Cortico-Ponto-Cerebellar Pathway in Rats. An Anatomical Study of the Somatosensory Cortical Input to the Cerebellum.

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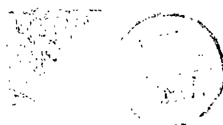

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ABSTRACT

Cortico-ponto-cerebellar pathway is one of the major subcortical pathways linking sensory cortical areas with distant motor regions of the brain. In this thesis I focus on the projections from the primary somatosensory cortex in order to understand the principles in the organisation of this pathway in rats.

Primary somatosensory cortex in rats contains aggregates of granule cells in layer IV, named "barrels" by Woolsey and Van der Loos (1970). There are about 32 whiskers and an equal number of large cortical barrels within the posteromedial barrel subfield (PMBSF) in the primary somatosensory cortex. In addition to its cortical representation the peripheral organisation of the whiskers is replicated in other stations within the afferent pathway, the trigeminal nuclei and the thalamus. This organisation is easily identified both anatomically and physiologically. Therefore rat somatosensory system can serve as a model system to study sensory-motor integration.

I mapped terminals from small regions of the primary somatosensory cortex of rats (within and surrounding the PMBSF) in the pontine nuclei using the anterograde tracers Phaseolus vulgaris leucoagglutinin, biocytin and biotinilated dextran-amine. The results demonstrate that cells in layer Vb of all the cortical barrels project to the ipsilateral pons. Most barrel columns terminate within the same set of pontine nuclei with slight medio-lateral shifts in their termination zones. The most rostral, smaller barrel columns and the cortical regions representing intervibrissal fur project bilaterally. This dichotomy is not present in corticotectal projections. Both small and large barrel columns as well as the less granular zones of the somatosensory cortex project to the deep layers of the ipsilateral superior colliculus. Retrograde tracing with fluorescent latex beads demonstrated that at least 70% of layer Vb cells in the primary somatosensory cortex in rats send collaterals to the deep layers of the superior colliculus and the pontine nuclei.

Pontine nuclei project as mossy fibres onto the cerebellar cortex. These projections are predominantly contralateral but there are also ipsilateral mossy fibre terminals. Some pontine cells in cats are known to send axons that collateralise within the cerebellar white matter and supply the ipsilateral as well as the contralateral cerebellar cortex (Rosina and Provini, 1982). The experiments in the third chapter were designed to analyse whether and to what extent the axons of the pontine cells in rats, which receive

somatosensory cortical inputs related to the whiskers, bifurcate. Whisker sensitive patches in lobules VII, IX and Crus I of the cerebellar cortex were injected with differently coloured fluorescent beads. Both sides of pontine nuclei contained large numbers of retrogradely labelled cells, a small percentage of which was double labelled.

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I. Introduction

The cerebellum is a large brain structure which receives sensory and motor information from the cerebral cortex and subcortical structures but projects mainly to descending motor structures. Lesions of the cerebellum produce impairments in smooth execution of movements in humans and other animals. In primates, the role of the cerebellum in visual control of movement has been emphasised, because vision is the dominant sense in these animals.

Although the cerebellum is known to co-ordinate movement, how that coordination is achieved is not completely understood. One approach to further our knowledge about cerebellar functions is to learn more about its afferent inputs.

Rats are "whisker animals" and touch is the dominant sense used in guiding their behaviour (Richardson, 1910; Vincent, 1912; Hutson and Masterton, 1986; Guic-Robles, 1989; Glickstein et al., 1992). The whisker system is a uniquely appropriate model for studying anatomical principles underlying sensory-motor coordination for two main reasons.

Firstly, whisker representations in the trigeminal nuclei, thalamus and primary somatosensory cortex reflect the peripheral organisation of the whiskers on the face and they are clearly visible in histological preparations (Belford and Killackey, 1979; Bates and Killackey, 1982; Van der Loos, 1976; Land and Simons, 1985a; Woolsey, 1967; Woolsey and Van der Loos, 1970).

Secondly, the punctate nature of whiskers makes it possible to record responses of cells to deflection of a single whisker. It was, thus, possible for a number of electrophysiological studies to show that there is a precise somatotopy in the afferent stations of the whisker sensory pathway, as well as in some of its efferent targets.

The importance of the primary somatosensory cortex in behaviour of rats has been well documented (Hutson and Masterton, 1986; Guic-Robles, 1992). The cortical tactile information is relayed to a number of structures, but the focus of this thesis is the cortico-ponto-cerebellar pathway. The principal aim of the work presented here is to describe and analyse the way in which the cortical information related to whiskers reaches the cerebellum.

The introductory part of this thesis will first describe whiskers and their role in behaviour, followed by the known anatomical details of the whisker sensory system. The next part will give an account of the materials and methods used in the experiments and the last three chapters represent the actual experiments.

1) Vibrissae

Vibrissae are tactile receptors, a specialisation of hair in mammals. Unlike body hairs, vibrissae are present at birth and have a continuous growth cycle.

According to Pocock (1914) all mammals (except anteaters) have facial vibrissae. The most prominent group of facial vibrissae are mystacial vibrissae, or whiskers, placed on the upper lip. In lions the pattern of whiskers is specific for an individual, almost like fingerprints. In rodents, the number and position of whiskers on the face is a constant feature. The largest whiskers on the rat's face are organised in 5 parallel rows with 5-8 whiskers in each row (Vincent, 1912; Zucker and Welker, 1969).

Figure 1.: Rat's face with rows of whiskers (A-E).

In common rodents (rats, mice and rabbits) tactile hairs on the muzzle are the most prominent but these animals also have tactile hairs on the lower lip, brow, cheeks and carpal joints (Vincent, 1912; Pocock, 1914).

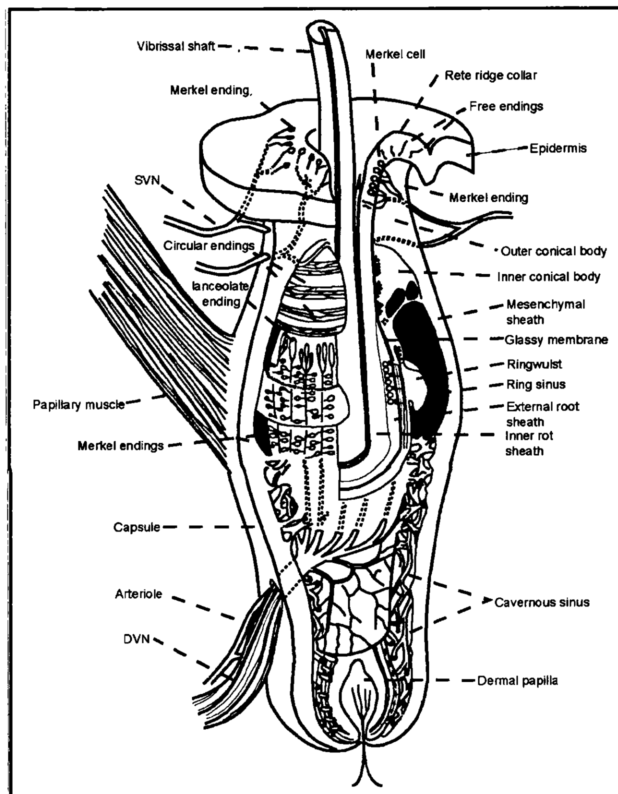
Whiskers have a longer and more complex follicle than the body hairs (Patrizzi and Munger, 1966). Rice, Mance and Munger (1986) adopted the term "follicle-sinus complex" (F-SC) to describe a vibrissal follicle, because this structure is, in fact, made up of two separate components. The actual hair follicle is derived from the epidermis and surrounded by a sinus, derived from dermis. This sinus has two distinct parts. Its

lower end is made of connective tissue and, due to its appearance, usually called "cavernous sinus". The upper end of the sinus, in the upper third of the follicle, is referred to as a "ring sinus".

Sensory receptors are situated in the middle (at the level of "ringwulst") and upper part of the vibrissal follicle (at the level of "rete ridge") and are innervated by sensory nerve fibres. The growing part of the tactile hair is the papilla which is embedded in the base of the follicle.

Each vibrissa follicle is surrounded by a layer of erectile and connective tissue.

Figure 2.: A diagram showing the main parts of the tactile hair follicle (adapted from Rice and Munger, 1986; redrawn by E. Jenkinson).



The sensory innervation to the follicle is provided by the infraorbital branch of the trigeminal nerve. Cell bodies of these fibres are located in the ipsilateral Gasserian ganglion. The smallest cells of the trigeminal ganglion innervate the skin between whiskers (Arvidsson and Rice, 1991). Compared to the whisker follicles, the density of innervation of the intervibrissal skin in rat is relatively low (Rice and Munger, 1986). Each of the larger whisker follicles receives 100-200 myelinated fibres which end in a variety of receptors: Merkel, Ruffini, Lanceolate, Pacinian corpuscle (Rice et al., 1986). There are two components in the innervation of the rat vibrissal follicle-sinus complex: one is the deep vibrissal nerve (DVN) and the other is superficial vibrissal nerve

(SVN). The superficial component is made up of several smaller fibres which travel in the dermis. This dual innervation arises from separate ganglion cells (Waite and Jacquin, 1992). Both cell types give rise to thickly myelinated axons that can detect single vibrissa deflection, as well as the direction, velocity and extent of vibrissal displacement (Waite and Jacquin, 1992). The only difference between the two cell types is that SVN cells are not responsive to pulling on the hair shaft and do not discharge after axotomy.

DVN enters the lower third of the follicle to terminate at the level of cavernous and ring sinuses. SVN ascend to the dermis between vibrissae and branch before entering the follicle. They end in either the epidermis surrounding the hair shaft (rete ridge) or the inner conical body. Although DVN and SVN terminals do not overlap in the follicle both of these nerves are connected to the same types of receptors: Merkel, Ruffini, lanceolate and free nerve endings.

1.1. Whisking, mystacial muscles

In some rodents whiskers are in constant motion. In the albino rat whiskers sweep across the surface of the object that the animal explores at a rate of about 5-11/second (W. I. Welker, 1964). This movement of whiskers ("whisking") consists of protraction and retraction and is co-ordinated with small head adjusting movements (W. I. Welker, 1964). The whisking pattern is as complex in golden hamster (L.E. Wineski,1985), but a little bit faster. The mystacial vibrissae in the hamster move at about 16 sweeps/second.

Two types of muscles of the mystacial region control whisking: extrinsic and intrinsic muscles. Both muscle types are innervated by branches of the facial nerve (Dörfl, 1982, 1985).

Extrinsic muscles insert on the skull outside of the mystacial region. They belong to the group of facial muscles which move upper lip and wing of the nose as well as the whole of the mystacial pad. These muscles are: M. levator labii superioris, M. maxillolabialis, M. transversus nasi and M. nasalis. Their fibre bundles are inserted into the corium between the rows of whiskers.

Intrinsic muscles have no attachments on the bone. They look like slings and connect two adjacent follicles belonging to the same row. The sling is wrapped around the

inferior part of the more rostral follicle and inserts its extremities into the conical body of the caudal follicle and the neighbouring corium. Intrinsic muscles act as protractors of vibrissae.

1.2. Information Coding

To find out what it is that whiskers communicate to the brain, Zucker and Welker (1969) recorded from the cells in the trigeminal ganglion. These neurones respond to the deflection of whiskers and their position; they can detect onset and termination of the whisker deflection as well as its amplitude, duration, velocity, repetition rate and temporal pattern.

The spatial distance between whiskers ensures that each can be stimulated independently (Dykes, 1975, study on cats), which would suggest that each whisker acts as a separate tactile receptor. However, "whisking" involves the whole of the mystacial pad, and objects are explored with simultaneous palpation with almost all of the whiskers. It was, hence, put forward that the mystacial whiskers together form a "sensory grid" (Wineski, 1983) where the position of the particular hair on the face is important in the system's ability to precisely locate a point of stimulation in the environment. During whisking the spatial distance between tips of some whiskers may even be increased which, even if the whole of the mystacial pad is used as a sensory organ, enables each tactile hair to be stimulated separately.

The question of how rats and other animals use their mystacial vibrissae is still a problematic issue. It is generally agreed that the longest, more posterior whiskers differ from the smaller, more anteriorly placed whiskers. Small, anterior whiskers move in very restricted excursions and are protracted even in their resting position. They are better suited for monitoring the immediate area around nose and mouth without impairing function of these organs. Larger whiskers are the ones that engage in scanning the environment and provide more sensory information (Carvell and Simons, 1990). Recently, Brecht et al. (1997) challenged this view. They agree that there is a functional difference between long mystacial vibrissae ("macrovibrissae") and the short, rostral ones ("microvibrissae"), but they suggest that the macrovibrissae function as a "distance detector array providing head-centred spatial information". Microvibrissae, in their view, are important in palpating objects and recognising shapes.

1.3. Behavioural significance of whiskers

Whiskers are the principal sensory organ for tactile exploration of the environment in rats. Cats and sea lions also use their whiskers as a very sensitive touch organ (Dykes 1975; Dehnhardt, 1994). For example, Broughton in 1823 showed that the blind kitten could learn a maze made out of books which he had arranged on the floor. He concluded that the animal was using its whiskers to avoid obstacles.

Small (1898) trained rats to run a wire mesh reproduction of the Hampton Court maze. This maze had two correct paths to the food box, one of which was shorter. Rats would be placed at the entrance of the maze and the time measured from the start until they reached the food box. He found that blinded animals could learn to negotiate the maze as well as the intact animals, and concluded that touch and perhaps kinesthesis must have provided essential cues for learning the maze.

In order to establish which senses the rat uses for maze learning Watson (1907) made a maze which was similar to Small's, whose paths were enclosed with walls erected on each side of the runway. Watson found that animals which were blinded, anosmic, or deafened could learn the maze as well as intact animals. Removing the whiskers had a drastic effect at first. The animals clung to the floor and bumped into walls. But about 48 hours later these animals could successfully accomplish the task again. Watson concluded that the essential cues for running the maze were provided by the kinaesthetic sense.

Stella Vincent (1912) was the major contributor to clarifying the role of the vibrissae in the behaviour of rats. She questioned Watson's conclusions arguing that in his experiments the animals were able to use the tactile cues provided by their whiskers, noses or other parts of the body. In order to establish the importance of the tactile cues, she built a maze which was elevated from the floor and whose walls could be removed. In this situation blind rats without whiskers performed very poorly. When they did manage to run from the start to the food box they adopted abnormal body postures in order to remain continuously in close contact with the floor. If the whiskers were cut on one side the animals would walk in a way that would keep the intact whiskers in contact with the wall of the apparatus.

In another experiment Vincent trained rats to differentiate between surfaces of different textures and showed that whiskers play a role in roughness discrimination.

Two years prior to Vincent's publications Florence Richardson (1910) described her experiment in which rats were trained to jump across a gap. She reported that blind rats would jump across the gap only if they could feel the other side of it with their whiskers. When these animals had their whiskers touched with a pencil by the experimenter they would try to stretch across the gap.

Hutson and Masterton (1986) published a classical study of the rat's use of vibrissal cues. They removed all but one whisker (C1) on both sides of the face. In the first experiment they tested whether rats could detect the oscillations produced by a varying stream of air directed vertically onto the tip of the vibrissa. In the second experiment they studied whether rats could detect a change in the rate of the oscillation. The third experiment was done on blinded rats which had been trained to jump a gap in an elevated runway.

For the first two experiments Hutson and Masterton used a conditioned suppression technique to determine whether rats could still detect the input from the mystacial vibrissae after removal of their primary cortical representation (the posteromedial barrel subfield -PMBSF). Thirsty rats were trained to lick a spout for their daily water requirements. The spout was fixed so that the animals had to keep their heads in an appropriate position to be rewarded. When they reached the natural frequency for licking (6-7 licks/sec) the tip of the vibrissa was deflected (5 mm amplitude, 5 Hz oscillation) by an oscillating air stream which was presented for 5 seconds. At the end of the air stream presentation the animal would receive a mild foot shock. Trained animals stop licking when they detect the air stream. They resume licking after the foot shock. If the stimulus is below threshold the animals continue to lick throughout its presentation. This method is useful for measuring sensory thresholds in experimental animals.

Under these conditions it was found that a single vibrissa has a threshold sensitivity of ~1 mm tip movement and that this sensitivity extends from 0.5- ~16 Hz.

The next experiment was done to determine whether rats can discriminate between different frequencies of oscillation. The animal would be presented with easily detectable "safe" frequency (0.8-2 Hz, 7 mm deflection amplitude) which was then replaced by a "warning" (higher) frequency before a foot shock was delivered. A pause in licking was taken as evidence that the animal detected a change in frequency. The threshold value for detecting the change was about 3.8 Hz.

Trained animals in both of these experiments received surgical ablations of the contralateral PMBSF. Postoperative testing showed that the animals retained the ability to detect the presence of the oscillations as well as a change in frequency.

In the third experiment blinded rats had to use their vibrissae to palpate the far side of the gap before attempting to jump across it. Trained animals could jump gaps of up to 15 cm. Bilateral removal of vibrissae, bilateral lesions of PMBSF and a combination of cortical ablation with ipsilateral whisker removal result in complete unwillingness of the animal to cross the 15 cm gap. They would continue to jump gaps of 13 or 14 cm using tactile information from their noses.

The study shows that there are at least two different ways in which the whisker information can be used. The animal can detect deflection of the vibrissae which is not a result of its own motor activity. This phenomenon is sometimes referred to as "passive" palpation. "Active" palpation requires the animal to actively brush the whiskers against a surface of interest. Processing of these two different types of information probably uses different circuits through the brain. For active palpation it is essential that the cortical representation of whiskers be intact, whereas for passive palpation this is not necessary.

Active palpation was required from the animals in a study by E. Guic-Robles (1989, 1992) who trained blinded rats to discriminate between rough and smooth surfaces using tactile cues. Rats were placed onto an elevated Y-shaped platform from which they had to stretch across the gap, palpate strips of sand paper glued to the front edges of the landing platforms and jump onto the platform with the smoother edge. The gap was gradually increased so that the animals could touch the platforms only with the tips of their whiskers. Rats learned to discriminate between surfaces using only vibrissal cues. Bilateral lesions of the primary somatosensory cortex abolish the animals' ability to discriminate between rough and smooth surfaces.

In our lab, jumping across the gap task has been studied for a number of years (Legg and Lambert, 1990; Glickstein et al., 1992; Jenkinson and Glickstein, 1997). Animals are required to jump in both light and in dark across a gap of variable size. When allowed to use visual cues the animals can be trained to jump distances of 20-40 cm (Legg and Lambert, 1990). In the dark they use their whiskers to gauge the distance which they can safely reach with whiskers fully protracted, about 16 cm. Shaving the whiskers does not impair jumping in the light. Rats refuse to jump 16 cm in the dark if

their whiskers are shaved (Jenkinson and Glickstein, 1997). Interruption of the efferent pathway from the whisker representation in the somatosensory cortex to the cerebellum, at the level of the cerebral peduncle, has the same effect. The animals retain their ability to jump much larger distances in the light using visual cues. Peduncle lesions effectively block information from the posteromedial barrel subfield from reaching pons and therefore the cerebellum. Cortico-cortical pathways, which are not affected by this procedure, appear not to be critical for this behavioural task.

2) Anatomy of the Somatosensory System

2.1. Ascending Pathway: From Whisker Pad to the Cerebral Cortex

2.1.1. Trigeminal Ganglion

Sensory information from the whisker follicles first reaches the trigeminal (semilunar) ganglion situated in the base of the skull via the maxillary branch of the trigeminal nerve.

Physiological (Zucker and Welker, 1969) and anatomical studies (Arvidsson, 1982) show that cells in the trigeminal ganglion are somatotopically organised. Cells innervating whiskers in row A are in the medial part of the ganglion. Cells innervating whiskers in row E lie in more lateral parts. Rostrally placed whiskers are innervated by the cells in ventral parts of the ganglion; caudal whiskers by the cells in the dorsal parts of the ganglion.

Physiological studies of the response properties of the cells in trigeminal ganglion concentrated on the cells sensitive to movement of the large whiskers (Zucker and Welker, 1969; Gibson and Welker,1983 a,b; Lichtenstein et al., 1990). Most whisker sensitive cells are not spontaneously active. They respond to movement of a single whisker and are very sensitive to the direction of whisker deflection. Responses of these cells are slowly adapting and are probably related to Merkel cell receptors in the root sheath of the tactile hair. There are also some rapidly adapting cells which show only limited directional sensitivity and these responses are probably due to lanceolate receptors. A very small percentage of cells (less than 10%) require very high velocity of movement or high threshold stimuli for activation. These cells may be the ones which give rise to free nerve endings.

2.1.2. Sensory Trigeminal Nuclei

Central processes of the ganglionic cells carry vibrissal information to the next station: the sensory trigeminal nuclei in the brainstem. These nuclei, extending from the pons to the spinal cord, are composed of mesencephalic, principal sensory and spinal trigeminal nucleus (Olszewski, 1950). The spinal trigeminal nucleus can be cytoarchitectonically subdivided into three subnuclei: oral, interpolar and caudal.

Trigeminal afferents enter the pons where they divide into an ascending and a descending branch. The ascending branch terminates in the principal sensory nucleus whereas the descending branch terminates in the spinal trigeminal nucleus. There is evidence that many trigeminal fibres bifurcate to innervate both the principal and spinal trigeminal nuclei (Hayashi, 1980).

Whiskers are represented both in the principal and in the spinal trigeminal nuclei (Hayashi, 1980; Arvidsson, 1982; Bates and Killackey, 1985; Jacquin et al., 1986 a,b). In the neonatal rat brain sections reacted for succinic dehydrogenase there are clusters of cells which replicate the peripheral organisation of the vibrissae and smaller sinus hairs on the face (Belford and Killackey, 1979; Bates and Killackey, 1982). Ma and Woolsey (1984) named these clusters "the barrelettes". The barrelettes were present in the principal and two divisions of the spinal trigeminal nuclei, interpolaris and caudalis. Direct application of horseradish peroxidase (HRP) to the trigeminal nerve (Arvidsson, 1982) revealed that the oral part of the spinal trigeminal nucleus also receives projections from the whiskers.

Somatotopy

Neurones innervating whiskers terminate in the trigeminal sensory complex in a highly ordered pattern. Each single whisker has its representation and there is very little overlap with the neighbouring whiskers. The general map of these projections as proposed by Arvidsson (1982) is shown in figure 3.

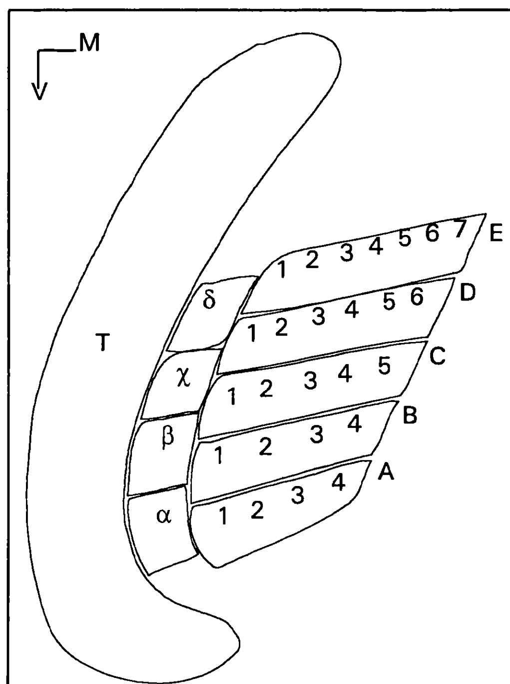


Figure 3:
Schematic drawing of the whisker representation in the trigeminal sensory complex (adapted from Arvidsson, 1982);
T= trigeminal tract
A-E and α–δ = rows of whiskers
M= medial, V= ventral

As evident in the diagram, the whiskers positioned more dorsally on the face are represented ventrally and caudal whiskers have their representations more laterally in the trigeminal sensory complex.

Response properties

Response properties of the cells in principal and spinal trigeminal sensory nuclei differ. Most cells in the principal sensory nucleus (about 70%, Jacquin et al., 1988) respond to whisker stimulation. About 80% of whisker sensitive cells in this nucleus respond predominantly to a single whisker and half of them are also directionally selective. Cells in the oral subnucleus of the spinal trigeminal nucleus can be either projection or local circuit neurones. Projection neurones typically have unimodal but converging receptive fields (several whiskers, for instance). Local circuit neurones have more restricted receptive fields, often responding to a single whisker or guard hair (Jacquin and Rhoades, 1990).

Similarly, in the subnucleus interpolaris of the spinal trigeminal nucleus, local circuitry neurones are responsive to manipulation of whiskers, guard hairs and painful stimuli. Very often whisker sensitive cells respond to manipulation of a single vibrissa only. A large number of projection neurones respond to whisker manipulation, but their receptive fields encompass several whiskers (Jacquin, 1986).

Subnucleus caudalis is generally thought of as part of the pathway for temperature sense and pain. However, its function may be more complex since it projects to all other trigeminal nuclei (Jacquin et al., 1990). Hallas and Jacquin (1990) proposed that subnucleus caudalis functions as a modulator for the response properties of cells in all the trigeminal nuclei.

Connectivity

Projection neurones within the principal trigeminal nucleus send their axons mainly to the contralateral ventroposteromedial (VPM) and posterior (Po) thalamus. They do not collateralise in the brainstem (Fukushima and Kerr, 1979; Chiaia et al., 1991a). These trigeminothalamic fibres travel in the medial lemniscus. A much smaller projection from the principal trigeminal is directed towards the cerebellum (Watson and Switzer, 1978) and superior colliculus (Huerta et al., 1983).

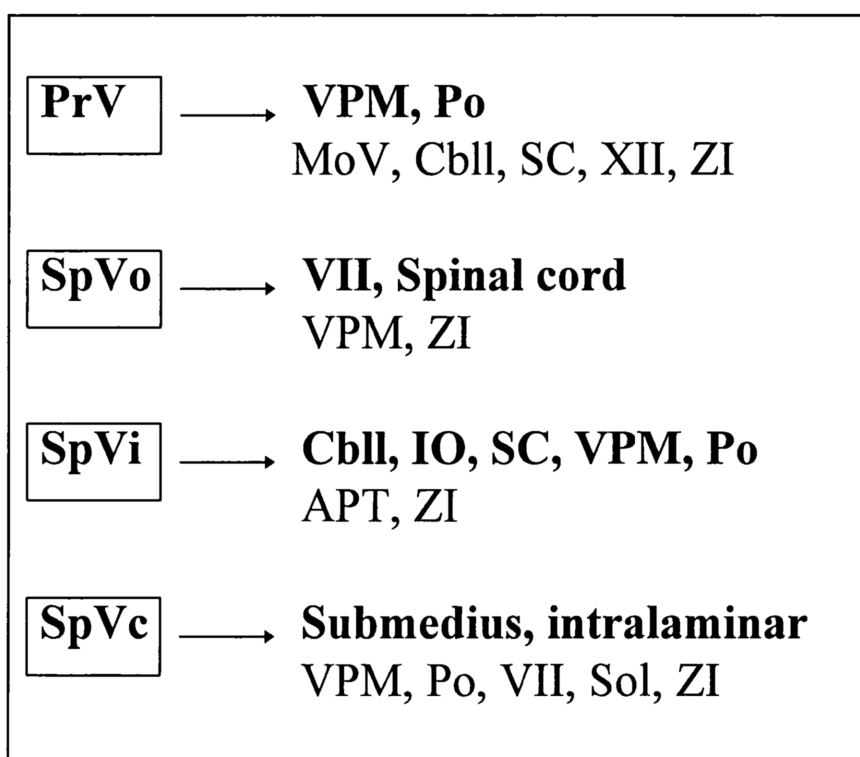
Subnucleus oralis of the spinal trigeminal tract also projects to the contralateral ventrobasal thalamus via the medial lemniscus. These fibres send collaterals in the brainstem and to the spinal cord (Jacquin et al., 1990). The strongest projection from this subnucleus is to the facial nucleus (Erzurumlu and Killackey, 1979).

Subnucleus interpolaris projects strongly to the cerebellar cortex, both directly, with its axons terminating as mossy fibres, and indirectly via inferior olive as climbing fibres. It also projects to the superior colliculus (Huerta et al., 1983) and to the contralateral VPM and Po (Fukushima and Kerr, 1979) of the thalamus. Some cells in the interpolar subnucleus collateralise and project to two targets: thalamus and superior colliculus (Jacquin, Mooney and Rhoades, 1986); thalamus and cerebellum (Bruce et al., 1987); superior colliculus and inferior olive (Huerta et al., 1983).

Subnucleus caudalis is continuous with the upper cervical dorsal horn at its posterior end. Rostrally it extends to the level of the obex. This nucleus has a laminar structure like the dorsal horn: marginal layer (lamina 1), substantia gelatinosa (lamina 2) and magnocellular layer (lamina 3 and 4). Vibrissal afferents terminate in a circumscribed fashion in layers 3 or 4 in the caudal subnucleus. Terminals from the subnucleus caudalis are found in the thalamus, mostly in nucleus submedius and intralaminar nucleus, although there are some projections to the VPM and Po (Craig and Burton, 1981; Fukushima and Kerr, 1979).

Figure 4: A simplified diagram summarising trigeminal efferents, according to Waite and Tracey (1995). Bold font stands for major connections.

Abbreviations:
APT = anterior pretectal nucleus; Cbll = cerebellum; intralaminar = thalamic nucleus; IO = inferior olive; MoV= motor n. of trigeminal; Po = posterior thalamic nucleus; Sol = n. of solitary tract; SC = superior colliculus; submedius = thalamic nucleus; VPM = ventroposteromedial thalamic nucleus; ZI = zona incerta; VII = facial nucleus; XII = hypoglossal nucleus.



2.1.3. Thalamus

Two large thalamic nuclei receive whisker sensory information relayed from the trigeminal nuclei: ventroposteromedial (VPM) and posterior (Po) thalamic nuclei. VPM and Po belong to a group of thalamic nuclei which receive somatosensory inputs. The majority of trigeminal fibres terminating in these two thalamic nuclei originate in the principal and spinal interpolar nuclei.

VPM is part of the "ventrobasal complex", a term coined by Rose (1935). It projects exclusively to the somatosensory cortex. The other nucleus of this complex is the ventroposterolateral nucleus (VPL) and is situated just lateral to the VPM. VPL receives projections from the dorsal column nuclei (cuneate and gracile) which relay the somatosensory information from the trunk and limbs. Ventrobasal complex in rats consists only of thalamocortical cells (Harris and Hendrickson, 1987), in contrast with primates whose VB thalamus contains interneurones as well (Williams and Faull, 1987).

Afferent inputs to the VPM are somatotopically organised, so that the nose is represented medially and caudal face more laterally. Particularly large and striking is

the representation of the facial vibrissae. Representation of the most dorsal row of whiskers (A) is found in the caudal portion of the VPM, and the most ventrally positioned row of whiskers (E) has its representation in the rostral part of the VPM.
In cytochrome oxidase preparation thalamic cells in the VPM form darker staining cylinders throughout its rostro-caudal extent. The spatial organisation of these cylinders resemble peripheral organisation of the mystacial vibrissae. These cylinders were first discovered in mice (Van der Loos, 1976) and named "barreloids". Barreloids were subsequently seen in cytochrome oxidase preparations of VPM in rats (Land and Simons, 1985a). The vast majority of the trigeminal inputs to the barreloids originates in the principal trigeminal nucleus (Williams et al., 1994); input from the spinal trigeminal nucleus to the same region is very sparse and mostly confined to the periphery of the barreloids and interbarreloid septa.
Somatotopic organisation in the VPM was confirmed with recording studies. The large caudal whiskers activate cells in dorsal and lateral pats of the VPM whereas cells in the ventromedial parts of the VPM respond to small, rostral whiskers (Waite, 1973 a). Most of the vibrissae related VPM neurones respond to movements of a single whisker (Waite, 1973 b). However, extracellular recordings in urethane anaesthetised rats demonstrated that the receptive fields of some of these neurones incorporate several whiskers (Ito, 1988; Armstrong-James and Callahan, 1991) and single VPM neurone can respond to discrete stimulation of up to 20 whiskers in awake or lightly anaesthetised animal (Nicolelis and Chapin, 1994).
These results seem to be at variance with the traditional view that the tactile information from each individual whisker is conveyed via separate channels in the VPM to the somatosensory cortex. Nevertheless, a typical VPM cell responds at a short latency to only one whisker in its "centre receptive field" (Diamond et al., 1992 a,b). This response property seems to be related to the afferent input from the principal trigeminal nucleus (Chiaia et al., 1991 a,b).
VPM projects to the ipsilateral primary somatosensory cortex. Its terminals are densest in layer IV, but are also present in layers Vb and VIa, as demonstrated by anterograde tracer Phaseolus vulgaris leucoagglutinin (Lu and Lin, 1993). More specifically, these terminals are aligned with the cortical barrels.

Po thalamic nucleus is a large structure which borders the VPM medially, and extends further caudally than the VPM. It is a very complex nucleus functionally. The rostral part of it, Pom, contains representation of the entire body surface (Fabri and Burton, 1991) and its afferents are somatotopically organised. Diamond, Armstrong-James and Ebner (1992) mapped the somatosensory responses in the rostral part of the rat Pom and showed that there is the whole body map within this nucleus. Region representing face and whiskers, for example, lies close to and parallel with the border of the VPM. Within that region caudal vibrissae are represented more dorsally and rostral vibrissae more medially. Row A of whiskers is represented closer to the border with VPM, whereas row E is represented more medially.

In a recent anatomical study it was demonstrated that the Pom receives very sparse projection from the trigeminal nuclei. Only 7% of cells in the principal and about 17% in the spinal interpolar nuclei were found to project to the Pom (Chiaia et al.,1991 a). Pom receives strong descending input from the primary somatosensory cortex (Hoogland et al., 1987). This anatomical connection plays a very important role in shaping the response properties of the Pom cells. Cooling of the primary somatosensory cortex completely abolishes responsiveness of the Pom, but has no effect on the VPM cells (Diamond et al., 1992).

Pom cells that are sensitive to whisker stimulation typically respond to a number of whiskers (average size of the receptive fields was 5.1 whisker- Diamond, Armstrong-James and Ebner, 1992) and at longer latency than the VPM cells. Another difference between these two thalamic nuclei is the pattern of the projection to the primary somatosensory cortex. Terminals from the cells in the Pom are found mainly in layers I and Va in the barrel areas of the primary somatosensory cortex, and in layers I-V in the interbarrel areas (Koralek et al., 1988; Lu and Lin, 1993).

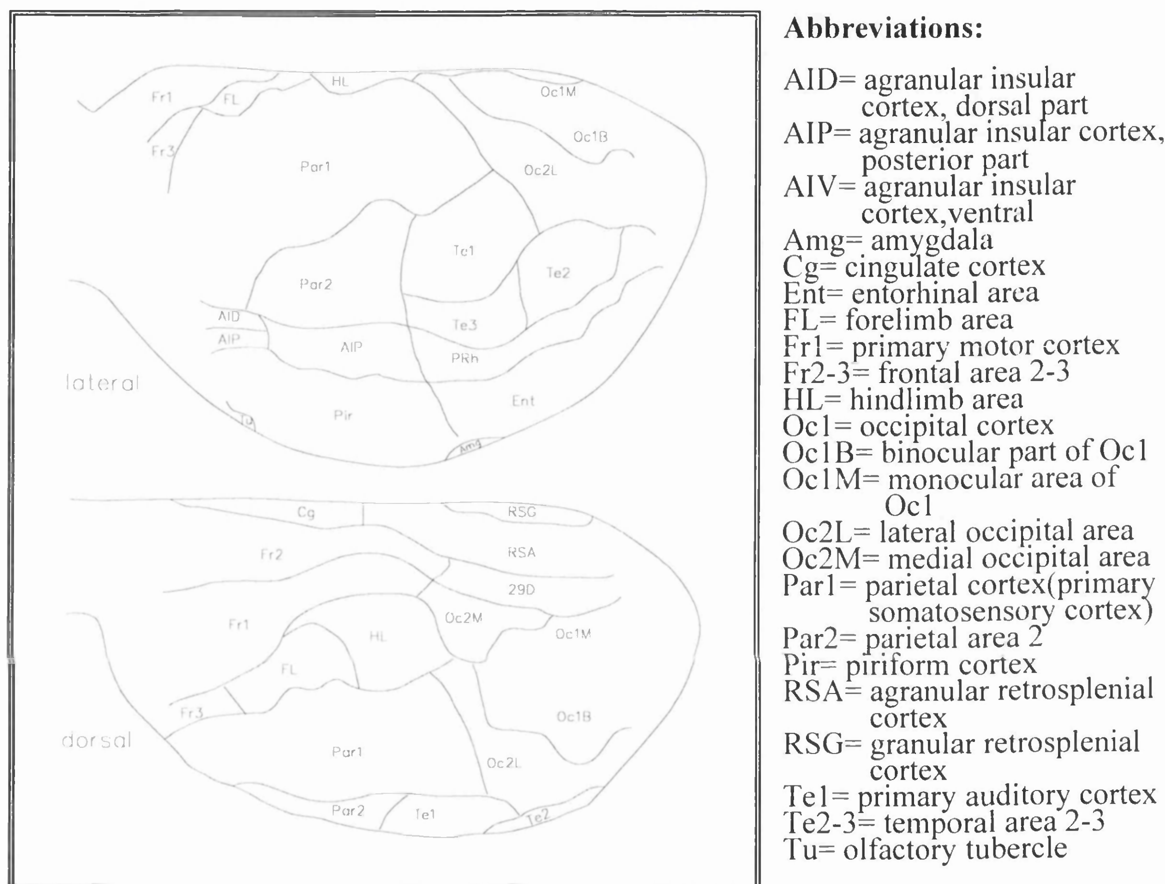
2.1.4. Somatosensory cortex

The final station for the afferent information from the whisker follicle is the somatosensory cortex.

Rat somatosensory cortex consists of primary (SI) and secondary (SII) somatosensory areas, named Par 1 and Par 2 in the map of the rat cortex by Zilles and Wree (1985). This map was made using computerised image analyser in an attempt to define the

boundaries of different areas on the basis of objective cytoarchitectonic criteria. Two other areas, representing front paw and leg also belong to the primary somatosensory cortex. In Zilles and Wree's map these areas are named FL for the forelimb and HL for the hindlimb.

Figure 5: Cytoarchitectonic division of the rat cortex as seen on lateral and dorsal view of the left hemisphere (Zilles and Wree, 1985; Zilles, 1985).



Abbreviations:

AID= agranular insular cortex, dorsal part
AIP= agranular insular cortex, posterior part
AIV= agranular insular cortex,ventral
Amg= amygdala
Cg= cingulate cortex
Ent= entorhinal area
FL= forelimb area
Fr1= primary motor cortex
Fr2-3= frontal area 2-3
HL= hindlimb area
Oc1= occipital cortex
Oc1B= binocular part of Oc1
Oc1M= monocular area of Oc1
Oc2L= lateral occipital area
Oc2M= medial occipital area
Par1= parietal cortex(primary somatosensory cortex)
Par2= parietal area 2
Pir= piriform cortex
RSA= agranular retrosplenial cortex
RSG= granular retrosplenial cortex
Te1= primary auditory cortex
Te2-3= temporal area 2-3
Tu= olfactory tubercle

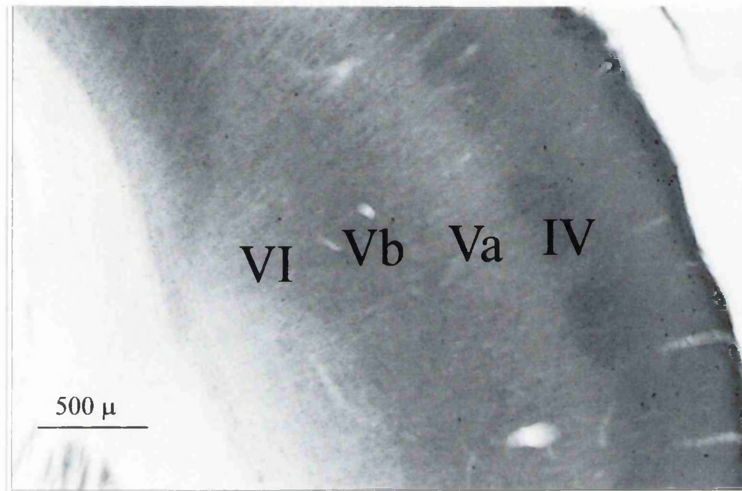
This map is widely used today, but somehow terms SI and SmI seem to be used more than Par 1. In this thesis I use older terms, SI for the primary and SII for the secondary somatosensory cortex.

Like all mammalian cortex, somatosensory cortex in rats is laminated. Borders between some of the laminae, like that between layers III and IV, are easy to recognise. Mostly, though, the changes from one layer to the next are gradual and not sharp. Cortical layers in different cortical areas vary in thickness. For instance, primary somatosensory cortex is characterised by thick layer IV, whereas in motor cortex this layer is much less developed.

Convention today is to divide cortex into 6 layers. In a cytochrome oxidase processed coronal section of a rat brain the most noticeable feature is a dark staining layer IV or granular layer, containing stellate cells. Below it is layer V whose sublamina Va is slightly paler than the sublamina Vb. Va and Vb laminae both contain large pyramidal cells.

The borders between other layers are not as sharp.

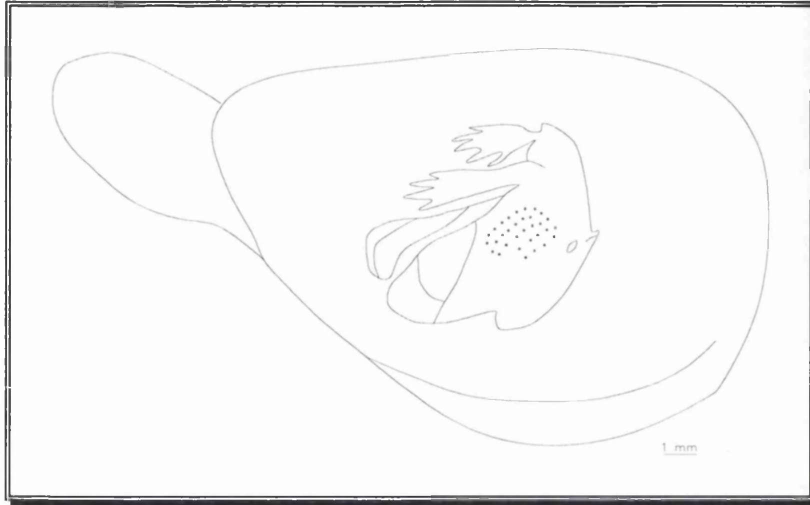
Figure 6: A photomicrograph of a coronal section through a rat brain, processed for cytochrome oxidase.



Rose (1912) and Droogleever Fortuyn (1914) described a sensory cortical area in rat that is characterised by its cell rich layer IV although at the time it was not possible to prove the sensory nature of this area. Woolsey (1958) mapped the topographic organisation of the somatosensory and motor cortex in several mammals (rat, rabbit, cat and monkey) using gross evoked potentials. He found that the somatosensory maps of the body in all the species studied have a similar orientation. The animal's head is placed ventrolaterally on the cortex, facing rostrally, and the hindlimbs are placed dorsomedially. Thus, the representation of a rat's body in the somatosensory cortex is inverted, the top of the head being represented at the lateral end and the hindlimb and tail at the medial end of the area. Distal parts of the paws and lower jaw are represented anterior to more proximal parts of the body. The striking feature of this

body map is the greatly augmented area representing face and whiskers in particular (see figure 7).

Figure 7: The whole body representation of the rat in the left primary somatosensory cortex (adapted from C. Welker, 1976).



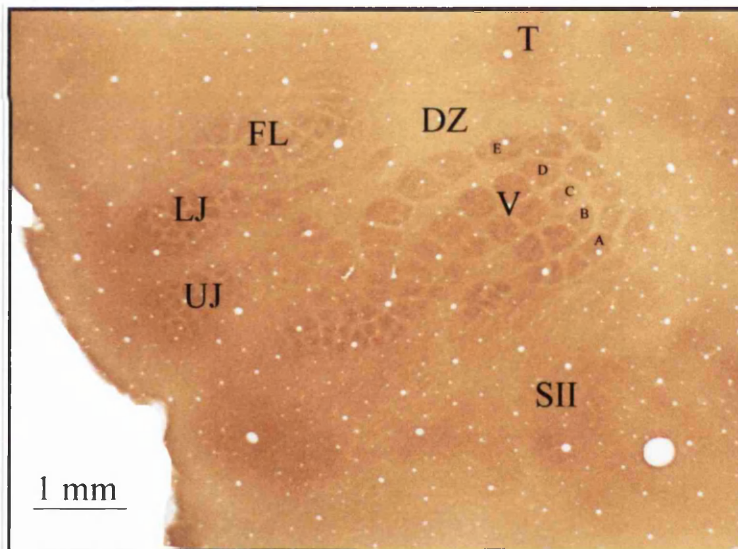
Primary somatosensory cortex (SI) in a rat is dominated by the presence of cell aggregates in layer IV which were first described in detail by Woolsey (1967) and Woolsey and Van der Loos (1970). They noticed darker staining structures in an area of a mouse cortex that receives its input from the face. The authors called them "barrels" due to their appearance in coronal sections. Each barrel consists of a barrel "side" and a "hollow" centre. Cells in the barrel "side" are densely packed compared to the barrel "hollow". Barrels are separated by relatively cell sparse zones, called "septa". Barrels in rats differ from those in mice because their centres are not cell sparse. Some authors prefer to call them aggregates (Chapin and Lin, 1990) although the functional properties of both structures are similar.

C. Welker (1971) counted 220 of these aggregates in the cortex of albino rat, which corresponded to the number of tactile hairs counted on the contralateral side of the face. Most rodents have barrels. They are also found in other species like Australian opossum. Barrels in rat cortex are first apparent on the 3rd day after birth in material stained with succinic dehydrogenase (Killackey and Belford, 1979). By day 4 all normal cortices have formed barrels. They are also present in the grossly abnormal brains of reeler and microcephalic mice (Welt and Steindler, 1977).

Primary somatosensory cortex in rats is a heterogeneous structure (Chapin and Lin, 1984). Barrels form a densely stained *granular zone* which is surrounded by a thin 100-300 μm strip of lighter staining tissue referred to as *perigranular zone*. A *Dysgranular zone*, 1-2 mm wide area in the centre of SI, separates the head and body representation. Another dysgranular area is present between SI and SII (Chapin and Lin, 1990). There is also a *transitional zone* which is located between the somatosensory and motor cortical areas. These zones can be defined not only on the basis of histological appearance, but also on differences in neural connectivity and electrophysiological properties.

Another feature of the rat primary somatosensory cortex is that a small part of it functionally overlaps with motor cortex. This particular area contains representations of fore- and hindlimb (Sapienza et al., 1981; Sanderson et al., 1984) and receives input from VPL and VL thalamic nuclei (Donoghue et al., 1979). This functional overlap is complete in the hindlimb area, but only partial in the forelimb region.

Figure 8: Photomicrograph of the cytochrome oxidase stained section of left cerebral hemisphere.



