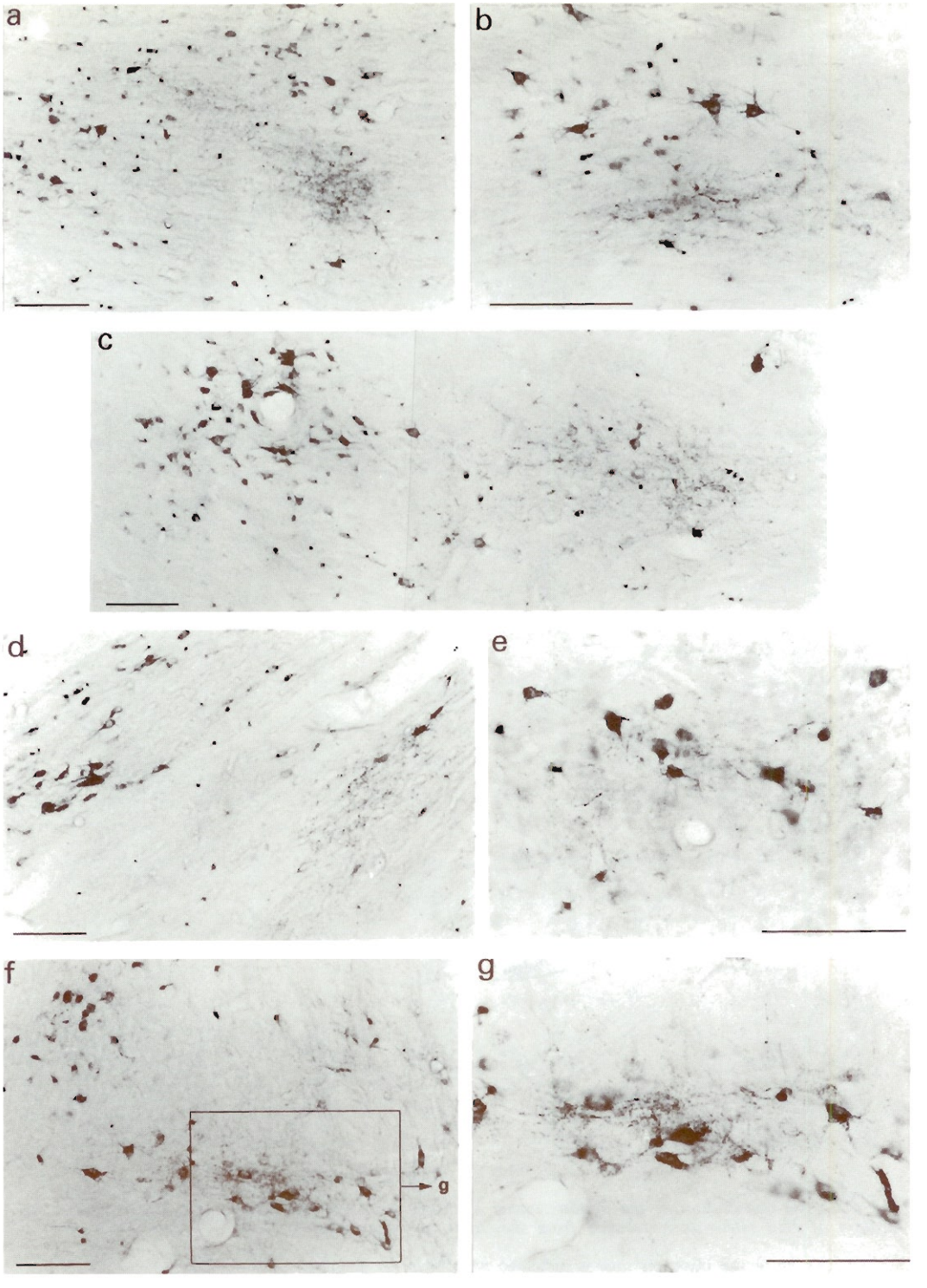

Fig. 3. – *Photomicrographs showing partial overlap between anterograde and retrograde labelling after injections of visual cortical areas (A-D) and second somatosensory area (E-G).*

A: Injection involving areas 17, 18 and 19 (cat ccol 320, see also Fig. 6). B: Injection of PMLS in cat ccol 354, see also Fig. 7). C, D: Injection restricted to area 17 (cat ccol 333, see also Fig. 6). E: Injection of SII (cat ccol 331). F and G: Injection of SII (ccol 357, see also Fig. 9). Bars represent 100 μm.

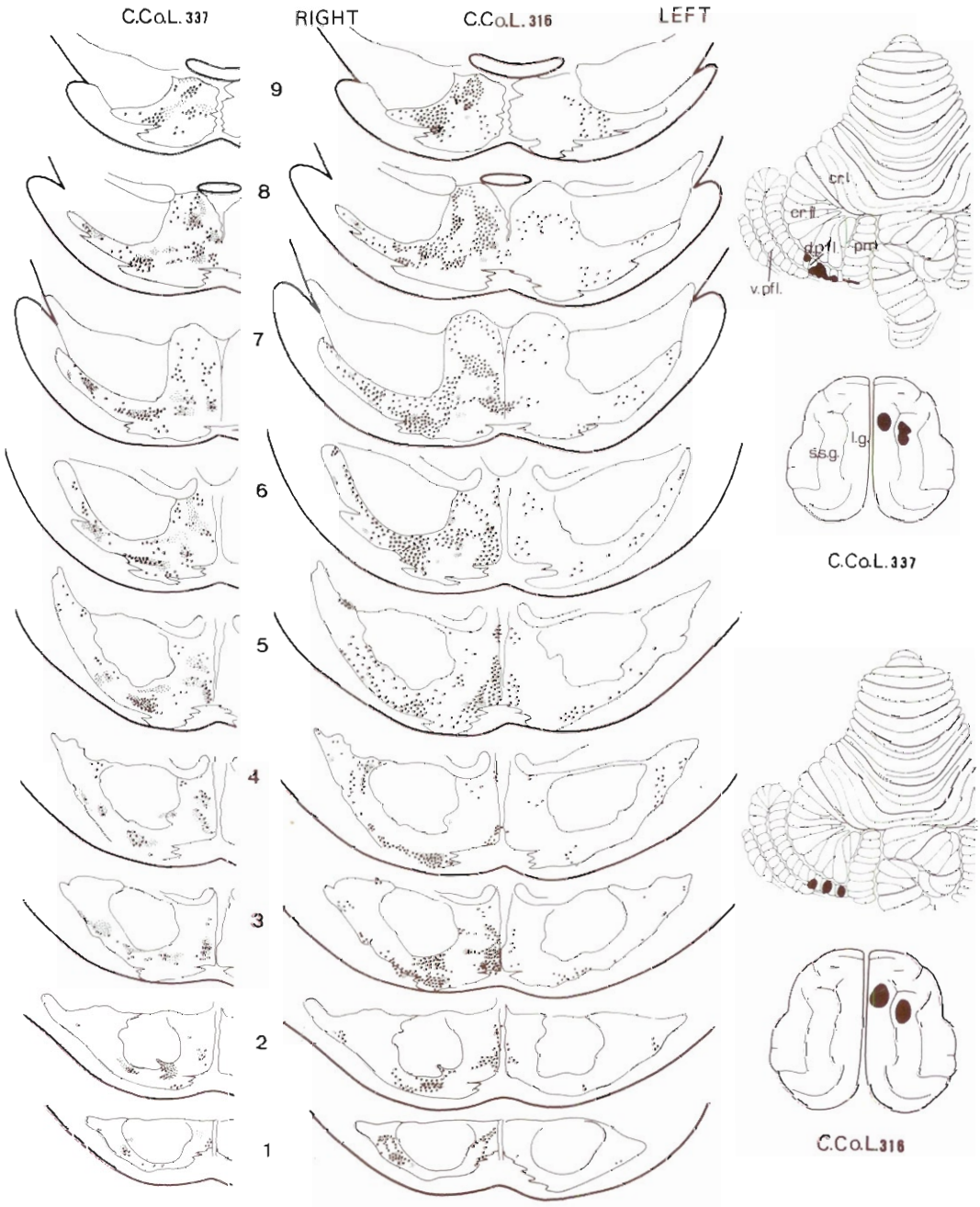

Fig. 4. – *The distribution in the pontine nuclei of fibres from the parietal association cortex and the location of cells retrogradely labelled from the dorsal paraflocculus.*

The staining at the injection sites is indicated with black in diagrams of the cerebral hemispheres and the cerebellum. Labelled cells are shown as *heavy dots* and terminal anterograde labelling as *fine stippling* in drawings of nine equally spaced transverse sections through the pontine nuclei. Note the strong tendency for *divergence* in the corticopontine projection and *convergence* in the pontocerebellar as witnessed by numerous patches of anterograde labelling and groups of retrogradely labelled cells at almost all rostrocaudal levels. Overlap between anterograde and retrograde labelling is quite marked and occurs at multiple sites and at all levels. For abbreviations see legend to Fig. 1.

ling. More significant, however, is probably that a major fraction of the anterograde labelling is overlapping with retrogradely labelled cells.

In two other cases (*cats ccol 332 and 323*, not illustrated) with injections closely similar to those in Fig. 4, the anterograde labeling is more massive than in the other cases, while retrograde labelling is less. Nevertheless, the same distribution of considerable, but partial overlap is observed in the pontine nuclei.

In a last case in this group, we also tried to decide whether areas 5 and 7 would contact different groups of pontocerebellar neurons projecting to the dorsal paraflocculus. In *cat ccol 356*, Fig. 5, a lesion was placed in the most anterior part of the right suprasylvian gyrus, while an injection of WGA-HRP was placed immediately posterior to the lesion. According to Hassler and Muhs-Clement (27), the lesion is located within area 5, while the staining due to the injection is confined to area 7. The parafloccular injection comprises the 2.-5. folium. Two main findings are apparent from this case. Firstly, terminal degeneration as visualized with the Fink and Heimer method (24) is distributed largely differently from the anterograde labelling in the pontine nuclei (Fig. 5). Often patches of degeneration and WGA-HRP labelling are found to be adjacent, with some degree of overlap. Secondly, both patches of terminal degeneration and anterograde labelling *overlap* with retrogradely labelled cells although the overlap appears to be somewhat more marked for patches of degeneration (area 5).

Comments on cases with parietal cortical injections. — There is a high degree of overlap between terminal regions of fibres from the parietal association cortex, and neurons projecting to the medial folia of the dorsal paraflocculus. Furthermore, the projection to the pontine nuclei from areas 5 and 7 terminate differently, suggesting that these cortical areas may influence different groups of neurons in the cerebellar cortex.

3. *The visual cortex.* — Four cases with injections in different visual cortical areas and in the medial part of the dorsal paraflocculus will be described and illustrated.

In *cat ccol 320* (Fig. 6) multiple cortical injections led to staining of parts of areas 17, 18 and 19 (43). Mainly lower- and central visual field representations are affected (32, 52). There is some staining of underlying white matter. The cerebellar staining involves the 2., 4. and 6. folium of the dorsal paraflocculus. In the pontine nuclei anterograde labelling is found mainly rostrally distributed in bands and patches. Roughly, there appears to be two bands of terminations curving around the ventral aspect of the peduncle (Figs. 6, sections 7-9). Groups of retrogradely labelled cells are at many places found adjacent to anterograde labelling, and often there is also some *overlap*. The degree of overlap is nevertheless lower than what was found after injections of the parietal cortex (Fig. 4 and 5).

In *cat ccol 333* (Figs. 3*c*, 3*d* and 6) the cortical injection is confined to area 17 on the medial wall of the hemisphere representing lower and central parts of the visual field. The parafloccular injection involves three folia. The anterograde labelling is less extensive than in the former case, and apparently restricted to