Abbreviations: V= vibrissae; FL= forelimb; LJ= lower jaw; UJ= upper jaw; T= trunk; DZ= dysgranular zone; A,B,C,D,E = rows of large whisker barrels; SII= secondary somatosensory area.

Figure 8 illustrates the precise somatotopy by which neighbouring points on the periphery are represented on neighbouring points on the cortex. This is particularly clear in the representation of whiskers and digits. In contrast to this precise somatotopy, common fur on the face, including fur amongst the large whiskers, has completely separate representation. Pidoux et al. (1979) found the common fur representation in an area they thought to be outside the barrel cortex. In fact, that area is part of the barrel cortex and represents small vibrissae. The discrepancy arises from the definition of the "barrel cortex". Pidoux et al. apply this term only to the largest cortical barrels representing the largest mystacial vibrissae.

Nussbaumer and Van der Loos (1985) found that two strips of cortex in mouse, bordering dorsal and ventral row of large vibrissal barrels, are responsive to manipulation of the common fur amongst the whiskers. The ventral part of the furry skin is represented in the strip of cortex just anterior to row E. The dorsal part of it is represented in an area just posterior to row A. The more rostral, smaller barrels respond not only to the stimulation of the corresponding vibrissa but also to the stimulation of the skin surrounding it. Even the large barrels, representing facial whiskers with order number of 5 or more (located further lateral and anterior), responded to stimulation of the common fur surrounding the isomorphic vibrissa.

Welker (1971) did not find the representation of the common fur in rats, perhaps due to the depth of the barbiturate anaesthesia.

2.1.4.1. Postero medial barrel subfield

The area representing the facial whiskers in the primary somatosensory cortex is greatly enlarged when compared to the other parts of the body. C. Welker (1971) found that the whisker representation occupies about 20% of the primary somatosensory cortex. The large caudal whiskers have larger cortical representation than the smaller, more rostrally placed ones. This increase reflects the differences in the density of innervation: follicles of the larger whiskers are more densely innervated (Lee and Woolsey, 1975).

Postero medial barrel subfield (PMBSF) is a distinct part of the barrel cortex which contains the largest barrels. These barrels are arranged in an array of 5-7 rows (A-E) with up to 8 barrels in each row. There are also 4 large, caudal barrels positioned between rows (α–δ, Woolsey and Van der Loos, 1970), which correspond to four

caudal, straddling whiskers. This arrangement is an exact replication of the peripheral organisation of the mystacial vibrissae. C. Welker (1971) recorded from cells in layer IV under deep barbiturate anaesthesia. She found that each of the barrels within this area responds to mechanical manipulation of a single whisker on the contralateral side of the face.

Simons (1978) showed that most cells (about 85%) in layer IV respond to movement of only a single (principal) whisker. Cells in layers above and below layer IV have receptive fields that are responsive to movement of principal and several adjacent whiskers (Armstrong-James and Fox, 1987).

Durham and Woolsey (1977) in a 2-DG study showed increased activity in all cortical layers associated with a single intact whisker. Barrels thus resemble the functional cortical columns, as demonstrated by Mountcastle for somatosensory cortex in cats (1957) and by Hubel and Wiesel for visual cortex in monkeys (1968). Barrels in layer IV can be, therefore, considered as anatomical correlates of functional cortical columns (Woolsey and Van der Loos, 1970).

Cytoarchitectonic differences between parts of SI are reflected in the response properties of their cells. Under barbiturate anaethesia neurones in the barrel or granular zones in the cortical representation of the vibrissae respond to light mechanical stimulation of contralateral whiskers (Welker, 1971; Chapin and Lin, 1984). Neurones in the interbarrel (septal) areas respond to high intensity multiwhisker manipulation. Neurones in the large dysgranular zone (separating whisker and hand regions of the SI) are not responsive (Welker et al., 1984; Chapin and Lin ,1984). This issue will be further addressed in the discussion part of the first experimental chapter.

The receptive fields of most cells in barrel cortex incorporate several whiskers, but there is always a dominant input from a single whisker (Simons, 1978). Armstrong-James and Fox (1987) defined this dominant input as a centre receptive field whisker (CRF). Other whiskers contributing to the formation of a receptive field were defined as surround receptive field (SRF) whiskers. The influence of the surround whiskers is excitatory. Short latency responses to peripheral stimulation in barrels are always associated with the CRF input.

2.1.4.2. Supplementary somatosensory cortex (SII)

The supplementary somatosensory cortex (SII) of the rat also contains a body map and is situated at the posterolateral border of the SI (Welker and Sinha, 1972). This region was much less studied, but it is known that the face area is a mirror image of the face representation in SI and that the two face representations are spatially close to each other. In mice, the face is represented most anteriorly and the hindlimb and tail most posteriorly. The representations of paws and mystacial vibrissae are particularly large (Carvell and Simons, 1986). The receptive fields of cells within SII are larger than the receptive fields within the same body representations in SI. Whisker sensitive cells respond usually to several adjacent whiskers. In the trunk and limb areas the cells frequently respond to auditory as well as somatosensory stimuli (Carvell and Simons, 1986, 1987).

Cytoarchitectonically, SII is more uniform in appearance than SI, because there are no barrels in layer IV.

2.1.5. Barrel cortex Afferents

Thalamic afferents

As was mentioned earlier, two thalamic nuclei provide input to the barrel cortex: ventral posterior medial (VPM) and the rostral part of the posterior nucleus (Pom). Input from the VPM is restricted to the barrel area only, whereas Pom projects to barrel and interbarrel area of the barrel cortex.

A recent study by Land et al. (1995) examined in detail the precision of the relationship between barrels and barreloids. In this study authors used retrogradely transported HRP to find cells of origin of thalamic terminals in a particular barrel. They found that majority of cells giving rise to terminals in a given barrel comes from the isomorphic barreloid. A few cells in the neighbouring barreloid (usually in the same row) may participate in this projection.

Terminal boutons from VPM cells are grouped and fill the centres of individual barrels in layer IV. Some VPM terminals are also found in the upper layer VI, lower layer V and supragranular layers (Lu and Lin, 1993).

Pom projects to the barrel area of SI in a more diffuse fashion. Terminal boutons are found mostly in upper layer V and in layer I. The main target of the Pom fibres is the

interbarrel area of SI. The greatest density of the terminal boutons in the interbarrel area is in layer IV and lower layer III, but there are also terminals in upper layer V and layer I (Lu and Lin, 1993).

These two nuclei provide the cortex with different kind of somatosensory information because they receive different afferent input. The trigeminal complex projects to both VPM and Pom. The cells of origin are largely segregated within the trigeminal complex and only a few cells sends collaterals to both nuclei (Chiaia et al., 1991). Pom may also receive an indirect somatosensory input from the deep layers of the ipsilateral superior colliculus (Roger and Cadusseau, 1984) which in turn receives projections from the contralateral trigeminal complex (Rhoades, 1989). Cells in VPM and Pom have different response properties. VPM cells respond to a light mechanical stimulation of contralateral whiskers. The responses are most vigorous to stimulation of a principal whisker and somewhat weaker to manipulation of the surrounding whiskers (Armstrong-James and Callahan, 1991; Diamond et al., 1992 a,b).

Cells in the Pom respond to more intense multiwhisker stimulation (Belford et al., 1987), although single Pom neurones can also respond to the light mechanical stimulation (Chiaia, 1991b; Diamond, 1992a,b). The receptive fields of cells in the Pom are much larger than the receptive fields of the VPM cells.

Callosal input

Primary somatosensory cortex receives callosal afferents from the homotopic contralateral cortex. This input is restricted to the dysgranular zones of the SI. Barrels (granular areas) do not receive or send any callosal fibres (Wise and Jones, 1976; Akers and Killackey, 1978).

Callosal fibres enter SI in bundles. They ascend from the white matter up to layer I where they ramify and spread out, sometimes covering neighbouring "barrel column". The majority of callosal terminals are found in layers III and V, although other layers also receive callosal input (I, II and VI). Terminals in layer IV are markedly absent. The cells of origin of callosal projections are principally from layers III and V of homotopic contralateral cortex, although a few cells in layers II, IV and VI contribute to this projection as well (Wise and Jones, 1977 a).

Ipsilateral cortical afferents

Primary somatosensory cortex does not receive input from other ipsilateral cortical areas apart from the projections originating in layer IV cells of the granular (barrel) areas which terminate in the surrounding interbarrel septa (Hoeflinger et al., 1995).

2.1.6. Barrel Cortex Efferents

Corticocortical pathways

i) Ipsilateral hemisphere

Several areas within the ipsilateral hemisphere receive projections from the parietal cortex (Akers and Killackey, 1978). These are: SII, motor cortex, the zone that is intermediate between granular cortex and SII, anteromedial border of granular cortex and an area posterior and medial to granular cortex. The cells of origin of these projections are in the dysgranular area.

Cells in layer IV of granular cortex project ipsilaterally to the surrounding septal area (Hoeflinger et al., 1995).

ii) Contralateral hemisphere

Granular cortex does not project to the contralateral hemisphere. Dysgranular cortex, though, projects to the homotopic area of the contralateral primary somatosensory cortex and to SII (Akers and Killackey, 1978). The cells of origin of these projections are mainly in layers III and V, although other layers are also involved (Wise and Jones, 1976). The projections to the contralateral hemisphere arise from a distinct group of layer V cells. They are not collaterals of the corticospinal neurones (Catsman-Berevoets et al., 1980). Collaterals of corticospinal neurones are distributed only within the same hemisphere and to various subcortical structures (Catsman-Berevoets and Kuypers, 1981).

Corticothalamic pathway

Each cortical area projects reciprocally to every thalamic nucleus from which it receives afferents (Jones, 1985). Rat barrel cortex, therefore, should have projections to VPM, Pom, reticular and central lateral nucleus. This distribution of corticothalamic

terminals was recently confirmed by Bourassa et al. (1995). Small amounts of biocytin were injected into layers V or VI of the barrel cortex and projections of single corticothalamic fibres were reconstructed. It was found that the majority of cells in the upper layer VI project to VPM and that their terminal arbours form rostro-caudally oriented bands. Cells in the lower layer VI project predominanly to Pom but they also send collaterals to VPM. Cells in layer V send their projections to Pom or the central lateral nucleus and these projections arise as collaterals of the main axons which travel towards the brainstem. Reticular thalamic nucleus receives collaterals from corticothalamic fibres originating in layer VI. Since Bourassa et al.'s study did not relate injection sites to the barrel structure of the cortex it is not possible to conclude whether there are differences in thalamic projections between dysgranular and granular zones.

Good and Killackey (1991) used fluorescent microspheres to map retrogradelly labelled cortical cells following injections in VPM and Pom. They found that cells in lower layer VI project only to Pom and there were very few double labelled cells. It is possible that the smaller collateral fibres do not incorporate or transport fluorescent spheres as well as the larger diameter fibres. These authors, however, suggest that the septal layer VI cells project to both VPM and Pom, whereas barrel layer VI cells project to VPM only.

The relationship between cortical barrels and thalamic barreloids is precise. Hoogland, Welker and Van der Loos (1987) used Phaseolus and HRP as tracers and showed that efferents from a given barrel column in mice form rod-like termination sites in the ventrobasal complex. Projections appear to be topographycally organised so that the more medial rows of barrel columns project to more ventral regions of the ventrobasal complex. Furthermore, each barrel projects to a corresponding barreloid in the VPM but also to the barreloids belonging to the same arc (barreloids from each row with the same order number: i.e. A1, B1, C1, etc.). When HRP is injected into a single cortical barrel, retrogradely labelled cells in the VB thalamus appear to outline the shape of the barreloid. Hoogland et al. (1987) concluded that the hollow of one barrel receives projections only from its isomorphic barreloid.

Results of a similar study in rats are somewhat different. Land et al. (1995) employed HRP as a tracer and used sections stained with cytochrome oxidase to identify the barreloids in the thalamus. Their plane of sectioning is parallel to the medial border of

the VPM and rows of barreloids seem to be easily identified. When HRP was injected in lamina IV of the barrel cortex and the injection site was restricted to the centre of the barrel, majority of retrogradely labelled cells (95%) was found within the corresponding barreloid. The densest orthograde axonal terminals from the same injection site appeared in the close proximity to the retrogradely labelled cells. These results led the authors to conclude that thalamocortical and corticothalamic connections in the rat vibrissal system are highly reciprocal.

Corticotectal pathway

The superior colliculus is a midbrain structure which co-ordinates sensory information so that eyes and head of the animal can be accurately turned towards the stimulus. It contains visual, somatosensory, auditory and motor map which are all in spatial register (Dräger and Hubel, 1976). This projection is the subject of the second experimental chapter in this thesis. Here I briefly state that there is a substantial projection from the primary somatosensory cortex of a rat to the intermediate and deep layers of the superior colliculus (Wise and Jones 1977 b; Killackey and Erzurumlu, 1981; Killackey et al., 1989). This projection is organised in patches which are aligned rostrocaudally thus forming columns. Electrophysiological studies have shown that there is a precise somatotopy in projections from SI to superior colliculus (Kassel, 1982). For example, projections from the forelimb region of the SI terminate in the areas of SC which are responsive to peripheral stimulation of the forelimb. Receptive fields of collicular cells are larger than receptive fields of the cortical cells, so projections from a number of cortical sites will converge on a single collicular site.

The areas of representation of the whiskers in the intermediate and deep layers of the superior colliculus is relatively larger than that of the rest of the body (Dräger and Hubel, 1976). Especially large are representations of the whiskers that cross the visual field.

Corticospinal pathway

There is a striking somatotopy in the projections from the SI cortex to the spinal cord and trigeminal complex in rats (Wise et al., 1979). Hindlimb representation of the SI projects to the lumbar segment of the spinal cord. Forelimb area projects to the cervical

enlargement of the spinal cord. Neck and posterior head representation project to the rostral cervical spinal cord. Head and face region of the SI project to the medullary trigeminal complex.

Cells of origin of the corticospinal tract are found in the deep lamina V (Vb).

Corticopontine pathway

This is one of the major pathways in the mammalian brain. Projections from the somatosensory cortex to the pontine nuclei is central to this thesis and will be described in detail in the first experimental chapter.

2.2. Descending Pathway: From the Barrel Cortex to the Cerebellum

Pontine nuclei and the cerebellum are two focal structures in this thesis. In this section I shall describe their anatomical and physiological properties as a final introduction to the experimental chapters.

2.2.1. Pontine nuclei

Pons was first described by the XVI century Italian anatomist Constanzo Varolio (1591) who dissected the brain from below. He thought that the great fibre bundle overlying the protuberance resembled a bridge, with the brainstem flowing underneath it. In mammals with large cerebral cortex the pons and the cerebellum are large as well. Matano et al. (1985) showed that ventral pons makes about 37% of the volume of the brainstem in man, 21% in Old World monkeys and up to 6% in prosimians.
Pontine nuclei receive projections mainly from the cortex, although there are some subcortical and spinal sources of pontine afferents. Pontine nuclei send their projections exclusively to the cerebellum. In most mammals the majority of the mossy fibre input to the cerebellum originates in the pons. It is therefore important to understand the principles of organisation of this structure in order to understand how the cerebellum works. So far we have not been able to say how the information reaching pons differs from the information leaving it. Numerous sources of afferents and their possible convergence suggest that pontine nuclei may be an integrative centre, which provides the cerebellum with important information for sensory guidance of movement (Stein and Glickstein, 1992).

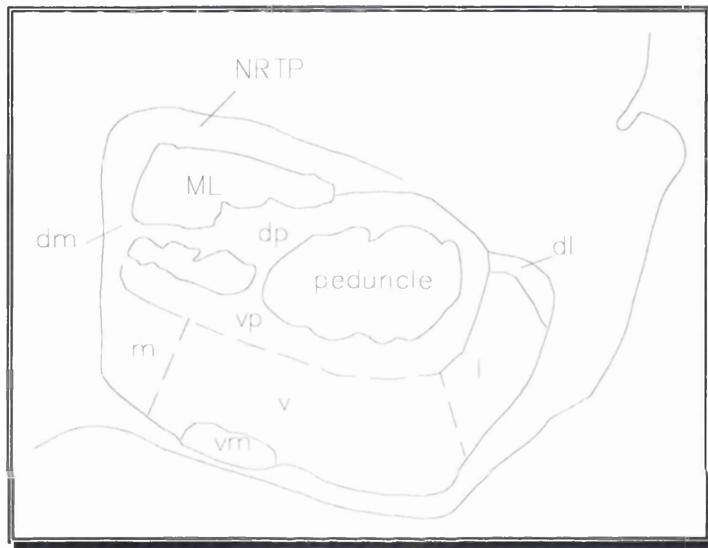
Pons - cytoarchitecture

Several studies addressed the question of the organisation of the pontine nuclei in various species (Brodal and Jansen, 1946; King et al., 1968; Mihailoff and King, 1975; Cooper and Fox, 1976; Mihailoff et al., 1981 b).
These studies employed the conventional Nissl and Golgi methods and are in general agreement regarding division of the pontine nuclei into 4 principal groups: medial, ventral, lateral and peduncular. Several smaller subnuclei have also been identified: intrapeduncular, median, dorsomedial and dorsolateral nuclei. These divisions are

useful only for descriptive purposes because there are no real cytoarchitectonic boundaries between them. The nomenclature was devised by Brodal and Jansen (1946) in the study of the cat pons. They named the nuclei according to their position relative to bundles of corticofugal axons passing through the pons.

Mihailoff and colleagues published two studies on the cytoarchitecture, cytology and synaptic organisation of the pontine nuclei in the rat. In the first study they used Nissl and Golgi stained tissue (Mihaillof, McArdle and Adams, 1981). A subsequent study was done at the electron microscope level (Mihailoff and McArdle, 1981).

Figure 9: A diagram of a section through the rat pons showing nuclear subdivisions



Abbreviations:

dl = dorsolateral
dm = dorsomedial
dp = dorsal peduncular
l = lateral
m = medial
ML = medial lemniscus
NRTP= Nucleus Reticularis Tegmenti Pontis
v = ventral
vm = ventromedial
vp = ventral peduncular

In this thesis I further subdivided the ventral peduncular nucleus into a lateral, central and medial portion in order to aid more accurate description of terminal projections from the barrel field.

The pontine nuclei in rats stretch from the interpeduncular nucleus at the rostral end to the trapezoid body at the caudal end. Dorsally, the pontine nuclei are bordered by descending fibres of the cerebral peduncle and ascending fibres of the medial lemniscus.

Ventrally, at caudal levels of the pons, the nuclei are bordered by the fibres of brachium pontis.

There are two general categories of neurones in the pontine nuclei as described by Cajal (1909), neurones with long axons and neurones with short axons. The first class

represents the *projection* neurones which provide the mossy fibre input to the cerebellum. The other is made of *intrinsic* neurones whose axons terminate within pontine grey (opossum- Mihailoff and King, 1975; monkey- Cooper and Fox, 1976). These observations were made on the basis of examination of Golgi stained sections of adult and embryonic tissue. According to the literature, the number of the intrinsic neurones in rat, cat and monkey is small but it seems to rise higher up the phylogenetic scale (Brodal and Bjaalie, 1992).

Projection neurones in rats (Mihailoff et al.,1981 b) appear in Golgi preparation as a varied population of neurones. They have round, oval or spindle shaped bodies and range in size from 8 - 20 μ. There are usually four to seven dendrites that come out of the cell body. One of the important features of the pontine neurones is that the dendrites of a single cell can cover terminal fields of several pontine afferent systems (Mihailoff et al., 1981 b).

Intrinsic cells in the rat are represented by small neurones with spherical or multiangular body with only 2-4 main stem dendrites. These dendrites are very thin and tortuous. Axons of these cells give rise to small plexus of thin beaded branches in the vicinity of the cell of origin.

Border and Mihailoff (1985) showed that some small neurones in rat pons stain positively for the enzyme glutamic acid decarboxylase and thus demonstrated that a small population of pontine cells are indeed interneurones. A similar finding was reported in cats and monkeys (Brodal et al., 1988).

Pons- physiology

Relatively little work has been done on the electrophysiological properties of pontine neurones. In cat, Rüegg and Wiesendanger (1975) found that neurones sensitive to electrical stimulation of the sensorimotor cortex were also responsive to stimulation of the pericruciate and supplementary somatosensory cortex. In contrast, Baker et al. (1976, 1983) described cells in the cat pons which were selectively activated by only one form of sensory stimuli. Cells activated by a visual, auditory or tactile stimulus were found in distinct clusters and not one cells responded to more than a single sensory stimulus.

In rats (Potter, Rüegg and Wiesendanger, 1978), about 55% of pontine neurones were sensitive to stimulation of a single cortical site only, but the rest received converging inputs from two or more cortical areas. The greatest input to the pontine nuclei seems to be from the primary somatosensory cortex. Neurones responsive to stimulation of the somatosensory cortex, for example, were not always found within anatomically defined corticopontine projection zones.

The difference in electrophysiological results may arise from different stimulating parameters and type of anaesthesia. Another reason may be that the investigators did not record from the same or comparable pontine nuclei. Convergence of input that some researchers record may be due not only to the overlapping corticopontine termination zones, but also to the fact that dendrites of some neurones extend into territories of neighbouring afferent systems (Mihailoff et al., 1981b).

Receptive fields of pontine visual cells in cats are large, sometimes encompassing a complete hemifield (Baker et al., 1976; Glickstein and Gibson, 1976). Majority of visual cortical input to pons in cats originates in areas 18 (Glickstein et al., 1972) and 19 (Brodal 1972), but there is also a small projection from the area 17. Since the receptive fields of these visual cells are much smaller, Baker et al. (1976) suggested that a number of cortical visual cells converge onto the same pontine cell and so produce much larger receptive field. Nearly all visual pontine cells studied were sensitive to movement of the target in a particular direction, but the exact speed of movement was not so important. Unlike cortical visual cell, pontine cells were not sensitive to stimuli like bars and edges. They were much more responsive to multiple dots on the screen.

Pontine Afferents

a) Corticopontine projections

The great majority of pontine afferents arise in layer Vb cells of the neocortex (Wise and Jones, 1977a).

In rats all parts of cortex project to the pontine nuclei (Legg et al., 1989; Wiesendanger and Wiesendanger, 1982 a). The largest input comes from somatosensory and visual cortices (Wiesendanger and Wiesendanger, 1982 a,b; Mihailoff et al., 1978; Legg et al., 1989).

In primates, large injections of the WGA-HRP in the pons label neurones primarily in the premotor, supplementary motor, primary motor and somatosensory areas, the posterior parietal cortex, parts of the extrastriate visual areas and in the cingulate gyrus (Glickstein et al., 1985). Striate cortex in monkeys appears not to send its projections to the pontine nuclei (Glickstein et al., 1980, 1985). This is in contrast to the findings of similar experiments in rat (Legg et al., 1989; Wiesendanger and Wiesendanger, 1982 a,b), cat (Bjaalie and Brodal, 1983; Brodal, 1968) and rabbit (Wells et al., 1989). Projections from cortex are precisely organised, but quite divergent. The smallest lesions or injections of tracers in cortex appear as multiple, well defined *patches* of terminal labelling in various parts of the pontine nuclei. These patches are described as columns, slabs or lamellae. They are most prominent in monkey and less so in the cat (Brodal and Bjaalie, 1992).

There is also a degree of topography in the corticopontine projection. Major divisions of the cortex project to segregated parts of the pontine nuclei in rat (Wiesendanger & Wiesendanger, 1982 a,b), rabbit (Abdel-Kader, 1968), cat (Brodal, 1968) and monkey (Brodal, 1978 a,b; Dhanarajan et al., 1977; May and Andersen, 1986; Schmahman & Pandya, 1989). Generally, more rostral areas of cerebral cortex project more medially within pons; the more caudal ones project to the more lateral zones within pontine nuclei.

In rats, projections from the motor cortex are found mainly in the medial portions of the pontine nuclei. Those originating in the visual cortex terminate more laterally in the pons and projections from the somatosensory cortex are found centrally (Mihailoff et al., 1985; Wiesendanger and Wiesendanger, 1982 a,b). Furthermore, cytoarchitectonically or functionally defined cortical subregions project to different areas within pontine nuclei in a topographic manner, with a degree of overlap. Mihailoff et al. (1978) studied projections from the forelimb (FL) and hindlimb (HL) area of the primary somatosensory cortex to the pontine nuclei in rats. They found that the FL area projects to the rostral end of the pons and that the terminals form patches of label in 5 longitudinally oriented columns. These patches were mainly in the ventral, ventromedial and ventrolateral pontine nuclei. The HL area projects to the same nuclei in 5 rostrocaudally oriented columns. The only difference is that the HL area projects further caudally.

Physiological studies confirm a strong input to pons from somatosensory,visual and auditory regions of cortex. Single unit recording studies in cats (Allen, Korn and Oshima, 1969) found that pontine cells can be activated at a short latency by stimulating sensorimotor cortex (1.7 -5.2 ms) and cerebral peduncle (1.0 - 2.4 ms). These results, together with the fact that there is a negligible number of inhibitory neurones, suggest that the pontine neurones should be able to transmit high-frequency information from the cerebral cortex to the cerebellum (Alen and Tsukahara, 1974).

b) Subcortical afferents to the pontine nuclei

Input to the pontine nuclei from sources other than neocortex is quantitatively much smaller. In cat this input probably represents only 10% or less of the total projections that pons receives. The input from subcortical structures is somewhat more substantial in rats. Subcortical pontine afferents arise mainly from visual structures (superior colliculus, pretectal nuclei and ventral lateral geniculate), somatosensory relay nuclei (dorsal column nuclei and trigeminal nucleus), hypothalamus and deep cerebellar nuclei. (Mihailoff et al., 1989). The topography of some but not all of these projections has been described.

Subcortical sources of pontine afferents in cats were divided in two groups (Aas, 1989). One group of nuclei sends diffuse and widespread projections to the pontine nuclei. Structures in this group include the reticular formation, the raphe nuclei, the locus coeruleus and the periaqueductal grey.

The other group of subcortical nuclei projects to pons in a topographic manner. Visual layers of the superior colliculus, for example, project to a restricted region in the ipsilateral dorsolateral pons of a rat (Redgrave et al., 1987), cat (Kawamura and Brodal, 1973) and monkey (Harting, 1977). Deep layers of superior colliculus in rats project to caudal pons, its peduncular and lateral nuclei (Burne et al., 1981). This projection is topographically organised, so that medial superior colliculus projects mainly to the peduncular areas, whereas lateral part of the superior colliculus projects to the lateral pontine nucleus. Deep cerebellar nuclei, dorsal column nuclei, sensory trigeminal nuclei and the spinal cord also project to restricted, largely segregated regions within pons.

Deep cerebellar nuclei in rats project to the contralateral pons via the superior cerebellar peduncle. The main input is derived from cells in the lateral (dentate) nucleus whose

axon terminals form three longitudinal columns. Caudal part of the dentate nucleus projects to rostral parts of the pontine nuclei and rostral parts of the dentate project to more caudal pontine regions (Angaut et al., 1985). Projections from the interposed nucleus are largely restricted to the ventral peduncular pontine nucleus (Watt and Mihailoff, 1983). The medial (fastigial) cerebellar nucleus provides very sparse input mainly to the dorsomedial pontine nucleus. Lee et al. (1989) demonstrated that pontine terminals arise as collaterals of the fibres travelling to the thalamus or the inferior olivary nuclei.

Input from the deep cerebellar nuclei partially overlap in some regions with terminals from the cerebral cortex (Lee and Mihailoff, 1990). The greatest overlap is found with projections from the motor face representation and forelimb somatosensory area. There was very little overlap between projections from the sensory face area and the deep cerebellar nuclei which was confined to the ventral pontine nucleus.

Recent electrophysiological study (Beretta et al., 1991) showed that the cells in the lateral cerebellar nucleus largely inhibit pontine cells, but just over 40% have an excitatory effect. The inhibitory crebellopontine projection may be a part of the feedback pathway which regulates the excitability of the pontine cells (Beretta et al., 1991).

Pontine Efferents

All projection neurones within pontine nuclei send their axons to the cerebellar cortex and some collateralise to the deep cerebellar nuclei in rats (Mihailoff, 1993, 1994). Pontocerebellar axons travel in the middle cerebellar peduncle, brachium pontis, and terminate largely in the granular cell layer of the contralateral cerebellar hemisphere as mossy fibres. There are also mossy fibre terminals in the posterior vermis and ipsilateral hemisphere.

Mihailoff et al. (1981 a) examined the pontocerebellar projection pattern in rats using WGA-HRP as a retrograde tracer. They looked at retrogradely labelled cells following injections into lobulus simplex, crus I and II and paramedian lobule. They found that rostral pons projects to the simple lobule, mostly ipsilaterally. The majority of retrogradely labelled cells were found in the ventral, dorsomedial, ventromedial and lateral pontine nuclei. Projections to Crus I originated from both sides of the pons, but the contralateral component was much stronger. Medial, ventral and lateral nuclei of

the pons provided the majority of this input. Projections to Crus II and paramedian lobule appear to originate in the same regions of the pontine nuclei, namely the peduncular and ventral pontine nuclei. The majority of cells were found in the contralateral pons, but ipsilateral nuclei contribute to this projection as well.

Another anatomical study based on the retrograde transport of WGA-HRP (Azizi et al., 1981) showed that projections to the posterior vermis in rats arise from both sides of the pons. There is some topography in arrangement of the pontocerebellar projection neurones, so that more rostral regions of pons provide input to more rostral lobules of the cerebellar vermis. Lobule VI receives projections from cells scattered throughout the rostrocaudal extent of the pons. Most of its input, though, is derived from the medial, lateral and ventral nuclei in the rostral pons. Lobule VII receives its projections from medial, lateral, ventral and dorsolateral nuclei in the middle of the rostrocaudal extent of pons.

Caudal pons, particularly its ventral regions, provide input to lobule VIII.

Cells in the peduncular, dorsolateral and dorsomedial nuclei in the middle and caudal pons provide input to vermal lobule IX.

Location of pontine cells projecting to either posterior vermis or the hemispheres appear to overlap, at least partially, with the pontine zones receiving cortical or subcortical inputs related to tactile, auditory and visual stimuli. It is, therefore, possible to say that Crus I, Crus II and paramedian lobules in rats receive information from the face sensorymotor cortex (Mihailoff et al., 1981 a). Similarly, vermal lobules VI and VII, receive input from those pontine nuclei which receive auditory input from the superior an inferior colliculi (Azizi et al., 1981). However, all the posterior vermal lobules may also receive some tactile inputs related to cortical face and limbs representations.

Small injections of anterograde tracers in the pons show that the mossy fibre terminals are arranged in parasagittal strips (Serapide et al., 1994). There were 5 strips in the vermal lobule VII and 3 strips in the paraflocculus following injections in the lateral, medial and ventral pontine nuclei. With larger injections this zonation is lost.

Caudal pons projects to lobule VIII. Lobule IX has a more diverse input from pons. Sublobules IXa and IXb receive input from medial and lateral nucleus in the rostral pons as well as peduncular and dorsolateral nuclei in the caudal pons. Sublobule IXc receives projections from dorsolateral and dorsomedial nuclei of the caudal pons.

2.2.2. The Cerebellum

The cerebellum's role in coordination of movement was first formulated by Flourens (1824). He noticed that the animals with cerebellar lesions can still move but their movements were not smooth and regular. Similar observations were made by Gordon Holmes (1939) who described in detail the deficits suffered by soldiers in the First World War. These men sustained gunshot injuries which affected the cerebellum. The major symptoms they had were atonia, ataxia, intention tremor and errors of rate, force and coordination of movement. Today, it is largely agreed that the cerebellum plays an important role in sensory guidance of movement.

In order to control or co-ordinate movement the cerebellum needs sensory information. Snider and Stowell (1944) were the first to show that the responses to tactile, auditory and visual stimuli could be evoked in cerebellar cortex in cats and monkeys. This sensory input to the cerebellum is provided by a number of structures - the spinal cord, vestibular and trigeminal nuclei, cerebral cortex and tectum. Cerebellar efferents are almost completely directed towards the structures controlling movement.

Gross anatomy of the rat cerebellum

Looking at the posterior surface of the rat cerebellum one can easily recognise the long, relatively straight chain of folds in the middle, called vermis, which is bordered on each side by a hemisphere (figure 10). The vermis of the anterior lobe is not as distinct from the hemispheres as in the posterior lobe. The transitional region between vermis and hemispheres is referred to as the intermediate cerebellum or pars intermedia.

The striking feature of the cerebellum is the number of fissures transversing its surface. Two major fissures divide cerebellum into lobes. The primary fissure divides the anterior from posterior lobe; posterolateral fissure separates the posterior from the flocculonodular lobe. Each of the lobes is further subdivided into lobules which themselves have subdivisions- folia (leaves, lat.). Larsell (1952) described the cerebellum in adult rats and his terminology is largely used today. He named vermal lobules by Roman numerals I-X (starting from the most anterior lobule) and hemispheric lobules, being simply vermal extensions, carry the same names, with H as a prefix to denote "hemispheric" (i.e. HVI). However, this terminology is not always strictly followed. Many of the names like *lobulus simplex*, *crusI* and *crus II*,

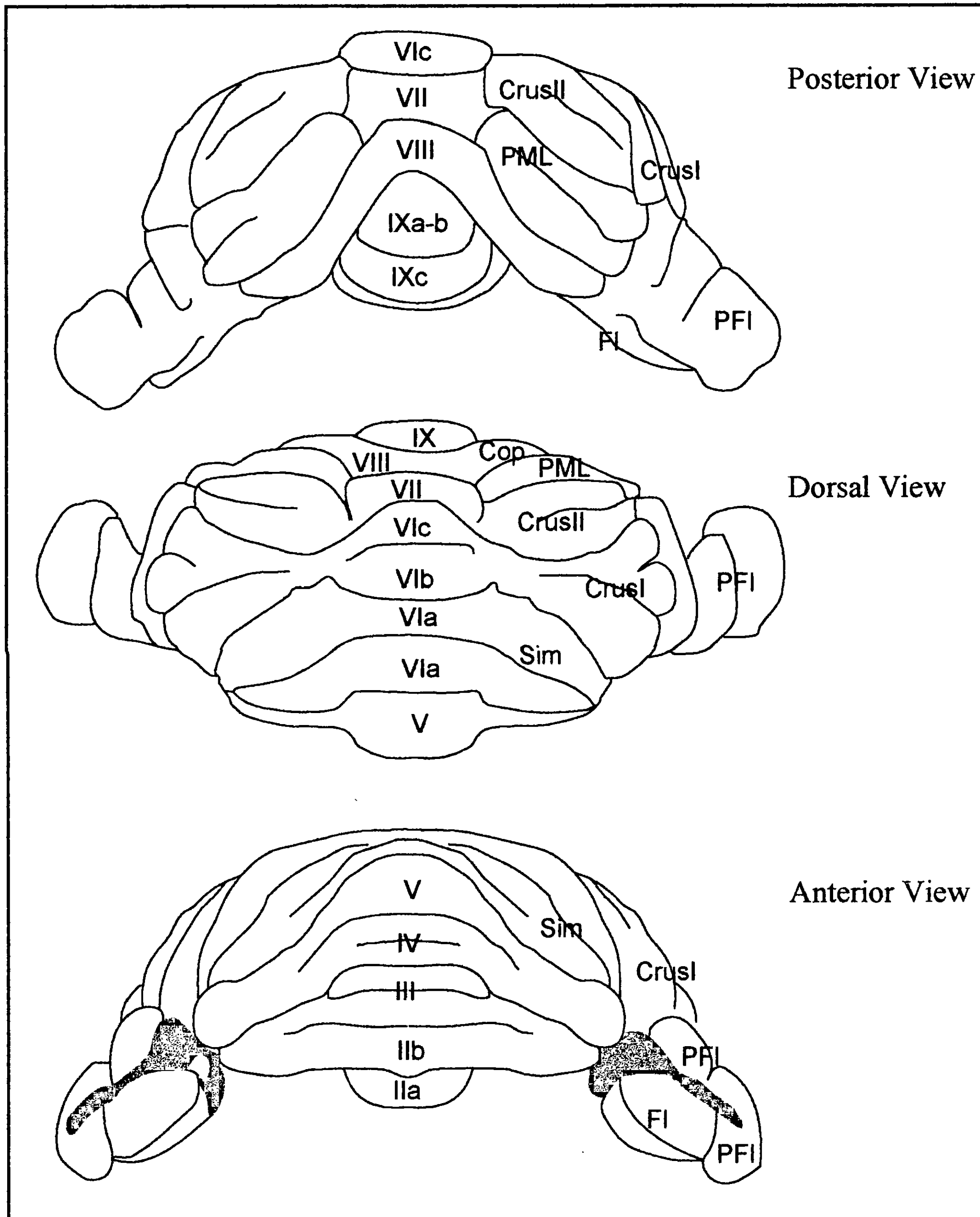
paramedian lobule were originally devised by Lodewijk Bolk (1906) and are still in use. Bolk showed that despite many convolutions, the cerebellar cortex forms a continuous sheet and that the principles of its organisation are the same in all mammals. First of all, Bolk showed that the vermal cortex forms an uninterrupted sheet from its rostral to its caudal end. This continuity follows in the medio-lateral direction, between vermis and hemispheres, in the anterior lobe and lobulus simplex of the posterior lobe. In the rest of the posterior lobe vermis and hemispheres are discontinuous in part, but the hemispheric lobules still run as a continuous chain of folds.

Continuity of the cerebellar lobules is best seen in the midsagittal section (see figure 11).

Like the cerebrum, the cerebellum is composed of cortex and white matter. Deep within the white matter, on each side of the midline, there are three groups of cells. They make up the deep cerebellar nuclei: medial (fastigial), interposed and lateral (dentate) nucleus (Korneliussen, 1968). The interposed nucleus can be further subdivided into anterior and posterior parts.

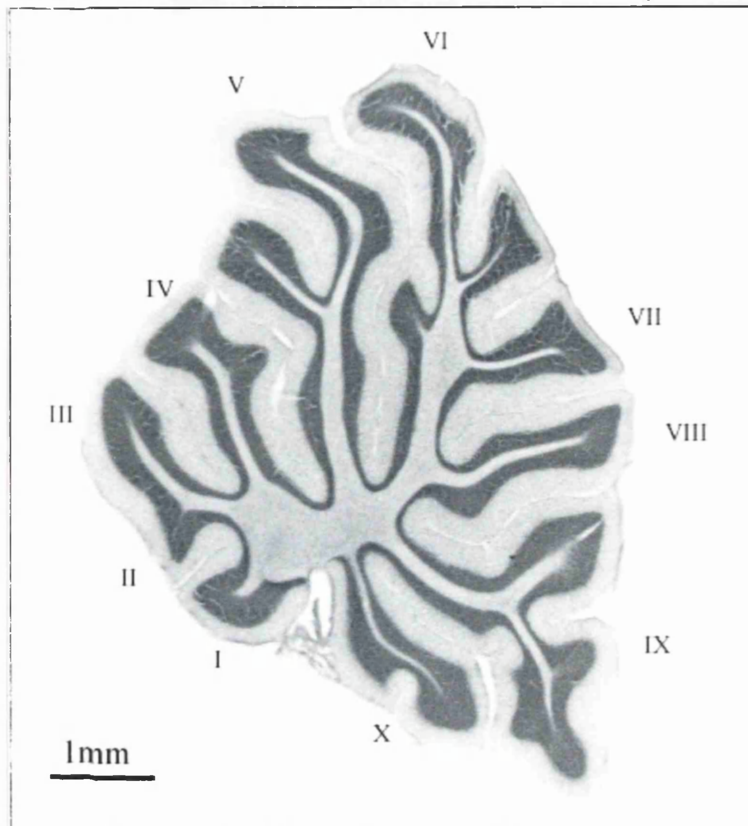
Information to and from the cerebellum travels in the three pairs of large fibre tracts or peduncles: superior (brachium conjunctivum), middle (brachium pontis) and inferior (restiform body) cerebellar peduncle. The middle cerebellar peduncle is the largest of the cerebellar peduncles. It contains mainly cerebellar afferents originating in the pontine nuclei. The inferior cerebellar peduncle consists of the afferent and some efferent fibres. The afferent component is made of spinocerebellar, trigeminocerebellar, cuneocerebellar, reticulocerebellar and olivocerebellar tracts. The efferent part of the inferior cerebellar peduncle carries efferent fibres from vermis and vestibulocerebellum on their way to the vestibular nuclei. Some fibres from the fastigial nucleus travel in this peduncle as well. The majority of efferent fibres from the deep cerebellar nuclei travel in the superior peduncle and pass to red nucleus, thalamus and brainstem (Daniel et al., 1987; Haroian et al., 1981). Some of the deep nuclear cells project to more than a single target (Bentivoglio and Kuypers, 1982).

Figure 10: A diagram of the posterior, dorsal and anterior aspect of the rat cerebellum with marked lobules. Shaded areas in the anterior view represent the white matter. Vermal lobule X (nodulus) is situated on the ventral surface of the cerebellum and therefore not visible in this figure (adapted from Voogd,1995).



Abbreviations: Cop= copula pyramidis (hemispheric lobule VIII);
Fl= flocculus; PFl= paraflocculus;
PML= paramedian lobule; Sim= lobulus simplex;
Roman numerals II-IX denote vermal lobules.

Figure 11: A midsagittal section of the rat cerebellum stained with cresyl violet.



Cytoarchitecture

Despite its convoluted appearance the cerebellar cortex is very simply constructed. There are only five cell classes in the cerebellar cortex (Cajal, 1909-1911) which are distributed in three layers: stellate cells, basket cells, Purkinje cells, Golgi and granule cells.

The most superficial, molecular layer, contains basket and stellate cells, dendrites of Purkinje and Golgi cells and the axons of granule cells- the parallel fibres. Granule cells' axons ascend to the molecular layer, bifurcate and run parallel to the long axis of the cerebellar folium, hence the name. Parallel fibres terminate on the Purkinje and Golgi cells' dendrites as well as on basket and stellate cells.

Underneath molecular there is a layer of large Purkinje cells. These cells have their dendritic trees organised so that they spread out in a single dimension, perpendicular to the main axis of the cerebellar follium. Purkinje cells are the only output of the cerebellar cortex and their action is inhibitory (Ito and Yoshida, 1964). Purkinje cell axons traverse the granule cell layer on their way to the subjacent white matter and travel to one of the deep cerebellar or vestibular nuclei.

The deepest cortical layer is granule cell layer. It is made of densely packed, small neurones with a few larger, superficially placed, Golgi cells. Apart from these cellular

elements, the granular layer contains terminal branches of mossy fibre afferents and also climbing fibres, which travel through this layer on their way to the molecular layer. There is another cell class in the granular layer, named the "brush cell", which has only a single, short dendrite (Mugnaini and Floris, 1994). It receives inputs from mossy fibres, but it's output is not yet known.

Purkinje cells are the only projection neurones of the cerebellar cortex. The rest of the cells are intrinsic inhibitory neurones with complex synaptic patterns.

Cerebellar Afferents

Cerebellar afferents terminate in two different ways in the cerebellar cortex. One set of afferents terminate as mossy fibres on the granule cells. The other set of afferents terminate directly on Purkinje cells as climbing fibres. Both types of afferents are excitatory, but their sources differ and they have different function.

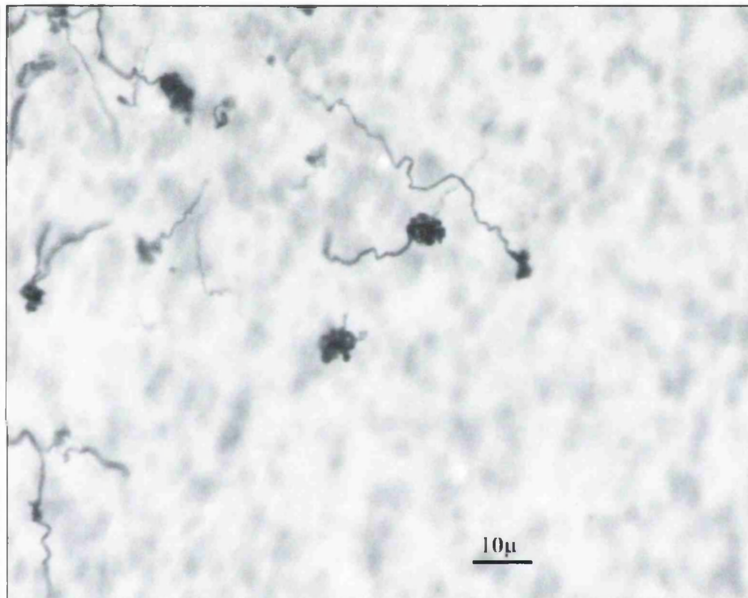
The mossy fibre system originates in a number of structures: pontine nuclei, reticular nuclei of the medulla, dorsal column nuclei, trigeminal nuclei and the spinal cord. In the granular layer mossy fibres form grape- like terminal sites, called mossy fibre rosettes (Figure 12). Mossy fibre rosettes are at the centre of a complex synaptic site, named cerebellar glomerulus, where they form excitatory contacts with up to 20 granule cell dendrites. Within the cerebellar glomerulus there are also terminals of Golgi cell axons which make inhibitory synapses with granule cells dendrites.

Axons from granule cells ascend into the molecular layer as parallel fibres and contact dendrites of Purkinje cells. Each Purkinje cell is contacted by a vast number of parallel fibres and each granule cell giving rise to a parallel fibre in turn receives converging mossy fibre input. Activation of a mossy fibre elicits a characteristic excitatory response in the Purkinje cell- a simple spike. This is a single, rapid action potential which involves the usual voltage sensitive channels, $Na+$ and $K+$, in the cell body and axon membrane.

Climbing fibres have only a single source, the inferior olivary nuclei. Olivary cells give rise to axons which branch up to 10 times and a single climbing fibre will contact only one Purkinje cell. Climbing fibres wrap themselves around soma and dendrites of Purkinje cells, but make synapses only with the proximal dendrites. This association is extremely powerful which is evident by the fact that a single action potential via

climbing fibre elicits a very strong excitatory postsynaptic potential in the Purkinje cell body and its dendrites. Purkinje cell response to activation of a climbing fibre is called a complex spike. It is manifested by a burst of spikes which are followed by a long depolarisation phase in which there may be some more spikes. This activity is caused by Ca++ sensitive channels in the cell's dendritic tree, as well as the usual Na++ and K+ channels in the soma and axonal membrane.

Figure 12: Mossy fibre rosettes in the cerebellar hemisphere as demonstrated with Phaseolus vulgaris leucoagglutinin.



Functional Subdivisions

On the basis of afferent inputs and efferent connections the cerebellum can be divided into three functionally different parts (Larsell, 1937). Vestibulocerebellum (flocculonodular lobe) is the oldest part of the cerebellum. It is considered important in eye movements and balance maintenance, because of its reciprocal connections with the vestibular nuclei. Spinocerebellum is made of anterior lobe, lobulus simplex and vermal lobule VIII and derives its name from the source of its afferent input. Its efferent projections are directed towards the fastigial and interposed cerebellar nuclei. Spinocerebellum controls the ongoing execution of limb movement.