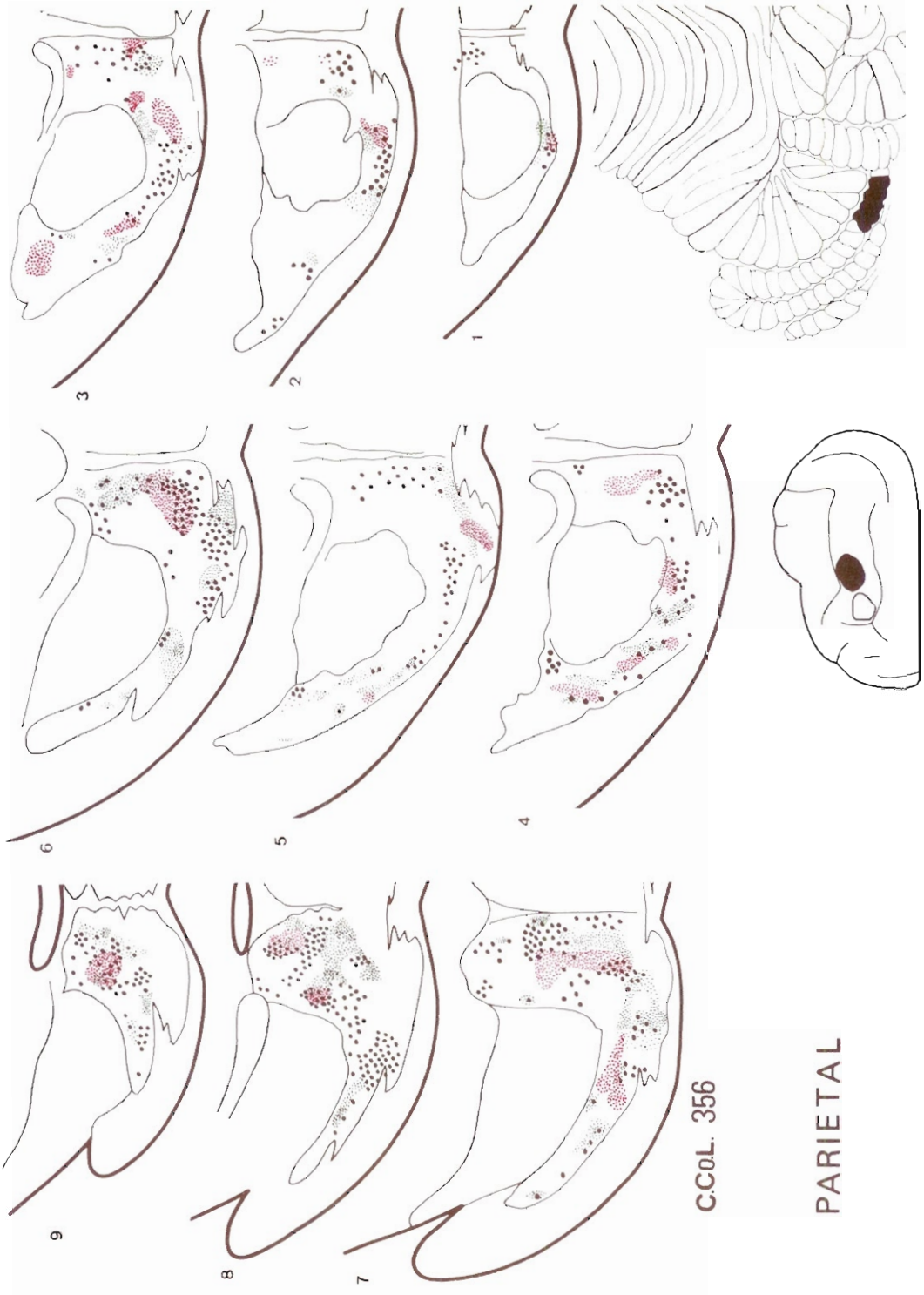

Fig. 5. – *Experiment with double anterograde tracing from two adjacent sites in the parietal association cortex combined with retrograde tracing from the dorsal paraflocculus.*

Conventions as in Fig. 4, except that *red stippling* shows terminal degeneration caused by the cerebrocortical lesion in area 5 (solid red), while *black stippling* shows terminal labelling from the cerebral WGA-HRP injection in area 7 (solid black). Note the clear separation between anterograde labelling and terminal degeneration, and that fibres from both cortical sites overlap considerably with retrogradely labelled cells. Boundaries of areas 5 and 7 according to Hassler and Muhs-Clement (27).

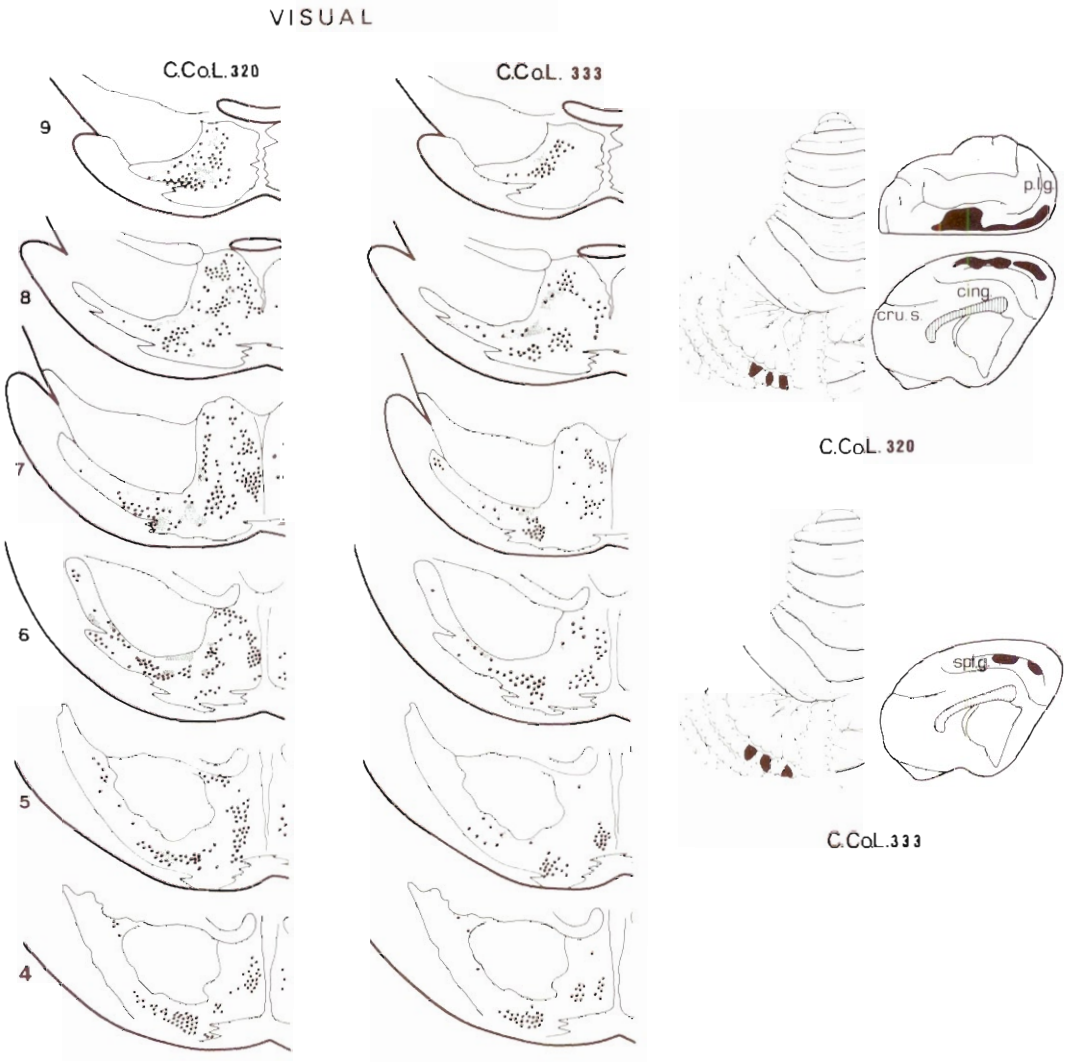

Fig. 6. – *The distribution in the pontine nuclei of fibres from parts of visual cortical areas 17, 18 and 19 and location of pontine neurons retrogradely labelled from the dorsal paraflocculus.*

Conventions as in Fig. 4. Although overlap is present in many places, it is less pronounced that after injections of the parietal cortex (Fig. 4). For abbreviations see legend to Fig. 1.

a band (and patches) close to the peduncle. Although patches of anterograde labelling and groups of retrogradely labelled cells is often found adjacent to each other, the degree of *overlap* is very modest.

In *cat ccol 354* (Figs. 1b, 3b and 7) injections are placed in both cerebral hemispheres. On the right side the injection lead to staining confined to the medial bank of the suprasylvian sulcus corresponding closely to the PMLS (posteromedial

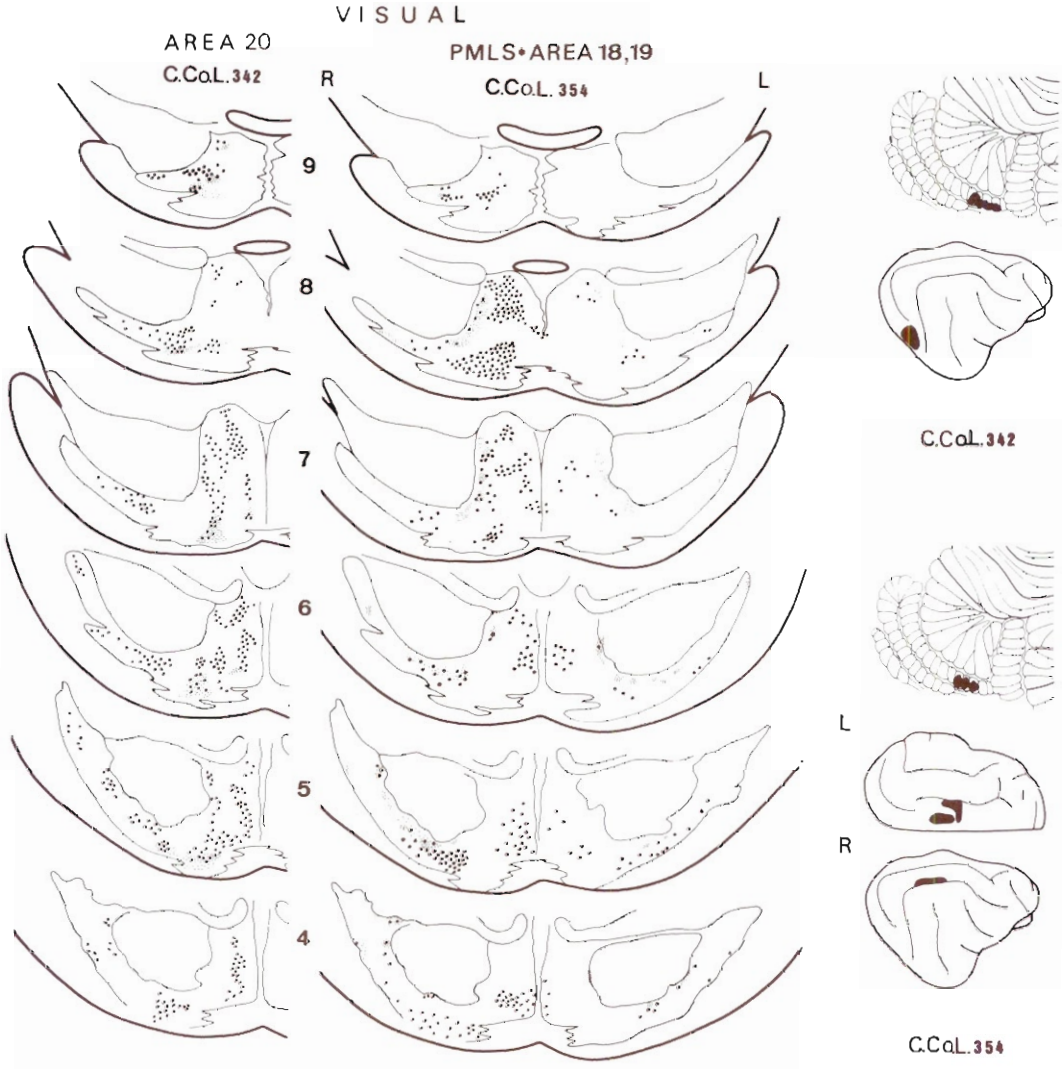

Fig. 7. – *The distribution in the pontine nuclei of fibres from visual cortical area PMLS (ccol 354, right side) and area 20, and location of pontine neurons retrogradely labelled from the dorsal paraflocculus.*

Conventions as in Fig. 4. Although area 20 and PMLS project to different parts of the pontine nuclei, both projections overlap with neurons projecting to the dorsal paraflocculus.

lateral suprasylvian visual area) of Palmer and co-workers (44). The same amount of tracer was injected the middle part of the lateral gyrus of the left hemisphere, with subsequent staining of rostral parts of areas 18 and 19. The parafloccular injection involves three folia. In the right pontine nuclei *overlap* between groups of retrogradely labelled cells and anterograde labelling from the PMLS is present at several levels. The last case in this group, *cat ccol 342* (Fig. 7, Table 1), has

an injection that gave staining confined to area 20 (28). The parafloccular injection is again restricted to the medialmost few folia. Anterograde labelling in the pontine nuclei ipsilateral to the cortical injection is distributed more ventrally in the rostral pons than what is seen in the cases with injections of other visual areas. This is in agreement with the findings of Bjaalie and Brodal (4). Most of the anterogradely labelled patches *overlap* with retrogradely labelled cells.