Phylogenetically the youngest part of the cerebellum, the neocerebellum, receives its major input from the cerebral cortex via pontine nuclei and is sometimes referred to as pontocerebellum or cerebrocerebellum. It is made of hemispheres in both anterior and posterior lobes and vermal lobules VII and IX. Pontocerebellum projects to the motor and premotor cortices via dentate nucleus and thalamus. Due to these connections, pontocerebellum is thought important in planning and initiation of movement.

Parasagittal zonation

Afferent and efferent cerebellar fibres appear to form compartments within the cerebellar white matter. These compartments run through the entire rostrocaudal extent of the cerebellum, and are known as parasagittal zones.

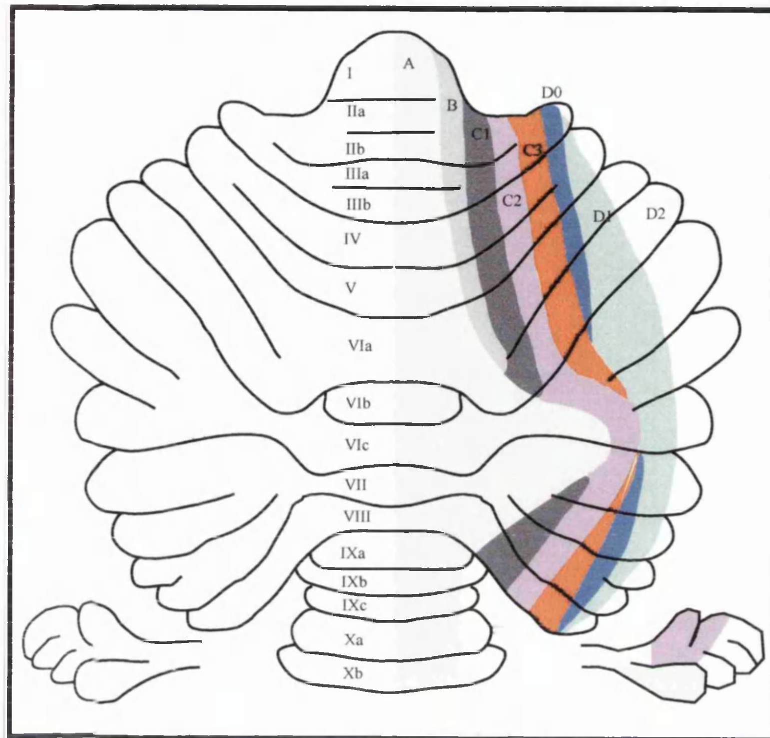
The base for this parasagittal zonation was laid by Klimoff (1899) who described the pattern of corticonuclear connectivity in rabbits. He studied Marchi preparations of brain sections and found that the vermis projects only to the fastigial nucleus, whereas hemispheres project to the interpositus and lateral cerebellar nuclei. The projections are strictly ipsilateral. Later lesion and stimulation studies (rabbits, monkeys- Jansen and Brodal, 1940; rats- Goodman et al.,1961, 1963) confirmed that there are three anatomical compartments within the cerebellar cortex. One compartment consists of fibres connecting vermis and the fastigial nucleus; the other is made of fibres connecting the intermediate cerebellum (paravermal area) with the nucleus interpositus and the third compartment is made of fibres connecting the hemisphere with the lateral (dentate) nucleus.

The work of Jan Voogd (1969) in cats and ferrets, however, showed that there are 4 major zones (A, B, C and D) consisting of 7 longitudinal strips of the cerebellar cortex

(figure 13). In his Nauta and Häggquist stained sections, Voogd noticed that Purkinje cell fibres originating in different parts of the cerebellar cortex tend to be compartmentalised and separated from each other in the white matter. Fibres within each compartment were usually of the same calibre. The borders between the compartments are aligned with borders between deep cerebellar nuclei. Voogd initially described seven compartments or zones. In the vermis of the anterior lobe and simple lobule there are two zones: zone A medially and zone B laterally. Zone A projects to the fastigial nucleus and zone B to the lateral vestibular nucleus. In the hemisphere there are three C zones and two D zones. C1 and C3 zones project to the anterior interposed nucleus, C2 projects to the posterior interposed nucleus. D1 zone projects to the caudal lateral cerebellar nucleus and D2 projects to the rostral part of it. Just like cortico-nuclear pathways, anatomical pathways between cerebellum and inferior olivary nucleus are very precisely organised. The inferior olive is made up of a group of cells situated just above the pyramidal tract in the medulla. It extends about 2.5 mm in the antero-posterior dimension, starting about 1.5 mm caudal to obex. The inferior olive can be divided into three major nuclei: medial accessory nucleus, principal olive and dorsal accessory nucleus. Minor inferior olivary nuclei are the nucleus β, the dorsal cap, the ventrolateral outgrowth and the dorsomedial cell column (Gwynn et al., 1977; Azizi and Woodward, 1987). All these subdivisions are part of the medial accessory nucleus. Cells in inferior olive projecting to the cerebellum are arranged into small groups. Cells within each group all send axons to innervate the same longitudinal zone of the cerebellum. They also send axonal collaterals to the cerebellar nuclei. Efferent cerebellar fibres from the longitudinal strips in turn project to those regions in the cerebellar nuclei that receive input from the same group of cells in the inferior olive.

Parasagittal zonation in rats has been described in great detail by Buisseret-Delmas and Angaut (1988, 1989, 1993). They used WGA-HRP to trace both anterograde and retrograde connections of small regions of the rat cerebellar cortex. Their findings are summarised in the following diagram.

Figure 13: Parasagittal zones in the cerebellar cortex of albino rats, as defined on the basis of cortico-nuclear and olivo-cerebellar projections (adapted from Buisseret-Delmas and Angaut, 1993).



Climbing fibre afferents to the A zone in the anterior lobe originate from cells in the caudal medial accessory olive (MAOc), the subnuclei a and b (according to nomenclature by Gwynn et al., 1977). Zone A of the posterior lobe receives olivary projections from the medial region of the MAOc, subnuclei b, c and β, but vermal lobules VIII and IX receive projections from subnuclei a and b in addition. Olivary input to lobule X is derived from MAO- dorsal cap (dc) and ventrolateral outgrowth (vlo).

Zone B of rat cerebellum stretches only to the level of vermal lobule VIa and receives olivary projections from the caudal dorsal accessory olive (DAO).

Three C zones are present in the rat cerebellum. C1 and C3 subzones receive input from the rostral part of DAO, whereas C2 receives projections from the rostral part of MAO.

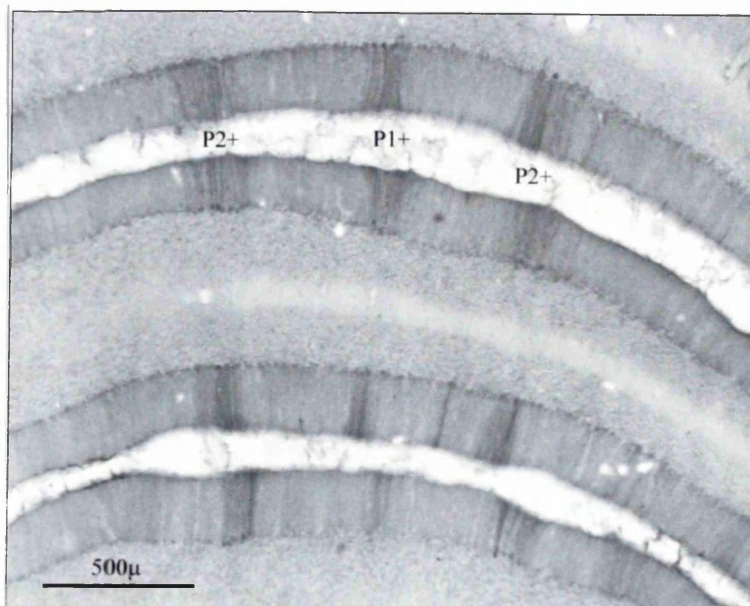
Finally, three D zones are recognised in the rat cerebellum, D0, D1 and D2. Ventral lamella of the principal olive (PO) projects to two of these subzones: medial part of it

projects to D0 and lateral part of it projects to D2. Zone D1 receives projections from the cells in the dorsal lamella of the PO.

Parasagittal zonation can be demonstrated with immunocytochemistry. A subset of Purkinje cells in rats contains a protein which can be recognised by monoclonal antibody, mabQ113 or "anti-zebrin I" (Hawkes et al.,1985). This protein ("zebrin I") is present in the whole of the Purkinje cell, its soma, axon, dendrites and axonal terminals. Another protein, named zebrin II has been discovered by Brochu et al. (1990) and identified as aldolase C (Hawkes, 1992). The zonal distribution of zebrin I and zebrin II immunopositive Purkinje cells is identical (Hawkes and Leclerc, 1987). Zebrin II has much lower molecular weight (36 kD) than zebrin I (120 kD) and appears to be found in fish and birds, as well as mammals. The function of either of these proteins is yet unknown.

There are 7 stripes of zebrin positive Purkinje cells, which alternate with zebrin negative stripes (figure 14). The first stripe, zebrin positive (P1+), is placed at the midline and runs the whole lenght of the cerebellar vermis, from lobule I-X. Two other P+ stripes are seen in vermis and further four in the hemispheric lobules. This zonation is not equally evident in all the lobules of the cerebellum because the staining is much weaker in the anterior lobe and in lateral parts of the hemispheres.

Figure 14: A photomicrograph of a section through the rat cerebellum showing zebrin stripes.

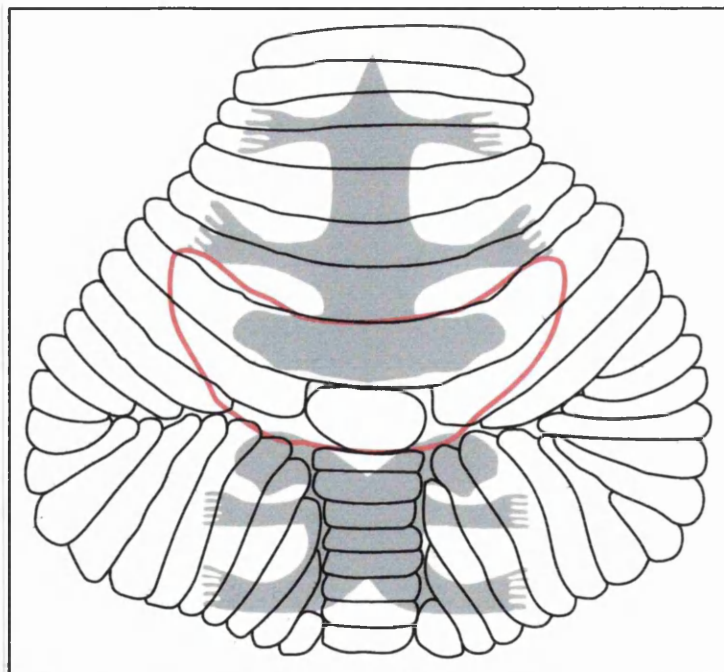


Localisation of function

The cerebellum was regarded only as proprioceptive and vestibular structure until the pioneering work of Snider and Stowell (1944) was published. They described the evoked potential responses in the cerebellar cortex of cats and monkeys following peripheral tactile, visual and auditory stimulation. When reconstructed onto the surface diagram of the cerebellar cortex the tactile response areas formed complete body maps in the anterior and posterior lobe of the cerebellum. Visual and auditory region was situated between these maps, but there was some overlap with the anterior head representation.

In each lobe there are two somatotopical maps, mirror images of each other. In the anterior lobe these mirror images are joined facing each other, whereas in the posterior lobe they face the opposite sides (see the following diagram).

Figure 15: Diagrammatic reconstruction of electrophysiologically defined regions of the cerebellar cortex as viewed from above (adapted from Snider, 1958). Areas shaded in grey represent tactile regions; area enclosed within the red line represents overlapping visual and auditory region of the cerebellum. Rostral end of the cerebellum is at the top of the figure.



There was a gap in the literature regarding topographical organisation of inputs from different sensory modalities until Shambes et al. (1978 a,b) and Joseph et al (1978) published their findings in the rat cerebellum. They showed that tactile receptive fields in the granular layer of the cerebellar cortex can be found in hemispheric lobules Crus I, Crus II, paramedian lobule and lobulus simplex. Crus I receives projections from head and upper face; Crus II receives input from perioral structures whereas the paramedian lobule receives input from the entire body. The caudal folium of lobulus simplex receives projections from gingiva, lips, incisors and vibrissae. The most remarkable feature of these projections is that they appear to be organised as patches on the cerebellar surface. The adjacent patches in the cerebellar cortex do not necessarily receive projections from the adjacent parts of the periphery. Also, the same part of the periphery can be represented several times in different cerebellar folia or within the same folium. The authors refer to this topographical arrangement as "fractured somatotopy" (Shambes et al., 1978 a). Vermal lobule IX also receives tactile projections, mainly from vibrissae and other facial structures (Joseph et al., 1978). This topographic arrangement is not specific for rodents. Kassel et al. (1984) described similar arrangement for cutaneous projections to the posterior cerebellar hemisphere in cats.

Tactile responses can also be recorded in the Purkinje cell layer. Saint-Cyr and Woodward (1980) recorded responses of Purkinje cells following peripheral stimulation of head, snout, forelimb and hindlimb of a rat. They found some somatotopy in the representation of different parts of the body on the cerebellar cortex. The strongest responses of Purkinje cells to stroking the top of the head were recorded in vermal lobules IV-VII and paravermal lobules II-VIIb. Some cells in paramedian lobule also responded to this stimulus. Tactile stimulation of the snout elicited responses mainly in vermal lobule VI, but also in other vermal lobules (from III-VIIa), as well as in the lateral portion of the lobulus simplex. Forelimb responsive cells were found in vermal lobules IV-VIa, whereas hindlimb sensitive cells were mainly found in vermal lobules II-IV. Purkinje cells which were specifically activated by rat's facial whiskers were recorded in vermal lobule VII (Thomson et al., 1989).

Multiple whisker representations in the rat cerebellum were also demonstrated by means of increased uptake of radioactively labelled 2-deoxyglucose following peripheral stimulation (Sharp and Gonzalez, 1985). The most active regions were

found in paramedian lobule, Crus I and II, lobulus simplex and HIV and HV of the anterior lobe. Areas sensitive to manipulation of whiskers appear as small, discontinuous patches of increased metabolic activity in these cerebellar lobules, mainly ipsilateral to the stimulation site.

The phenomenon of "fractured somatotopy" was first described twenty years ago. The underlying principle for this phenomenon has not been discovered yet. Whether the same topographical representation applies to other sensory modalities is also unknown.

II. MATERIALS AND METHODS

The experiments in this thesis are presented in three chapters. In the first experimental chapter I used anterograde tracers: Phaseolus Vulgaris Leucoagglutinin (PHA-L, Vector), Biocytin (Sigma) or Biotyinilated Dextran Amine (BDA, Molecular Probes). In the experiments which are described in other chapters I used rhodamine or fluorescein labelled latex microspheres (Lumafluor Inc.) as retrograde tracers. Protocols for histological processing of the tissue are specific for each tracer and will be presented at the end of this section.

The number of animals used in all of the experiments is 45. All were male Lister hooded rats, body weight between 250-400g. The animals were kept in individual cages on food and water ad libitum.

Surgery

The anaesthesia was induced in a chamber saturated with 3-4% Fluothane in oxygen. When the animal was sufficiently relaxed it was placed into a Kopf stereotaxic headholder for small animals, with an anaesthetic mask covering the nose. For cortical injections the incisor bar was set at 3.3 mm above the ear bars, thus achieving the flat skull plane (Paxinos and Watson, 1982). Surgical anaesthesia during the experiment was maintained with dose of 1.5-2% Fluothane in oxygen.

Under aseptic surgical conditions a skin incision was made and the skull opened using a dental burr.

In pilot experiments it was determined that the borders of PMBSF are located between 0.5-3.7 mm posterior and 4.0-7.0 mm lateral to bregma (β). The position of each of the individual barrels within the PMBSF relative to β was also determined in these pilot experiments and those sterotaxic coordinates were used in the experiments presented here. In several cases the placement of the injection pipette was confirmed with prior recording. Barrel C2, for example, was injected at the following coordinates: AP= 2.9 mm posterior to β; ML= 6.0 and DV= 1.1 mm ventral from the pial surface. Recording electrode and pipette were angled to 35° from the vertical.

Electrophysiological recording was done using tungsten microelectrodes of 2-5MΩ impedance. The cells within the PMBSF were tested for response to gentle mechanical manipulation of the contralateral whiskers. When a desired barrel column was

identified its position relative to β was recorded in order to assist placement of the injecting pipette. All the cortical injections were made into the left hemisphere. Experiments in the second experimental chapter required different head restraint for each set of injections. The superior colliculus was injected with head in the flat skull position. Pontine injections were done with the incisor bar raised 5 mm above the intraaural line (as in the atlas by Pellegrino and Cushman, 1967) and the injecting pipette angled to 55° from the vertical, parallel with the midline. In these experiments all the injections were done on the left side of the brain.

Coordinates for injections were:

PONS

AP= from 0.5 to 2.0 mm anterior to EB0

ML= from 0.5 to 1.8 mm lateral from the midline

DV= from -0.9 to +0.2 mm from the DV coordinate for the EB0

SUPERIOR COLLICULUS

AP= from 5 to 6.5 mm behind β

ML= from 1to 1.8 mm lateral from the midline

DV = 3.5 mm below the level of cortex

For cerebellar injections in the third experimental chapter a caudal approach was used. The skin was incised and neck muscles were deflected to expose the occipital bone. A hole was drilled using a dental burr and the bone in the midline was removed to reveal cerebellar vermis. The opening was larger for the Crus I injections and more rostral. To inject whisker areas of cerebellar lobules IX and Crus I, I used detailed maps of Joseph et al. (1978) and Shambes et al. (1978 a,b). There are three patches of whisker sensitive areas in each hemivermis of cerebellar lobule IX, measuring about 1.5 mm^2 in total. The first patch borders the midline and ends about 400 μm lateral; the second patch extends from 0.7 - 1.2 mm lateral from the midline and the third whisker sensitive region extends from 1.6 - 1.9 mm lateral to the midline. I tried to inject the middle patch in each hemivermis of lobule IX . Two whisker sensitive patches were found in Crus I. The first one starts at the crown of the folium and extends 1.1 mm laterally. The second whisker representation starts at about 2 mm from the medial end of the

folium and extends further two mm laterally. I aimed to inject the more medial whisker representation in Crus I.

Whisker responsive patches in vermal lobule VII were described by Thomson et al. (1989). They found most responses to whisker stimulation at 200 μm and 1 mm lateral to the midline. In this study I aimed to inject this lateral zone in the vermal lobule VII.

Following the completion of the experiments each animal received a dose of analgesic (0.03 ml of Vetergesic). Postoperative recovery was rapid and uneventful.

Injection

Anterograde tracers

Capillary glass cannulae were pulled on a vertical pipette puller and the tips broken using fine watchmakers forceps to an inner diameter of 10-16 μm. Prior to injection the pipette was vacuum-filled with the tracer and a fine silver wire was inserted into it for electrophoretic injection. The wire was connected to the positive pole of the current source and the earthing electrode was attached to the temporal muscle, exposed by incision of the skin. A positive current of 2-3 μA was applied in pulses (0.5 second on/ 0.5 second off), over a period of 5-20 minutes.

The above parameters were the same for each of the tracers.

Phaseolus vulgaris leucoagglutinin ("Vector") was used as 2.5%. solution in 0.9% saline.

Biotinilated dextran amine ("Molecular Probes"; molecular weight 10,000) was dissolved in 0.9% saline to obtain a 5% solution.

Biocytine ("Sigma") was used in concentration of 5%, dissolved in 0.05M Tris buffer (pH=7.5).

Gerfen and Sawchenko (1984) found that PHA-L is only taken up and transported by axons if the tracer is applied by iontophoresis. The same method of application was used for BDA and biocytin, although these tracers can be applied using pressure (Brandt and Apkarian, 1992; Dolleman-Van der Weel et al,, 1994).

The mechanism of transport of PHA-L is still not completely understood. Gerfen and Sawchenko (1984) showed that the tracer is taken up by neuronal perykarya and dendrites as well as astrocytes in the vicinity of the injection site. It is not incorporated by endocytosis and does not enter the lysosomal system. Gerfen and Sawchenko suggested two possibilities of how the tracer might be taken up. During iontophoresis

neuronal membranes might become temporarily permeable so that the tracer gains entry. Alternatively, iontophoresis may disrupt association between dendrites and astrocytes, thus opening a site for entry of the tracer into intracellular compartment. The same authors report that PHA-L is not taken up by fibres of passage.

The tracer is stable in the injected animal for quite a long time. Wouterlood and Groenewegen (1991) showed that even after 4-5 weeks the injection site as well as terminals do not change in appearance. With longer survival times the tracer gradually disappears.

Biotinilated dextran amine (BDA) and biocytin are also very sensitive anterograde tracers (Dolleman Van der Weel et al., 1994) which require much less histological processing than the PHA-L. BDA is transported in retrograde direction as well, but is not considered a reliable retrograde tracer. Unlike PHA-L it is readily taken up by the fibres of passage (Brandt and Apkarian, 1992).

Majority of the experiments in the first experimental chapter were done with PHA-L as a tracer. However, in some studies I used two tracers (PHA-L and BDA) and injected them in different barrel columns in the same animal. The intention in these experiments was to reduce the overall number of the animals needed for completion of the study but also to help resolve the question of overlap in cortico-pontine projections.

Retrograde tracers

Pressure was used to inject the fluorescent tracers into superior colliculus, pontine nuclei and cerebellar cortex. The glass pipette (tip size 30 μm) was vacuum filled with the latex microspheres. The pipette was connected to a pico spritzer by a length of plastic tubing. This arrangement allows for controlled injections of small quantities of the tracer.

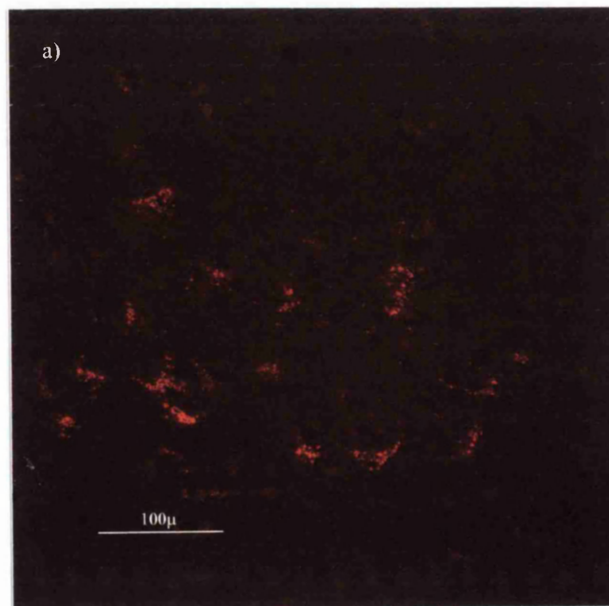
The superior colliculus and pontine nuclei of animals described in the experimental chapter 2 were injected with a total of about 1.2 -1.5 μl of red or green latex beads in multiple penetrations. In preliminary experiments I determined that an injection of 100 nl of this tracer produces a circumscribed injection site of about 300 μm in diameter. Since larger amounts of tracer at one site damage the tissue and do not increase the effective injection site, a standard procedure was to make 12 injections into each of the

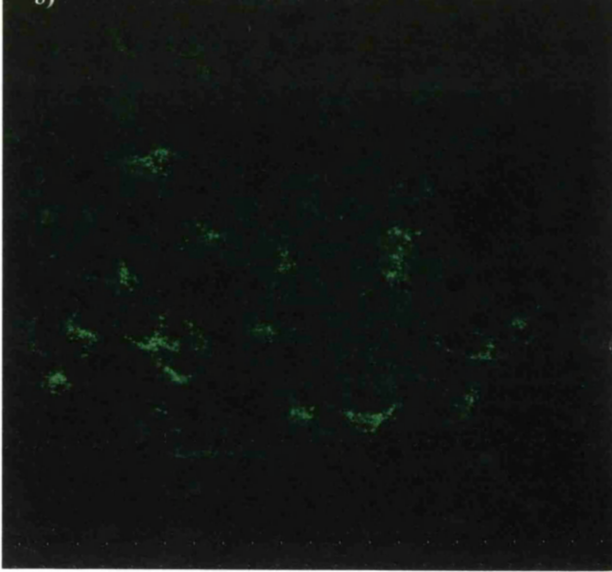
structures. The aim was to label primarily those regions which receive cortical inputs- roughly the caudal two thirds of the pons and rostral superior colliculus.

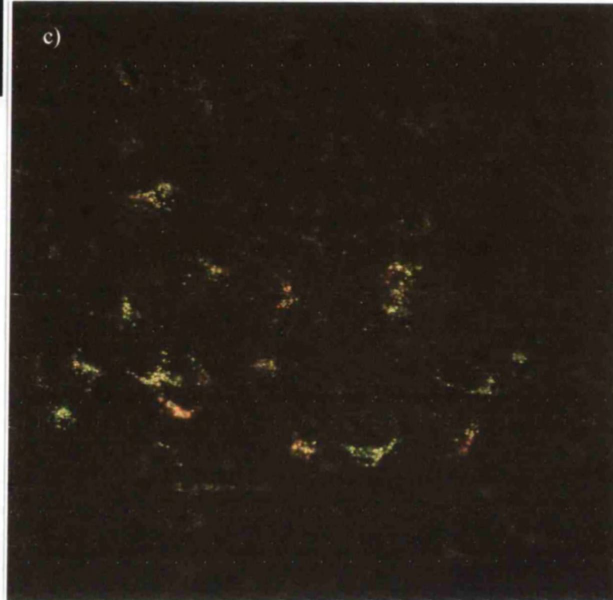
For cerebellar cortical injections (experimental chapter 3) the tip of the pipette was advanced to a depth of 0.7 mm, measured from the point of touching cerebellar cortex. The pipette was angled to be parallel to the long axis of the cerebellar lobule. About 100 nl of the tracer was injected at the injection site in steps of 2.5 nl.

Fluorescent beads are latex spheres whose diameter is about 20 nm and are labelled with rhodamine or fluorescein. Strongly labelled cells are visible with immersion objectives at 10x magnification or greater. Each colour is easily distinguishable, especially at higher magnifications. Rhodamine beads fluoresce red under green illumination (wavelength 540 nm), and fluorescein labelled beads fluoresce green under blue illumination (wavelength 430-490 nm). In a preliminary experiment I mixed beads of both colours and injected them into cerebellar lobule VII and looked at retrogradely labelled pontine cells. Each of the pontine cells was double labelled (figure 16).

Figure 16: Photomicrograph of double labelled pontine cells in case where the latex beads were mixed prior to injection into cerebellar cortex. The same image is viewed in red (a), green (b) and combined channels (c).







Survival times

Transport of PHA-L and BDA is relatively slow. It is reported that PHA-L travels 5-6 mm/day in the central nervous system (P. Bolam, 1992). Cases with cortical injections of PHA-L or BDA were perfused 8 days following the operation. Biocytin travels faster, about 20-40 mm/day , but is subject to enzymatic degradation with longer survival times. The literature varies on the optimal survival times. I allowed 48 hours on the basis of work by Tomihiro and Mamoru (1993) who found labelled corticospinal terminals in the rat in that period.

Animals which received fluorescent tracer injections were perfused 4 days after the injection. Preliminary experiments of varying survival times, from 24 hours to 8 days, showed that the maximum number of retrogradelly labelled cells in pons and inferior olive (after cerebellar injections) were present from day 3 onwards. No improvement in quality or amount of labelled cells was seen after 3 days.

Perfusion/Fixation

Animals were terminally anaesthetised with Fluothane in CO_2 and transcardially perfused with the following:

-1000 ml 0.9% saline with 1ml of heparin (1000 units/ml)

-1000 ml fixative- 4% paraformaldehyde in 0.1M phosphate buffer (PB) pH=7.4

<u>or</u>

4% paraformaldehyde + 0.05% glutaraldehyde in 0.1M PB (for biocytin and BDA)

-500 ml 10% sucrose in 0.1M phosphate buffer, pH=7.4

A transverse cut in the stereotaxic plane was made in the brain, prior to the removal from the skull, just anterior to β.

The injected cerebral hemisphere was dissected from the rest of the brain and flattened in a Petri dish filled with 20% buffered sucrose. The flattened hemisphere was weighted with a glass slide and additional 15 g weight. This procedure allows for the whole barrel field to be seen in 2-3 sections, when the brain is cut.

The brain was allowed to sink in 20% buffered sucrose overnight.

Histological Processing

The brains were sectioned on a freezing microtome at 50 μm and sections were collected into 0.1 M PB or phosphate buffered saline (PBS), pH=7.4 - 7.5.

For cases presented in the first experimental chapter a series of coronal sections at 150 μm apart was processed immunohistochemically to reveal structures labelled with a specific tracer. Following the completion of the immunohistochemistry, sections were mounted onto gelatine coated slides and air dried over night. The following day the sections were dehydrated through a series of graded alcohols (50, 70, 95, 100, 100%) and cleared in Xylene or Histoclear. DPX was used as medium for coverslipping. Some cases were counterstained with thionin or cresyl violet.

The injected cerebral hemisphere for each case was cut in the plane parallel to the pial surface. Alternate sections (every 100 μm) through the injection site were reacted for cytochrome oxidase to reveal the barrel structure in layer IV.

In cases presented in the second experimental chapter the whole brains were sectioned in the coronal plane. One section at every 150 μm was mounted for fluorescent microscopy onto subbed slides. A parallel set of sections was reacted with cytochrome oxidase to reveal the barrel pattern in layer IV of the primary somatosensory cortex. The sections were allowed to air dry overnight and were only briefly dehydrated in alcohol and cleared in Xylene (up to 1 minute) the following day in order to prevent damage to the latex beads. Slides were then coverslipped using Fluoromount. Some sections were examined with fluorescent microscope as soon as they were dry, with no other histological processing.

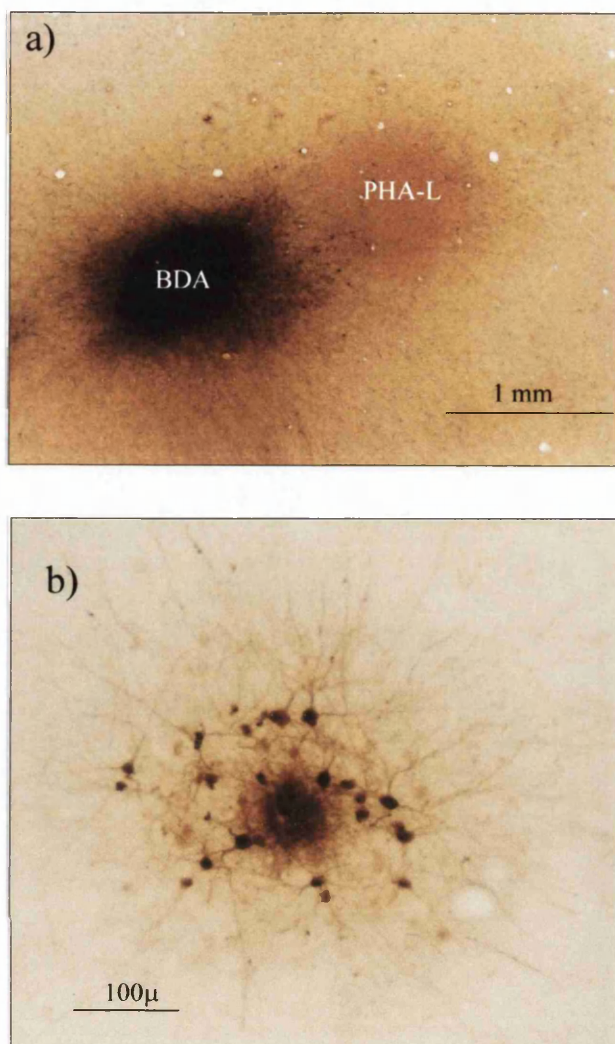
Brains of the animals that received injections of fluorescent microspheres in the cerebellum were usually embedded in gelatine prior to cutting. In some cases the cerebellum was dissected from the rest of the brain and embedded separately. The cerebellar sections were then cut in parasaggital plane at 50 μm, and the rest of the brain was cut in coronal plane. Every third section (150 μm) was mounted onto gelatine coated slides and briefly dehydrated in 100% alcohol, cleared in Xylene and coverslipped using Fluoromount.

Injection Sites Reconstruction

Cerebral cortical injection sites were reconstructed onto a series of camera lucida drawings of the sections that had been processed for cytochrome oxidase to reveal the barrel field in layer IV. The sections processed for tracer were superimposed onto drawings of adjacent sections processed for cytochrome oxidase using major vessels and artificial markers as guides.

The size of the injection site was defined as a diameter of the densely stained area within which the labelled cell bodies could be seen. There is usually a zone of diffusion of the tracer around this area but previous studies showed that there was no uptake of the tracer from this wider zone (Gerfen and Sawchenko,1984). The sizes of the injection sites are measured in sections through the cortical layers V and VI and are expressed as average of those values.

Figure 17 : Representative injection sites in the primary somatosensory cortex: a) Phaseolus vulgaris leucoagglutinin and biotinilated dextran amine; b) biocytin.



njection sites in cases where fluorescent microspheres were used as tracers were plotted on an X-Y plotting system. The system is composed of linear potentiometers mounted on the microscope stage and connected to a pen plotter (Hewlett Packard). Magnification can be adjusted by varying the gain of the voltage that drives the pen. The position of the pen is calibrated to the position of the cross lines in the microscope eye piece. Each point under the cross hairs could be translated to dots made by the plotter pen. The injection sites in the cerebellar cases were subsequently reconstructed onto a series of drawings of either coronal or parasagittal cerebellar sections, using original brain sections.

Latex microspheres are strongly adherent so there is little diffusion around the injection site. The injection sites are therefore restricted and uniform in diameter among experimental cases.

In cases with pontine nuclei and superior colliculus injections the injection sites were plotted onto outlines of each section using the plotting system described. Diagrams were made from these original tracings.

Terminal Label Reconstruction

Sections through the pons were drawn for each case that is described in the first experimental chapter, using camera lucida, and labelled fibres and terminals were drawn at relevant positions. In order to compare the cases, label from each case was transferred onto a set of standard drawings of pontine nuclei. The standards show all the sections through the pontine nuclei sampled at 150 μm. This standards were made using one of my original experimental cases which had been stained with cresyl violet. Cytoarchitectonic borders and nomenclature of the pontine nuclei which appear on the diagrams is adopted from Mihailoff et al. (1981 b).

In order to reconstruct the location of the retrogradelly labelled cells in the primary somatosensory cortex, following the injections in pons and superior colliculus, I first examined the CO-processed series of sections to establish the location and the extent of the barrel field. The sections in which there was a marked contrast between granular and dysgranular zones were selected and drawn using camera lucida. Major blood vessels were also included in these diagrams. Adjacent unprocessed sections were then examined with a fluorescent microscope that was connected to the x-y plotter system. After drawing the outline of the sections the position of labelled cells was marked. The plotter also has a counter attached so the number of labelled cells could be counted. In order to record cells labelled with both tracers it was necessary to flip often between the filters to establish whether both types of spheres were present within the same cell.

A number of sections was also examined and photographed with a confocal microscope. At high power magnification the confocal microscope's laser bleaches the label and the tissue leaving a dark rectangular shape on the section. This proved to be an additional help in determining precisely the origin of the scanned images.

Fluorescent label in cases which received cerebellar injections was also plotted using the x-y plotting system. When the plotting was finished the sections were counterstained with cresyl violet and coverslipped. Using camera lucida, details of the section were drawn onto original plots to help with reconstruction. Finally, label was transferred onto drawings of a standard set of pontine sections, as described earlier. A parallel set of sections was additionally examined with aid of a confocal microscope and photographs were taken of those images.

A) Phaseolus vulgaris leucoaglutinin (PHA-L) procedure

1. Rinse sections 3 x 10 minutes in TBS+

2. Add Anti-Phaseolus vulgaris leucoagglutinin (E+L) IgG, ("Vector", cat. no. AS-2224, 1mg) and leave to react for 12 - 16 hours at room temperature, or 24-36 hours at 4 °C. Dilute antibodies to 1 : 2000 with TBS+

3. Rinse sections 3 x 10 minutes in TBS+.

4. Add Biotinylated anti - goat IgG produced in rabbit, ("Vector", cat. no. BA5000, 1.5mg) diluted to 1:200 with TBS+ (125 µl in 25 ml TBS+) , and leave to react for 90 - 120 minutes at room temperature with gentle agitation.

5. Rinse 3 x 10 minutes in TBS+.

6. Add ABC solution from "Vector" (cat. no. PK 6100). Mix 1 drop of solution A with 1 drop of solution B in 10 ml of TBS+. Prepare this solution at least half an hour prior to the reaction and react sections for 30 - 60 minutes.

7. Rinse section 6 times in TB

8. React the sections for DAB for 30 minutes (same as for HRP , only dissolved in TB)

9. Rinse sections 6 times in TB

10. Air dry the sections and counterstain with cresyl violet (0.3%) or thionin (0.05%).

11. Coverslip

SOLUTIONS

- **TB** = 0.05M tris buffer

6.06 g trizma base dissolved in 750 ml distilled water. Titrate with HCl to pH = 7.6 .
Add distilled water to 1l.

- **TBS** = 0.05M tris buffer with saline

6.06 g trizma base ("Sigma", cat. no. T8524, 100 g)
9 g NaCl
Dissolve above ingredients in 750 ml distilled water (pH should be about 10) and titrate
with concentrated or 0.5M hydrochloric acid until pH reaches 7.6 , then add distilled water to 1liter.

- **TBS+** = 0.05M tris buffer with strong saline

6.06 g trizma base
29.22 g NaCl
5ml triton x 100 ("Sigma", cat. no. T8787, 100 ml)
Dissolve above ingredients in 750 ml distilled water and titrate with hydrochloric acid to pH = 8.6.
Add distilled water to 1l.

- **DAB** = Dissolve 50 mg of DAB in 100 ml Tris buffer (pH = 7.6) and filter the solution.

Add 20 μl of 30% H_2O_2 .

B) Biotinilated dextran amine (BDA) protocol

1. Rinse sections 3 x 10 minutes in 0.1 M phosphate buffered saline (PBS) with triton (0.3%).
2. React sections in ABC solution for 2 hours on the shaker (5 drops of A + 5 drops of B in 25 ml of working solution - 0.1M PB with triton).
3. Wash sections in 0.1 M PB for about 20 minutes (4 changes).
4. React sections in DAB for up to 15 minutes (0.05% DAB in 0.1M PB, with 0.006% H_2O_2).
5. Wash 3 times in 0.1 M PB buffer.
6. Mount onto subbed slides.
7. Dehydrate and counterstain, coverslip.

C) Biocytin protocol

1. Rinse sections 3 x 10 minutes in 0.1 M phosphate buffered saline(PBS) with triton (0.3%).
2. React sections in ABC solution for 2 hours on the shaker (2drops of A + 2 drops of B in 10 ml of working solution - 0.1M PBS with triton).
3. Wash sections in 0.1 M PBS for about 20 minutes (4 changes).
4. React sections in DAB for up to 15 minutes (0.05% DAB in 0.1M PB, with 0.006% H_2O_2).
5. Wash 3 times in 0.1 M PB buffer.

6. Mount onto subbed slides.

7. Dehydrate and counterstain, coverslip.

D) Proticol for double anterograde tracing (PHA-L & BDA)

1. Wash sections 3x10 minutes in 0.05M TBS-T

2. Incubate the sections in ABC "Elite" 1:200 (1 drop of A+1 drop of B in 10ml of working buffer)

3. Wash in 0.05M TBS-T, 3x10 minutes

4. Incubate in DAB-Co chromogen for 10-15 minutes to obtain a purple-black labelling

5. Wash in 0.05M TBS , 5 times

6. Incubate overnight in anti-PHA-L (1:4000)in TBS-T

7. Wash sections in TBS-T, 3x10 minutes

8. Incubate 1 hour in anti-goat IgG (1:50 in TBS-T)

9. Wash sections in the working buffer , 3x10 minutes

10. Incubate in ABC "Elite" 1:400 in TBS-T buffer, for 30-60 minutes

11. Wash in 3x10 minutes in TBS-T

12. React with DAB about 10 minutes

13. Wash in TBS buffer 5x

14. Mount on gelatine subbed slides from TBS

Colours: BDA= purple-black
PHA-L= brown

SOLUTIONS:

- TB = 0.05M tris buffer (6.06 g Trizma in 1l distilled water, pH=7.6)
- TBS = 0.05M tris buffer with 0.9% saline
- TBS-T= 0.05M phosphate buffer with 0.9% saline with added Triton X-100 (0.3%)

- DAB

50 mg DAB dissolved in 100 ml of Tris buffer with 33.5µl of 30% H_2O_2

- DAB-Co

50 mg DAB dissolved in 100 ml Tris buffer with 1 ml of 1% Cobalt acetate and 33.5 μl of 30% H_2O_2

Embedding in gelatine

Gelatin was prepared by dissolving 10 g of gelatine ("Sigma") in 100 ml of distilled water (heated to 42^0 C). When dissolved 10 g of sucrose (BDH) was added to the solution. The brain was placed into a suitable vessel and covered with gelatine. When the gelatine was set the block was taken out of the vessel and placed in 20% buffered sucrose. The following day, the gelatin block was hardened in the fixative (4% paraformaldehyde) for 2 hours. After hardening, the block was rinsed in 20% buffered sucrose for about 20 minutes and stored in 20 % buffered sucrose until ready to cut.

Cytochrome oxidase staining

Dissolve 50 mg of DAB and 35 mg cytochrome C ("Sigma") in 100 ml 0.1 M phospate buffer (pH= 7.2-7.6) using ultrasonic bath. React free folating sections either at 4°C overnight or at room temperature for four hours (it may take longer, depending on the initial reaction speed). Rinse twice in 0.1 M phospate buffer, mount onto subbed slides and air dry. Dehydrate slowly (50, 70, 95, 100, 100% alcohol), clear in Xylene and coverslip with DPX.

Zebrin Protocol

1. Rinse section three times in TBS+.

2. React free floating sections with mabQ113 antibodies in TBS+ (diluted 1:150) for 12-24 hours at room temperature or 48 hours at 4°C.

3. Rinse three times in TBS+.

4. React with secondary antibodies (biotinilated anti-mouse), diluted to 1:200 in TBS+, for 1-2 hours at room temperature.

5. Rinse three times in TBS+.

6. Incubate sections in ABC solution ("Vector"), for 30 minutes.

7. Rinse sections 6 times in 0.05M phospate buffer, pH=7.2

8. React with DAB (see previous page).

9. Rinse sections in 0.05M phospate buffer, mount onto subbed slides, air dry overnight

10. Dehydrate and coverslip with DPX.

III. Cortico-pontine termination zones

INTRODUCTION

There is a very strong anatomical connection between the cerebral cortex and pontine nuclei. In man, for example, an estimated five million fibres project subcortically from the occipital and posterior parietal cortex (Stein et al.,1987). Great majority of these fibres synapse in the pontine nuclei and only about one million proceeds further in the pyramidal tract. Even these fibres send collateral branches to the pontine nuclei (Ugolini and Kuypers, 1986).

Cortical efferents terminating in the pontine nuclei travel in the cerebral peduncle. In rats, fibres originating in different cortical areas occupy different parts of the cerebral peduncle (Glickstein et al., 1992). Fibres from the most rostral regions of the frontal lobe occupy the most ventromedial pole of the peduncle. Fibres from the occipital and temporal cortical areas travel in the most dorsolateral part of the peduncle. Somatosensory fibres are found between these two extremes, in the middle portion of the peduncle. What happens to this spatial organisation once the fibres reach their target cells in the pons? A number of studies in different species agree that projections from functionally and cytoarchitectonically distinct cortical areas remain segregated at the pontine level.

Wiesendanger and Wiesendanger (1982 a,b) studied projections from several cortical areas in rats, using HRP and tritiated amino acids as anterograde tracers. They found that sensorimotor and visual cortices provide the bulk of the input to the ipsilateral pontine nuclei. Termination zones are spatially organised so that the more rostral areas of cortex project more medially within pontine nuclei. Similar organisation has been reported in monkeys (Dhanarajan et al. 1977; Brodal 1978 a,b; Wiesendanger et al.,1979).

Corticopontine terminals appear as patches in coronal sections through the pontine nuclei. Some of these patches seem to extend in rostro-caudal direction and therefore resemble columns. In cat and monkey (Brodal 1968, 1978 a,b) the projections from sensory and motor cortex appear to form two longitudinal columns of terminals within

pontine grey. The areas of pons that receive sensory input are spatially segregated from those receiving motor input. This spatial gradient is particularly marked in monkeys. Mihailoff et al. (1978) studied projections from the representations of fore and hind limb in the primary somatosensory cortex of rats and identified five longitudinal terminal columns in the pontine nuclei. Both of these areas contributed to all terminal columns. Terminals from the hind limb area were found further caudally within each of the columns than the terminals from the fore limb area. Most of the corticopontine projections from the fore and hind limb areas were found to be ipsilateral but there was also a small contralateral projection zone in the medial nucleus from the fore limb area and in the ventral pontine nucleus from the hindlimb area.

Another study (Mihailoff et al., 1985) focused on the relationship between pontine terminals from rat sensory and motor areas of the cortex related to the face. The somatosensory face area projects to several pontine nuclei in the ipsilateral pons and to the contralateral medial pontine nucleus. Motor face area projects to non-overlaping regions within the pontine nuclei with larger contralateral component. Almost all of the termination zones within the ipsilateral pons are mirrored on the contralateral side.

In the most recent study Panto et al. (1995) described projections from small parts of primary motor and somatosensory cortex in the rat utilising WGA-HRP as a tracer. They reported a clear segregation between motor and somatosensory termination zones in the ipsilateral pontine nuclei. Generally, motor representation of a particular part of the periphery terminates rostral to its somatosensory projections. Some injections of the motor cortex showed terminal fields in the contralateral pons as well.

Aims and framework of the present study

All the cortical information that reaches pontine nuclei is relayed to the cerebellar cortex as mossy fibres. It is generally accepted that the cerebellum uses this information to accurately and quickly guide movements. However, the precise nature of the information from the cerebral cortex and whether it is modified within the pontine nuclei is poorly understood. An anatomical study of the pontine projections from small, electrophysiologically well defined cortical areas would contribute to our understanding of this issue.

The previous studies have shown that there is a spatial arrangement among the cortico-pontine terminals such that projections from functionally different and spatially distant cortical areas remain segregated within the pontine nuclei.

In this chapter I focused on the rat primary somatosensory cortex, especially the posteromedial barrel subfield (PMBSF), in order to establish whether there is a finer topographic organisation of corticopontine terminals. Large barrels in the PMBSF represent the largest whiskers on the rat's face. Each barrel is principally activated by light mechanical stimulation of a particular whisker (C.Welker, 1976).

In contrast to this "one-to-one" relationship between large cortical barrels and the long, facial whiskers, cells in the smaller barrels are responsive not only to manipulation of the whiskers they represent but also to manipulation of the hairs surrounding the whiskers (mice-Nussbaumer and Van der Loos, 1985; rats - Pidoux et al., 1979). Furthermore, deep cutaneous and joint movements responses can be elicited from cells in perigranular and dysgranular zones of the primary somatosensory cortex in awake rats (Chapin and Lin, 1984).

Earlier anatomical studies that focused on the connectivity of the PMBSF established that the barrel areas (granular zone) receive thalamic input from the ventroposterior medial (VPM) nucleus (Killackey, 1973; Wise and Jones, 1978). Interbarrel area (perigranular zone) seemed free of the VPM input but it received callosal and ipsilateral cortico-cortical projections (Akers and Killackey, 1978; Wise and Jones, 1978). However, Koralek et al. (1988) showed that the interbarrel area also receives thalamic input from the posterior (Pom) nucleus. Lu and Lin (1993) confirmed this and showed that layers V and III of the barrel area receive input from the VPM and Pom, whereas the interbarrel area receives input exclusively from the Pom.

So, within the primary somatosensory cortex in rats and mice there are subtle cytoarchitectonic and functional differences. These differences may be reflected in the way these regions project to their targets, in particular to the pontine nuclei.

Detailed description of the projections from the PMBSF to a number of cortical and subcortical areas in mice is available (Welker et al., 1988). However, projections to the pontine nuclei have not been studied in detail.

The experiments in this chapter were designed to answer the following questions:

1) Do all the barrel columns within the PMBSF project to the pontine nuclei?

2) Does a single barrel column project to more than one cytoarchitectonically defined area within pontine nuclei?

3) Is there overlap between projections arising from different barrel columns or group of barrel columns?

4) Do peri- and dysgranular zones project to the pontine nuclei in the same fashion as the granular zones?

In this set of experiments I have used three very sensitive anterograde tracers which clearly label the whole pathway, down to the axon terminals.

RESULTS

All of the cortical barrel columns within the PMBSF project to the pontine nuclei. Each barrel column projects to at least two restricted zones within the pontine nuclei. Pontine terminals from different barrel columns overlap considerably. The main "whisker" area is within the ventral peduncular nucleus. Pontine projections originating in larger PMBSF barrel columns differ from those originating in smaller ones. Smaller barrel columns, as well as the perigranular zones, project to a greater number of pontine nuclei and their projections are bilateral.

Twenty male Lister Hooded rats, ranging in weight from 250-400 grams were used in these experiments. All the injections were made into the left cortical hemisphere. Three different tracers were used: Phaseolus vulgaris leucoagglutinin (PHA-L), biocytin and Biotinilated Dextran Amine (BDA). Their similarities and differences are described in the Materials section of this thesis. Most of the experiments were done using PHA-L. In one animal (96-01) two different anterograde tracers were injected into neighbouring sites (PHA-L and BDA) thus producing two experimental cases (10 and 14). All of the other animals received a single injection.

A complete account of the animals and the details of the injection sites are given in the following table.

Table I-1. List of experimental animals including placement and nature of the tracer and the size of the injection sites

Case no.	Animal no.	Injection site	Size of the inj.site (μm)	Tracer
1	96-06	A1 (+ PGZ)	188	BDA
2	95-32	B1,B2,C1	375	PHA-L
3	95-26	C2	454	Biocytin
4	94-45	D1	295	PHA-L
5	95-34	E1 (+ PGZ)	304	PHA-L
6	94-49	δ	333	PHA-L
7	93-28	δ / PGZ	363	PHA-L
8	95-29	A3,4 (+PGZ)	400	PHA-L
9	95-30	B4	159	Biocytin
10	96-01p	D1,E1,δ	909	PHA-L
11	95-17	D1/δ	652	PHA-L
12	95-16	D3,E2	636	PHA-L
13	93-29	E 2,3 (+PGZ)	772	PHA-L
14	96-01b	E3,4	800	BDA
15	96-12	D6,7; E6,7	530	BDA
16	96-17	E5	368	PHA-L
17	96-04	DZ-lateral	272	PHA-L
18	95-13	DZ -lateral	287	PHA-L
19	94-48	DZ-medial	409	PHA-L
20	94-44	Oc2L	249	PHA-L
21	95-03	FrI	266	PHA-L

PGZ = perigranular zone; DZ = dysgranular zone (Chapin and Lin, 1984)

The results are presented in four sections according to the placement of the tracer in experimental animals:

Group A = injections into PMBSF only (11 cases: 2,3,4,6,9,10,11,12,14,15,16)

Group B = injections into PMBSF and the perigranular zone bordering it (5 cases: 1,5,7,8,13)

Group C = injections into dysgranular zones of SmI (3 cases : 17,18,19)

Group D = injections into other cortical areas (2 cases : 20,21)

Results of each case are presented as 2-part diagrams. The first part of each diagram shows the injection site reconstructed onto a drawing of a flattened cortical section, processed for cytochrome oxidase. The second part presents transverse sections through the pontine nuclei at intervals of 150 μm. Labelled fibres and terminals are marked at the appropriate levels. Nomenclature and cytoarchitectonic division of the pontine nuclei are adapted from Mihailoff et al.(1981 b) with a slight modification. In

order to aid more precise reconstruction I divided ventral peduncular nucleus into two parts: central and lateral. This decision was formed on the basis of the appearance of the termination patches in the pilot experiments.

Group A

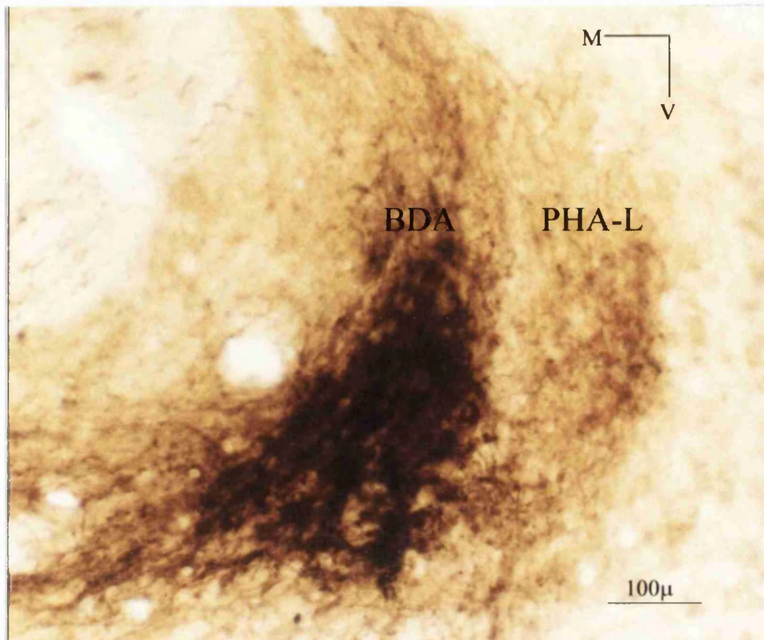
There are eleven cases in this group. The great majority of the pontine terminals are on the ipsilateral side. In three cases, however, the pontine label is bilateral (cases 14, 15 and 16). All the cortical barrel columns studied here project to the ipsilateral ventral peduncular nucleus. There are two patches of terminals within this nucleus, central and lateral, which are present in all of the cases. The rostro-caudal level at which these termination zones appear differ from one case to the next, but on average they start at about 800 μm from the rostral end of the pons. In four of the cases (4, 6, 10 and 11) the ventral peduncular nucleus is the only recipient of the cortical axons. Their common characteristic is that the injection sites were in the largest and most caudal cortical barrel columns: D1, E1 and δ.

In case number 2 the injection site involved large barrel columns B1 and C1 However, probably because of the involvment of the third barrel column (B2), termination zones in the ipsilateral pons include the dorsal as well as ventral peduncular nucleus. The same pontine nuclei receive cortical projections in cases 9 and 12 (barrel columns B4 and D3-E2, respectively). Barrel column C2 (case 3) projects to the ventral peduncular and medial pontine nucleus.

Labelled fibres and terminals first appear rostrally in the central patch of the ventral peduncular nucleus in a majority of the cases. Further caudally, the label occupies more lateral position within the ventral peduncular nucleus forming lateral ventral peduncular terminal patch. There is no simple pattern in the way the termination zones start or finish. The only obvious feature is that the terminals form rostro-caudal columns within the pontine nuclei which extend from 150-750 μm. There was some medio-lateral shift in the position of the terminal fields,depending on which barrels have been injected, but it was not possible to quantify this spatial gradients by comparison between cases. In one experimental animal I used two anterograde tracers in order to clarify this issue (cases 10 and 14). Figure I-8 shows the result of this double anterograde experiment. These cases show that the larger, more caudally situated cortical barrels project to more lateral positions within the ventral peduncular nucleus in both central and lateral terminal columns within this nucleus. This segregation is particularly clear in two sections where the fibres entering the pontine grey curve around the peduncle, forming the lateral ventral peduncular termination column (see figure I-1). This case also confirms that the smaller, more rostrally

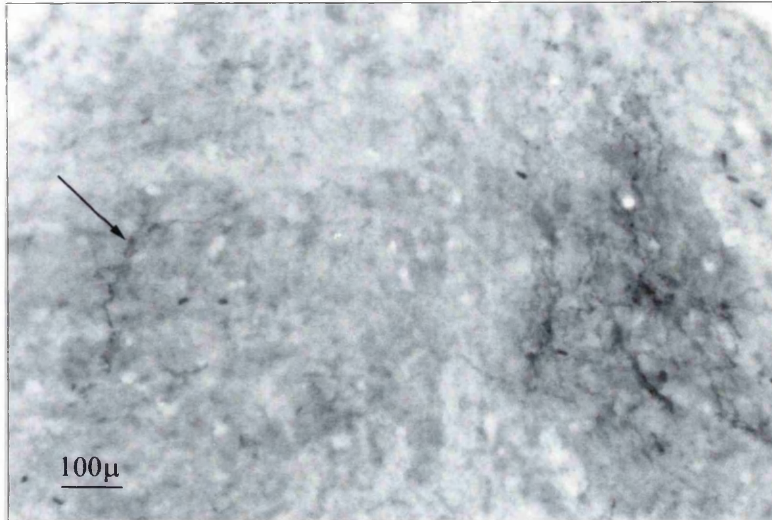
situated barrels project bilaterally to more patches in the pontine nuclei and their termination zones end further caudally.

Figure I-1: Photomicrograph of a section through the pontine nuclei in double anterograde tracing experiment. BDA (black) and PHA-L (light brown) labelled fibres in the lateral ventral peduncular nucleus at about 1.4 mm from the rostral end of the pons.



In three cases (14, 15, 16) that involved the most lateral cortical barrels (D 6-7 and E 3-7) there were labelled terminals in four or five pontine nuclei. At least two of the terminal patches are mirrored on the contralateral side. Nuclei receiving bilateral projections are: medial (case 14, 15), dorsal peduncular (case 14 and 15), ventral (case 16), dorsomedial (case 16) and ventral peduncular lateral (case 14) pontine nuclei. Fibres and terminals on the ipsilateral side are more heavily labelled than the fibres on contralateral side as illustrated in figure I-2.

Figure I-2: Bilaterally labelled terminals in the medial pontine nucleus in case 10 (14). Arrow points to the label on the contralateral side.



Figures I-3 to I-12 are diagrams of the cases in group A. Cases 10 and 14 are illustrated on the same diagram (Figure I-8).

All the diagrams are composed of two parts. The top part represents the PMBSF or primary somatosensory cortex. The bottom part is a series of sections through the pontine nuclei at 150 μm apart. Injection site and the terminal label are indicated in red stippling. The position and size of the area occupied by the dots in the pontine sections illustrates the position and the extent of the terminal labelling.

Abbreviations:

A-E, α,β,γ,δ = rows of mystacial whiskers

dM = dorsomedial pontine nucleus

DP = dorsal peduncular pontine nucleus

Lat. = lateral pontine nucleus

Med. = medial pontine nucleus

NRTP = Nucleus Reticularis Tegmenti Pontis

PED = pyramidal tract

VP (c, l, m) = ventral peduncular pontine nucleus (central, lateral and medial division)

Figure I-3: Diagram of the injection site and terminal pontine label in case number 2.

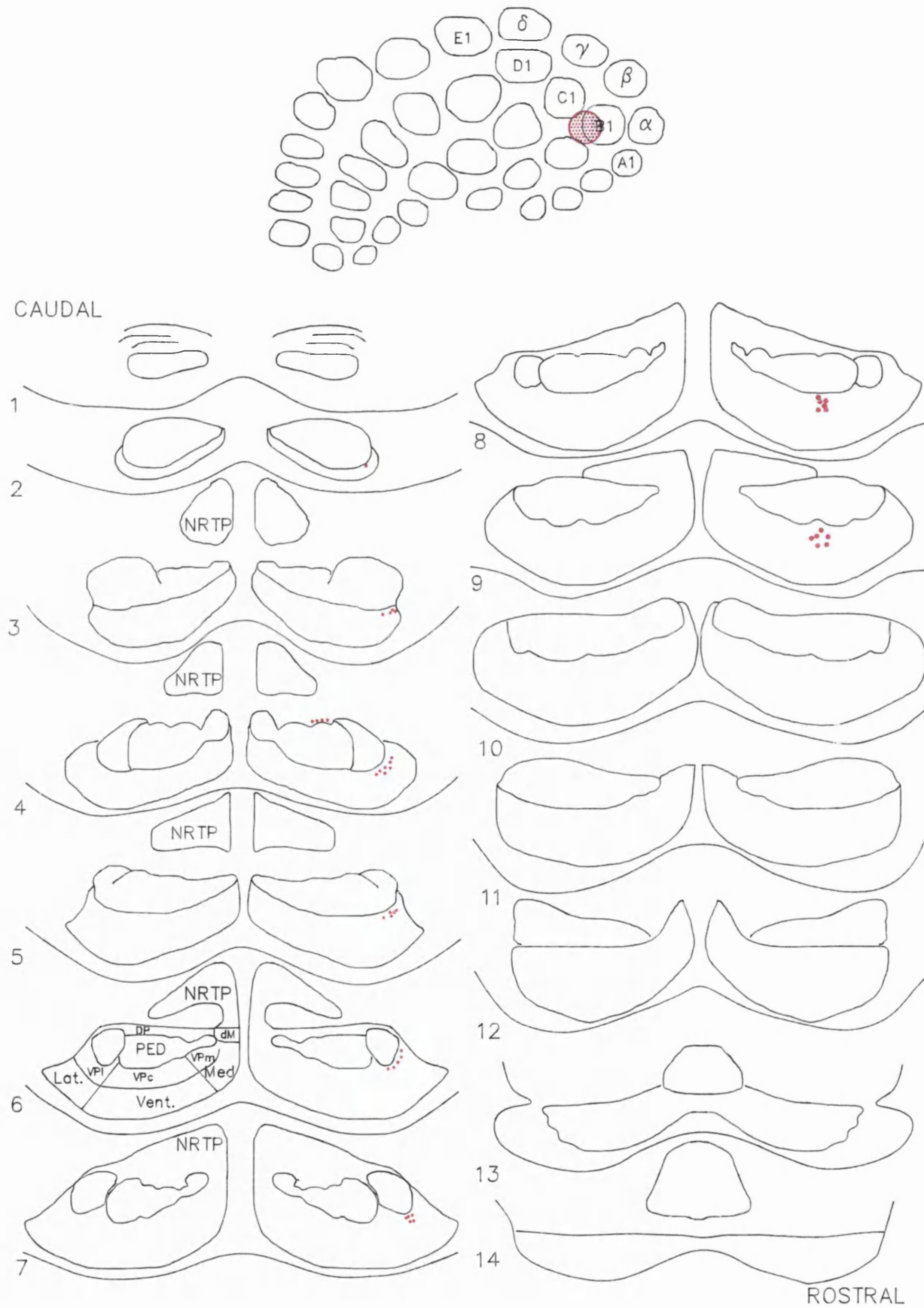


Figure I-4: Diagram of the injection site and terminal pontine label in case number 3.

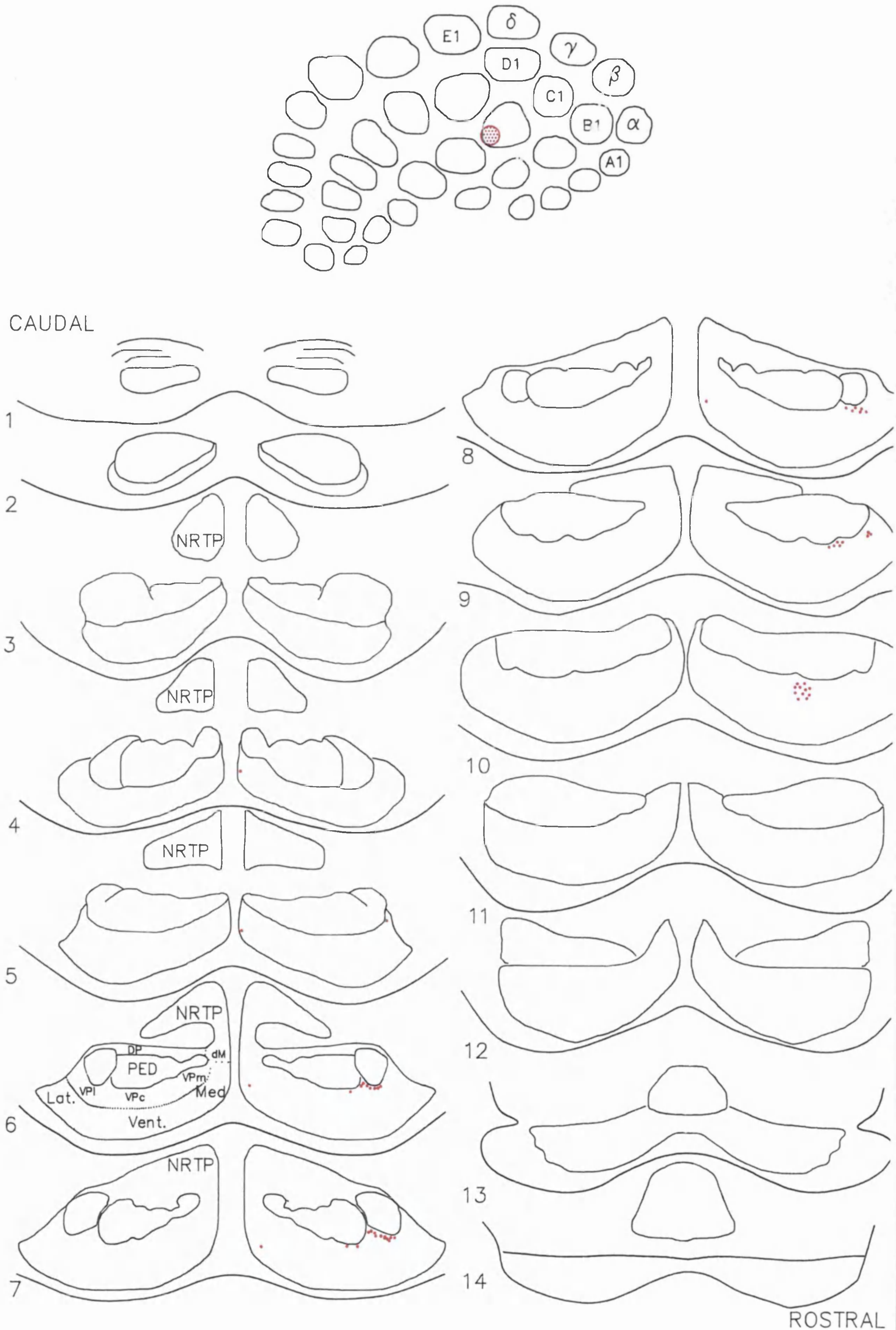


Figure I-5: Diagram of the injection site and terminal pontine label incase number 4.

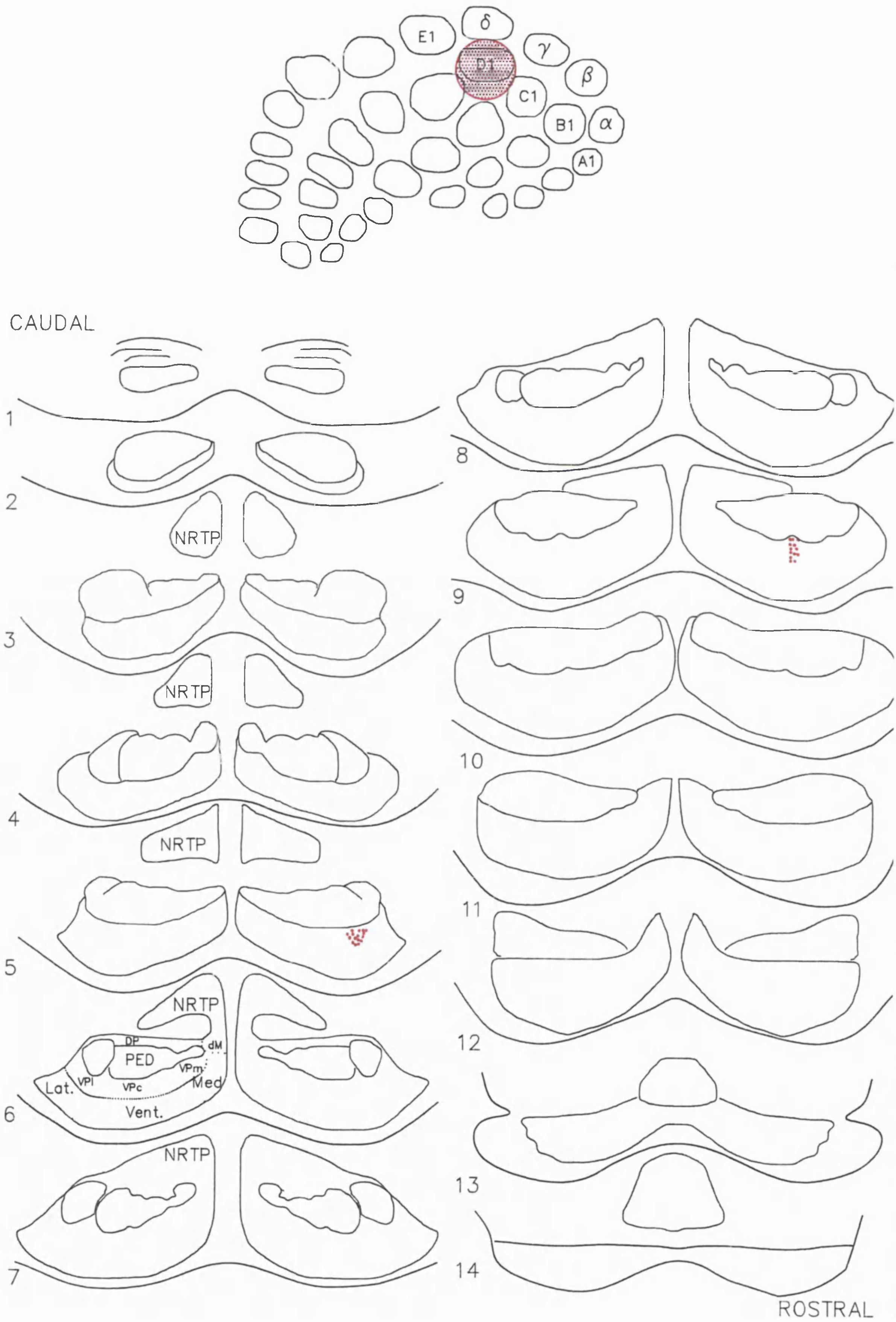


Figure I-6: Diagram of the injection site and terminal pontine label in case number 6.

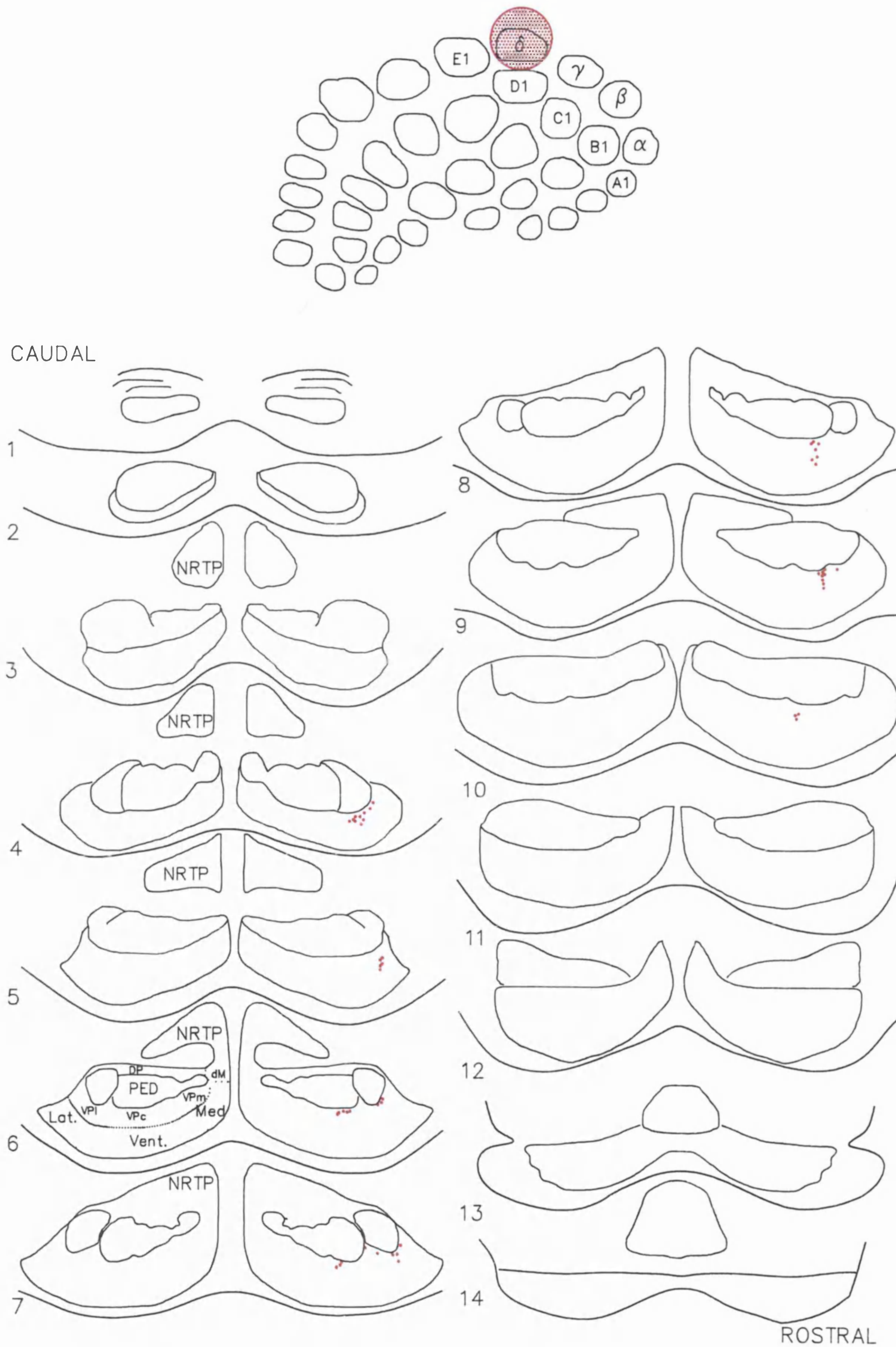
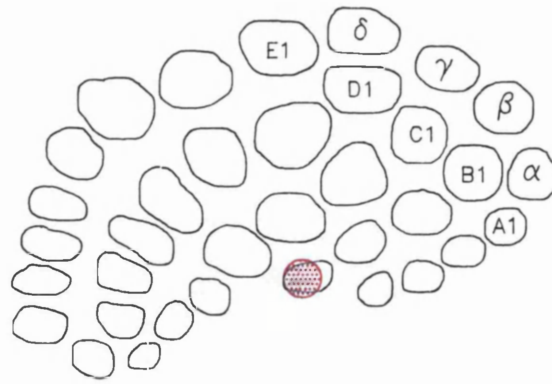


Figure I-7: Diagram of the injection site and terminal pontine label in case number 9.



CAUDAL

1
2
NRTP
3
NRTP
4
NRTP
5
NRTP
DP
PED
dM
VPm
VPl
Lat.
VPc
Med
Vent.
6
NRTP
7
8
9
10
11
12
13
14
ROSTRAL

Figure I-8: Combined diagram of the injection sites and terminal pontine label in cases number 10 and 14.

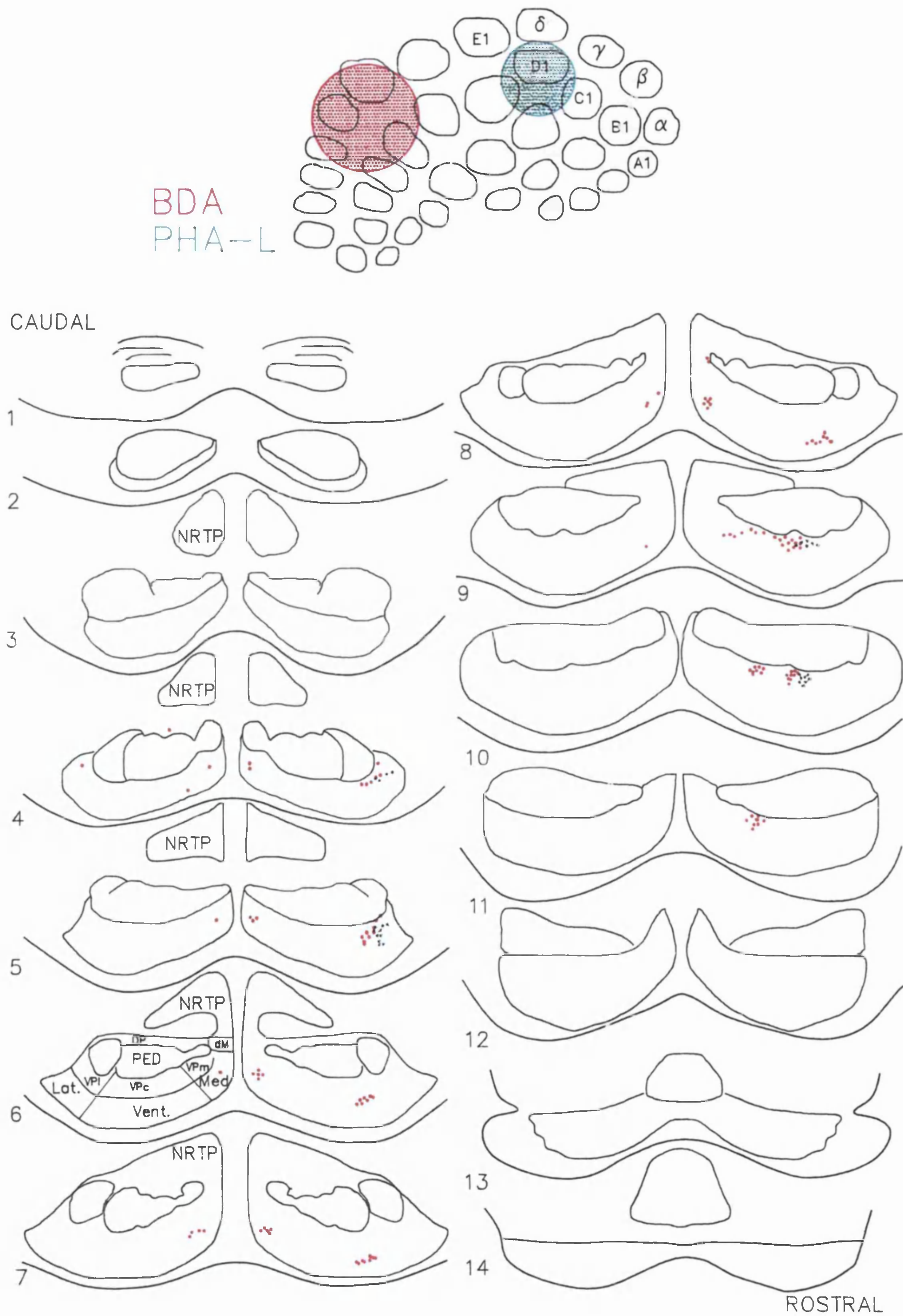


Figure I-9: Diagram of the injection site and terminal pontine labe in case number 11.

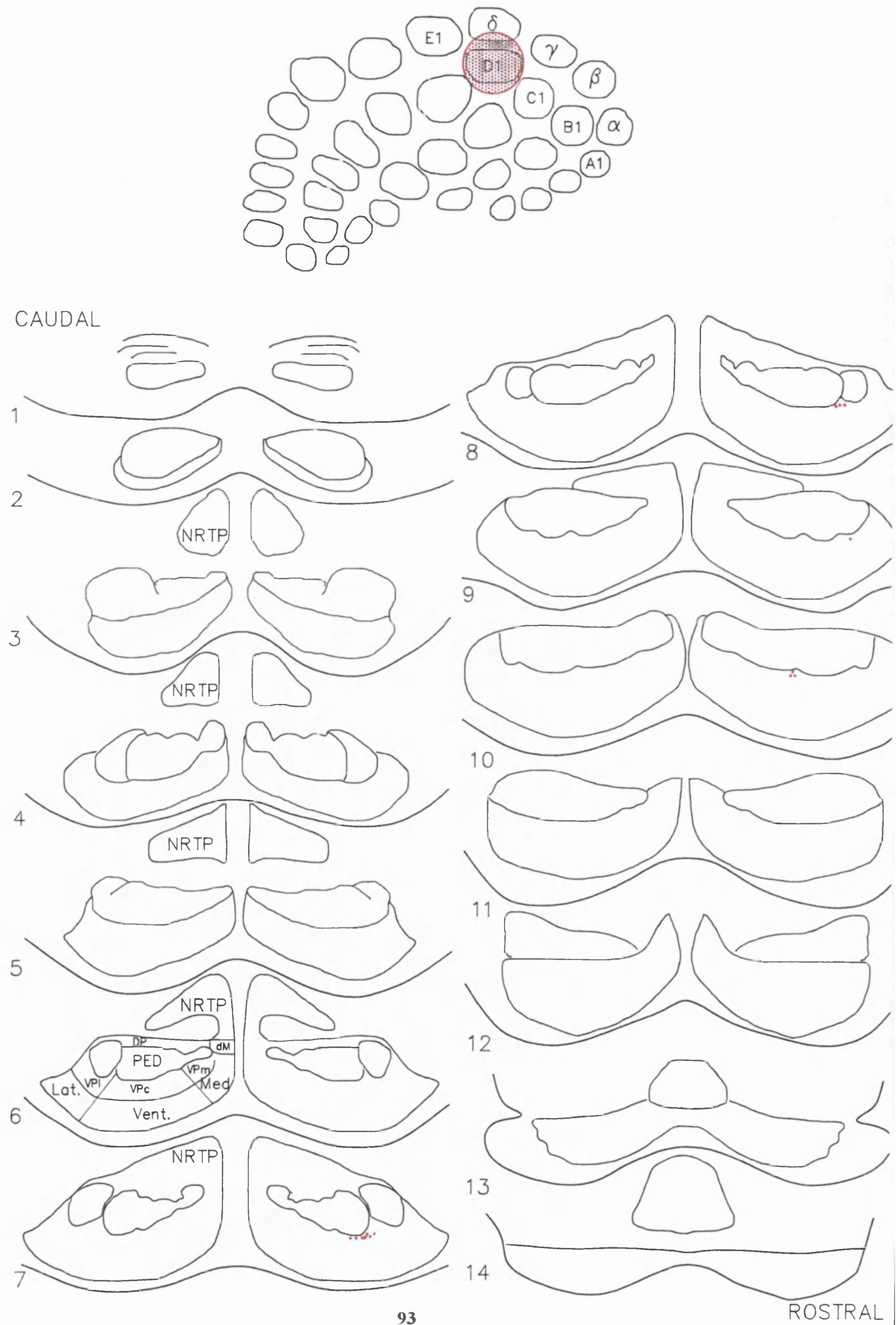


Figure I-10: Diagram of the injection site and terminal pontine label in case number 12.

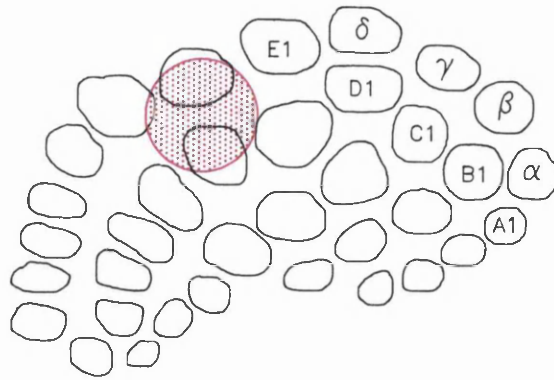


Figure I-11: Diagram of the injection site and terminal pontine label in case number 15.

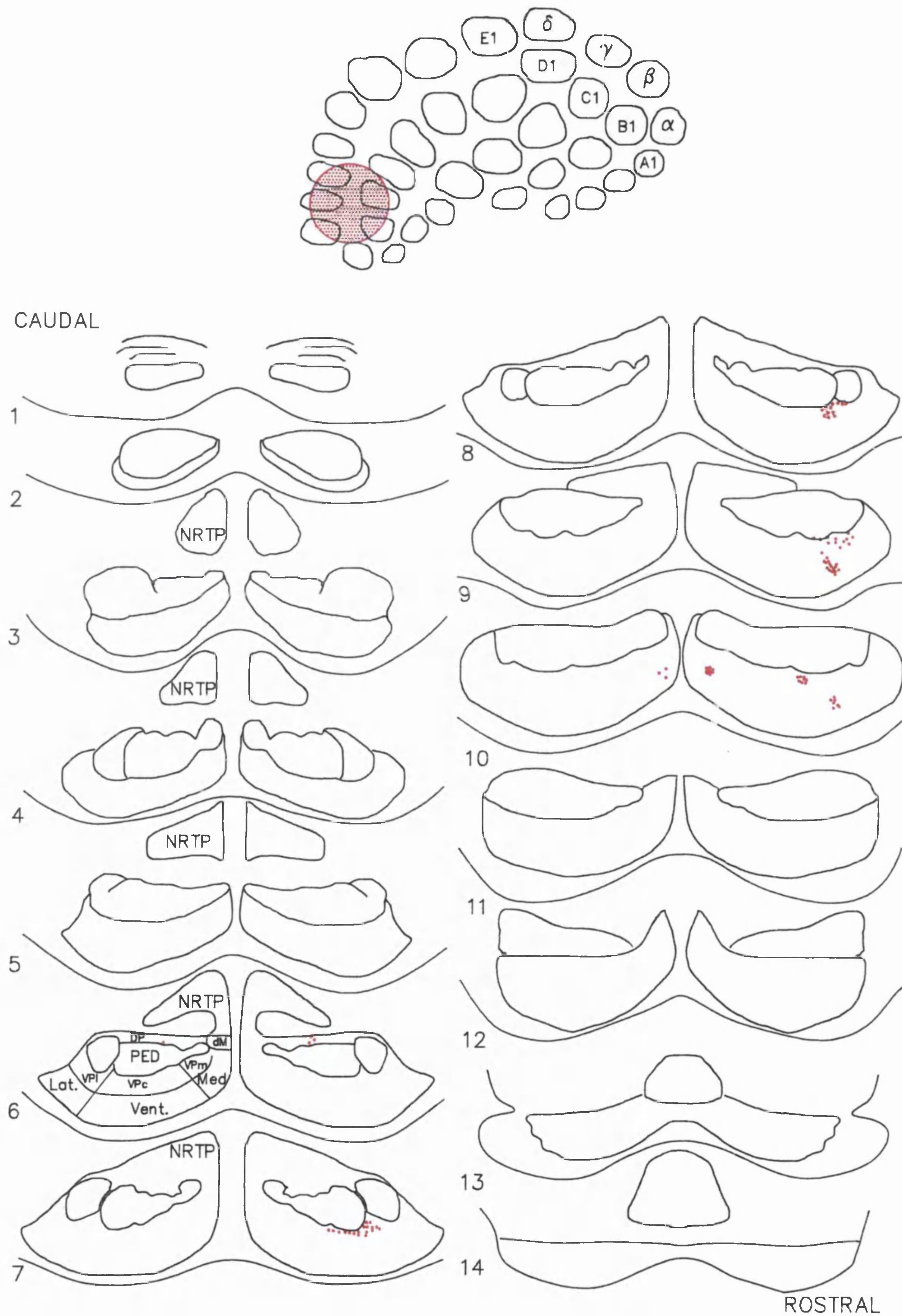


Figure I-12: Diagram of the injection site and terminal pontine labe in case number 16.

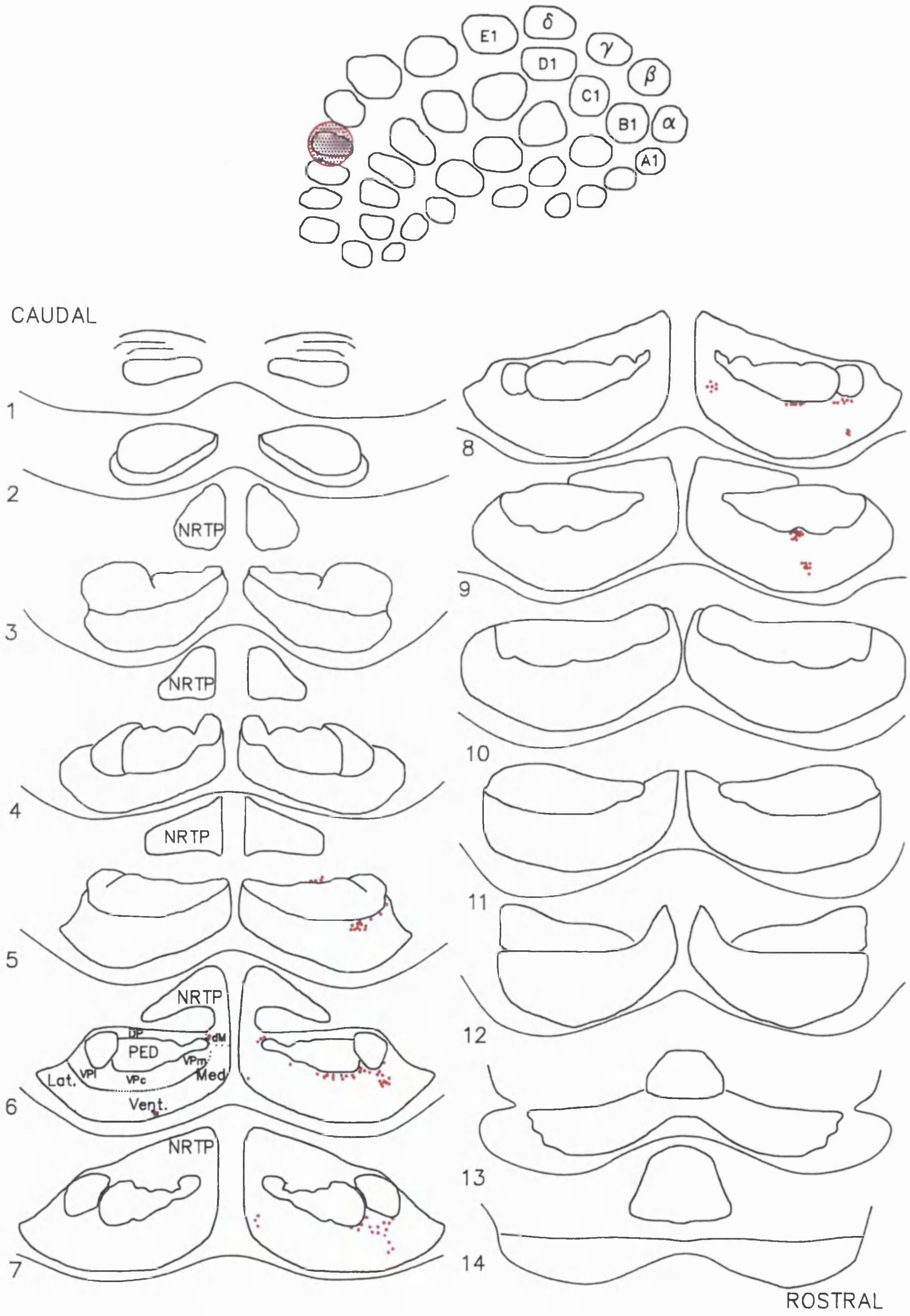


Table I-2: Summary of the results for the ceses in group A. List of pontine nuclei and levels at which labelled fibres and terminals are found.

Case number (injection site)	**Pontine nuclei in which label found**	**Level at which label found (μm from the rostral end of the pons)**	
		ipsilateral	**contralateral**
2 (B1,B2,C1)	ventral peduncular central	750-900	
	ventral peduncular lateral	1050-1800	
	dorsal peduncular	1500	
3 (C2)	ventral peduncular central	600	
	ventral peduncular lateral	750-1350	
	medial nucleus	900-1500	
4 (D1)	ventral peduncular central	750	
	ventral peduncular lateral	1350	
6 (δ)	ventral peduncular central	600-1200	
	ventral peduncular lateral	1200-1500	
9 (B4)	ventral peduncular central	1800	
	ventral peduncular lateral	1050-1200	
	dorsal peduncular	900-1050	
10 (D1, E1,δ)	ventral peduncular central	600-720	
	ventral peduncular lateral	1320-1440	
11 (D1/δ)	ventral peduncular central	600	
	ventral peduncular lateral	750-1500	
12 (D3, E2)	ventral peduncular central	750-900	
	ventral peduncular lateral	1050-1500	
	dorsal peduncular	1200	
14 (E3,4)	ventral peduncular central	720-840	
	ventral peduncular lateral	1320-1560	1560
	dorsal peduncular	1200-1680	1440
	medial	960-1440	840-1560
	ventral	960-1200	1560
15 (D6,7;E6,7)	ventral peduncular central	600	
	ventral peduncular lateral	600-1050	
	ventral	600-750	
	medial	600	600
	dorsal peduncular	1200	1200
16 (E5)	ventral peduncular central	750-900	
	ventral peduncular lateral	900-1350	
	ventral	750-1200	1200
	medial	900-1050	
	dorsomedial	1200	1200
	dorsal peduncular	1350	

Group B

There are five cases in this group. The tracers in these cases were injected into "outer" barrels of the PMBSF, belonging to rows A and E and spread from the barrels into the surrounding perigranular zone.

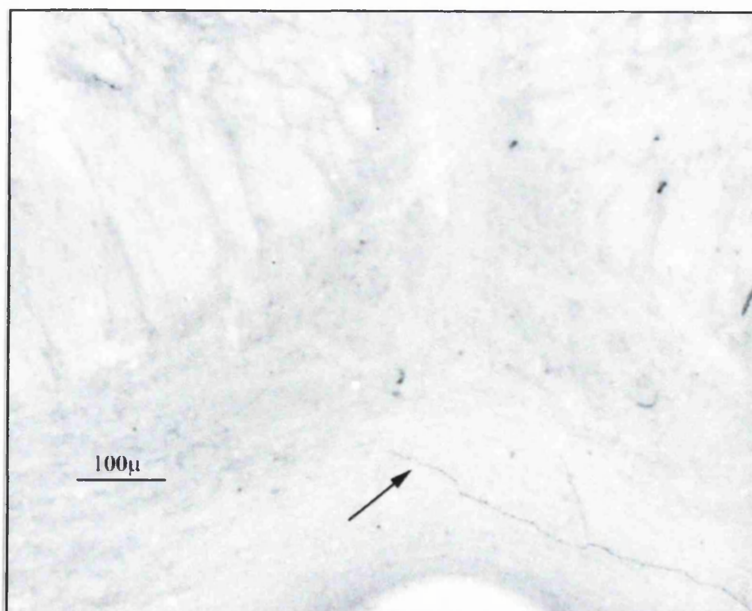
In four cases labelled fibres and terminals were found bilaterally in the pontine nuclei. Labelled fibres and terminals are found in the ipsilateral ventral peduncular nucleus in all the cases. In cases 1 and 5 terminals were also found in the ventral pontine nucleus. The medial pontine nucleus contains labelled fibres and terminals in cases 5, 8 and 13. Label in dorsal peduncular nucleus is found in cases 7 and 8.