Comments on cases with visual cortical injections. — In all cases with injection of visual areas combined with parafloccular injections there is some degree of overlap between retrograde and anterograde labelling. In addition, patches of anterograde labelling and groups of retrogradely labelled cells are often adjacent. Nevertheless, only a relatively minor part of all retrogradely labelled cells appear to be contacted by fibres from visual areas (see Figs. 6 and 7).

4. *The primary sensorimotor cortex (MI and SI).* — In four cases injections were placed in the pericruciate region, giving staining of large parts of the sensorimotor region. In *cat ccol 327* (Fig. 8) the injection involves the anterior and posterior sigmoid gyrus and the coronal gyrus, that is both SI and MI. For unknown reasons the amount of anterograde labelling is less than expected (as judged from previous studies), but the distribution is typical. Although there is some *overlap* at various levels between anterograde labelling and retrogradely labelled cell groups, none of the major groups of labelled cells are close to anterograde labelling.

Very similar observation are made in another case, *cat ccol 339* (Fig. 8), with staining restricted to the posterior sigmoid gyrus and adjacent parts of the coronal gyrus (SI). At caudal levels where the anterograde labelling is maximal, retrogradely labelled cells are mainly located more ventrally than the anterograde labelling.

Corresponding observations are made in two other cases with injections of large parts of the sensorimotor cortex, *cat ccol 303* (not illustrated) and *cat ccol 353* (Fig. 9).

The second somatosensory cortex (SII). — In two cases multiple injections were placed in the anterior ectosylvian gyrus, within the limits of SII (17, 55). In *cat ccol 357* (Fig. 9) the anterograde labelling is distributed in agreement with previous descriptions (7, 23). Some *overlap* between anterograde labelling an retrogradely labelled cells is seen at several levels in the right pontine nuclei.

Corresponding observations concerning location and degree of overlap were made in *cat ccol 331* (not illustrated).

Area 6 (supplementary motor area). — In two cases injection were placed in the medialmost part of the anterior sigmoid gyrus, that is, in area 6 (27). In both staining due to the injections involved mainly area 6 on the medial wall, probably within the supplementary motor area according to Woolsey (55). Cerebellar injections covered the medial three folia of the dorsal paraflocculus. In *cat ccol 353* (Fig. 9) the number of retrogradely labelled cells is fairly low. There is hardly any *overlap* with the anterograde labelling that is distributed as described before after area 6 injections (12, 14, 46). This case also shows for comparison

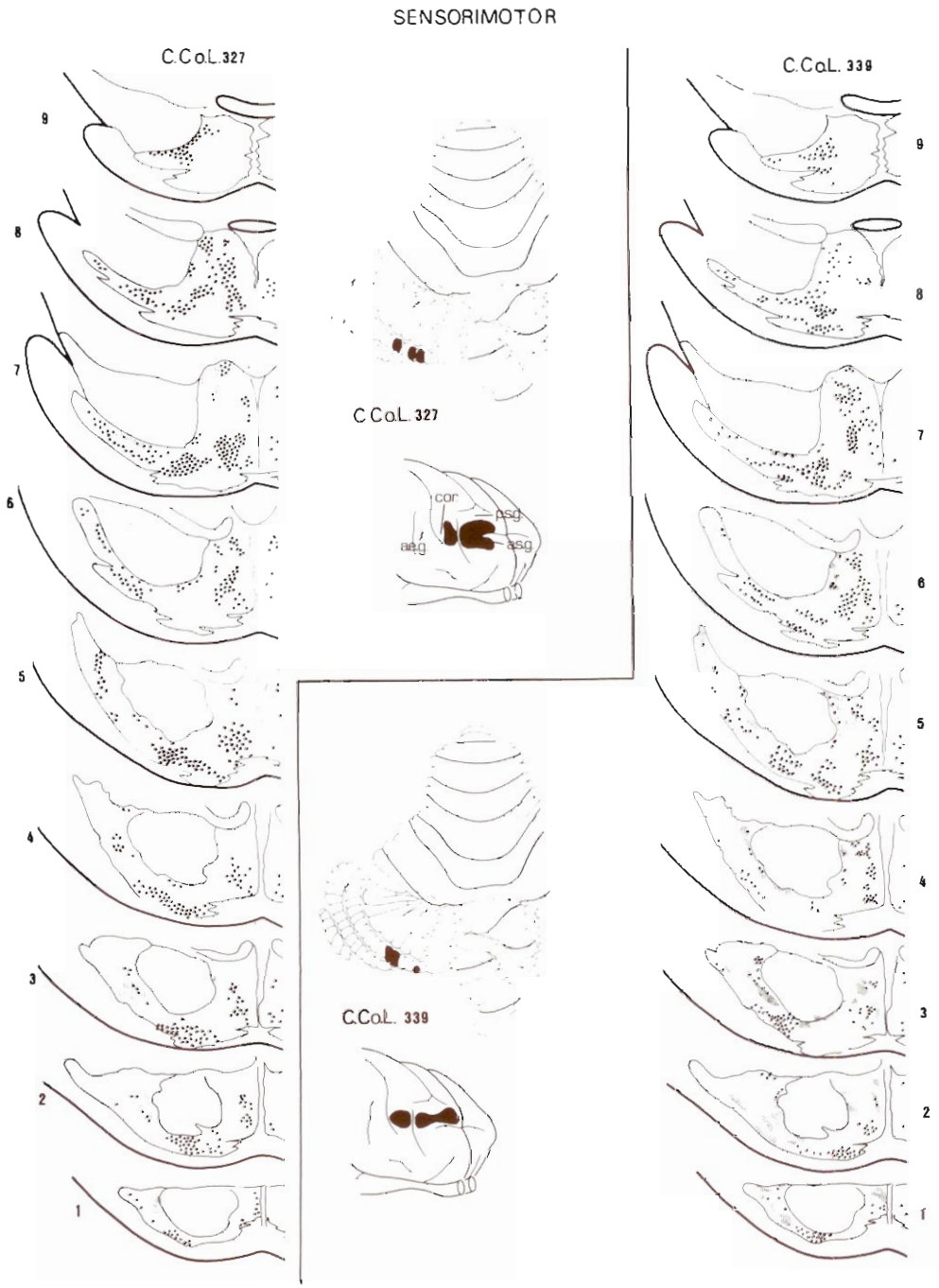

Fig. 8. – *The distribution in the pontine nuclei of fibres from the sensorimotor region and location of pontine neurons retrogradely labelled from the dorsal paraflocculus.*

Conventions as in Fig. 4. Note modest degree of overlap in both cases presented. For abbreviations see legend to Fig. 1.