Label in the contralateral pontine nuclei is present in the ventral nucleus (case 1), medial (case 5 and13), ventral peduncular (case 8) and dorsal peduncular nucleus (case 8).

Case number 7 (injection in δ/PGZ) differs from the rest in this group, because terminal label was restricted to the ipsilateral pontine nuclei.

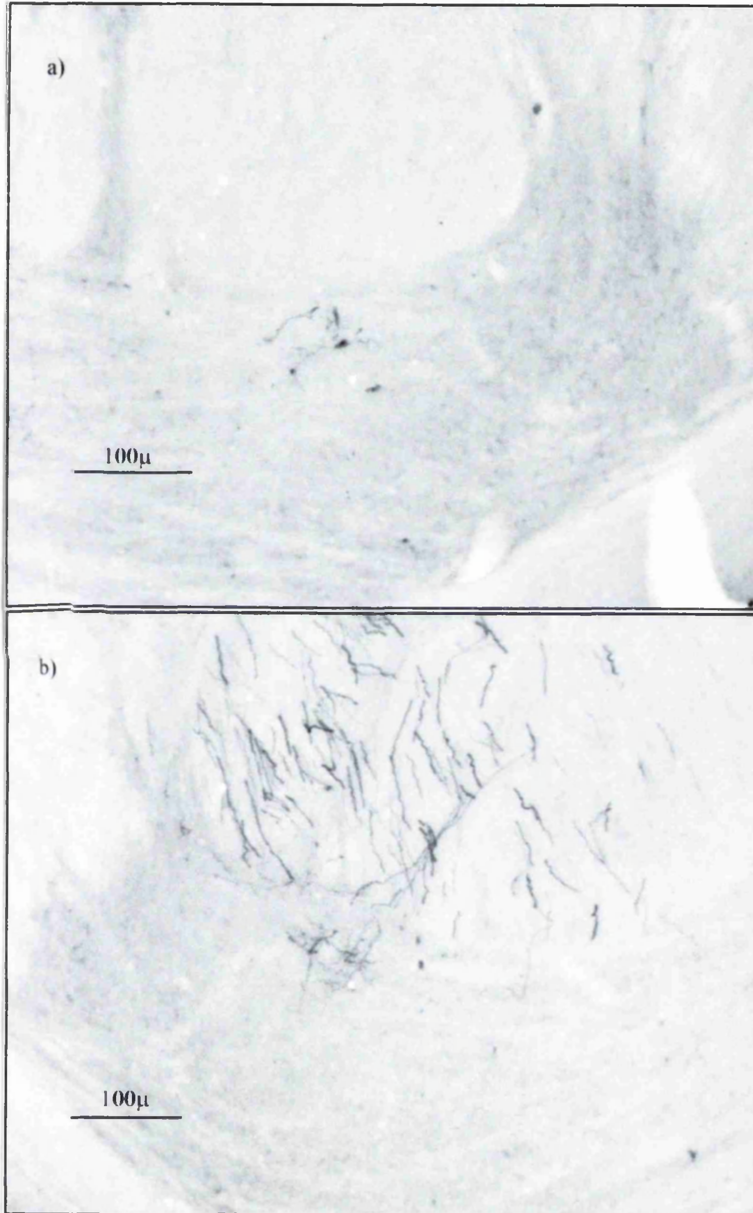
Fibres giving rise to the contralateral pontine terminals travel in the ipsilateral cerebral peduncle and cross the midline at the level of the pontine nuclei .

Figure I-13: Photomicrograph of a section through the pontine nuclei showing labelled fibre (arrow) traversing the ipsilateral pons on its way towards the contralateral side.



Terminal label in the contralateral pontine nuclei is sparse when compared to the dense ipsilateral terminals.

Figure I-14: Photomicrograph of the section through the pontine nuclei in case number 8 showing termination patches in a) contralateral and b) ipsilateral ventral peduncular nucleus. Note the difference in densities of the labelled fibres and terminals.



The following figures will illustrate each case in Group B.

Figure 15: Diagram of the injection site and terminal pontine label in case number 1.

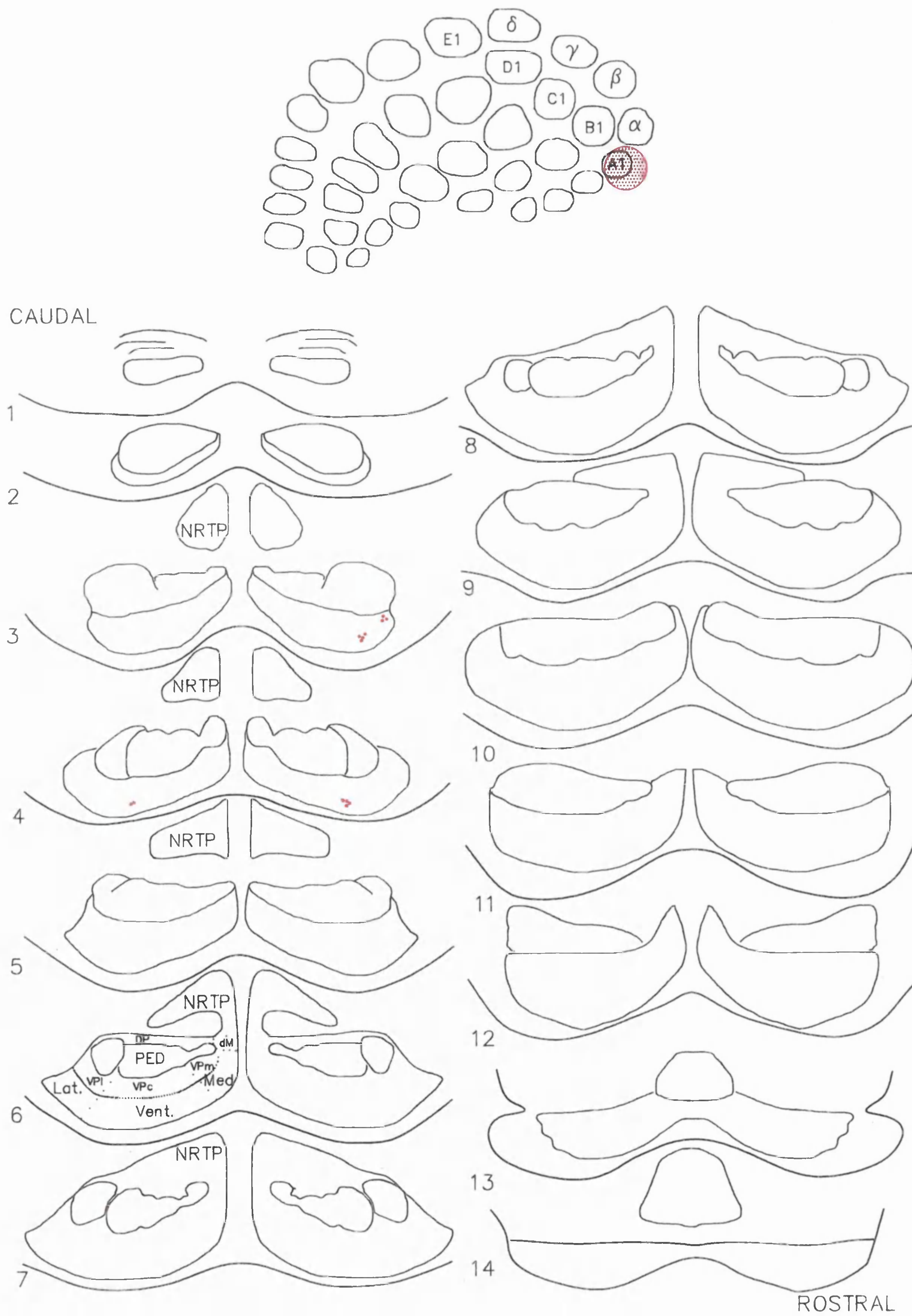


Figure I-16: Diagram of the inejction site and terminal pontine label in case number 5.

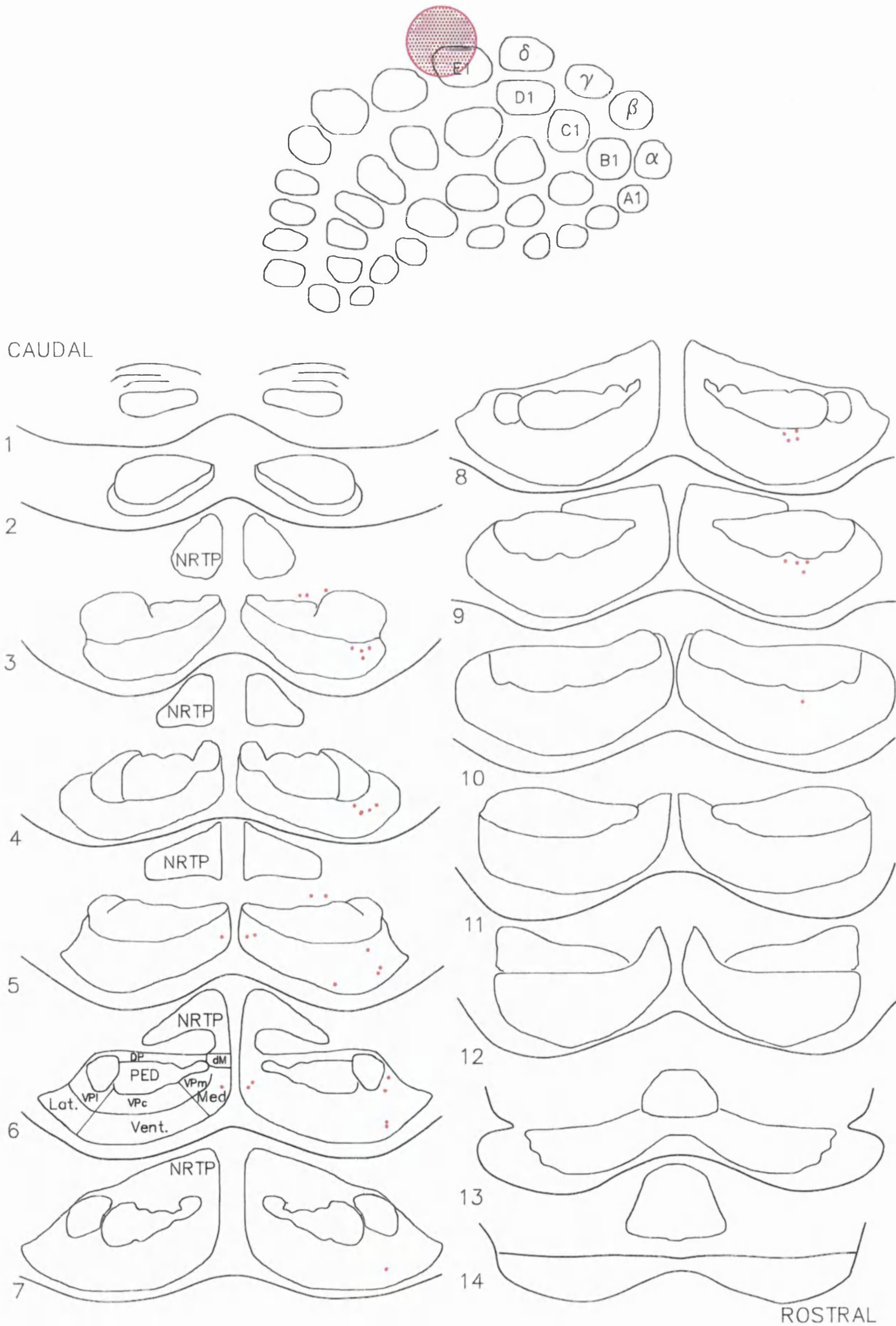


Figure I-17: Diagram of the injection site and terminal pontine label in case number 7.

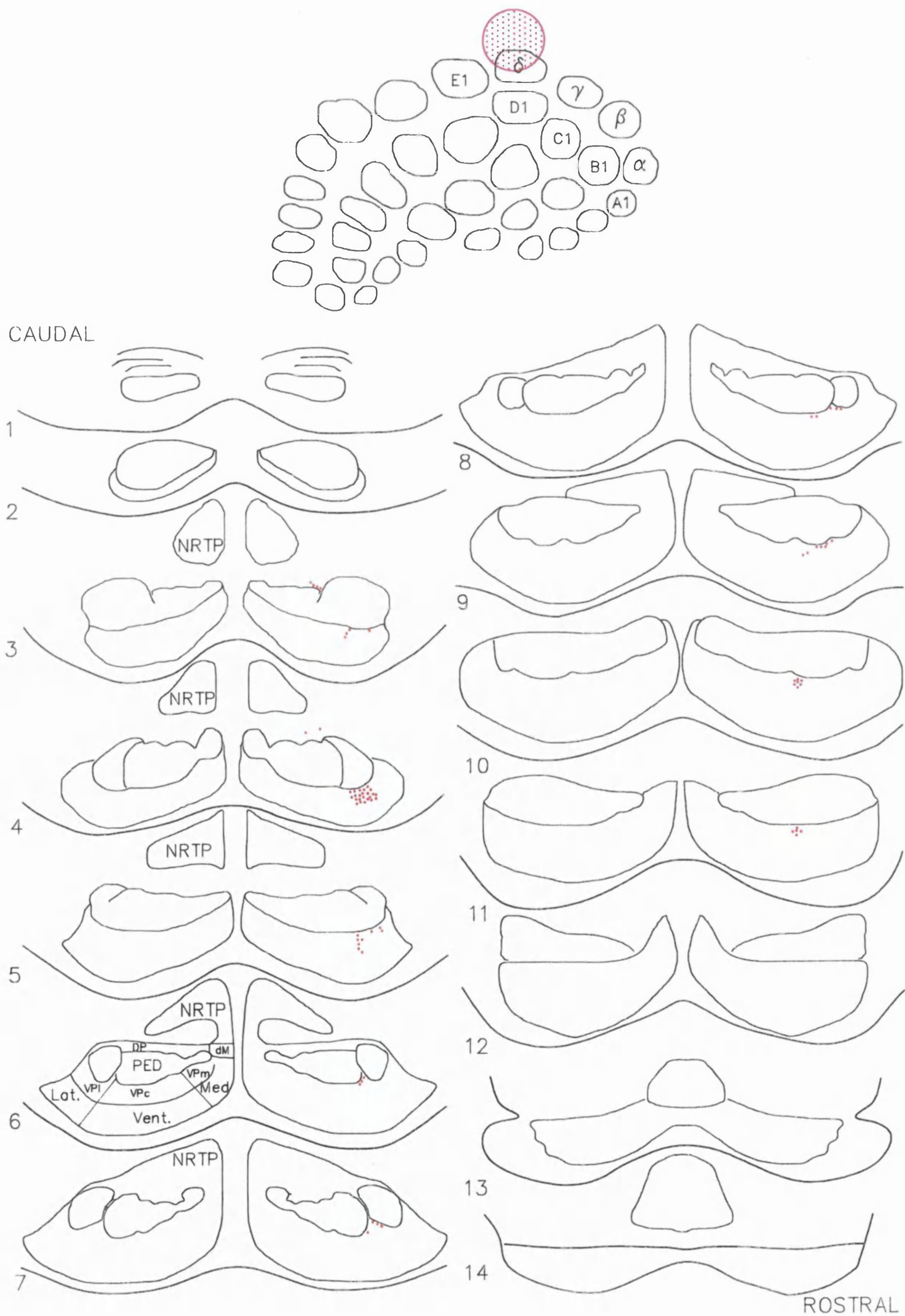


Figure I-18: Diagram of the injection site nad terminal pontine label in case number 8.

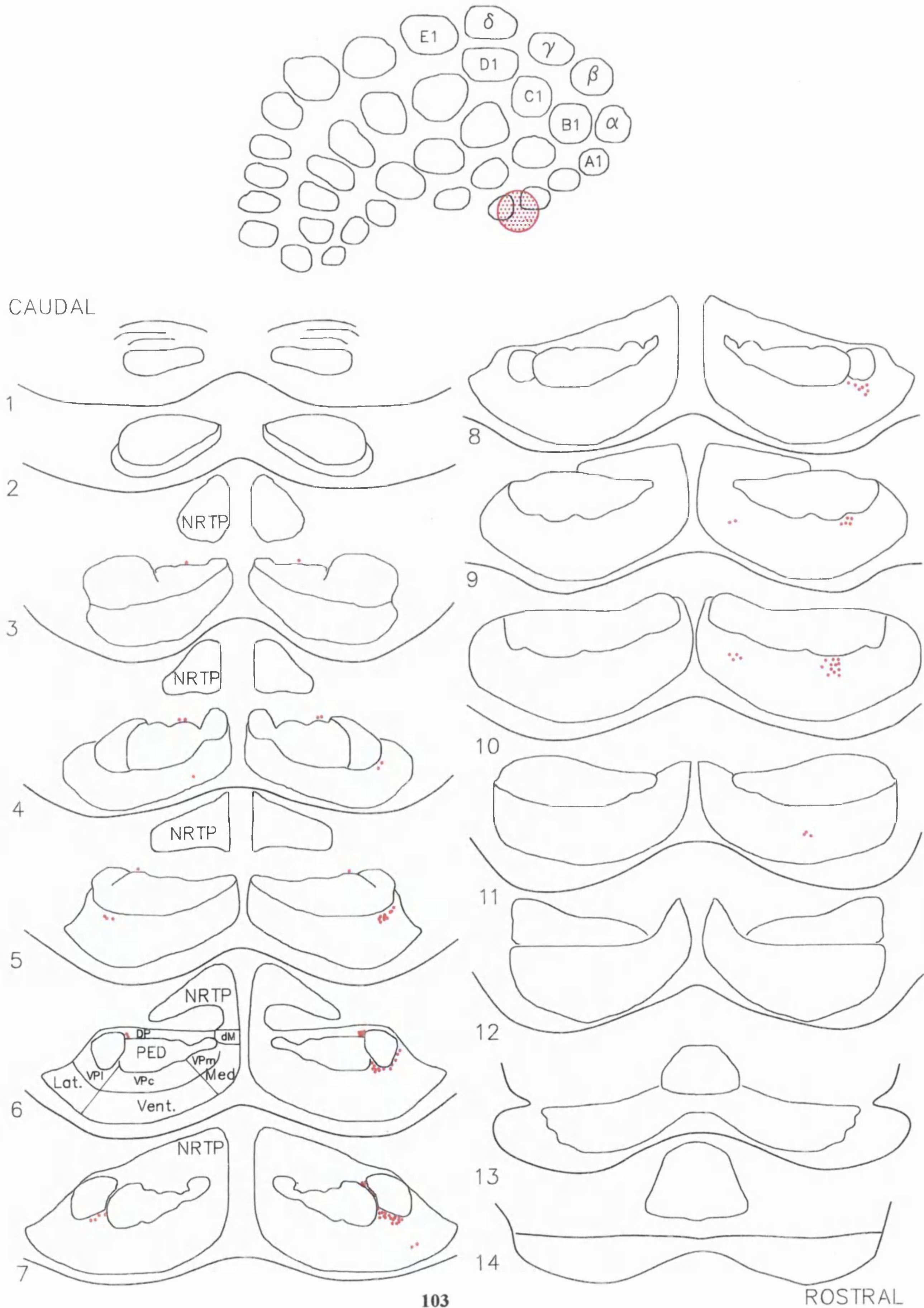


Figure I-19: Diagram of the injection site and terminal label in case number 13.

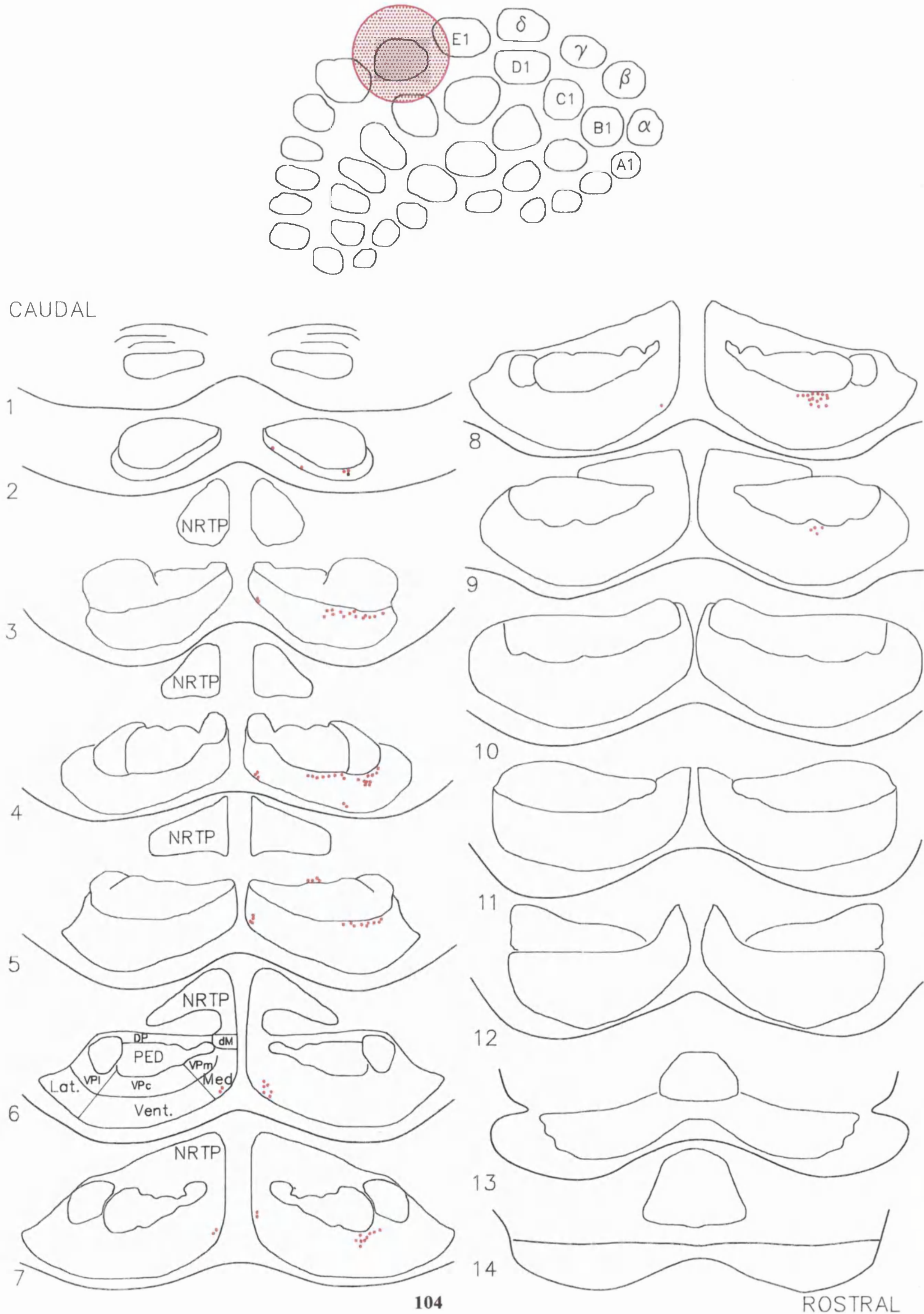


Table I-3: Summary of the results for cases in group B. List of pontine nuclei and levels at which the label was found.

Case number (injection site)	Pontine nuclei in which label found	Level at which label found (μm from the rostral end of pons) ipsilateral	contralateral
1 (A1/PGZ)	ventral peduncular lateral	1650	
	ventral	1500-1650	1500
5 (E1/PGZ)	ventral peduncular central	600-900	
	ventral peduncular lateral	1200-1650	
	dorsal peduncular	1350-1650	
	ventral	1050-1350	
	medial	1200-1350	1200-1350
7 (δ /PGZ)	ventral peduncular central	450-600	
	ventral peduncular lateral	750-1050, 1350-1650	
	dorsal peduncular	1500-1650	
8 (A3,4/PGZ)	ventral peduncular central	450-600	
	ventral peduncular lateral	750-1500	1050-1350
	dorsal peduncular	1200-1650	1200-1650
	medial nucleus	600-750	
13 (E2,3/PGZ)	ventral peduncular central	750-1050	
	ventral peduncular lateral	1350-1800	
	medial	1050-1800	900-1200

Group C

Cortical injections in this group involved only the dysgranular zones of the primary somatosensory cortex and in one case (17) the label spread to SII.

The terminal fields in this group are solely ipsilateral.

Both medial and lateral dysgranular zones of the SI projects to ventral peduncular and dorsal peduncular nuclei. The area on the border between SI and SII projects to three nuclei: ventral peduncular (lateral part of it only), lateral and ventral nucleus. Most of the terminals form columns, as in the previous cases.

Summary of the results for this group of experiments is given in the table below.

TABLE I-4 List of pontine nuclei and levels at which the label is found for Group C

Case number (injection site)	**Pontine nuclei in which label found**	**Level at which label found (μm from the rostral end of the pons) ipsilateral**
17 (border SI/SII)	ventral peduncular lateral	1350-1500
	lateral	1200, 1650
	ventral	1650
18 (DZ -lateral)	ventral peduncular central	600-750
	ventral peduncular lateral	1350-1500
	dorsal peduncular	900
19 (DZ -medial)	ventral peduncular central	450-750
	ventral peduncular lateral	750-1350
	dorsal peduncular	900-1200

The following diagrams will illustrate cortical injections and terminal label in each of the cases in Group C.

Figure I-20: Diagram of the injection site and terminal pontine label in case number 17.

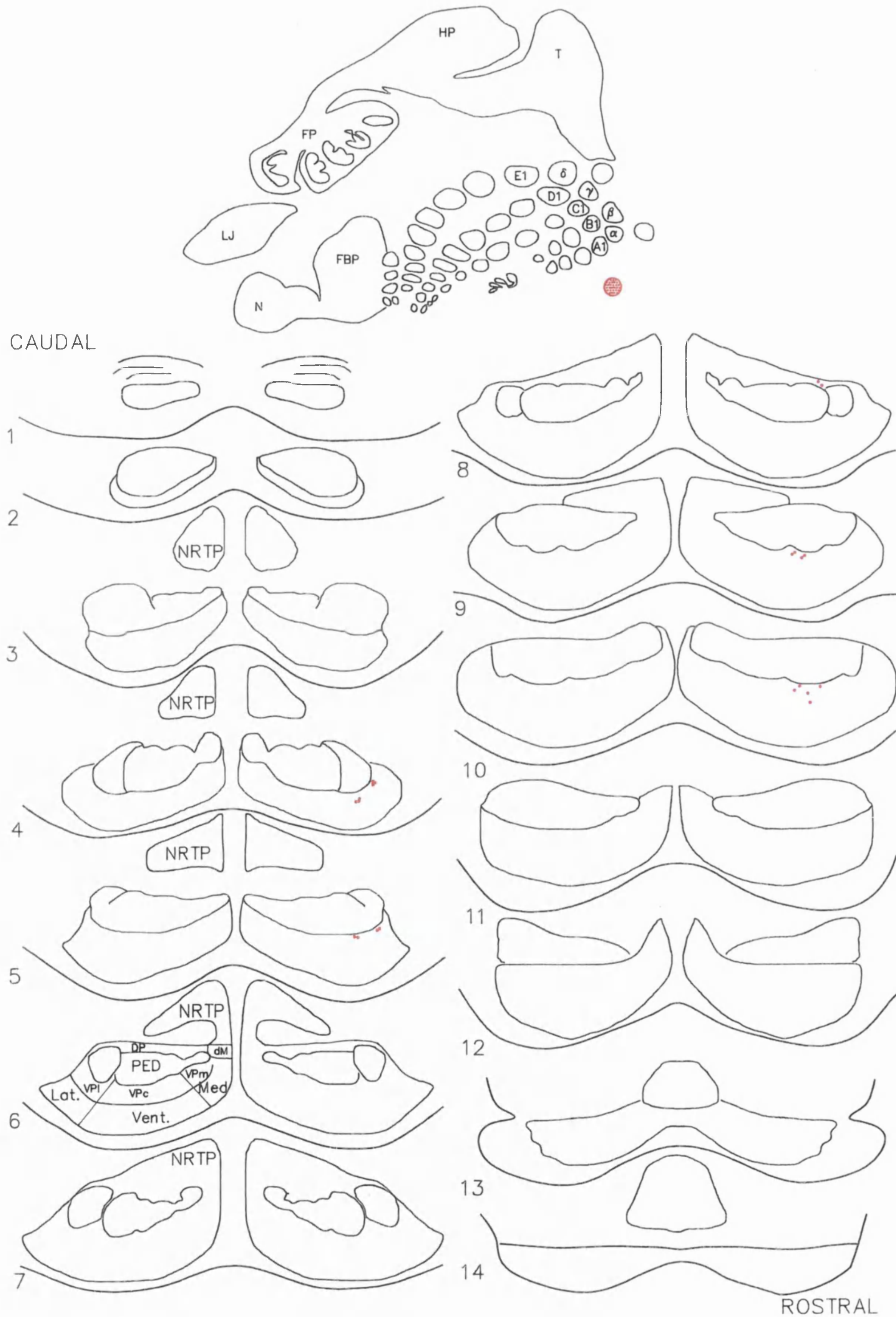


Figure I-21: Diagram of the injection site and terminal pontine label in case number 18.

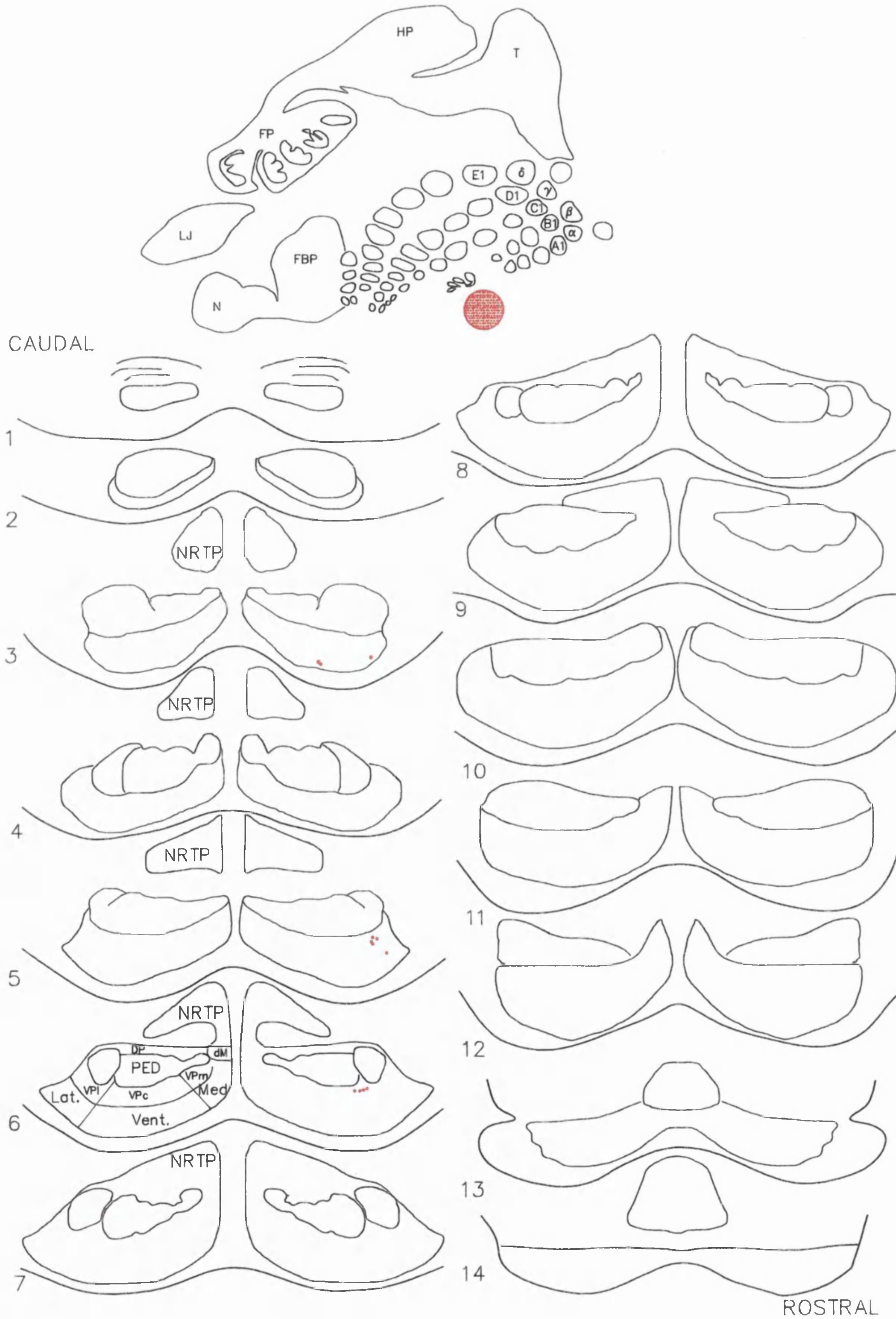
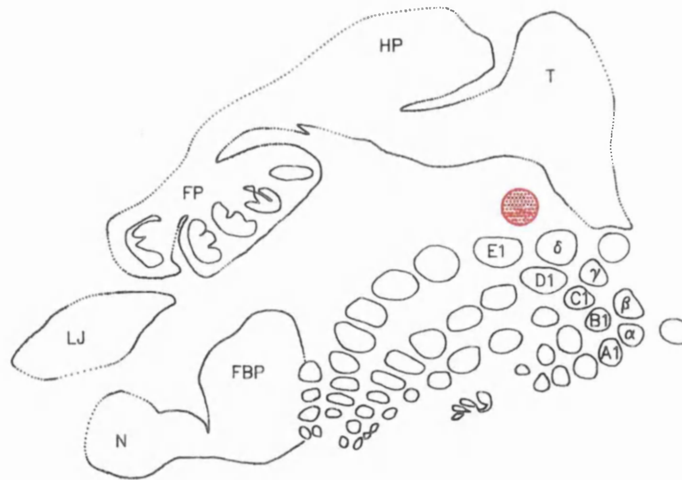


Figure I-22: Diagram of the injection site and terminal pontine label in case number 19.



CAUDAL

1

2

NRTP

3

NRTP

4

NRTP

5

NRTP

DP
PED
dM
VPm
VPl
VPc
Lat.
Med
Vent.

6

NRTP

7

8

9

10

11

12

13

14

ROSTRAL

Group D

There are two cases in this group. In one case I injected a visual area of the neocortex , called Oc2L in the Paxinos and Watson's atlas (1982), and in the other I injected primary motor cortex.

The results differ in two respects:

1) Oc2L (20) projects only to ipsilateral pons, whereas FrI (21) projects bilaterally

2) Oc2L projects only to the lateral part of the ventral pontine nucleus.

FrI projects to ventral peduncular, ventral and medial pontine nuclei. Projection to medial pontine nucleus is bilateral.

TABLE I-5: Summary of the results for Group D.

Case number (injection site)	**Pontine nuclei in which label found**	**Level at which label found (µm from the rostral end of pons) ipsilateral contralateral**
20 (Oc2L)	ventral (lateral)	450-1050
21 (FrI)	medial	1050-1200 1050
	ventral nucleus (lateral)	300-900
	ventral peduncular central	600-750
	ventral peduncular medial	450-600

The following two figures will illustrate each of these cases.

Figure I-23: Diagram of the injection site and pontine terminal patches in case number 20.

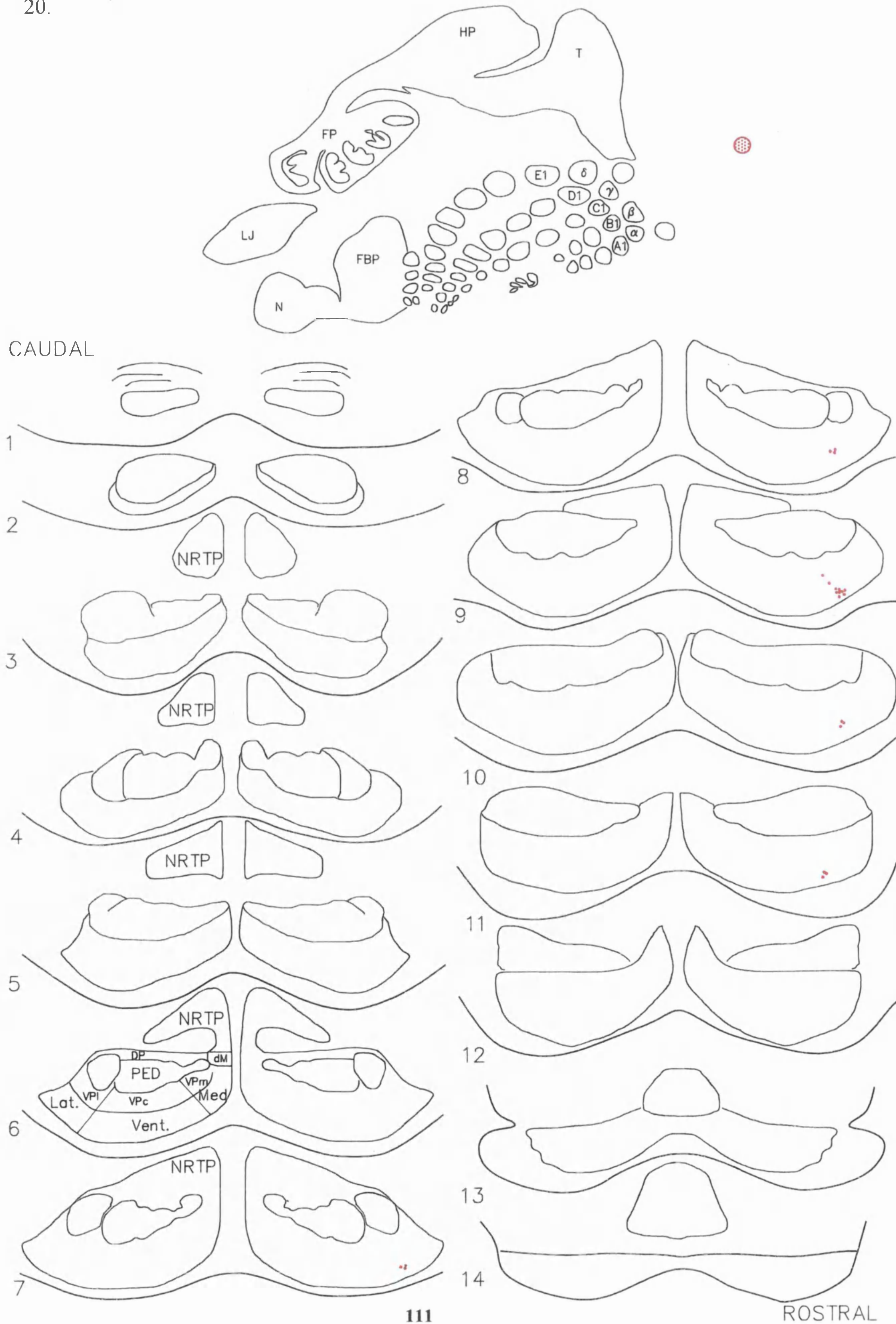
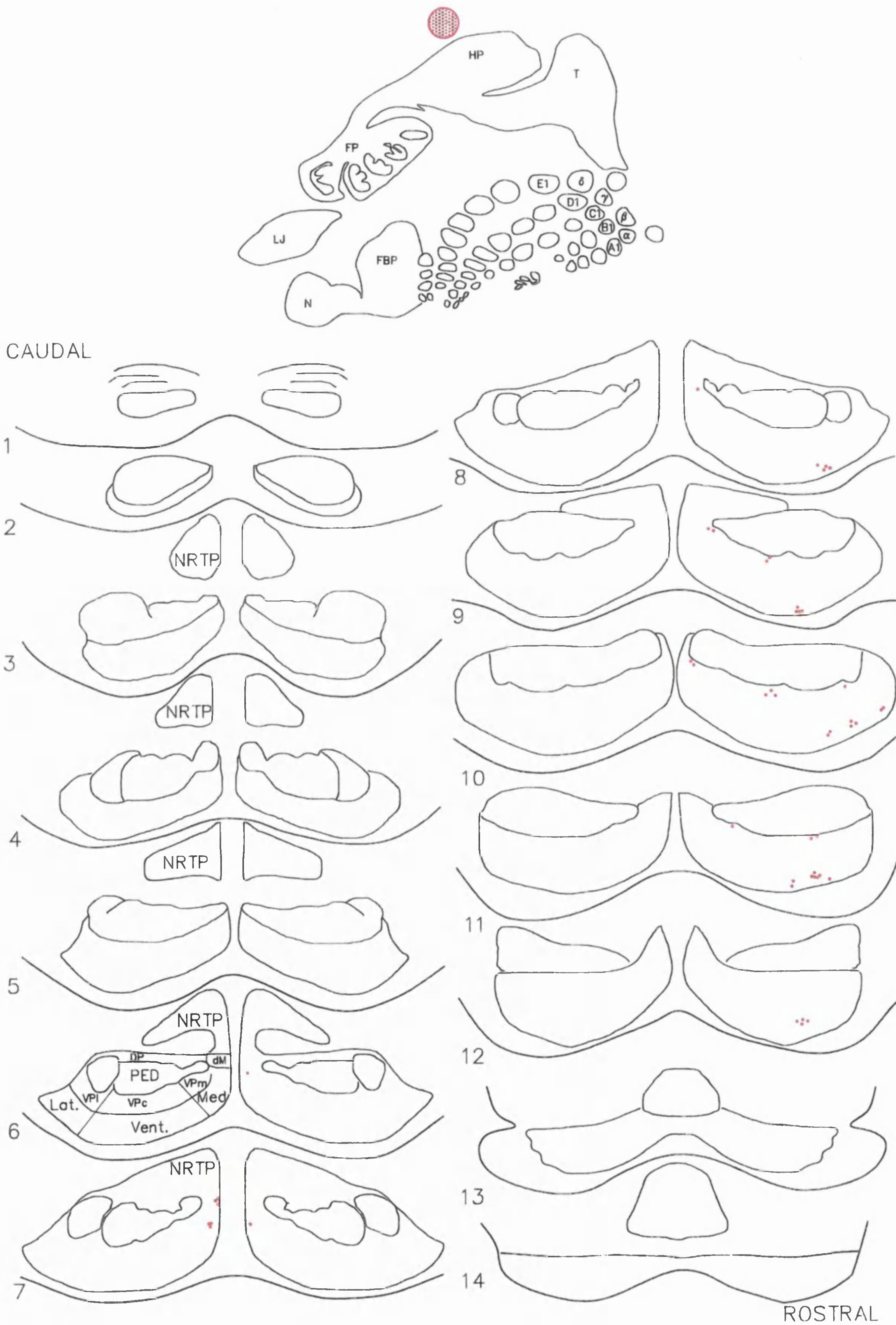


Figure I-24: Diagram of the injection site and terminal label in pons in case number 21.



Other Efferent Targets

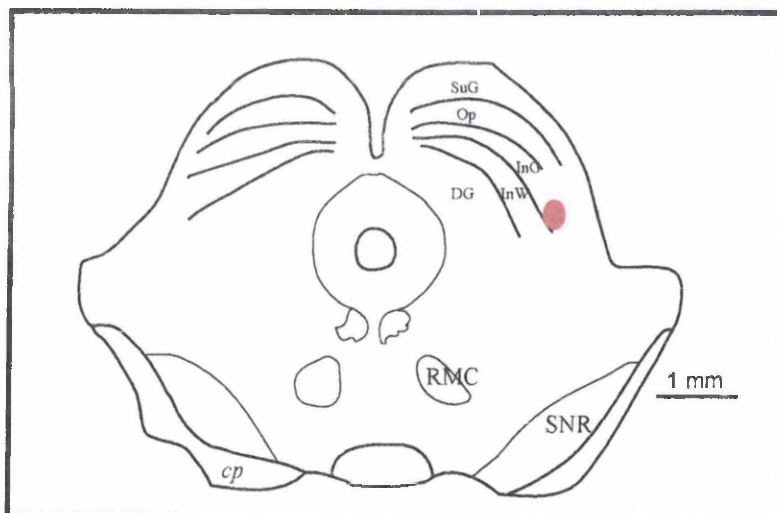
In addition to the pontine projections, PMBSF projects to a number of other subcortical structures. Here I present only a brief account of the projections to the ipsilateral colliculus and thalamus.

1) Projections to the superior colliculus

Terminal label was present in the intermediate and deep grey layer of the superior colliculus in all of the cases. The label was located mainly in the lateral part of the rostral superior colliculus. Labelled fibres and terminals appeared thinner and more faintly labelled than those in the pontine nuclei.

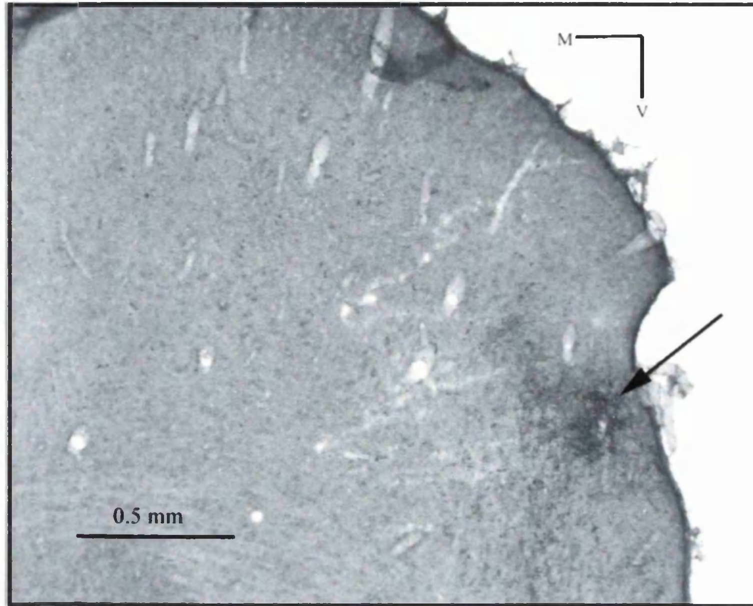
Figure I-25:

a) A diagram of the section through the superior colliculus in case number 10 with labelled terminal patch.



Abbreviations:
SuG = superior grey layer
Op = layer of the optic tract
InG = intermediate grey layer
InW = intermediate white layer
DG = deep grey layer
RMC = red nucleus, magnocellular
SNR = Substantia Nigra Reticularis
cp = cerebral peduncle

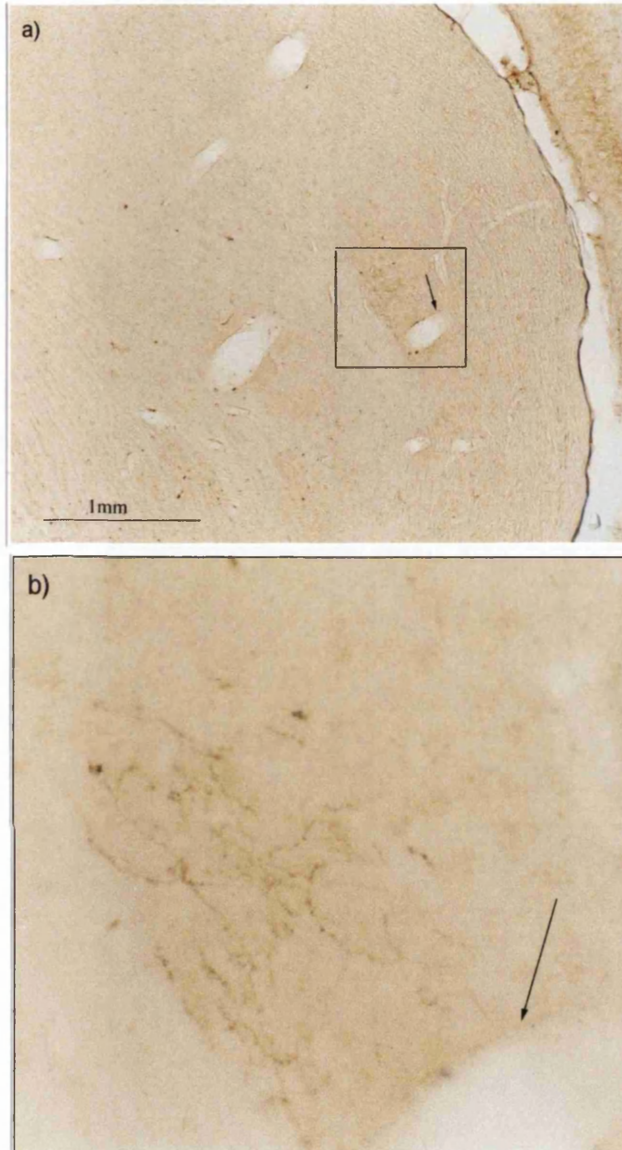
b) Photomicrograph of the unstained section through the superior colliculus at the same level.



2) Thalamic terminals

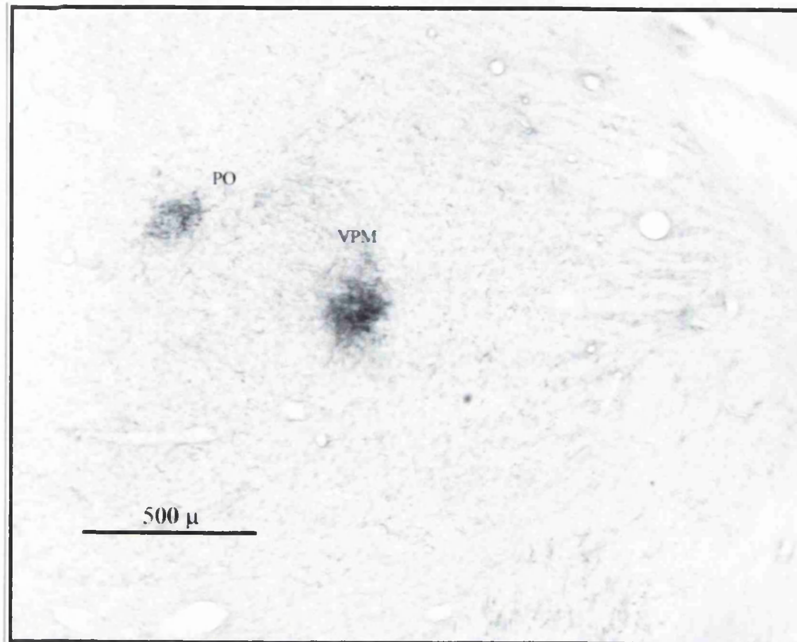
Thalamic terminals were present in all the cases. The label was found in VPM, Po and Rt thalamic nuclei in all the cases when the injections were placed in the primary somatosensory cortex. In one case where the injection site was placed in the visual cortical area (Oc2L, case number 20) DLG nucleus was labelled (see figure I-26).

Figure I-26 : Terminal label in dorsolateral geniculate nucleus. Photograph in b) is a higher resolution image of the area enclosed in a). Arrows point to the same blood vessel in each of the images.



Labelled fibres travel through the reticular nucleus to the VPM and Po. Both of these nuclei received cortical projections in all of the cases. Some terminals were also found in the reticular nucleus, but dense plexuses of terminal arborisation are characteristic for VPM and Po.

Figure I-27: Labelled fibres and terminals in VPM and Po in case number 15.



SUMMARY

Cortical barrel columns representing large mystacial vibrissae project to the ipsilateral pontine nuclei. Terminal arborisations are distributed in the caudal 2/3 of pontine nuclei. The pattern of termination can be described as patchy although most of the patches are aligned rostrocaudally, thus forming columns of up to 750 μm in length. There are 5 columns in the ipsilateral pons. Two of the columns are contained within the boundaries of the ventral peduncular nucleus: one is in the central part of the nucleus, the other at its lateral edge. Other columns are in the dorsal peduncular, medial and ventral pontine nuclei.

The ventral peduncular nucleus may be considered as the main "whisker" area of the pontine nuclei because it receives projections from all of the barrel columns in this study. Other pontine nuclei (medial, ventral and dorsal peduncular) receive projections from barrel columns but not as consistently as the ventral peduncular nucleus.

The largest cortical barrel columns, representing whiskers B1, C1, D1, E1and δ, project only to the ventral peduncular nucleus. All other barrel columns project additionally to one or more other nuclei: dorsal peduncular (cases 2, 9, 12, 15, 16), medial nucleus (cases 3, 14, 15, 16), ventral (cases 15, 16) and dorsomedial (case16).

Corticopontine termination zones start in the central ventral peduncular nucleus. Further caudally they shift laterally to form a terminal field at the lateral extreme of the ventral peduncular nucleus, curving around the peduncle fibres as they course through

the pons. Terminal patches in other nuclei are formed at various rostrocaudal distances as presented in tables.

Projections from different barrel columns overlap in the same pontine nuclei.

However, experiments with double anterograde labelling (96-01), shows that the more medial and caudal PMBSF barrel columns project to more lateral areas of the ventral peduncular nucleus.

Large PMBSF barrel columns project only to the ipsilateral pons. Contralateral projections are found :

a) in cases where the injection sites involved most lateral barrels within PMBSF with order number of 4 or greater (group A: 14, 15, 16);

b) in cases where the injection labelled neurones in the perigranular zone of the primary somatosensory cortex bordering the PMBSF (group B).

Labelled terminals on the contralateral side in these cases are found in at least one of the nuclei, but very often they are mirror images of the label on the ipsilateral side.

Fibres terminating in the contralateral pons travel in the ipsilateral peduncle and cross the midline at the level of pontine nuclei.

Corticopontine terminals are topographically organised so that the more rostral cortical areas project more medially within the pontine nuclei (i.e. cases 18 and 19; motor cortex vs. visual area Oc2L). Similarly, more rostral cortical barrels within the SI project more medially within the pontine nuclei and more lateral barrels project further caudally.

DISCUSSION

The major finding of this study is that there are differences in the corticopontine termination pattern between small parts of the primary somatosensory cortex in the rat. Layer V cells within the granular areas representing the largest facial whiskers and the surrounding perigranular zones within the PMBSF project only to the ipsilateral pontine nuclei. The main recipient of the cortical axons in this case is the ventral peduncular nucleus. Small barrel columns and the surrounding perigranular areas project bilaterally. Their terminals are found in ventral peduncular, dorsal peduncular, ventral and medial pontine nuclei. Perigranular zones bordering the PMBSF (parallel to barrel rows A and E) project bilaterally to the pontine nuclei in the same fashion as the smaller barrels. Dysgranular areas within the SI project to the ipsilateral pons only, largely to the ventral peduncular nucleus.

These anatomical findings will be discussed in relation to two electrophysiological studies of the primary somatosensory cortex. In the first study, Chapin and Lin (1984) defined the cytoarchitectonic zones within the rat SI cortex (see figure I-28). They described granular, perigranular and dysgranular zones and showed that the cells in each of these subdivisions have different response properties. Cells in the granular zones of the rat SI have small, well defined cutaneous receptive fields. Cells in the perigranular areas (among the PMBSF barrels) have larger receptive fields and typically respond to stimulation of several whiskers. Cells in the main dysgranular zone (about 300 μm medial to the whisker field) were completely unresponsive. The above description of the receptive fields was obtained in animals that were tested under barbiturates or Halothane anaesthesia and the peripheral stimulation was achieved with hand held probes. When more stringent conditions for stimulus and response properties were applied, as in a study by Armstrong-James and Fox (1987) in rats under light urethane anaesthesia, the results are osmewhat different. For example, cells in the granular layer of a barrel have similar receptive field sizes to the cells in the granular layer of interbarrel (septal) regions. The receptive fields in the granular barrel areas (at the lowest levels of responsiveness) involved on average 5.4 whiskers and in the septal areas 6.2. At the same level of responsiveness, the infragranular layers had even larger receptive fields encompassing 7.6 whiskers. The "centre receptive fields" for cells in granular barrel areas were, on average, slightly larger than a single whisker; for

granular septal areas the average was about 1.5 whiskers and for the cells in the infragranular layers more than 2.5 whiskers. The average "surround receptive fields" in granular barrel and septal areas were formed by 2.5 whiskers and in the infragranular layers by about 3.5 whiskers. These results agree more with the data Chapin and Lin (1984) obtained when recording from the awake animals. To summarise, they found that the cutaneous receptive fields were "larger, more complex and more volatile". Also, "granular zones exhibited predominantly cutaneous properties, whereas in dysgranular and perigranular zones a combination of joint and cutaneous receptive fields were found".

Despite these differences, the overall map of the body representation in the rat SI (figure I-28), as defined in the anaesthetised animals, still applies.

Nussbaumer and Van der Loos (1985) described the response properties of the cells in the primary somatosensory cortex in mice. The authors found two strips of cortex on the anterior and posterior border of the PMBSF in which cells responded both to cutaneous stimulation of the whiskers as well as the furry skin surrounding them. These two strips of cortex are probably within the perigranular zones of SI, as described by Chapin and Lin (1984), because they are about 100 μm in width and are immediately adjacent to the granular zones. Common fur between rows A-B and B-C was represented in a strip medial to the PMBSF, along the row E of cortical barrels. Fur between rows C-D and D-E was represented lateral to the PMBSF. Cells in another strip of cortex, just parallel to the row of "straddling" barrels (α, β, γ, δ), were responsive to the manipulation of several large whiskers. Cells in and around barrels representing more anterior whiskers were also responsive to multiple whiskers and common fur.

Armstrong-James and Fox (1987) also found cells in the rat which were responsive to the facial hair stimulation. These cells were rare and mostly found at the edge of the barrel field (parallel to row E barrels). There were also some cells in barrels E1, E2 and δ which showed convergent input from vibrissae and surrounding hairs. This finding is in contrast to results from study by Nussbaumer and Van der Loos (1985) which showed only whisker input to the cells within the PMBSF.

Figure I-28: Cytoarchitectonic zones in the rat primary somatosensory cortex (adapted from Chapin and Lin, 1984). GZ= granular zones (grey shading); DZ= dysgranular zones (yellow); PGZ= perigranular zones (white islands among the barrels within the PMBSF and between GZ and DZ in other parts).

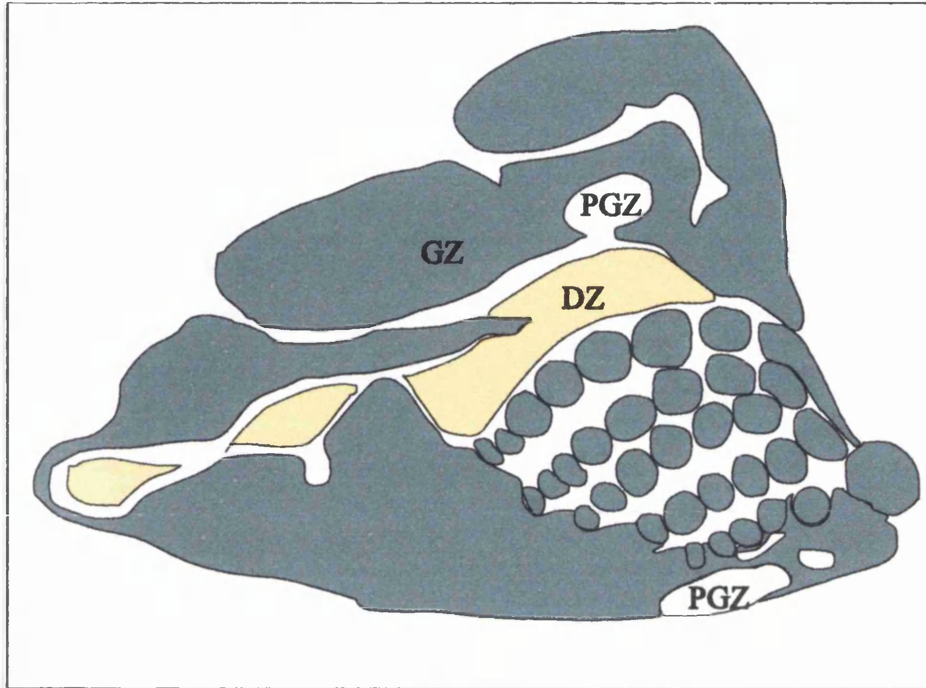
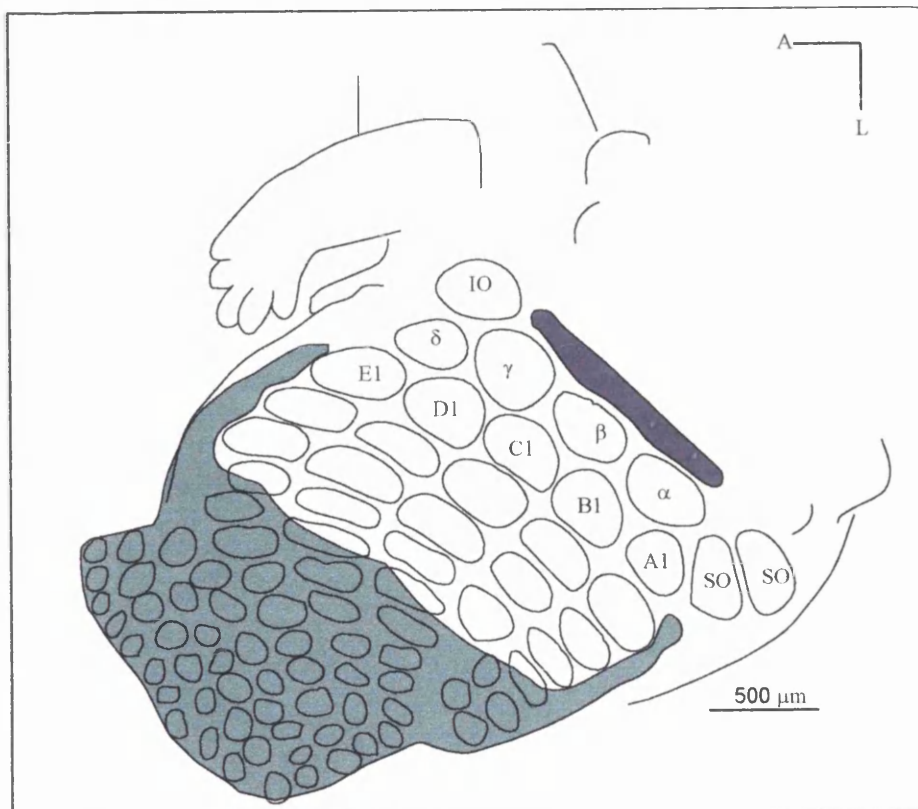


Figure I-29: Physiological map of the mouse somatosensory cortex (adapted from Nussbaumer and Van der Loos, 1985). Grey shading represents regions responsive to hairy skin on the face. Purple shading represents region responsive to manipulation of several whiskers. IO= infraorbital sinus hair; SO= supraorbital sinus hair: A1-E1 and λ–δ= rows of mystacial whiskers



The injection sites in cases 14, 15 and 16 in group A and cases 1, 5, 8 and 13 in group B were located in a region which seems identical to the finely stippled areas within mouse SI in figure I-29. The injected tracers labelled cells in the perigranular zones, but spread to the neighbouring barrel columns. These cells, responsive to whiskers and surrounding hairs, have bifurcating axons that project bilaterally to the pontine nuclei. This conclusion is further supported by the results of case number 7 in group B. The injection site in this case included part of barrel δ as well as perigranular cortex just posterior to it. Cells here are responsive to a number of large whiskers and project only to the ipsilateral pons. Therefore, it seems that function and not the cytoarchitecture of a particular cortical area determines the pattern of the projections to the pontine nuclei. The largest, most caudally situated barrel columns in the PMBSF (D1, E1, δ, B1 and C1) project only to the ventral peduncular nucleus. Most of the other barrel columns have more than a single target within the pontine nuclei.

Both of the large dysgranular areas within the SI project only to the ipsilateral pons, mainly to the ventral peduncular nucleus, thus resembling the pattern of projections of the large whisker barrels. This was not an expected finding considering differences in the response properties of cells in these two cytoarchitectonic areas. The apparent overlap between the terminal sites from the two cortical areas may not be significant, because the axons of the cells from the dysgranular areas may contact different pontine cells within the same nuclei. This issue may be resolved by using two anterograde tracers and electron microscopy. Even at the light microscope level used in the present study it was possible to show that there are subtle rostrocaudal and mediolateral shifts in location of the terminal patches from different parts of the PMBSF. The borders of the terminal patches may not be sharp, but there is segregation. The more posterior regions of the PMBSF (case 10-brown PHA-L terminals) project to the more lateral parts of the ipsilateral ventral peduncular nucleus. Further rostral PMBSF barrels (case 14-dark blue-black BDA terminals) project more medially and caudally within the same terminal column. This finding agrees well with Wiesendanger and Wiesendanger (1982) who concluded that the more rostral cortical areas project more medially within pons.

Terminal patches within pons can be aligned to form up to five terminal columns of about 750 μm in length. This finding has also been reported in earlier studies of the

pontine projections from the sensorymotor cortex ((Mihailoff et al., 1978; Panto et al., 1995).

Earlier literature reported only the medial pontine nucleus as receiving contralateral somatosensory cortical projections (Mihailoff et al., 1985). I found that label in the ipsilateral nuclei is mirrored on the contralateral side (cases 14, 15 and 16 in group A and cases in group B). The label on the contralateral side is weaker and hence less obvious than that on the ipsilateral side. The tracers used in my study are highly sensitive and at higher magnifications (100x or more) it is possible to identify a single fibre with terminal boutons. Fibres terminating on the contralateral side travel in the ipsilateral peduncle and cross the midline within pons. They could be collaterals of the fibres terminating within the ipsilateral pons, because they appear thinner and stain palely, but this question should be addressed in another anatomical study.

Functional implications

In summary, the information sent from the primary somatosensory cortex to the cerebellum varies, depending on which whiskers, and therefore barrel columns, are stimulated. The largest barrel columns, physiologically responsive only to whiskers, project predominantly to the ipsilateral pontine nuclei. Axons of layer V cells in the rostrolateral part of the PMBSF project bilaterally to the pontine nuclei. Some cells in the perigranular cortex bordering the PMBSF project to the pons in the same fashion. It seems plausible that it is the cells responsive to the manipulation of the facial hairs, rather than whiskers (or perhaps cells responsive to both), that project to greater number of sites within the pons on both sides of the midline. Sharp and Gonzalez (1985) used (^{14}C) 2-deoxyglucose method and showed that whiskers and common fur on the face have largely separate representations in a number of stations within the trigeminal pathway. At cortical level the facial hair representation was not exhaustively studied, but it exists and is not completely segregated from the whisker input (Pidoux et al., 1979; Nussbaumer and Van der Loos, 1985; Armstrong-James and Fox, 1987). The injection sites in my study certainly involved the regions in which the inputs from the facial hairs have been recorded.

Bilateral pontine projections would ensure that the information directed to the cerebellum would reach more cerebellar cells and would influence the motor output in a different way. Perhaps the importance of immediately attending to stimuli which

appear so close to the animal's face commands the need for a different representation within pontine nuclei. Strong bilateral activation of the cerebellar cortex may be the way of coordinating symmetrical or the whole body movement in contrast to largely unilateral activation which may be present with orientation movements.

Choice of tracer

All three of the tracers used reveal anterogradelly labelled fibres and terminals in detail. In the beginning of this study I chose PHA-L and majority of the experiments were done with it. PHA-L gives clearly labelled injection sites whose boundaries can be easily determined, especially in the infragranular layers. Labelled neurones at the injection site give the appearance of a Golgi preparation. However, immunohistochemistry takes a long time which limits the number of experiments that can be done. Biocytin and biotinilated dextran amine need much less histological processing and were used in some experiments. BDA is the preferred tracer because it resembles PHA-L in many respects, from the survival times needed for transport to the appearance of the labelled terminals. Another advantage of BDA is that it can be pressure injected, unlike PHA-L which must be deposited in the tissue by iontophoresis. Biocytin tends to produce much smaller injection sites and terminal label is often very weak. This is probably due to the short survival time that has to be employed when biocytin is used. Survival of 3 days or more results in enzymatic degradation of the tracer so it is suitable only for short neuronal tracts.
Combination of PHA-L and BDA has been used in double anterograde tracing studies (i.e. Dolleman-Van der Weel et al., 1994). I presented one of the cases where both of the tracers were injected into neighbouring cortical sites (listed as cases 10 and 14). In my experience it is very difficult to strike the right balance with immunocytochemical procedures to show each of the tracers in clearly distinguishable colours and to keep the background staining at low level. Also with very dense projections to similar areas it is possible that one of the tracers may obscure the other.

IV. Cells of Origin of Corticotectal and Corticopontine Projections within Primary Somatosensory Cortex of the Rat.

INTRODUCTION

The focus of this thesis is on the descending pathways from the whisker representation of the somatosensory cortex. In rats the somatosensory cortex receives a massive input from the whiskers and projects to several subcortical targets. Two of the major subcortical targets are the superior colliculus and the pontine nuclei. Is the same or different information sent to these target structures?

The superior colliculus lies on the roof of the midbrain. In non-mammalian vertebrates it is the principal target of the optic nerve. In mammals its superficial laminae are exclusively visual. The visual input comes directly from the eye and indirectly from the cerebral cortex. Cells in the deeper layers receive visual, somatosensory and auditory information and project to brainstem structures that are involved in the control of eye and head movement. Somatosensory inputs to these layers originate in the somatosensory cortex and the spinal cord. The superior colliculus receives tactile information largely from the specialised receptors in glabrous skin on the snout and vibrissae on the face (Abrahams et al., 1988).

Somatosensory information, like visual information, can be used to guide an animal during exploration, especially if touch is its primary sense. Therefore, in rat, whisker information may be used by the colliculus to coordinate eye, head and body movements in orienting responses.

Sensory information (regarding face and whiskers) from the intermediate layers of the rat colliculus reaches the cerebellar cortex as mossy fibres (Kassel, 1980). The exact pathway connecting these two structures is not known but there are a few candidates. The bulk of the mossy fibres would reach the cerebellum via dorsolateral (DL) pontine nuclei and Nucleus Reticularis Tegmenti Pontis (NRTP). Somewhat sparser mossy fibre input may be provided by the medial and lateral pontine reticular formation. Collicular input to the cerebellum may be mediated by the climbing fibre system as well because there is a projection from the superior colliculus to the inferior olive.

In this chapter I examine anatomical differences between the cortical projections to the pontine nuclei and superior colliculus and ask two questions:

1) Are there areal or laminar differences between cortical cells that project to superior colliculus and those that project to the pontine nuclei?

2) If somatosensory cortical cells project to both targets are they more likely to be in granular or dysgranular zones of the SI?

Earlier literature addressed these questions with somewhat contradictory results. Killackey et al. (1981) at first reported that rat corticotectal cells can be found only in the dysgranular zones of the primary somatosensory cortex. In a later paper (1989) the same authors found corticotectal cells to be present throughout SI. One aim of the present study is to establish the distribution of corticotectal cells within SI.

Axons of many cortical cells that project subcortically often branch and project to two or more target structures (Wise and Jones, 1977 a). Most studies in this field, i.e. Hallman et al. (1988), have focused on projections from the visual cortex. No study has directly compared outputs to the pons and superior colliculus from the somatosensory cortex.

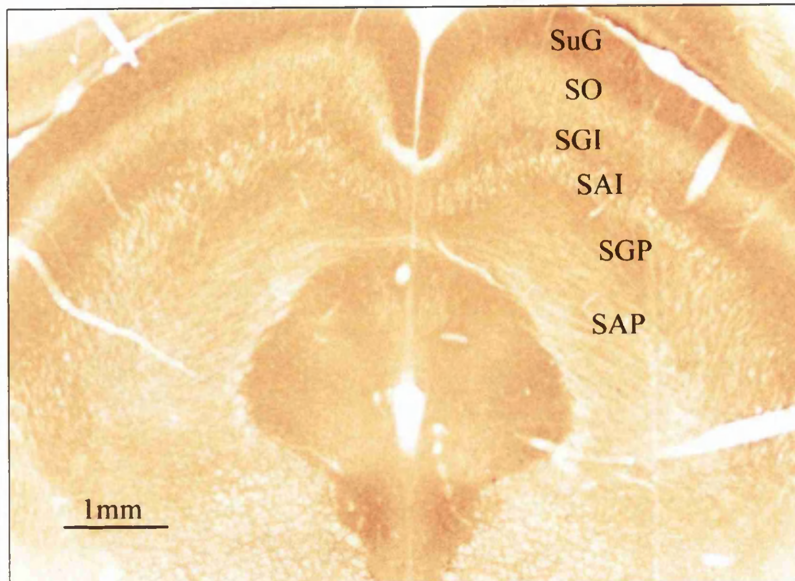
In the previous chapter I demonstrated a distinction between pontine projections from the PMBSF granular and bordering perigranular zones. Here I tested whether there is a similar bias in projections to the superior colliculus.

Superior Colliculus

Cytoarchitecture and Anatomical Connectivity

The superior colliculus is a midbrain structure of a striking, laminar appearance. Its laminae are easily distinguished in cytochrome oxidase preparations (fig. II-1)

Figure II-1: Cytochrome oxidase processed coronal section of a rat brain showing collicular layers.



Abbreviations:

SuG = superficial grey

SO= stratum nervi optici

SGI= str.griseum intermedium

SAI = str.album intermedium

SGP = str. griseum profundum

SAP = str. album profundum

There are seven layers in mammalian superior colliculus (Huber and Crosby, 1933):

1) Stratum zonale, composed of a thin layer of small myelinated axons immediately below the pia and a layer of small neurones and astrocytes.
2) Stratum griseum superficiale (superficial grey layer), composed of two types of cells: small with bushy dendritic trees confined to the upper part of the layer and larger neurones in lower part of the layer whose dendrites spread and can reach the upper part of the layer.
3) Stratum opticum, made up primarily of afferent and efferent collicular fibres but also containing some small and medium sized neurones. Dendrites of these cells arborise within the layer but their axons can reach deep collicular laminae.
4) Stratum griseum intermediale, the widest layer made up of cells of all sizes.
5) Stratum album intermediale, mostly made up of fibres with a few small and medium sized neurones.

6) Stratum griseum profundum, containing cells and fibres.
7) Stratum album profundum, lies next to the periaqueductal grey, largely made up of fibres.

Cells in the superficial and deep layers differ in their connections, response properties and functional roles.
The three superficial layers (zonal, superficial grey and optic) are strictly visual, receiving direct projections from the ipsi- and contralateral retina as well as primary and secondary visual cortex (Stein, 1981). These projections are organised in a precise retinotopic manner in all animals (Finlay et al., 1978). Other inputs to superficial layers come from the magnocellular division of the ventral lateral geniculate nucleus and parabigeminal nucleus (Linden and Perry, 1983).
Efferent fibres from the superficial laminae project to the deep layers of the ipsilateral SC, pretectum, dorsal and ventral lateral geniculate and lateral posterior nucleus of the thalamus (Perry, 1980).

The intermediate and deep layers of rat superior colliculus receive projections from many different structures (Cadusseau and Roger, 1985). The main inputs to the intermediate and deep layers of the superior colliculus arise from somatosensory and auditory systems (Westby et al., 1990). Somatosensory projections to these layers in hamsters come from ipsilateral somatosensory cortex and contralateral dorsal horn of the spinal cord, dorsal column nuclei, spinal trigeminal nucleus and the deep layers of superior colliculus (Rhoades, 1981).
The primary somatosensory cortex projects to the intermediate and deep grey layer of the rat superior colliculus in the form of multiple patches (Wise and Jones, 1977 b). These projections are somatotopically organised: head and face representation are in the anterolateral part of the superior colliculus; paws and the rest of the body are represented in the posterolateral part of the superior colliculus.
Visual afferents originate in cortical area 18 a, the nucleus of the posterior commissure and the parabigeminal nucleus. Auditory cortex, inferior colliculus and the nuclei of lateral lemniscus provide auditory input to deep layers. Other inputs to these layers originate in cingulate and motor cortex, zona incerta, substantia nigra, some hypothalamic, thalamic, pontine reticular and deep cerebellar nuclei.

Other afferents (i.e. spinotectal and trigeminotectal) also show patchy or column like termination patterns (Wise and Jones, 1977 a; Killackey and Erzurumlu, 1981). Trigeminal afferents terminate in the stratum album intermediale and stratum griseum profundum.

Collicular efferents are organised in two major tracts, tectospinal and tectopontine. The cells of origin of these projections are mainly in stratum griseum intermediale and deeper layers. Fibres of the tectospinal tract cross the midline as the predorsal bundle and descend to the upper spinal cord, where they terminate among the neck motoneurones. The tectopontine tract carries fibres which terminate in the dorsolateral pontine nuclei. In cats some of these fibres originate in the stratum opticum of the superior colliculus (Mower et al., 1979). Efferent fibres from the superior colliculus terminate primarily in the brainstem structures involved in the control of eye and head movements.

Maps

Each colliculus contains a representation of the contralateral visual field in its superficial layers. There is also a representation of the whole body surface-somatosensory map and a representation of auditory space in deeper layers. The somatosensory and auditory maps are in register with the visuotopic map in the superficial layers (Dräger and Hubel, 1976). The simplest description of their relationship would be that a point in visual space on the contralateral side excites cells in the superficial layers. Cells directly beneath in intermediate layers receive projections from the same part of the somatosensory or auditory space. These sensory maps are connected to a motor map in deep layers (intermediate white and intermediate grey) whose cells are active prior to saccadic eye movements (McHaffie and Stein, 1982).

Topographically, the nasal visual field and anterior tactile receptive fields are found in rostral superior colliculus. The inferior visual and ventral tactile receptive fields are placed laterally (Dräger and Hubel, 1976).

Physiology

Cells in the three superficial collicular layers respond only to visual stimuli. These cells have been most often studied and their response properties appear better understood

than the properties of cells in the deeper layers. Some of the cells in the superficial layers have binocular receptive fields, some are directionally selective, others prefer moving stimuli and some respond to a target that moves at a particular speed across the visual field (Ogasawara et al., 1984).

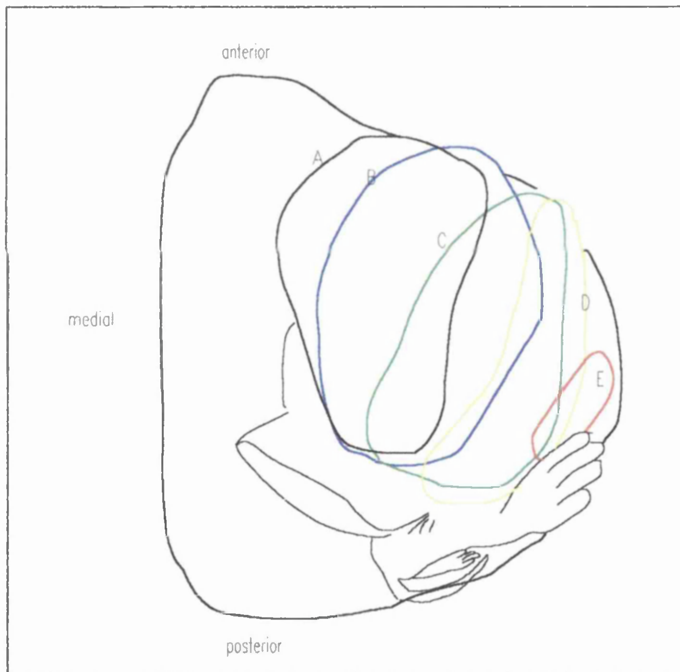
Cells in the intermediate and deep layers respond to visual, somatosensory and auditory stimulation. Visual cells in these layers share the response properties of the visual cells in the superficial layers. Data on the receptive field properties of somatosensory and auditory cells is much sparser. However, it is known that the majority of the somatosensory cells respond to light cutaneous stimuli, especially displacement of guard hairs or vibrissae, some respond to deeper cutaneous stimulation. The average somatosensory receptive field in cat colliculus is about 270 cm^2 (Meredith and Stein, 1986). In hamsters, somatosensory receptive fields are at least 30-40° (sometimes even 120° in diameter), whilst the corresponding visual receptive fields measure up to 10° (Finlay et al., 1978). About half of the cells in intermediate and deep layers in hamsters respond to more than one modality (Chalupa and Rhoades, 1977) and it seems that convergent sensory inputs determine their response properties (Meredith and Stein, 1983). Simultaneous presentation of different stimuli can enhance or inhibit responses of multimodal cells. Sometimes, a cell can be excited by one afferent and depressed by another. The mechanism for interactions among the afferents of the collicular cells is still not clear.

Pain and thermal stimuli can activate cells in deeper layers of rostral colliculus in hamsters (Stein and Dixon, 1978) although there is no evidence that superior colliculus of other mammals receives such input.

Electrical stimulation of cells in the intermediate layers of rats, as well as other animals, produces conjugate contralateral saccadic eye movements (McHaffie and Stein, 1982). Also, movements of ears, vibrissae, neck and limbs could be elicited at appropriate locations. For a long time the superior colliculus in primates was considered as a structure that is concerned only with eye movements. All of the other movements that could be seen following collicular stimulation in non-primates were attributed to species differences. Recent work by Freedman et al. (1996) demonstrated that head movements are elicited by electrical stimulation of the colliculus in monkeys as well. Therefore, head and eye movements together form orienting resoponses in all animals studied so far.

The most prominent feature of the somatosensory map in the deep layers of the superior colliculus of a mouse is the whisker representation (Dräger and Hubel, 1976). In mice whiskers overlap most of the visual field and their collicular representation is relatively greater than that of other parts of the body. Responses from a single whisker can be elicited over a wide area and there is a considerable overlap between representations of different whiskers. The largest area is occupied by representation of whiskers closest to the eye; thus whisker A1 has larger representation than whisker E1. Such specificity was not observed in hamsters (Finlay et al., 1978).

Figure II-2: Dorsal view of the mouse superior colliculus showing somatosensory map. Letters indicate rows of large mystacial whiskers and the area where each row is represented is demarcated by lines of different colours (adapted from Dräger and Hubel, 1976)



The superior colliculus in rats is involved in sensory-motor transformations needed for fast responses to novel stimuli (Dean et al., 1989). Stimulation of the output neurones produces coordinated eye, head and body movements towards or away from the stimulus. Which of these two responses is selected depends on stimulus characteristics and its position in space. A small object moving rapidly in the lower visual field is

likely to elicit orientation and approach movements. Large moving stimuli in the upper visual field tend to evoke avoidance and defence movements.

Since the cerebellum is also involved in rapid coordination of movements under sensory guidance it is important to understand and compare the nature of their respective afferents.

The experiments to follow were designed to ascertain the extent to which fibres from the somatosensory cortex branch to provide an input to the superior colliculus and the pontine nuclei. Fluorescent latex beads were injected into both structures and retrogradely labelled cells were analysed.

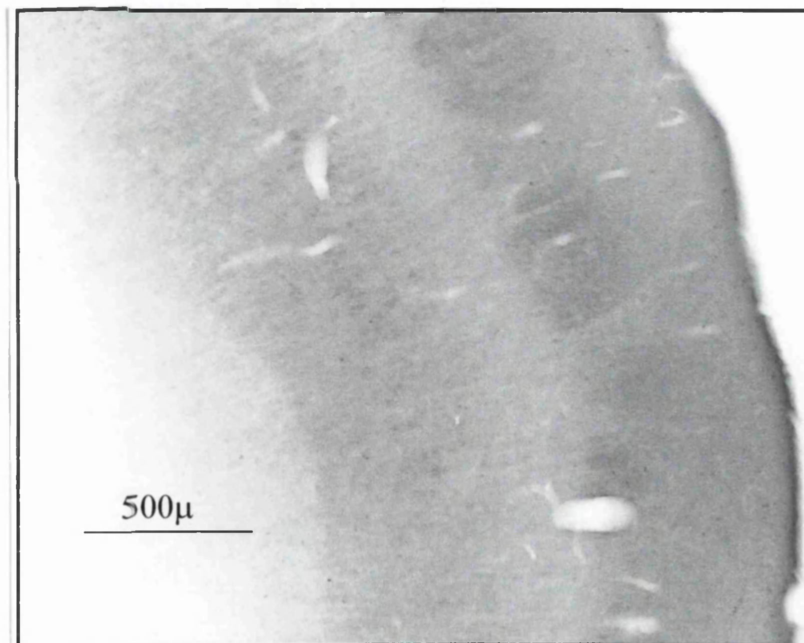
RESULTS

I used fluorescent latex beads to inject superior colliculus and pontine nuclei in 10 animals. Surgical and histological procedures were given in the Materials and Methods section. My aims in this chapter were:

- to establish areal and laminar location of corticotectal and corticopontine cells in SI
- to identify cells that project to both targets and estimate their numbers

Retrogradely labelled cells were distributed throughout the neocortex. I focused particularly on two areas of the primary somatosensory cortex which can be easily distinguished in coronal sections: the granular zone representing large mystacial whiskers and the dysgranular area that separates whisker barrels from the hand representation.

Figure II-3: Cytochrome oxidase processed coronal section with barrel field in case R96-29.



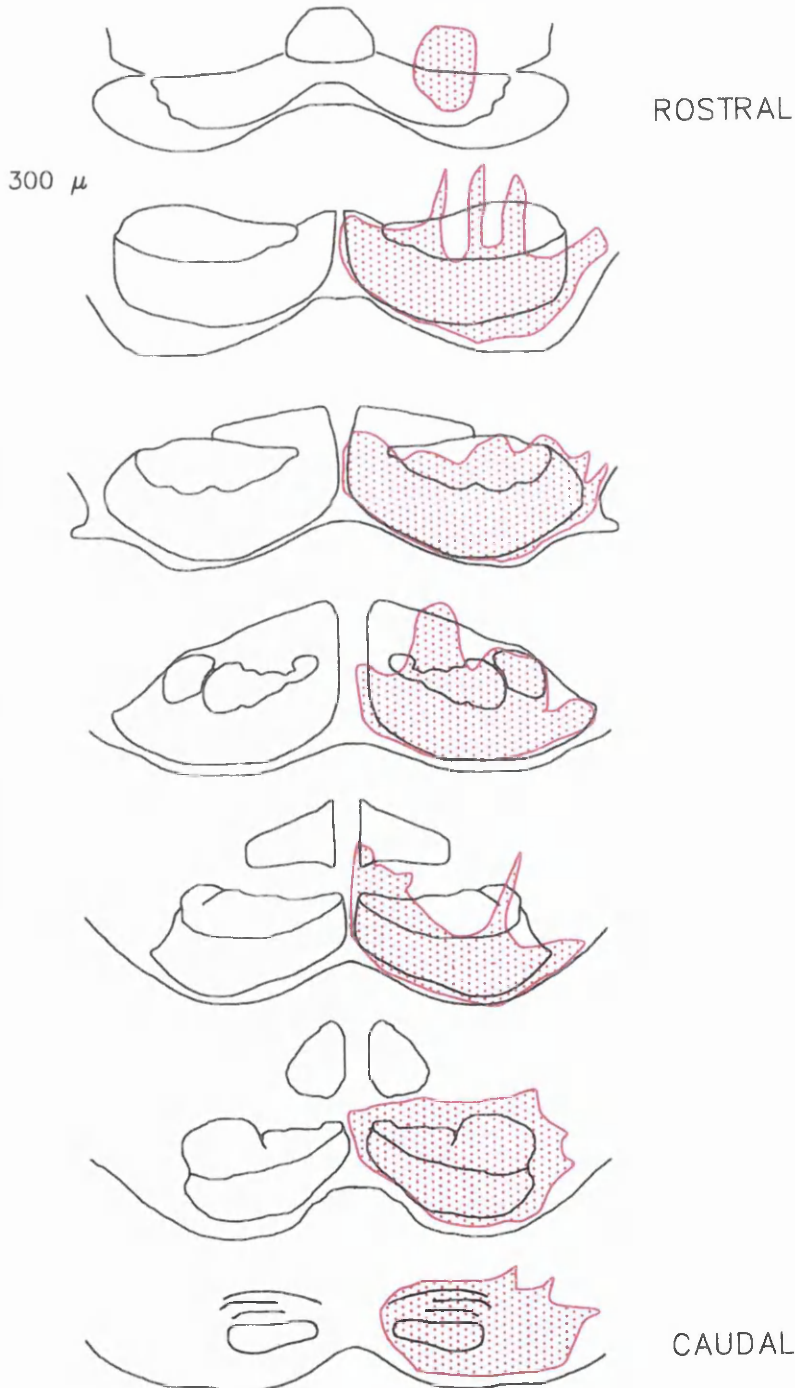
The results in all the cases were similar. The only difference among the experimental cases was the extent of the injection sites. Here I will present in detail one case where the injection sites were most complete.

Injection sites

In case R96-29 green fluorescent beads were injected into the superior colliculus and red beads into the pontine nuclei. The pontine nuclei were almost completely labelled by the tracer whereas only the rostro-lateral part of the superior colliculus contained primary label.

Figure II-4: A series of sections through pons (a) and superior colliculus (b) showing the spread of the beads at injections sites in case R96-29 (distance between sections 300 μ).

a)



b)

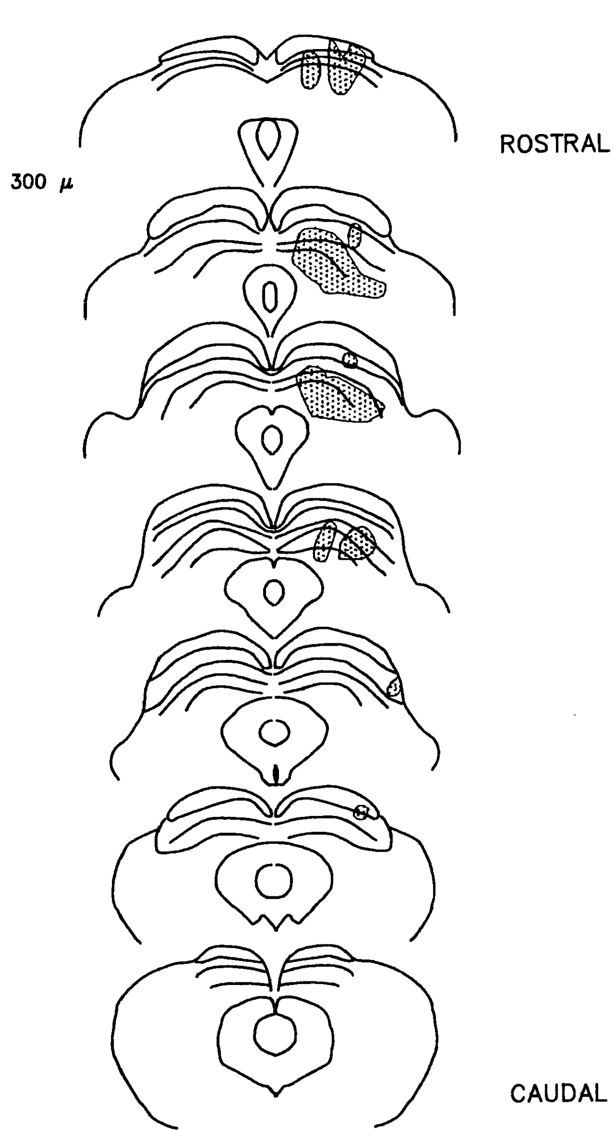
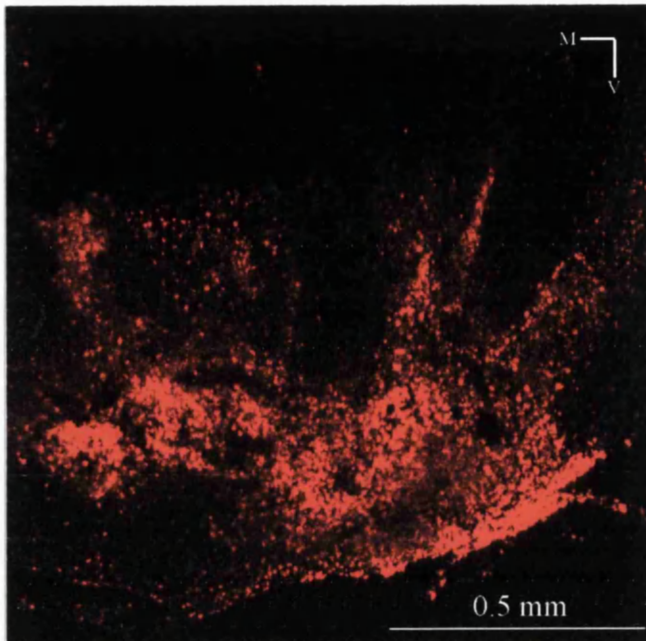
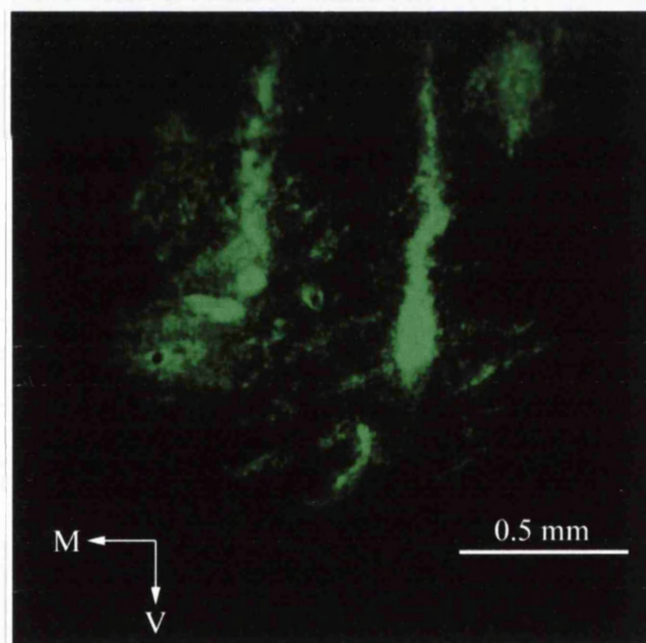


Figure II-5: Photomicrographs of sections through pons (a) and superior colliculus (b) at indicated levels. Multiple injection tracks can be seen on the photomicrographs which serve to demonstrate that the tracers do not diffuse far from the centre of each injection thus some axon terminals inevitably remain free from the label.



a) Injection site in the pontine nuclei approximately at level 5 of the diagram in figure II-4a.