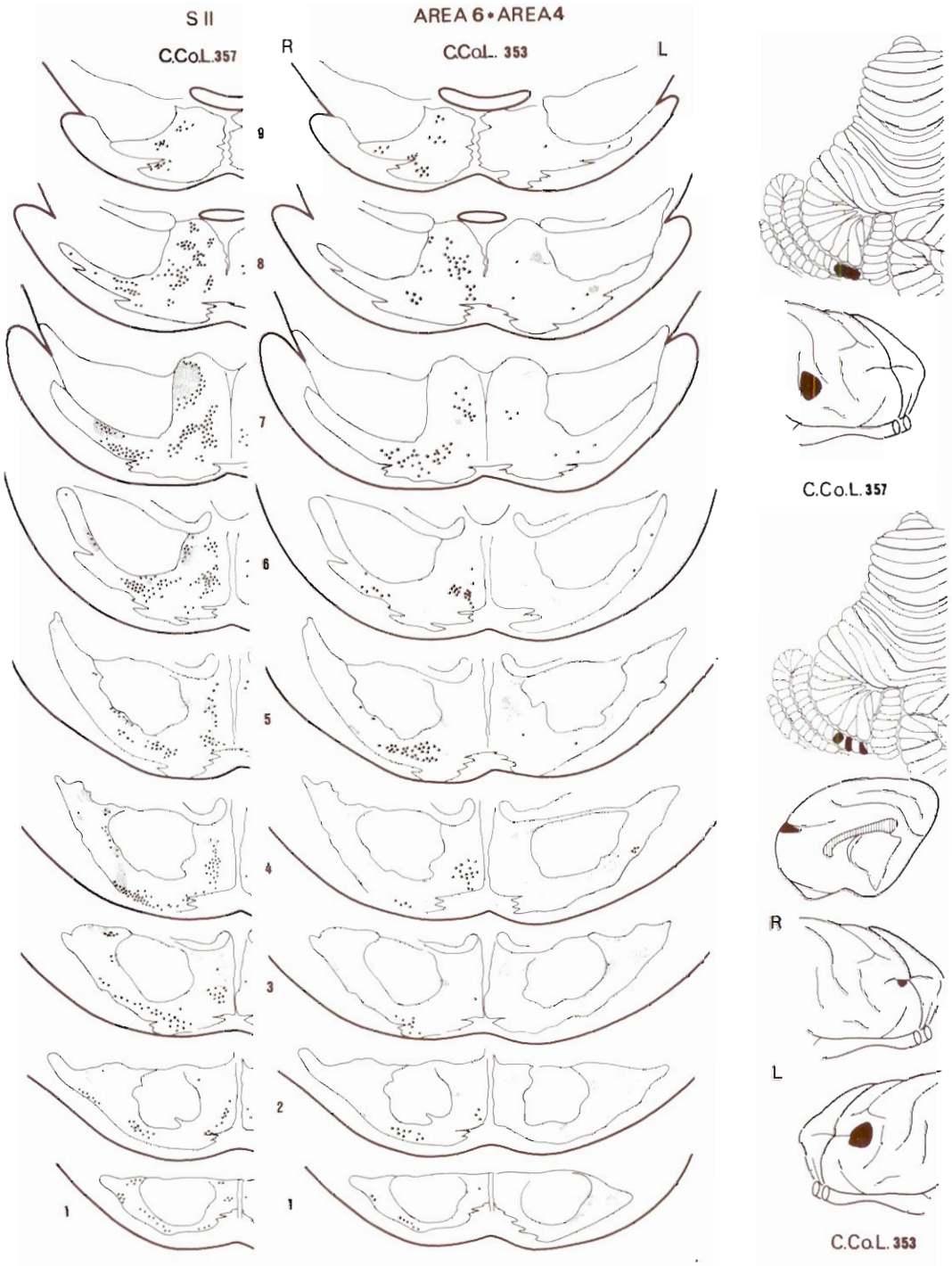

Fig. 9. – *The distribution in the pontine nuclei of fibres from the second somatosensory area (SII) and area 6 (supplementary motor area) and location of pontine neurons retrogradely labelled from the dorsal paraflocculus.*

Conventions as in Fig. 4. Overlap between fibres from SII and cells projecting to the dorsal paraflocculus can be seen at several places, while hardly any overlap occurs after injections of area 6. Cat ccol 353 also shows the differential distribution of fibres from the motor cortex (area 4) and area 6.

For abbreviations see legend to Fig. 1.

the termination of fibres from parts of the primary sensorimotor cortex, mainly area 4. As shown before (12, 14, 23) area 6 and 4 project to different parts of the pontine nuclei. In *cat ccol 329* (not illustrated) there are many more retrogradely labelled cells than in 353, but nevertheless hardly any overlap.

Comments on cases with injections in sensorimotor areas. — Overlap between the projection from the primary sensorimotor region and cells retrogradely labelled from the medial part of the dorsal paraflocculus is observed at several pontine levels, but is generally very restricted (Fig. 8). The impression is gained that a greater proportion of the anterograde labelling overlaps with cells retrogradely labelled from the dorsal paraflocculus after injections of SII than after injections of SI and MI (compare Figs. 8 and 9). Area 6 (at least the parts of it studied here) appears to have very limited access to the dorsal paraflocculus.

5. *The auditory cortex.* — In *cat ccol 363* (Fig. 10) multiple injections were placed within the limits of AI and AII of Woolsey (56). The ensuing staining is limited to these areas. The parafloccular staining affects three folia with very slight encroachment upon crus II. Modest amount of anterograde labelling is found mainly dorsolaterally in the caudal half, with some small patches more medially, in agreement with previous descriptions (10, 12, 23). There is no overlap between patches of anterograde labelling and groups of retrogradely labelled cells, and for the most part they are widely separated.

6. *The cingulate gyrus.* — In *cat ccol 335* (Fig. 10) four injections were placed in the middle and posterior parts of the cingulate gyrus. The staining does not go beyond this gyrus. The parafloccular staining affects several folia in the medial part. Heavy anterograde labelling forms a c-shaped band ventrally in the rostral part of the pontine nuclei (Fig. 10, section 7-9), which continues caudally in a ventral position (section 1-3) in agreement with previous descriptions (1, 11). There is no *overlap* between anterograde labelling and retrogradely labelled cells, and close apposition is only found a few places (Fig. 10, section 7). On the whole, fibres from the cingulate gyrus terminate ventral and medial to cells projecting to the dorsal paraflocculus.

DISCUSSION

The main findings of the present study are that the quantitatively dominant inputs to the medial part of the dorsal paraflocculus come from the parietal association cortex and all major visual cortical areas, with minor contributions from SII, SI and probably MI. No significant contributions could be found from the supplementary motor region in area 6, the auditory cortex and the cingulate gyrus. Furthermore, we show with double anterograde tracing that the projection from the parietal association cortex is topographically organized in a mosaic-fashion.

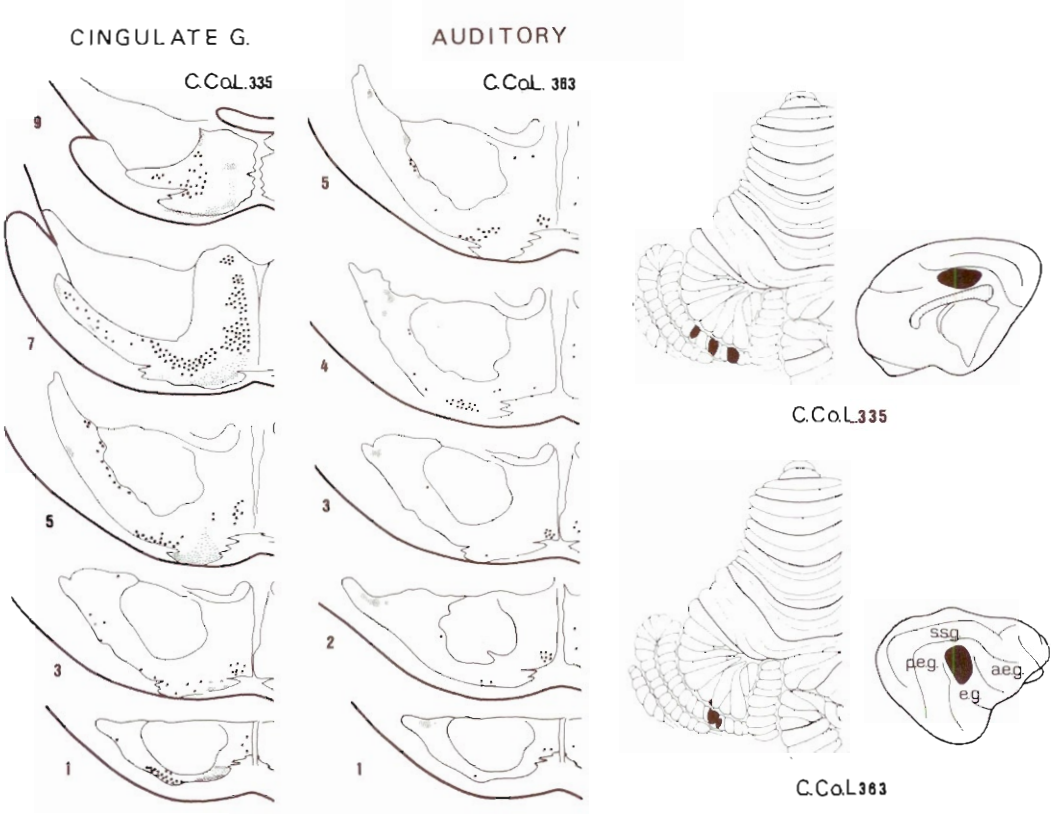

Fig. 10. – *The distribution in the pontine nuclei of fibres from parts of the cingulate gyrus and the auditory cortex, and location of pontine neurons retrogradely labelled from the dorsal paraflocculus.*

Conventions as in Fig. 4. Fibres from neither of these cortical regions appear to overlap significantly with neurons projecting to the dorsal paraflocculus.

For abbreviations see legend to Fig. 1.

Together with previous evidence of different termination of projections from functionally different cortical regions our findings suggest that there is a high degree of topographical order in the cerebrocerebellar connections to the dorsal paraflocculus.

There are problems of interpretation using the present double labelling approach. These have been discussed before (12-14) and will not be dealt with here. Suffice it to say that on the whole our findings of overlap probably represent underestimates, and that we do not put any weight on small differences between cases. In order to avoid being mislead by individual variations, for most cerebral regions we have repeated experiments.

It should also be emphasized that we have restricted ourselves to roughly the medial half of the dorsal paraflocculus, and our conclusions are therefore strictly speaking not applicable to lateral parts. In fact, by use of different fluorescent tracers we have recently been able to show that, although located in similar parts

of the pontine nuclei, neurons projecting to lateral and medial parts of the dorsal paraflocculus are clearly separated (41).

The pontocerebellar projection to the dorsal paraflocculus.

The distribution of labelled cells in the pontine nuclei in our cases are in general agreement with the findings of Gould (26) and Robinson and co-workers (45) in the cat, and of Hoddevik (29) in the rabbit. However, all the injections of Robinson and co-workers and most of those of Gould and Hoddevik included also parts of the ventral paraflocculus which we now know receives from other parts of the pontine nuclei than the dorsal paraflocculus (1). The complicated distribution of labelled cell groups after injections of the dorsal paraflocculus precludes any simple description from observations in single sections. Computer reconstructions from complete series of sections after paraffloccular injections, however, indicate that labelled pontine cells from largely obliquely oriented branching and twisting but continuous cylinders (41), rather than rostrocaudally oriented columns as previously described (29).

When comparing our findings in the present study with those obtained in other parts of the cerebellar hemisphere, certain differences become obvious. Firstly, as one moves from anterior to posterior within the hemisphere (anterior lobe, crus I, crus II dorsal paraflocculus and ventral paraflocculus), more and more of the labelled cells are found in the rostral part of the pons. Thus, after injections of the dorsal paraflocculus about 70% of all labelled cells are found in the rostral half of the pons, against 30% for crus I, 40% crus II and 50 for the paramedian lobule (13). There is also a simultaneous dorsoventral shift as evident from double labelling experiments, so that on the whole cells projecting to crus II is located more caudally and dorsally in the pontine nuclei than cells supplying the dorsal paraflocculus, that are located caudal and dorsal to cells projecting to the ventral paraflocculus (1). Secondly, the degree of crossing of pontocerebellar fibres is higher for the paraflocculus (about 90%) than for the crura (70-80%) in agreement with findings of Rosina and Provini (46) using two fluorescent tracers.