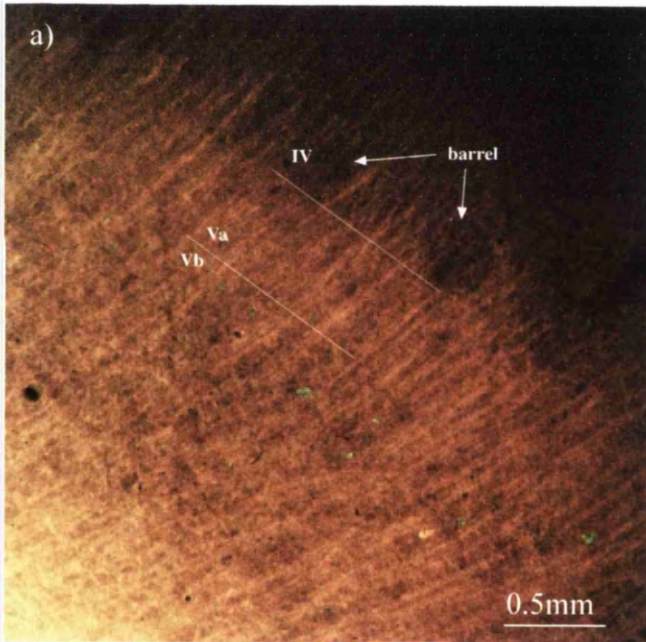
b) Injection site in superior colliculus at approximately level 3 of the diagram in figure II-4b.

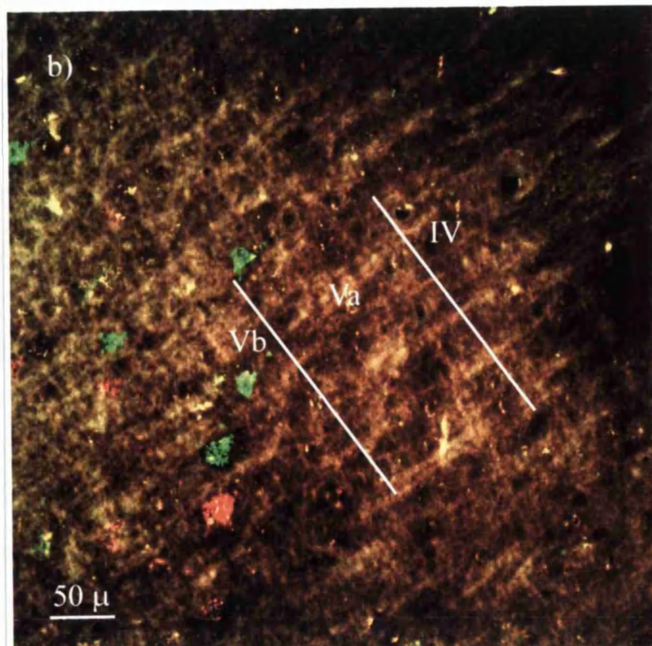
Retrogradelly labelled cells in SI

Cells labelled with latex fluorescent beads were found throughout neocortex of R96-29 and were present in great numbers in primary somatosensory cortex.

Both corticotectal and corticopontine cells were found in the lamina Vb of the SI Occasionally, a few corticotectal cells were found slightly superficially to the majority of other retrogradelly labelled cells in layer V of the SI. These cells were located either in the deepest part of lamina Va or very superficial lamina Vb.

Figure II-6: CO processed section with retrogradely labelled cells at lower (a) and higher (b) magnification. The histological processing causes some degree of autofluorescence and some of the label is lost. However, many labelled cells are still present although the red beads are somewhat harder to distinguish. Note that labelled cells are present both in barrel areas and in the interbarrel septa.





Using conventional fluorescent microscopy it appeared that about 30-40% of the corticotectal cells in layer Vb send collaterals to the pons (Table II-1).

Table II-1: Results of cell counts in one of the cases at the beginning of the study (case R96-24). This data shows that there were more corticopontine than the corticotectal cells in the region of the PMBSF in which the cells were counted. One reason for this finding might be that more cells in the SI do indeed project to the pontine nuclei than to the superior colliculus. It is also possible that the collicular injection site in this case did not label terminals of all corticotectal cells.

SECTION NO.	RED CELLS (corticopontine)	GREEN CELLS (corticotectal)	DOUBLE LABELLED
49	138	50	21
52	60	23	16
55	102	57	25
58	118	40	18
61	165	55	12
64	79	16	6
TOTAL	662	241	98
double labelled cells as %	**14.8%**	**40.66%**	

Analysis of the retrograde label using the confocal microscope allowed a more precise determination of the projections to these two structures.

In a number of digitised images of the case R96-29 I counted the number of red and green cells. Only the whole cell bodies present in the sample were counted.

In the field size of 250x250 µm (magnification 40x) there were on average 21 corticotectal cells (SD= 6.2) and 28 corticopontine cells (SD= 5.4). Of these cells there were 20 double labelled cells (SD= 5.9) which represents 95% of corticotectal cells and 75% of corticopontine cells.

The reason for the apparent discrepancy between the results from the conventional and confocal microscopy becomes clear when analysing high power confocal images. Fluorescent beads are seen as individual spheres and in some instances a cell which appears to contain only one class of microspheres proves to contain a few spheres of the contrasting colour.

Figure II-7: A series of images taken by the confocal microscope. First, there is an image seen with red channel only, then an image seen on green channel, and the last is a combination of the two. The same cells appear in both red and green images. When both channels are combined and tissue is viewed with a high power objective it is often possible to see beads of both colours next to one another.

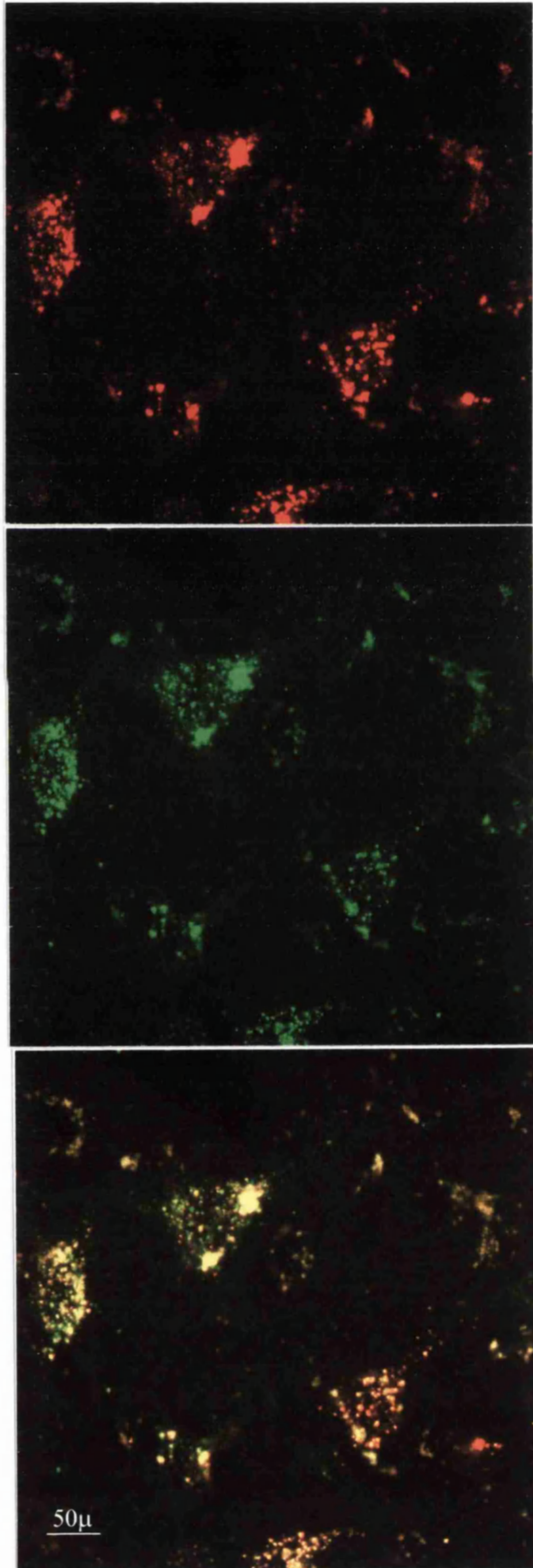
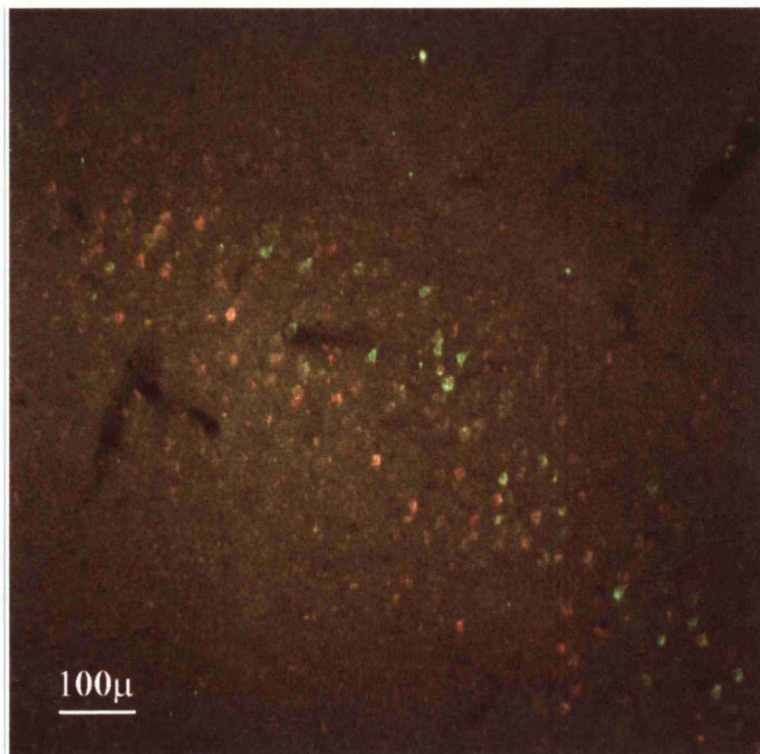


Figure II-8: A high magnification of the corticopontine cell with only red beads.

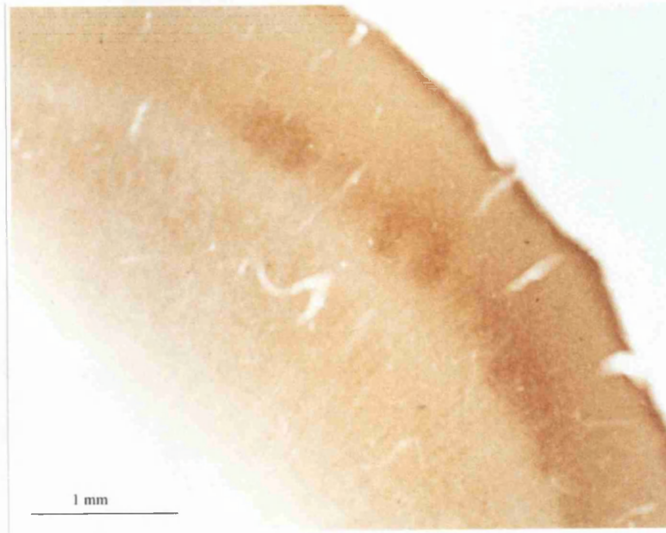
Both granular and dysgranular zones of the primary somatosensory cortex project to the pontine nuclei and the superior colliculus. Furthermore, nearly all of the cells in both divisions of the SI that project to the superior colliculus send a collateral to the pontine nuclei.

Figure II-9:

a) Retrogradelly labelled cells in granular part of the SI in case R96-29

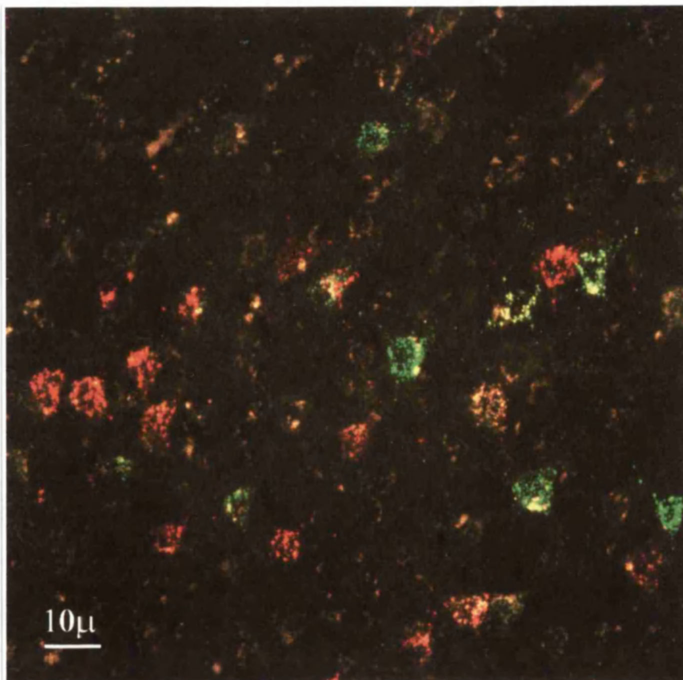


b) Adjacent section processed for CO to show barrels at the same level.

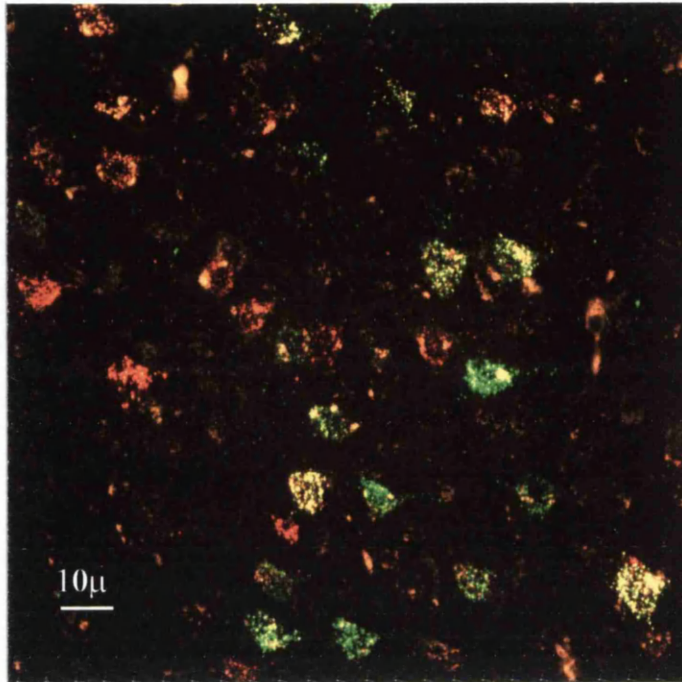


Although whiskers closer to the eye (i.e. whiskers in row A and long, more caudal whiskers in other rows) have larger representation in the superior colliculus, there was no discernible difference in label between corticotectal cells belonging to different rows of barrels.

c) Higher magnification photomicrograph of the labelled cells in granular part of the SI, more medial, larger barrels.



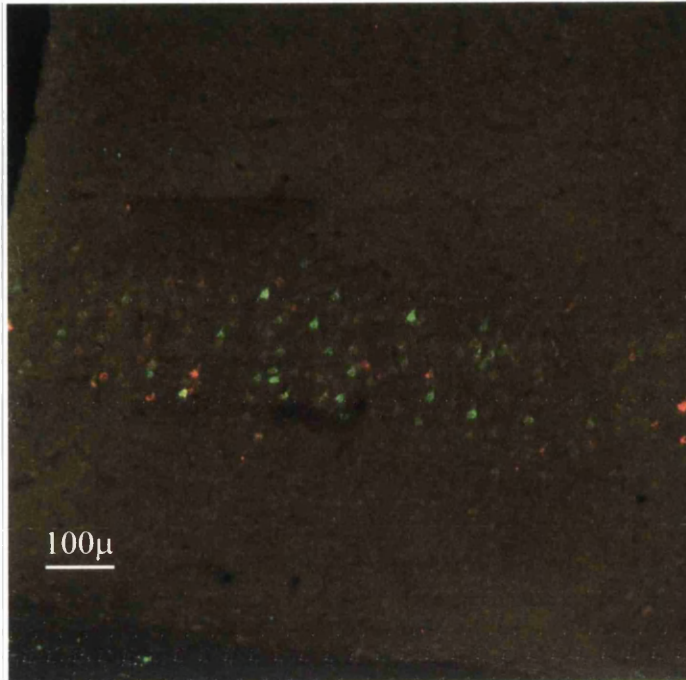
d) High power photomicrograph of the labelled cells in granular part of the SI representing whiskers closer to the nose (smaller, lateral barrels).



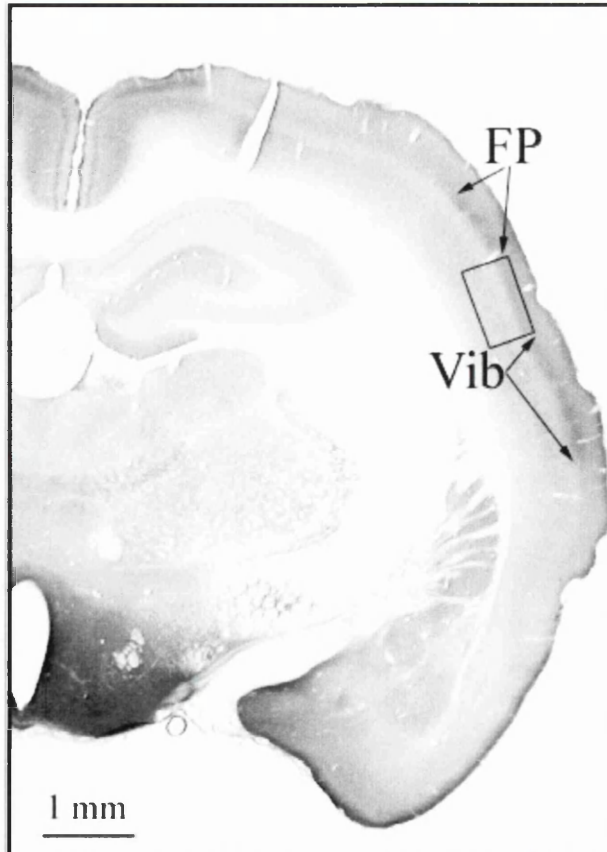
The following few images will illustrate retrogradely labelled cells in the dysgranular zone of the primary somatosensory cortex.

Figure II-10:

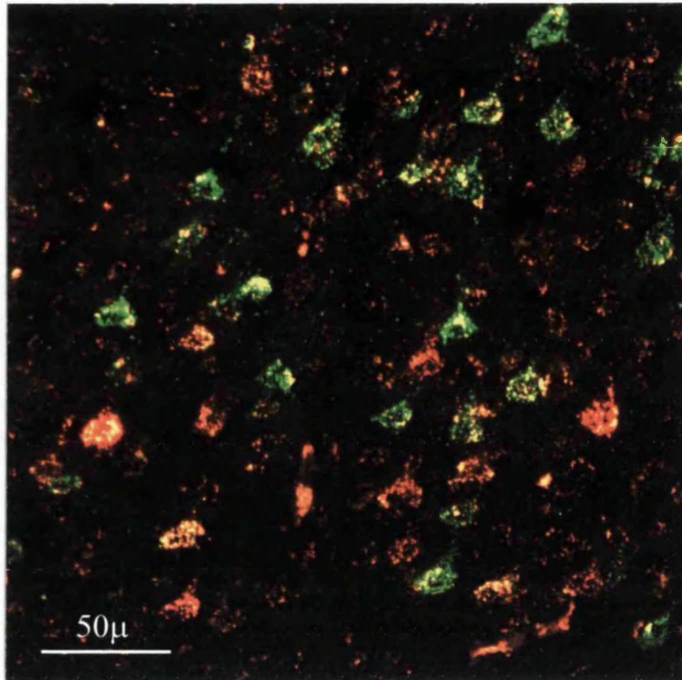
a) Low power view of retrogradely labelled cells in the dysgranular zone of the SI (area enclosed in the rectangle in b) in case R96-29.



b) Adjacent section processed for CO, showing the location of the dysgranular zone in SI. FP= forepaw, Vib= vibrissae



c) Higher power view of the labelled cells in the dysgranular zone of the SI.



SUMMARY

Virtually all of the cells in layer Vb of the rat primary somatosensory cortex that project to superior colliculus project to the pontine nuclei as well. Since the filling of both superior colliculus and pontine nuclei may not have been complete, it seems likely that nearly 100% of cells in the lamina Vb of the rat primary somatosensory cortex projects to both targets. Cells in both granular and dysgranular zones of the SI project to the deep layers of the superior colliculus.

DISCUSSION

Somatosensory cortex projects heavily to the intermediate and deep layers of the ipsilateral superior colliculus (Price and Webster, 1972; Wise and Jones, 1977 b). Mercier et al. (1990) suggested that around 25% of cells in layer Vb of the primary somatosensory cortex in a rat project to the superior colliculus. This relatively low estimate is probably due to the placement of the tracer (WGA-HRP) in the superior colliculus. Corticotectal projections from the barrel field are found specifically in the anterior and lateral part of the superior colliculus. If the injections of WGA-HRP were placed more medially or caudally they would spare the area that receives the largest cortical projections. Most of the injection sites in this study were centred over the rostrolateral part of the superior colliculus which resulted in a much larger number of retrogradely labelled cells in the primary somatosensory cortex.

Mercier et al. (1990) demonstrated that every cell in layer Vb projects to the pontine nuclei. So, the number of the corticopontine cells equals the total number of the layer Vb cells. Ratio between corticopontine and corticotectal cells would therefore represent the proportion of cortical cells projecting to the superior colliculus. From the cell counts in my study it appears that at least 70% of cells in layer Vb of the primary somatosensory cortex (dysgranular as well as granular areas) project to the superior colliculus. Since the latex microspheres may not have completely covered the entire target structures this number is probably an underestimate.

Hallman et al. (1988) studied collateralisation of layer V cells in the primary visual cortex of a rat. They found that corticotectal and corticopontine cells belong to the same class of pyramidal neurones in layer Vb- cells with medium to large soma, 5-6 basal dendrites and a single apical dendrite which extends to layer I. In this study the authors found that the number of double labelled cells vary, depending on the tracers used. When Fast Blue was used in combination with Diamidino Yellow or Nuclear Yellow there were 25-30% of double labelled cells. Fluoro-gold in combination with rhodamine microspheres produced about 60% of double labelled cells.

Mercier et al. (1990) using True Blue and Diamidino Yellow as tracers estimated that 25% of cells in the primary somatosensory cortex project to the superior colliculus and that this projection is a result of the bifurcating corticopontine axons. Thus these earlier results agree with those of Hallman et al. (1988) who used the same tracers.

However, as Hallman et al. suggested, on the basis of results using Fluoro-gold and rhodamine microspheres, even 60% of double labelled cells may be an underestimate. Fluorescent microspheres used in this study are readily taken up by the axon terminals Katz et al., 1984). They are not toxic and are quickly transported to the cell bodies. Combination of these tracers and confocal microscopy provided evidence that the vast majority of cells in the primary somatosensory cortex project to both superior colliculus and pontine nuclei.

The high percentage of cells projecting to both targets is surprising since the superior colliculus was not completely labelled with the tracer throughout its extent. Latex beads have very strong affinity towards each other and never spread further than half a mm or so from the centre of the injection. Also, the structure of the superior colliculus seems to further limit the spread of the tracer. Equivalent amounts of the same tracers in the pons produce slightly larger injection sites. Despite these limitations imposed by the technique used in all of the experimental cases a large number of corticotectal cells were labelled in the primary somatosensory cortex.
Earlier studies (Wise and Jones, 1977 b) reported that corticotectal cells in the primary somatosensory cortex of a rat form clusters of varying sizes. This phenomenon may simply be due to incomplete labelling of the terminals at the injection site.

The other finding of this study is that superior colliculus receives projections from both granular and dysgranular zones of the primary somatosensory cortex , as Killackey et al. reported in their later study (1989). Retrogradelly labelled cells were present equally in both areas. Since the granular and the dysgranular zones have different functional properties and connections the result is somewhat unexpected. Cells in different cytoarchitectonic zones of the rat primary somatosensory cortex (as described by Chapin and Lin, 1984) have differing response properties. Cells in granular zones have low threshold cutaneous receptive fields. Cells in the dysgranular zones of awake animals show multimodal convergence of cutaneous and joint movement receptive fields. In anaesthetised animals these cells are silent. The inputs to these two zones are also different. Dysgranular areas receive different input from thalamus (Akers and Killackey, 1978), contralateral hemisphere (Ivy and Killackey, 1981) and ipsilateral

hemisphere (Jacobson, 1979). Despite differences in inputs these two parts of the primary somatosensory cortex share common targets in pons and superior colliculus. Dräger and Hubel (1976) found that the A1 whisker has larger representation in the colliculus than the E1 whisker. Does this physiological phenomenon have an anatomical basis? Killackey and Erzurumlu (1981) suggested that the trigemino-tectal projections could be the underlying mechanism for such a physiological finding. They injected the superior colliculus and charted retrogradelly labelled cells in the spinal trigeminal nucleus- pars interpolaris and the principal sensory nucleus. The greatest density of the labelled cells was in the areas of these nuclei containing representations of dorsally and caudally situated whiskers.

The results of this study suggest that barrel columns representing different whiskers project to pons and superior colliculus in the same fashion. The percentage of double labelled cells in the medial and lateral barrel columns (as shown in figure II-9 c and d) was the same.

Functional considerations

Cortical barrel columns play an important role in the behaviour of rats; without them rats can not discriminate between rough and smooth surfaces (Guic-Robles, 1992) or jump accurately across gaps in the dark (Hutson and Masterton, 1986). Thus, the barrel fields are important for sensory discrimination and for using whisker information to guide movement. One obvious question to ask is how that information reaches motor structures in the brain. In the present study I found that the same whisker information from barrel cortex reaches both superior colliculus and pontine nuclei. The pontine nuclei serve as relay nuclei forwarding cortical instructions to the cerebellum. The cerebellum, in turn, has access to all of the descending motor pathways. The superior colliculus also serves to link sensory input to motor output. It is a complex structure "implicated in the sensory-to-motor transformation and generation of commands that produce orientation of the sensory apparatus" (Freedman et al., 1996).

At least 70% of layer Vb cells in the primary somatosensory cortex of a rat project to the superior colliculus. Most, perhaps all, corticotectal axons bifurcate to send a collateral to the pontine nuclei. The strong anatomical connection between cortex and colliculus suggests a functionaly important role for the cortical input to the colliculus. Despite this, the available literature is controversial.

The cortico-tectal relationship has been studied in two ways. One way is to stimulate cells in the cortical areas projecting to the colliculus and record responses of the target cells. The other method is to determine the effect of inactivated cerebral cortex on the response properties of the collicular cells. Most studies of the corticotectal pathway have dealt with inputs from the visual cortex to the superficial layers of the superior colliculus. One of the most widely cited is the work of McIlwain and Fields (1970). They stimulated visual cortex (area 18) in the cat and recorded the responses of visual cells in the superficial layers (stratum griseum superficiale and stratum opticum). Electrical stimulation produced initial burst of impulses from activated cells which was then followed by a period of inhibition lasting 50-100 ms. Following visual cortical stimulation responses of the collicular cells to visual stimuli were suppressed.
Rhoades (1980) used similar approach to that of McIlwain and Fields in recording from superficial and deep layers of hamster colliculus. He confirmed that stimulation of visual cortex or optic chiasm first excited the visual collicular cells in the superficial layers and then caused suppression lasting 50-200 ms. Likewise, stimulation of the somatosensory cortex or spinal cord suppresses responses of the tactile cells in the deep layers following an initial burst of activity.
On the basis of these reports it appears that both visual and somatosensory cortices influence collicular cells in a similar manner; an initial excitation is followed by a long depression. However, lesion studies point to a somewhat different conclusion.
Lesions of the visual cortex in cats (Rosenquist and Palmer, 1971), hamsters (Rhoades and Chalupa, 1978) and rabbits (Graham et al., 1982) do not abolish visual responsiveness but markedly decrease the incidence of directionally selective cells in the stratum griseum superficiale and stratum griseum intermediale of the superior colliculus. Cooling of the visual cortex in cat (Ogasawara et al., 1984) affects the cells in the superficial layers in at least three ways: the majority of cells showed depressed responses and loss of directional selectivity, binocularity and preference for moving stimuli; about 22% of cells stopped responding completely and 17% of cells were not affected.
What about visual cells in deeper layers?
There are fewer and less detailed studies of the effect of lesions of the somatosensory cortex. Both in cats (Clemo and Stein, 1986) and hamsters (Rhoades, 1981) there appeared to be no change in the response properties of the collicular cells following

ablations of somatosensory cortex. Rhoades (1981) reported that the incidence and the response characteristics of sensory neurones in deeper layers of hamsters remain the same as in the intact animal. For example, there were 38.6% somatosensory, 50.9% visual and 1.5% auditory neurones among the tested cells and these numbers did not change following cortical lesion. The size of the receptive fields remained unchanged. In a more recent study Clemo and Stein (1986) reported that cooling of the somatosensory cortex (area SIV) in the cat produced depression or even completely abolished responses of about 60% of the collicular tactile cells (intermediate and deep layers). The rest of the tested cells did not appear to be affected by inactivation of the somatosensory cortex. Cells with smaller receptive fields sensitive to hair displacements were the most affected. Cortical cooling slightly reduced the size of the receptive fields of some cells, but the general response properties in the rest of the field did not change.

There may be a number of reasons for this difference in the effect of inactivation of visual and somatosensory cortex. The stimuli that were tested may not have been appropriate for determining the precise effect of the cortical inactivation. The response properties of the somatosensory collicular cells are poorly understood compared to those of the visual cells and fewer parameters were measured. Also, about half of the cells in deeper layers (stratum griseum intermedium and below) are multimodal. In rodents, in which the vibrissal system plays a most important role in orientation and identification of objects, the majority of multimodal cells are somatosensory-visual (Dräger and Hubel, 1975). Meredith and Stein (1983) showed that the activity of visual cells in the deeper layers is profoundly altered by auditory and somatosensory stimuli. Cells that responded strongly to a particular visual stimulus could be completely depressed by an apperently ineffective non-visual stimulus. In other cases a weak response to a visual stimulus could be greatly enhanced by presenting non-visual stimuli. If the same principles are true for tactile cells is the deep layers, then lesion studies which used simple, hand held probes for tactile stimulation are probably not sensitive enough to detect the true effect of a cortical lesion on the response properties of the collicular cells.

Visual and somatosensory cortex differ in their tectal projections. Visual cortex projects to superficial collicular laminae in a strict retinotopic fashion (McIlwain and Fields,

1970) which is consistent with its strong influence on the response properties of cells in the superficial layers. Somatosensory corticotectal projections, though organised in a topographic manner, show considerable overlap between representations of different body parts (Dräger and Hubel, 1975). Since a large number of these cells receive inputs from several sources, their receptive fields are likely to be a product of that convergence. The exact contribution of each afferent may be difficult to detect.
Large receptive fields of tactile cells in deep laminae suggest that there is a considerable spatial convergence of somatosensory input. Together with the loose representation of body surface in deep layers this characteristic may form the basis for keeping the visual and somatosensory maps in rough register while the animal is moving (Finlay et al., 1978).

What does superior colliculus communicate to the cerebellum?

Stimulation of cells in the stratum griseum intermediale in a rat (Kassel, 1980) evoked responses in the granular layer of the contralateral cerebellar hemisphere. Interestingly, only the tactile information from the face appears to be conveyed to the cerebellar cortex . Areas receiving collicular projections were crus I, crus II, simple and paramedian lobule. These areas also receive tactile projections from the trigeminal nuclei (Watson and Switzer, 1978) and primary somatosensory cortex, via pontine nuclei (Bower et al., 1981).

How does collicular information reach cerebellum? There are two efferent pathways from the superior colliculus. One is ipsilateral tectopontine tract and the other is a medial efferent tract which crosses the midline and becomes predorsal bundle. The tectopontine tract gives off fibres that terminate in the lateral part of the Nucleus Reticularis Tegmenti Pontis (NRTP) and in the dorsolateral region of the pontine nuclei. The crossed pathway gives rise to terminals in the contralateral inferior olive and medial part of the Nucleus Reticularis Tegmenti Pontis.

The most likely route for the collicular efferents to reach cerebellar cortex is via the ipsilateral dorsolateral pontine region which sends a powerful mossy fibre input to the contralateral hemisphere.

Westby et al. (1990) found that the cells giving rise to the ipsilateral tectopontine projection in rats are primarily visual, whereas cells sending their axons in the predorsal bundle were largely somatosensory. The somatosensory cells were found in the

intermediate layers and responded strongly to vibrissal stimulation. If these cells were providing somatosensory input to the cerebellum their axons would have to relay in the NRTP and their mossy fibre terminals would be found largely in vermis. Although vermal tactile receptive fields exist, they are most readily found in the hemispheres (Shambes et al., 1978 a,b). Therefore, ipsilateral tectopontine pathway should be the main route for the somatosensory collicular efferents. In cat, a portion of fibres from the medial efferent bundle diverges and joins the lateral bundle on its way towards the pons (Stein et al., 1984). Although such diversion has not been reported in the rat, it may be present and could be one of the pathways for the somatosensory tecto-cerebellar projections.

Finally, the functional importance of the tactile information conveyed from the colliculus to the cerebellum is not known. Somatosensory information that reaches superior colliculus must differ from the somatosensory information that leaves it. Possible transformation, apart from the one due to the convergence of inputs from different sources, may involve encoding new head/body position relative to the stimulus following an orientation response.

The relationship between superior colliculus and cerebellum becomes more complex since the anatomical and physiological studies show that deep cerebellar nuclei exert their influence onto collicular cells (Westby et al., 1993).

In the cat cerebello-tectal projection originates mainly from the cells in the caudal half of the medial (fastigial) nucleus bilaterally and from the contralateral posterior interposed nucleus (Kawamura et al., 1982). Very few scattered cells in the lateral (dentate) nucleus project to the superior colliculus as well. Fibres arising from the fastigial nucleus terminate mainly in the superficial part of the layer IV. Fibres from the interpositus terminate largely in layer V, deep layer IV and layer VI.

In rats cerebello-tectal input originates mainly from cells in the lateral (dentate) nucleus and to a lesser extent from the caudal posterior interpositus (Gayer and Faull, 1988).

It has been demonstrated recently that the cells in the posterior interpositus excite the output cells of the superior colliculus in rats (Westby et al., 1993).

What is the role of the tecto-cerebello-tectal connection?

Generally, both superior colliculus and cerebellum are complex structures involved in coordination of movements, be it eye, head or the whole body movements. Superior colliculus is probably the site where it is decided which one of the behaviours is appropriate, so the animal orients the head towards (approach) or away (avoidance) from the stimulus (Dean et al., 1989). Some transformed sensory information from the superior colliculus is relayed to the cerebellum. This information is compared to the sensory inputs from the periphery via other pathways and a set of commands follows depending on the situation. If the animal turned its head away, cerebellum would possibly coordinate the whole body movements in running from the predator. If the animal approaches the stimulus, cerebellum may coordinate a different set of movements, for example, those necessary for retrieving food. However, all of the above is just a speculation. Physiological data to demonstrate the precise relationship between superior colliculus and the cerebellum in tecto-cerebello-tectal pathway is still lacking.

V. Cells of origin of ponto-cerebellar mossy fibres in regions of cerebellar cortex receiving whisker information: A retrograde tracing study.

INTRODUCTION

The cerebellar cortex of rats contains a fragmented somatosensory map of the body surface (Shambes et al., 1978 a,b; Joseph et al., 1978). The same part of the periphery can sometimes be multiply represented in spatially distant cerebellar lobules. Tactile information pertaining to whiskers is relayed to the cerebellum via both afferent cerebellar systems, mossy and climbing fibres.

In the vermis, whisker responsive patches are found in the Purkinje cell layer of lobule VII (Thomson et al., 1989) and the granule cell layer of lobule IX (Joseph et al., 1978). Whisker sensitive regions in the hemispheric cerebellar cortex were found in the granule cell layer of Crus I, Crus II, paramedian lobule (Shambes et al., 1978 a) and lobulus simplex (Shambes et al., 1978 b). Whisker related climbing fibre responses were also recorded in Crus I and II and lobulus simplex (Akaike, 1989). The total surface area devoted to whiskers and number of whisker sensitive patches in each of the lobules is different, as can be seen from figure 15, in the Introduction chapter. The largest whisker related areas in the cerebellar cortex appear to be in Crus I (Shambes et al., 1978 b).

Mossy fibre terminals originate in the spinal (pars interpolaris) and principal sensory trigeminal nuclei (Watson and Switzer, 1978). Other sources of mossy fibre terminals are the primary somatosensory cortex (Morissette and Bower, 1996) and superior colliculus (Kassel, 1980). Both of these structures project to the cerebellar cortex via a synapse in the pontine nuclei.

Trigeminal mossy fibre projections to the cerebellar cortex are organised somatotopically. For example, trigeminal neurones that are responsive to tactile stimulation of the upper lip send their axons to the regions of the cerebellar cortex also responsive to that particular stimulus (Woolston et al.,1981). Furthermore, tactile information from the SI pertaining to the upper lip reaches the same areas of the

cerebellar cortex as the direct trigeminal input (Bower et al., 1981). However, neighbouring parts of the body surface are not neccesserily represented in neighbouring patches of the cerebellar cortex (Shambes et al., 1978 a).
Electrophysiological data suggest that trigeminal mossy fibres bifurcate (Woolston et al., 1981). Trigeminal mossy fibre collaterals sometimes terminate within a single tactile patch, but they can also supply distant patches with similar receptive fields. Similar data on the ponto-cerebellar mossy fibre input is not yet available.
Anatomical studies of the pontocerebellar system in rats (Mihailoff et al., 1981; Mihailoff, 1983), using retrogradely transported tracers, showed that neighbouring pontine cells might project to different cerebellar lobules. This evidence was used to suggest that there may be a population of pontine cells which give rise to collateralising mossy fibres. It was also suggested that such pontine neurones might be the ones which collateralise whithin the cerebellar white matter and terminate in the symmetrical regions of the two hemispheres (Rosina et al.,1980; Rosina and Provini, 1984).

Electrophysiological studies in cats (Provini et al., 1968) and rats (Bower et al., 1981; Morissette and Bower, 1996) showed that somatosensory cortical input influences responses in the cerebellar granule cell layer. Direct electrical stimulation of SI in rats evokes responses in Crus IIa (Bower et al., 1981). A typical response to peripheral tactile stimulation in the granule cell layer of the cerebellar cortex consists of two components which differ in their latency and amplitude. The first component is a short latency (8-10 ms) response with an average amplitude of 1.1 mV. The second component is a longer latency (16-22 ms) response with an amplitude of 2.06 mV. Primary somatosensory cortex contributes significantly to the long latency response. Injections of lidocaine or ablation of SI considerably reduced the amplitude of the long latency component of the cerebellar response to tactile stimulation (Morissette and Bower, 1996) but the response was never abolished. The authors suggest that these results are probably due to mossy fibre inputs from motor cortex and colliculus and since decerebration at midcollicular level completely abolished the second phase of the cerebellar response to peripheral stimulation. It should be noted that some of the tactile regions in Crus II and paramedian lobule are responsive to bilateral and contralateral stimulation (Shambes, Gibson and Welker, 1978), so it is quite possible that ipsilateral SI contributes to variability of the latency of the second component of the tactile

response in the cerebellar cortex as well as maintaining the responsiveness when the contralateral cerebral cortex is ablated.

Primary somatosensory cortex projects to the cerebellar cortex via pontine nuclei. One of the conclusions in the first experimental chapter of this thesis was that there was convergence of cortical inputs to the pontine nuclei, since all of the PMBSF barrels project to 5 terminal patches in the ipsilateral pons and predominantly involve the same regions of the pontine nuclei. How is the whisker information, then, from the pontine cells distributed to the cerebellar cortex? Considering that there are whisker representations in many different cerebellar lobules and also that the number of granule cells on which the mossy fibres terminate is vast, it seemed likely that the axons of pontine neurones would bifurcate, perhaps many times, in order to provide the cerebellum with cortical somatosensory information. Studies using HRP indicated that there must be collateralisation in the pontocerebellar system, but were unable to provide firm evidence. Fluorescent retrograde tracers were used in cats (Rosina et al., 1980) and rats (Mihailoff, 1983) to examine the same problem, but their results were not comparable. In cats it was found that about 30% of pontine cells have bifurcating axons, whereas in rats the number of these cells appeared to be much smaller. In both studies the injection sites were large and the results were not related to any particular pontocerebellar system (i.e. visual, auditory etc.). I decided to re-examine the question of branching of the ponto-cerebellar axons using modern fluorescent tracers. The main guiding questions were:

1) Are the multiple whisker representations within a particular lobule innervated by the axon branches of a single relay neurone?

2) Are the separate whisker representations in Crus I, vermal lobule VII and uvula supplied by the same population of pontine neurones?

3) Which of the pontine nuclei contribute to vermal projections and which to the hemispheric projections?

4) Does the spatial arrangement of the retrogradely labelled pontine cells correspond with the distribution of cortico-pontine terminal patches?

RESULTS

A total of 15 animals were used in this study. The experiments were divided into two groups, depending on the placement of the injection sites. The first group consists of animals in which the cerebellar injection sites were in vermal lobules. The second group was formed by experiments in which the animals received bilateral injection of fluorescent latex beads into homotopic sites in Crus I. The first group was further subdivided into group A, in which the injection sites involved the same lobule (IX) but different side of the vermis and group B, where the injection sites were placed in different lobules (VII and IX) in the same hemivermis.
The placement of the injection cannulae in each case was determined on the bases of detailed electrophysiological maps by Shambes et al. (1978 a,b) and Joseph et al. (1978), as described in Materials chapter.
The criterion for establishing whether a cell is double labelled was set in a preliminary experiment, where solution of mixed beads was injected into cerebellar lobule VII. All the retrogradely labelled pontine cells contained beads of both colours (see figure 16, page 64).
High power confocal microscopy was used to collect and present the data.

1. Vermal injections

A) Injections into lobule IX (uvula)

Case 98-14 illustrates this group of experiments.

Injection sites

Red latex beads were injected into the left hemivermis and green beads in the right. The centre of the red injection site was about 525 μm from the midline. Centre of the green injection site was slightly more lateral, at 675 μm from the midline. The densest core of the injection on both sides has a diameter of 300 μm.

Figure III-1: Diagram of the parasagittal sections through the cerebellum in case 98-14.

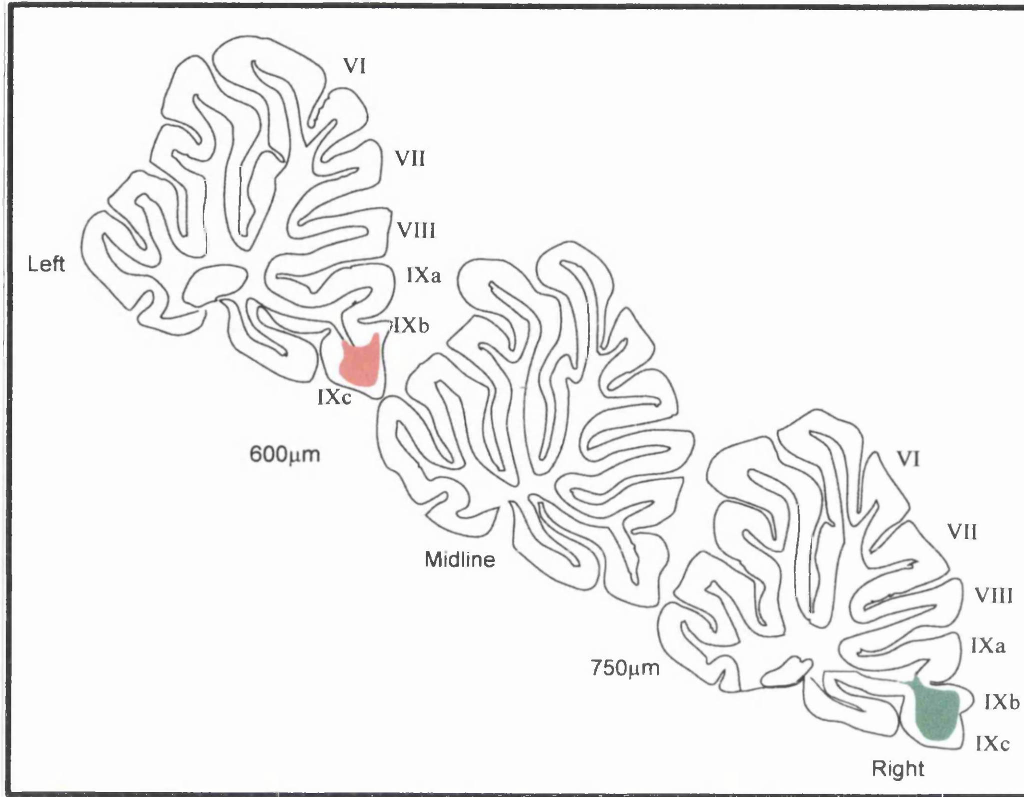
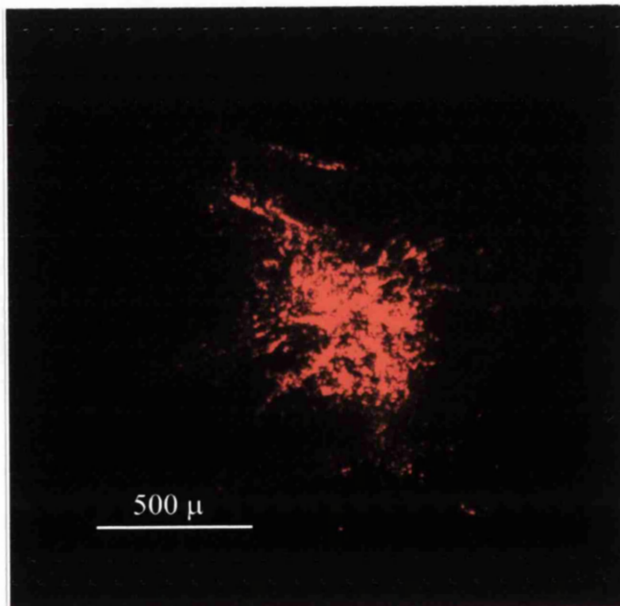
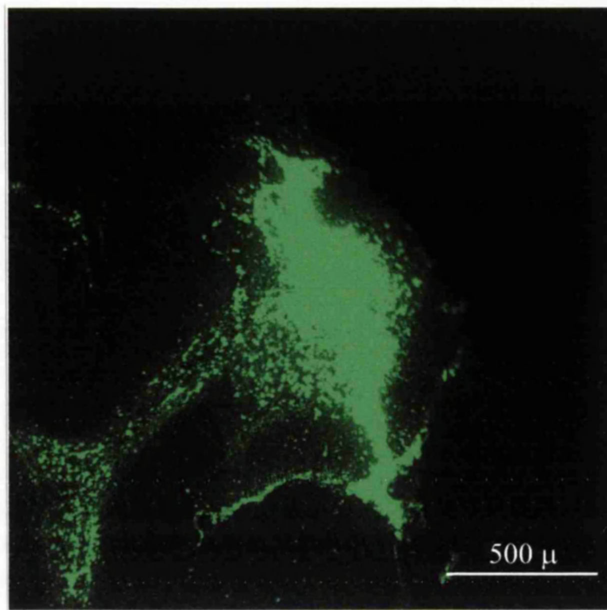


Figure III-2: Confocal images of the red (a) and green (b) injection sites

a)



b)



Olivary label

The olivary label resulting from each injection site is strictly contralateral and strikingly symmetrical. Both red and green cells were present in the caudal MAO: subnucleus a, subnucleus b and dorsal cap. The absence of retrogradelly labelled cells in the ipsilateral inferior olive confirms that the injection sites were strictly separate and did not cross the midline.