Cortical regions projecting to the dorsal paraflocculus.

The degree of overlap in cases with injections in parietal association cortex is striking (Figs. 4 and 5). This must obviously represent a major input to the dorsal paraflocculus. Furthermore, since both the corticopontine projections from this part of the cortex (Fig. 5) and the pontocerebellar projection to the dorsal paraflocculus (41) are topographically organized, it is possible that inputs from area 5 and 7 are directed to somewhat different parts of the dorsal paraflocculus. One might guess that the organization would be in the form of multiple patches in the cerebellar cortex receiving different set of inputs from the parietal cortex,

and with the same input repeated in several patches, in analogy with the "fractured somatotopy" described by Welker an co-workers (53, 54).

The kind of information relayed to the dorsal paraflocculus from parietal associations areas can only be a matter of speculation. Judging from properties of single neurons in primates, it might, for example, be information related to projected movements in extrapersonal space and to visual guidance of movement (see Hyvärinen (33) for data from primates). Although little is known about functional characteristics of areas 5 and 7 in the cat, careful studies of connections indicate that posterior parts of area 7 (in the middle suprasylvian gyrus) corresponds to area 7 in primates (42).

The other major cortical input to the dorsal paraflocculus comes from visual cortical areas, as shown previously (45). It should be emphasized that judging from the degree of overlap, the parietal association cortex would have stronger connections with the dorsal paraflocculus than the visual cortical areas. This seems to correspond to the electrophysiological results of Jansen (34) who evoked short latency (mossy fibre) responses in the dorsal paraflocculus primarily after stimulation of the parietal association cortex. Also Sasaki and co-workers (48) evoked mossy fibre responses in the dorsal paraflocculus after stimulation of the contralateral parietal cortex in the cat.

Our results would seem to indicate that several visual cortical regions, although terminating in part differently in the pontine nuclei (Figs. 6 and 7, see also (4)), all would be able to influence the dorsal paraflocculus. Again, it seems possible that information from, for example, area 18 and 20 would reach at least partly different regions in the dorsal paraflocculus. To what degree information from parietal and visual areas are integrated in the dorsal paraflocculus is so far unknown.

As to other cortical regions, our experiments indicate that SII may have access to the dorsal paraflocculus (although it would not be the main cerebellar target of SII), while connections from the primary sensorimotor region most likely are weak. Neither do limbic parts of the cortex (represented by the cingulate gyrus) have more than scant connections with the dorsal paraflocculus (Fig. 10). On the other hand, it was recently demonstrated that the cingulate gyrus, together with the medial mammillary nucleus, have strong connections with the ventral paraflocculus (1).

Altogether the dorsal paraflocculus (at least in the cat) must play a special role in processing information from posterior association cortex and visual cortex. While both crus I (14) and crus II (12) were found to receive their main inputs from the parietal cortex in agreement with physiological data (48), the ansiform lobule appears to be much less influenced from the visual cortex (although crus II apparently more than crus I). Crus I receives additional small inputs from several cortical regions (among them the orbital gyrus (14)), while Crus II has a significant input from area 6. The paramedian lobule receives strong connections from the sensorimotor region, but also from the parietal cortex (23). Each lobule of the cerebellar hemisphere thus seems to have its own unique combination of cerebrocortical inputs.

Also in the rat there is evidence of a strong connection from visual cortical areas to the paraflocculus (15, 16, 22). Certain differences appear also to exist, however. Thus, in the cat auditory inputs are unlikely to reach the dorsal paraflocculus as judged from physiological data (31, 50) and from the present findings (Fig. 10) while in the rat many cells in the paraflocculus can be activated by electrical stimulation of the auditory cortex (3). In the monkey, the dorsal paraflocculus appears likely to be a major target for inputs from the visual cortex (25).

The dorsal paraflocculus appears to receive few mossy fiber afferents from other brain stem nuclei (20, 37, 51), the most important outside the pontine nuclei probably being the reticulotegmental nucleus (30, 36). Thus, the dorsal paraflocculus would be heavily dominated from the cerebral cortex via the pontine nuclei. Its output is directed primarily to the dentate nucleus with additional termination in lateral parts of the nucleus interpositus posterior (19). Physiological studies (see (47) for a review) indicate that outflow from the dentate and interposed nuclei to the cerebral cortex via thalamic nuclei VL and VA is not only directed towards the motor cortex, but also to parietal association cortex. It is not known, however, which of these cortical regions are the major target of the dorsal paraflocculus.

SUMMARY

To reveal the organization and relative magnitude of connections from various parts of the cerebral cortex to the dorsal paraflocculus via the pontine nuclei, WGA-HRP was injected in the dorsal paraflocculus in conjunction with injection of the same tracer in various parts of the cerebral cortex in 17 cats. Termination areas of cortical fibres (anterogradely labelled) and pontine neurons projecting to the dorsal paraflocculus (retrogradely labelled) were carefully plotted in serial transverse sections. As an average of countings in ten cats, 90% of the labelled cells were found in the pontine nuclei contralateral to the injection, and the majority (70%) were located in the rostral half of the nuclei. The highest degree of overlap between anterograde and retrograde labelling was found after injections of the parietal association cortex (areas 5 and 7). In an experiment with double anterograde tracing, it was shown that both area 5 and 7 contribute substantially to the cerebral inputs to the dorsal paraflocculus. High degree of overlap also occurred after injections of several visual cortical areas (areas 17, 18, 19, 20 and the posteromedial lateral suprasylvian visual area, PMLS). Cases with injections restricted to individual visual areas indicate that they all contribute to the paraflocular input. Considerably less overlap occurred after injections of the primary sensorimotor region (SI, MI) and second somatosensory area (SII), while the supplementary motor area, the auditory cortex and gyrus cinguli probably have no or very restricted access to the dorsal paraflocculus.

It is concluded that the dorsal paraflocculus has its major cortical input from the parietal association cortex and the visual cortical areas. Since all the various

cortical regions studied project to largely different parts of the pontine nuclei, and overlap with neurons projecting to the dorsal paraflocculus takes place at numerous places, it follows that the pontine neurons projecting to the dorsal paraflocculus must consist of many subgroups differing with regard to their cortical input.

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