Figure III-3: Confocal image of olivary label in caudal MAO. The section in this figure corresponds to level 5/6 of the diagram in figure III-11.

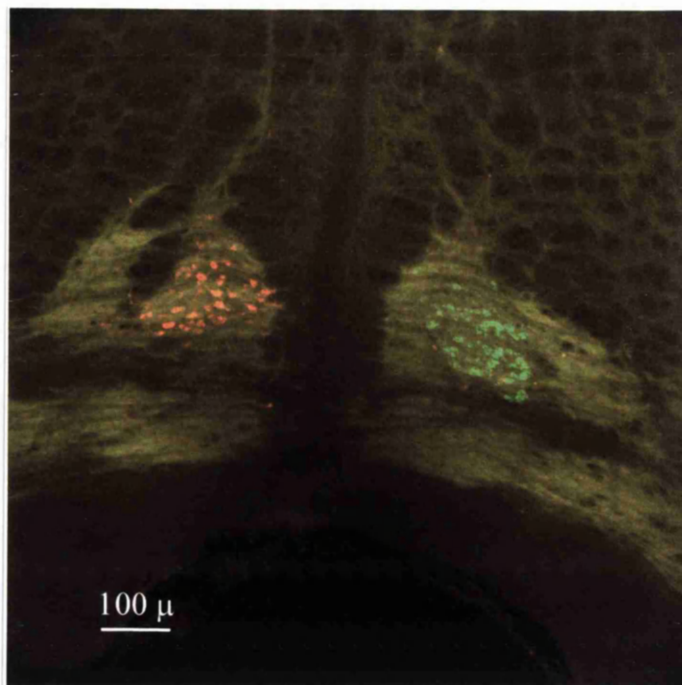
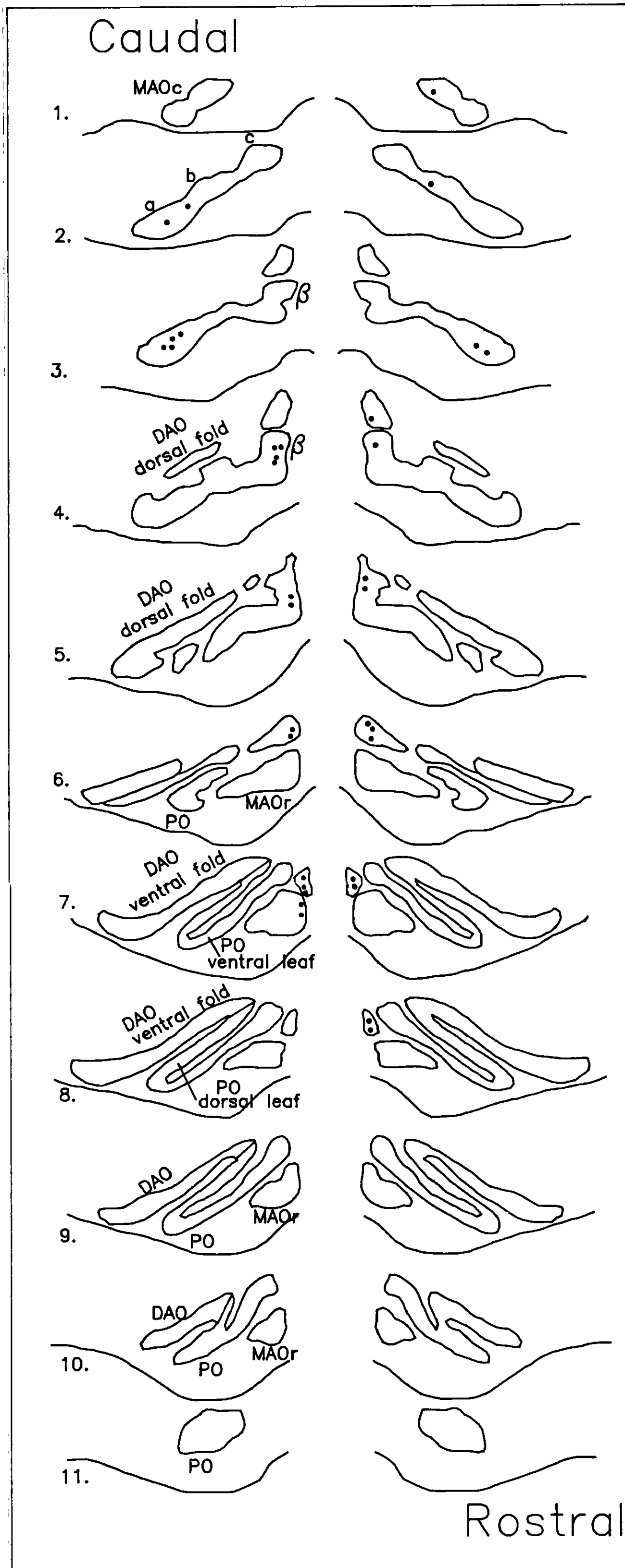


Figure III-4: Reconstruction of the olivary label in case 98-14.



Pontine label

Labelled cells were present throughout the rostro-caudal extent of the pons. The majority of pontine cells project to the contralateral hemivermis, but a few project ipsilaterally.

At most caudal levels red and green cells are present at symmetrical loci in the pontine nuclei. They are usually found in clusters. Most cells are found in the NRTP, medial nucleus, lateral nucleus and lateral portion of the ventral peduncular nucleus. Some cells were present in the dorsal peduncular nucleus as well.

Figure III-5: A diagram of the sections through the pontine nuclei with labelled cells in case 98-14.

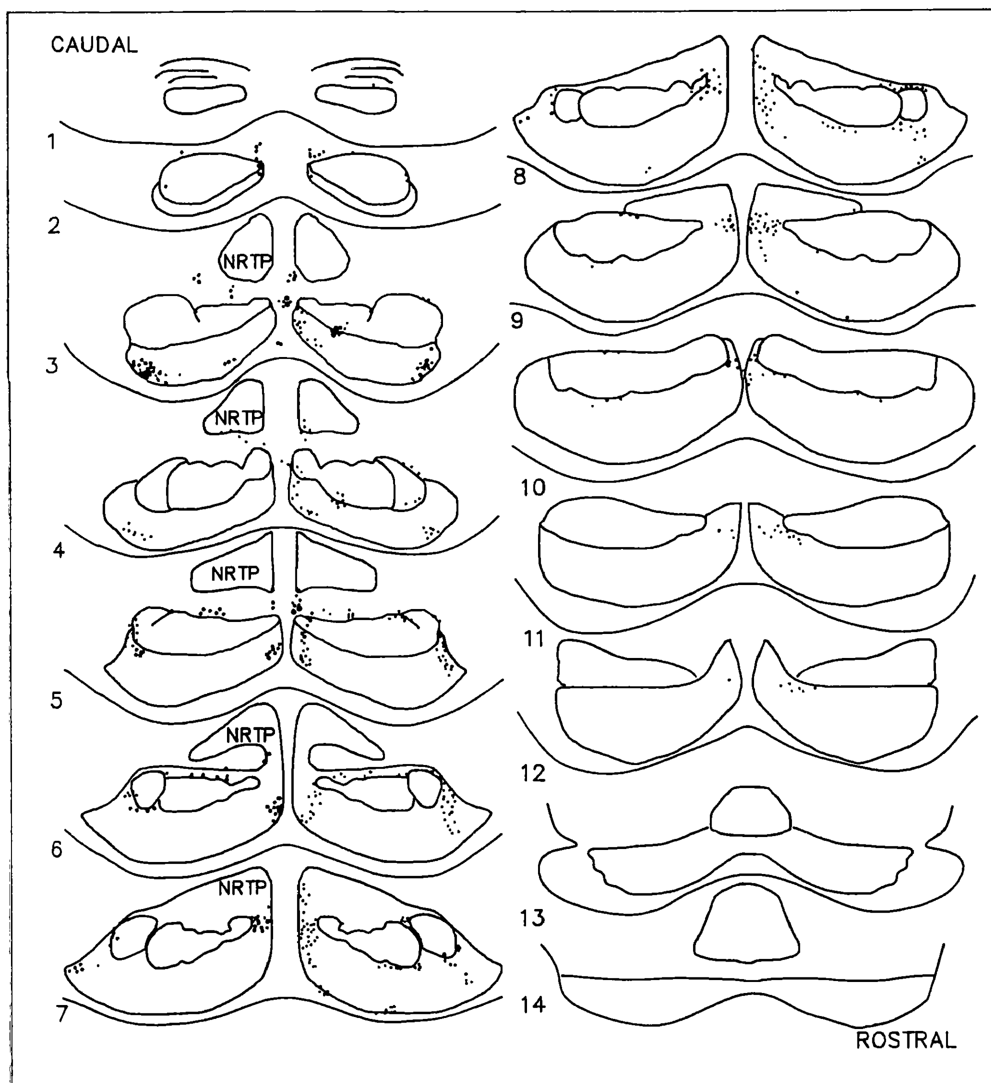
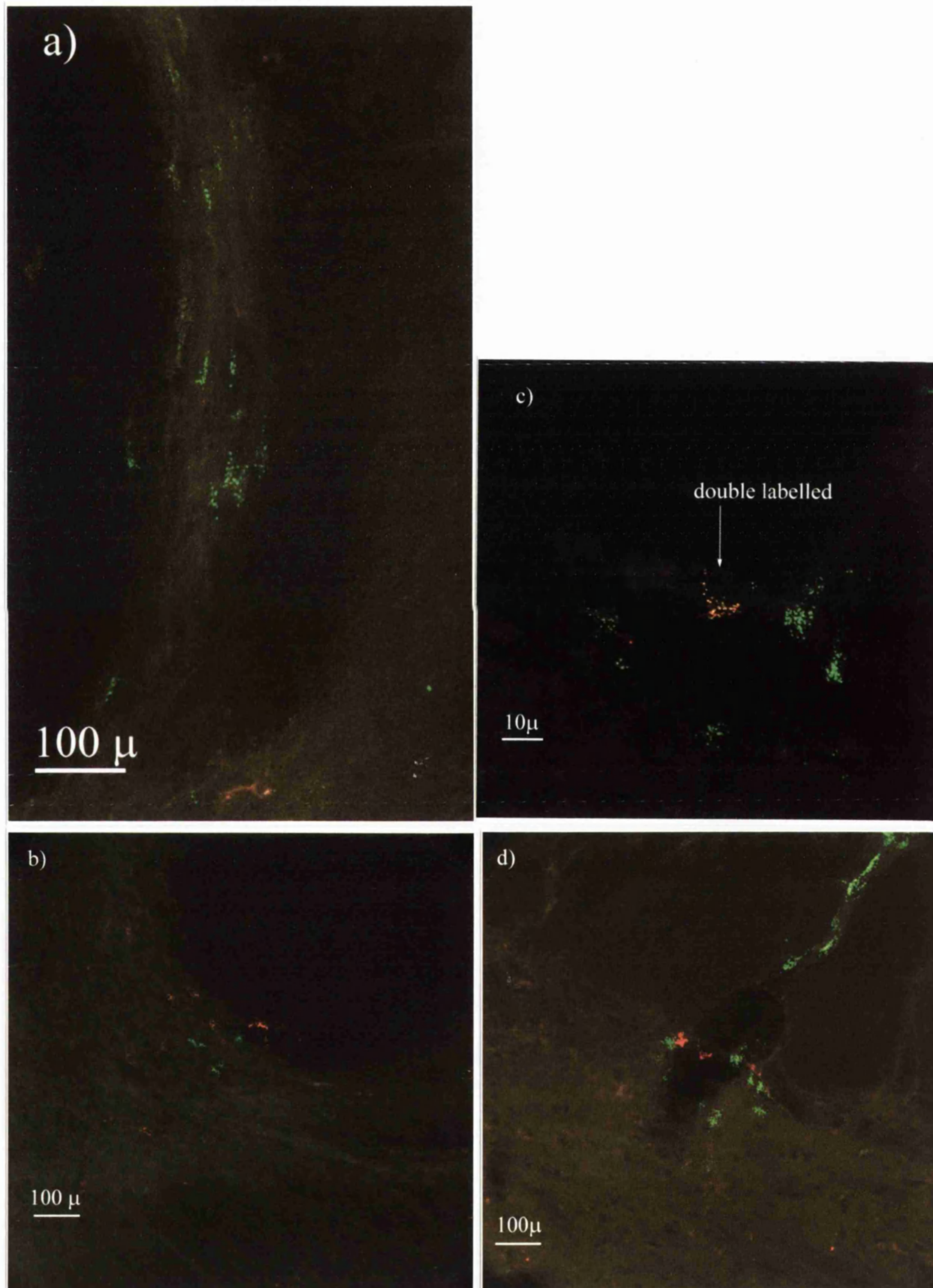
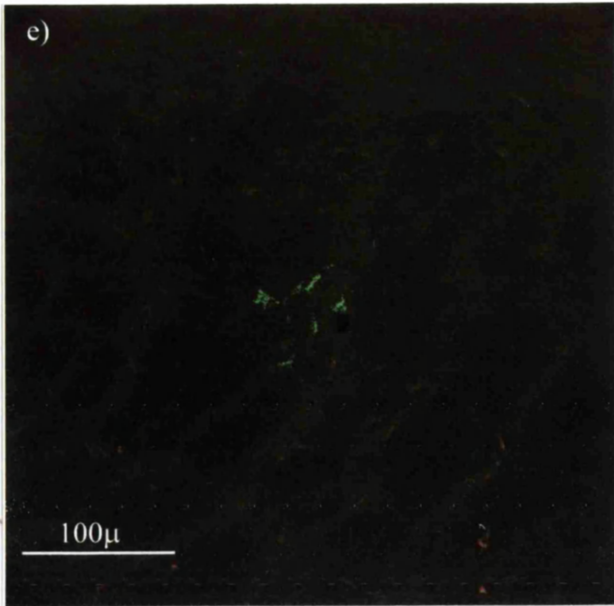
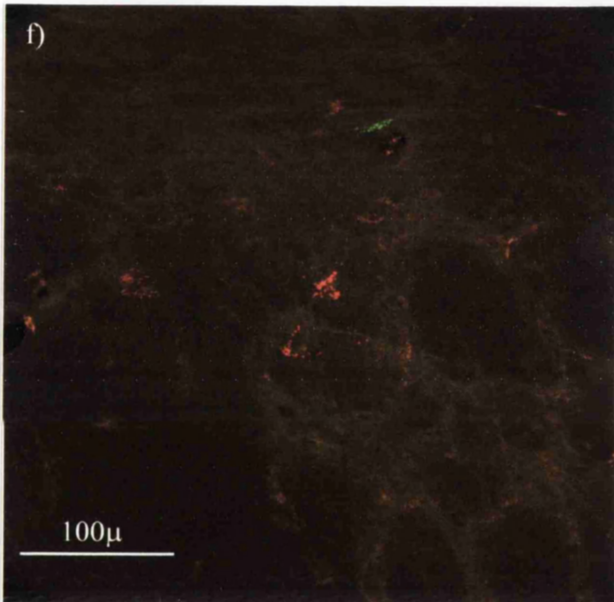
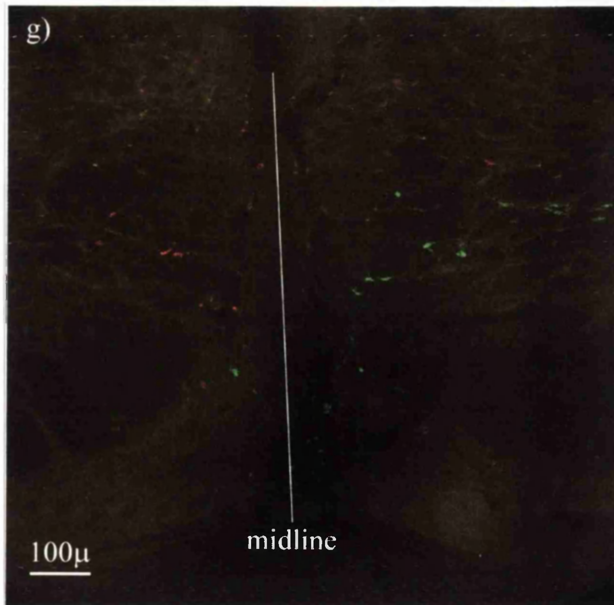


Figure III-6 : A confocal image of pontine cells in ventral peduncular lateral nucleus on the left (a) and right side (b) of the pons; ventral peduncular central, left (c) and right side (d); dorsal peduncular, left (e) and right side (f) ; medial pontine nucleus, bilaterally (g).




e)
100µ


f)
100µ


g)
midline
100µ

A few double labelled cells were present, in the caudal half of the pons. They were found in the lateral pontine nucleus, ventral peduncular central and dorsomedial pontine nucleus and in NRTP.

Table III-1: Results of counting the cells in the pontine nuclei in case 98-14

	Right side of the pons			Left side of the pons		
Section at level...	red	green	double	red	green	double
1	11	2	0	0	30	0
3	35	16	5	8	52	2
5	34	17	2	6	82	0
7	13	15	0	2	69	0
9	5	10	0	3	25	0
11	1	2	0	0	11	0
Total	99	62	7	19	269	2

From the cell counts presented in the above table it would appear that about 80% of cells giving rise to the mossy fibre terminals in each hemivermis of lobule IXb/c are in the contralateral pons. The number of double labelled cells is very small, representing only about 2% of all retrogradelly labelled cells.

B) Injections into lobule VII and IX

Case 95-38 represents these experiments.

The injections were made in the left hemivermis and the resulting injection sites were confined to lobules VII and IX, respectively. The cerebellar sections were cut in a parasagittal plane and the brainstem in a coronal plane.

Figure III-7: Diagrammatic reconstruction of the injection sites in case 95-38. Central section of the diagram is at the level of midline. Sections above and below it are 750 μm to the left and right, respectively, from the midline.

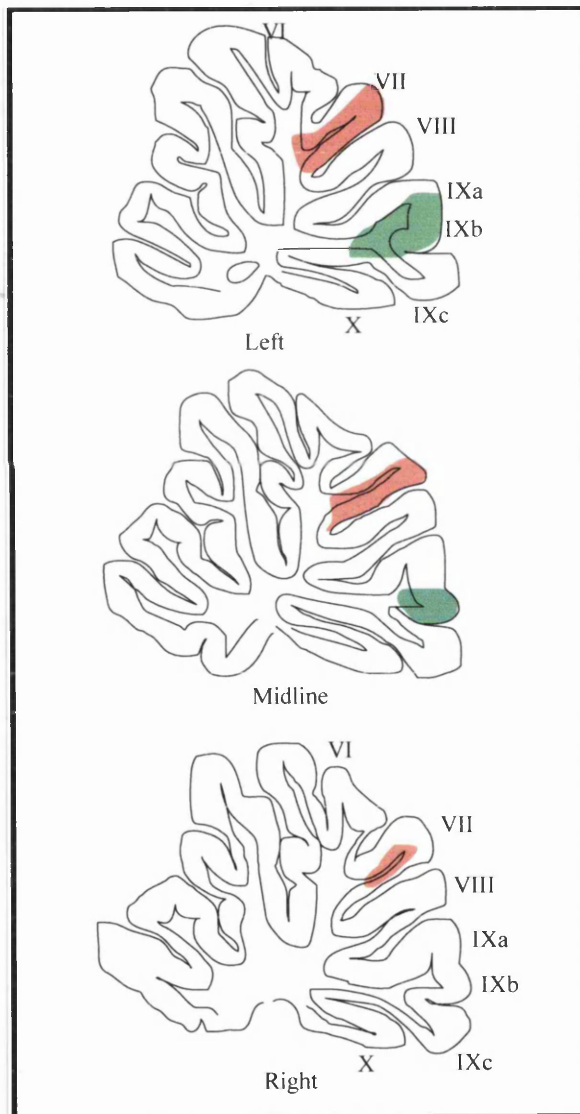


Figure III-8 : Confocal image of a section through the centre of red injection site.

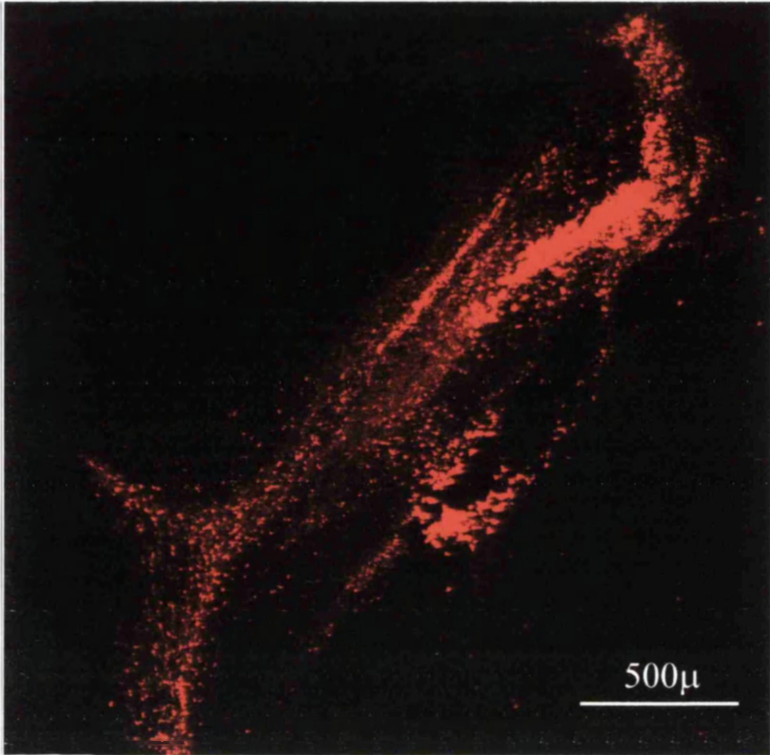
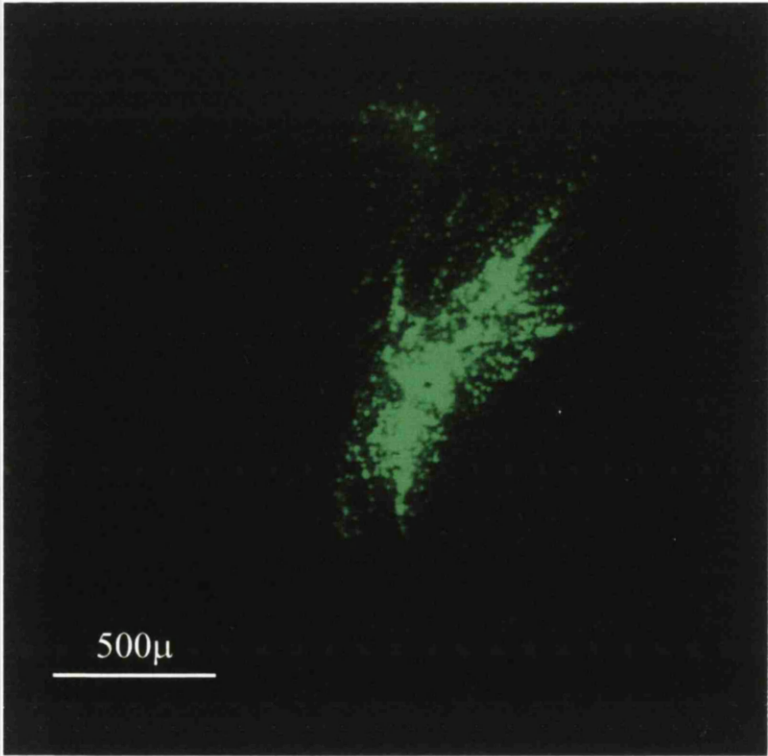


Figure III-9: Confocal image of a section through the centre of green injection site.



The red injection site in lobule VII occupies most of the left half of the vermal lobule VII, but spreads across the midline. Its size in mediolateral direction is about 1.5 mm. The green injection site spreads about 600 μm in mediolateral direction. It also spreads across the midline, but is mainly centred in the left hemivermis.

Olivary label

Retrogradelly labelled red cells were found predominantly in the contralateral MAOc (sublobule c, dorsal cap, subnucleus β), PO (ventral leaf, dorsal leaf), dorsomedial cell column and rostral MAO. Ipsilaterally, red cells are found in the subnucleus c of the MAOc and in the rostral MAO.

Retrogradelly labelled green cells were present in the contralateral MAOc (sublobule a, dorsal cap), PO (ventral leaf) dorsomedial cell column. Green cells on the ipsilateral side were found only at one level, in the dorsomedial cell column.

Figure III-10: A confocal image of red and green olivary cells in caudal MAO (ventrolateral outgrowth) on the right side. This image is from a section that would be at a level between sections 5 and 6 in the diagram (Figure III-11).

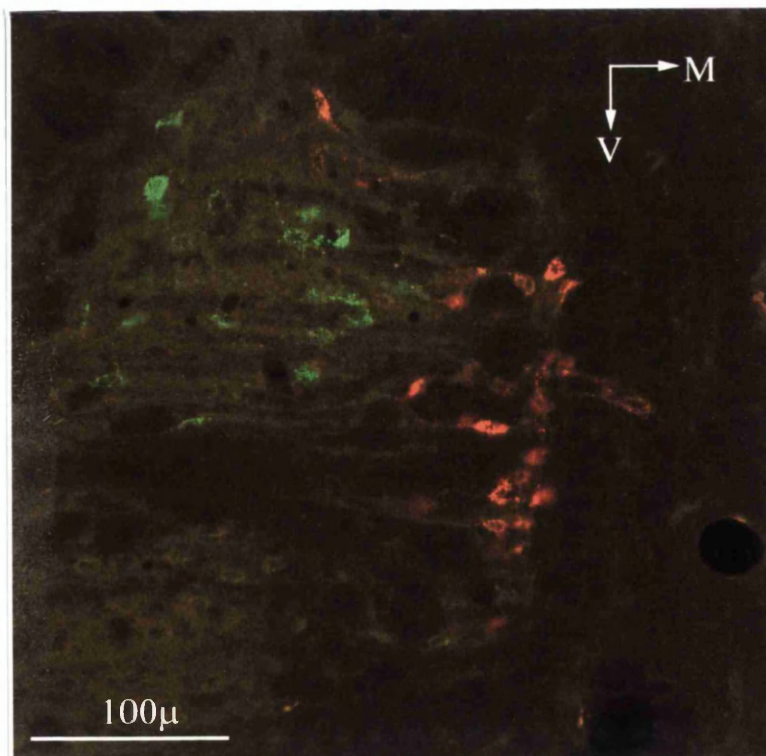


Figure III-11: A diagrammatic reconstruction of the olivary nuclei in case 95-38, with retrogradelly labelled cells at appropriate levels.

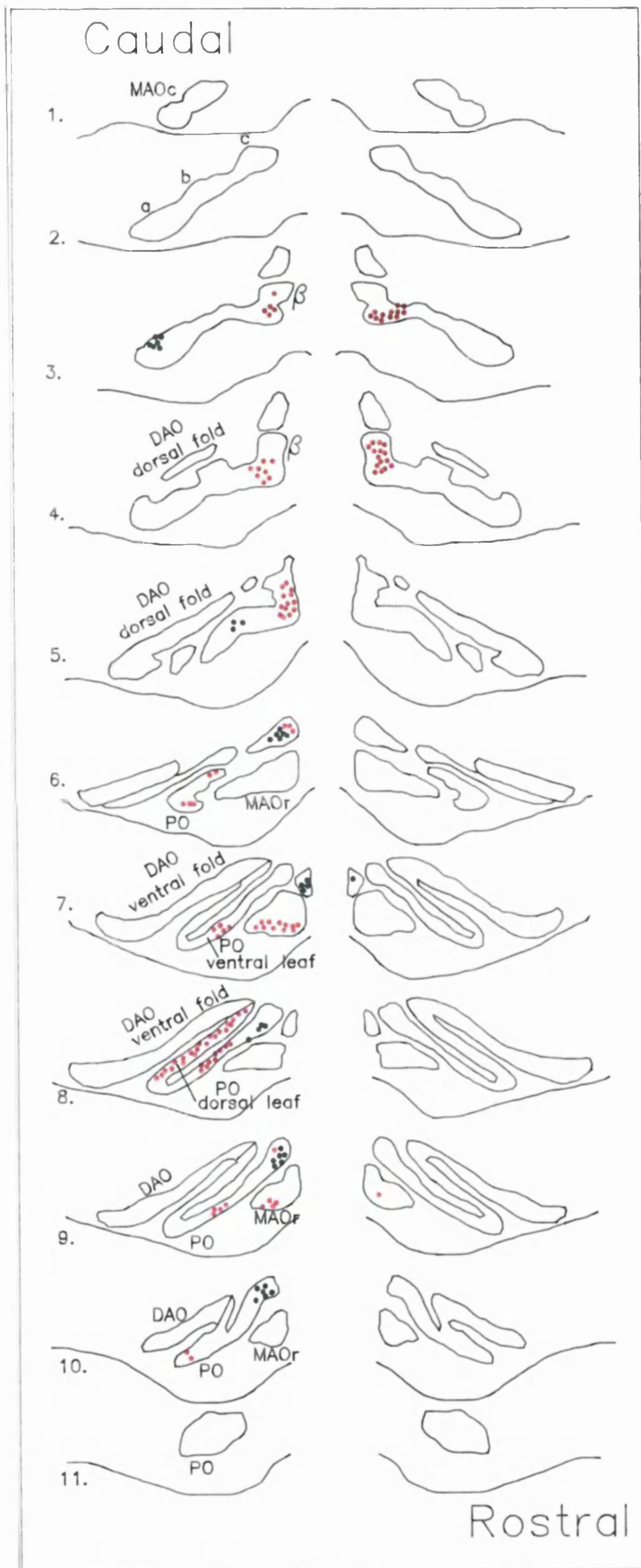
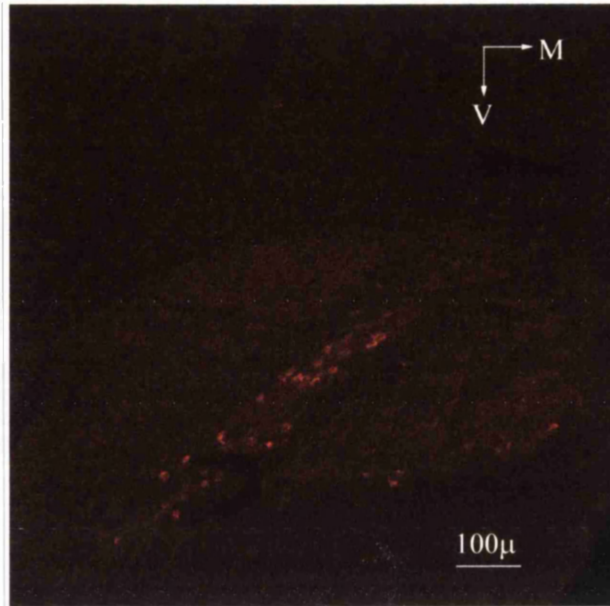


Figure III-12: A confocal image of the red olivary cells in the principal olive, right side. This image was from a section corresponding to approximately level 9 in the previous diagram.



As the figures show, there are more red labelled olivary cells and they are present in more olivary subdivisions. The fact that both red and green cells are found in the ipsilateral olivary nuclei confirms that both injection sites spread to the other side of the vermis.

<u>Pontine label</u>

In the pontine nuclei, green and red cells were abundant on both sides of the midline. The label was present throughout the rostro-caudal extent of the pons. Cells labelled with green or red beads were often found in close proximity, within the same pontine nuclei. However, there were only 4 cells in the whole of the pontine grey that were double labelled, containing beads of both colours.

Cells giving rise to mossy fibre terminals in lobule VII were found in nearly all pontine subdivisions. Most cells were found in the NRTP, medial peduncular, medial, ventral and lateral ventral peduncular nucleus.

Cells giving rise to mossy fibre terminals in lobule IX are found largely in the caudal pons, especially ventral peduncular nucleus (all subdivisions), dorsal peduncular and medial nuclei. Most of the patches formed by the green labelled cells resemble strongly the terminal patches of cortico-pontine axons.

Figure III-13: Diagram of the pontine nuclei with labelled cells.

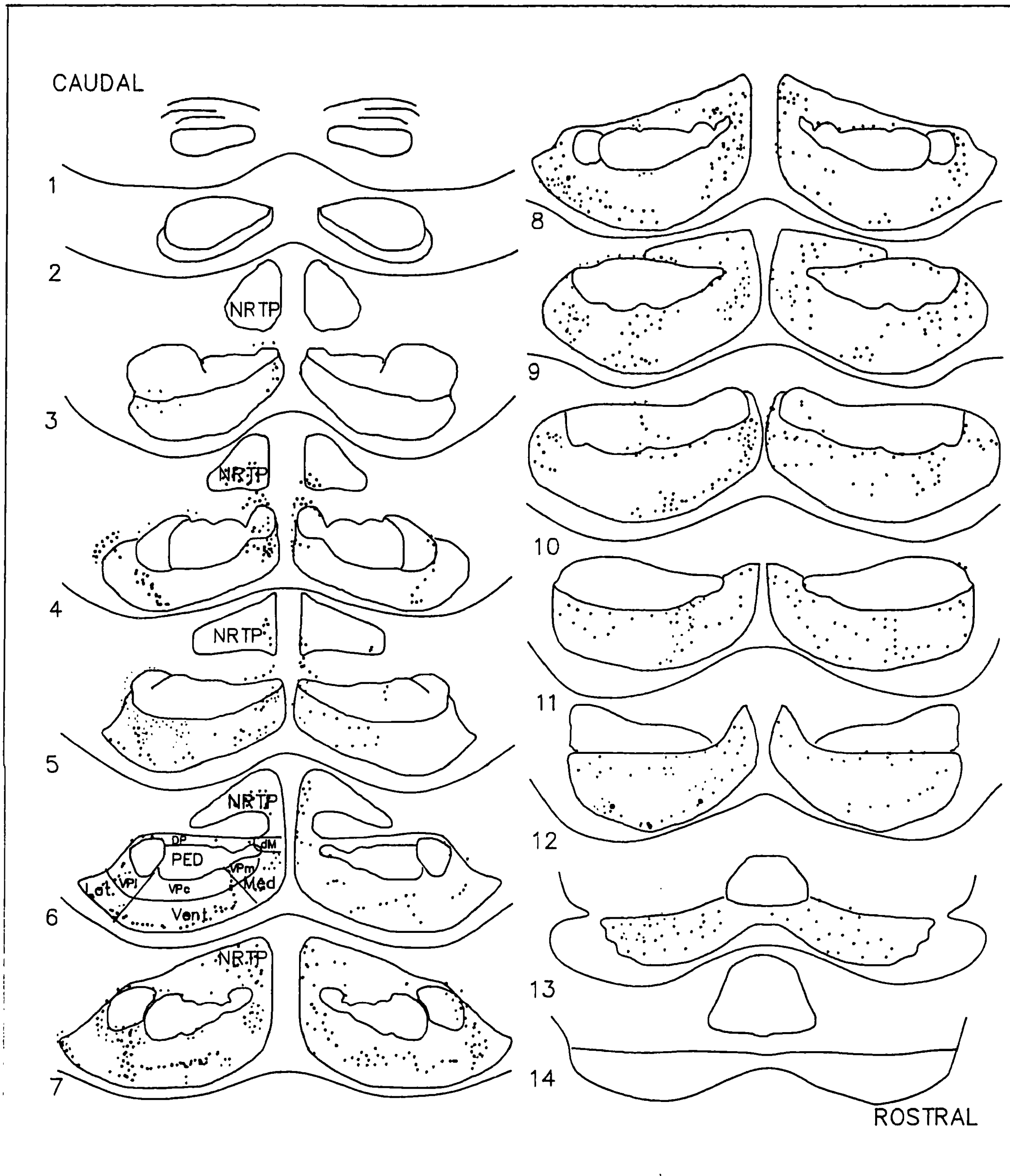
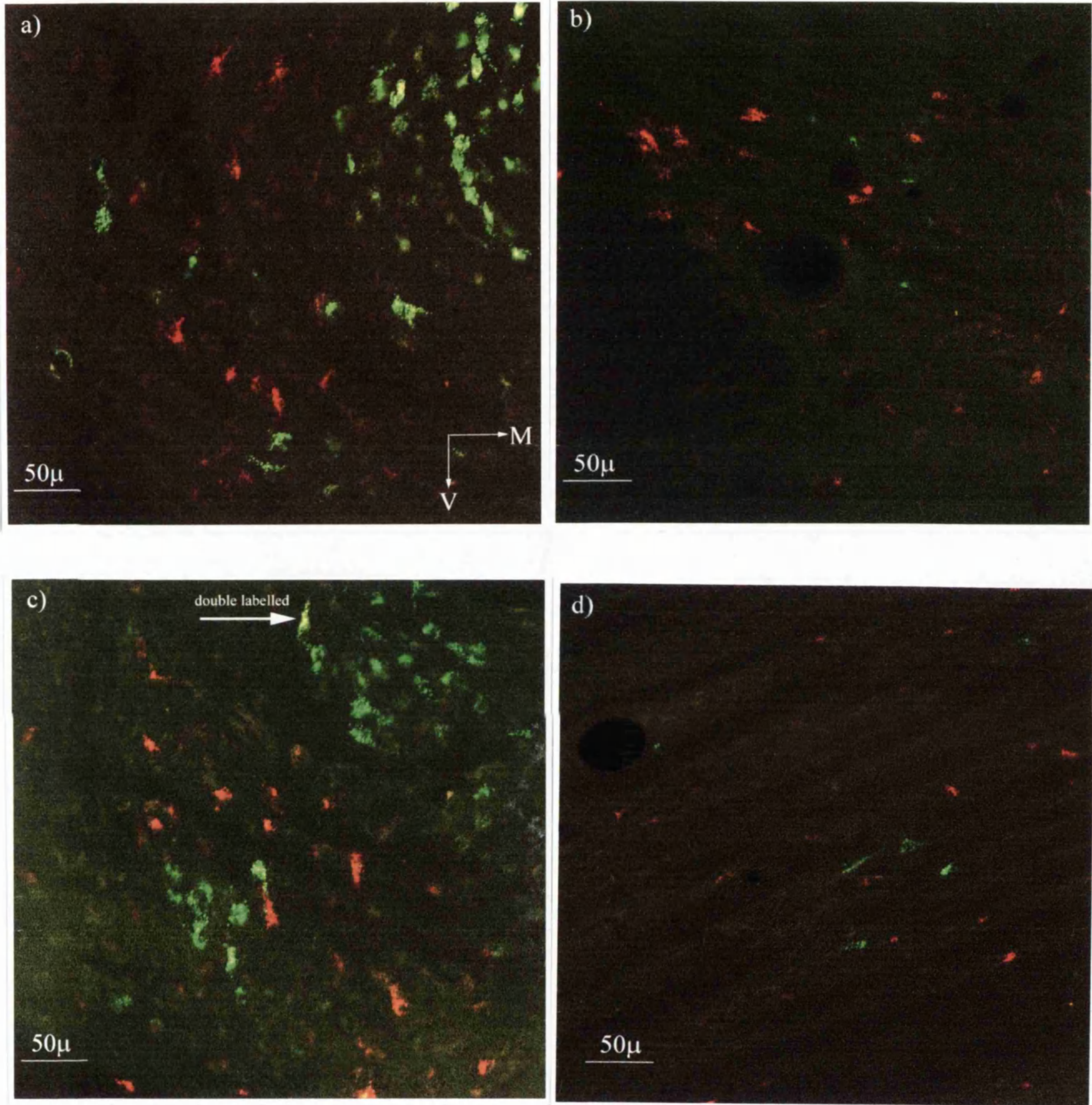


Figure III-14: Confocal images of labelled cells in the ventral peduncular lateral(a), dorsal peduncular (b), medial nucleus (c) and ventral nucleus (d) on the right side of the pons (contralateral to the injection sites).



One double labelled cell was present in the ventral peduncular lateral nucleus at the level 8 of the diagram. Three double labelled cells were found more rostrally, in the lateral and ventral nuclei, at approximately level 12 of the diagram.

Table III-2: Results of counting labelled cells in case 95-38.

	Right side of the pons			Left side of the pons		
Section at level...	red	green	double	red	green	double
4	95	23	0	82	5	0
6	88	46	0	55	4	0
8	157	27	1	102	4	0
10	116	14	0	101	1	0
12	127	20	3	72	4	0
Total	583	130	4	412	18	0

On the basis of cell counts in this case, it is possible to conclude that the number of cells in the pontine nuclei that simultaneously project to both vermal lobules VII and IX b is very low, no more than just over 0.5%.

2. Bilateral hemispheric injections- CrusI

Here I describe case 98-15 in detail.

Injection sites

Red fluorescent beads were injected in the left and green in the right cerebellar hemisphere. Each injection site is confined to Crus I, but green microspheres labelled this lobule almost completely, whereas the red injection site is more limited.

In rostrocaudal direction green microspheres spread to 900 μm and red microspheres to 600 μm.

Figure III-15 : Diagram of a series of coronal sections through the cerebellum showing red and green injection sites. Section 1. in the diagram is approximately 2.5 mm from the caudal end of the cerebellum; section 6. is about 1.2 mm from the rostral end of the cerebellum. Distance between sections in the diagram is 150 μm.

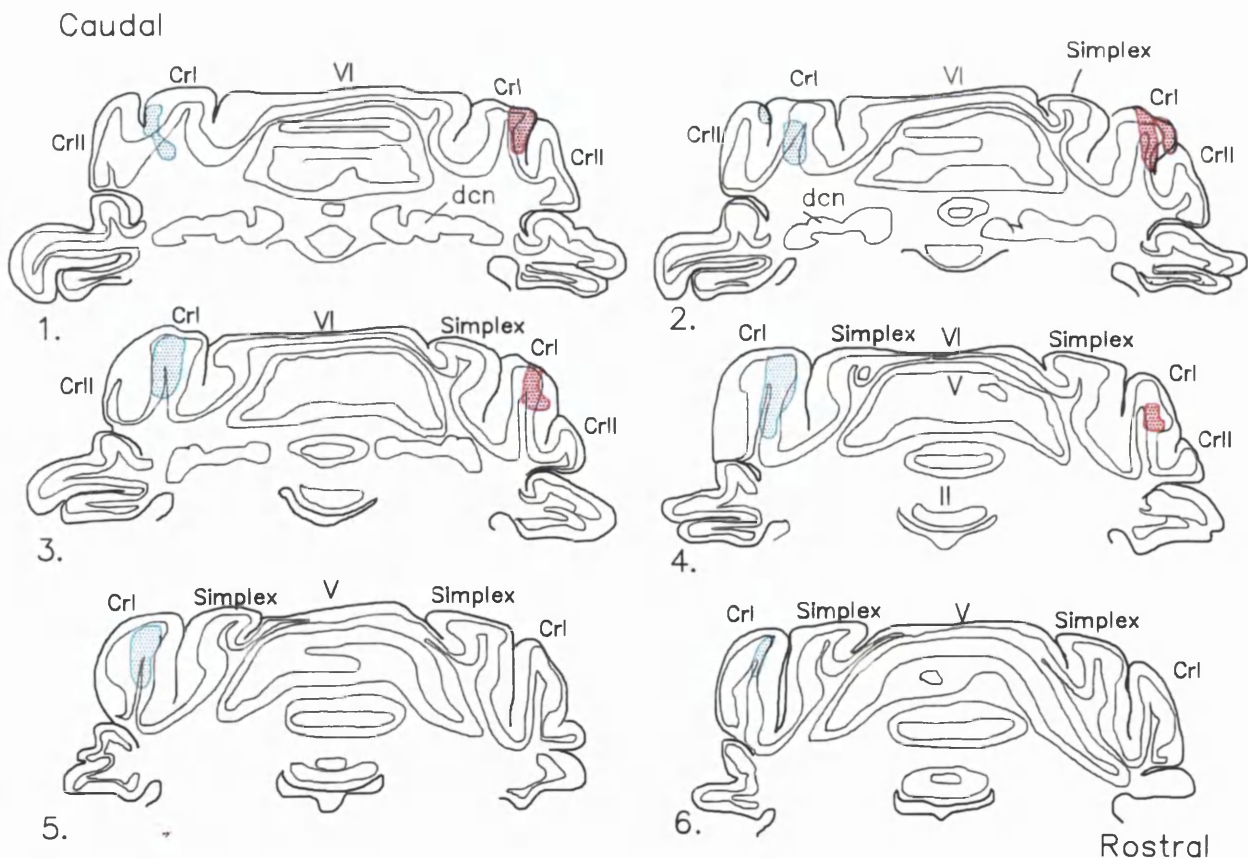


Figure III-16 a: Confocal image of the red injection site in case 98-15.

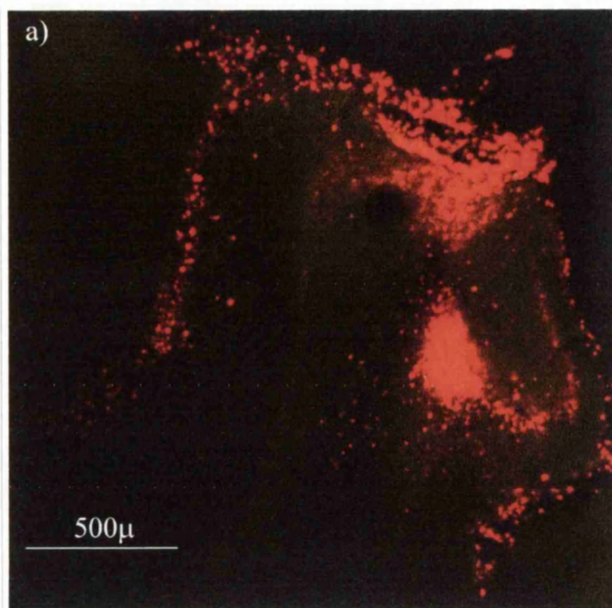
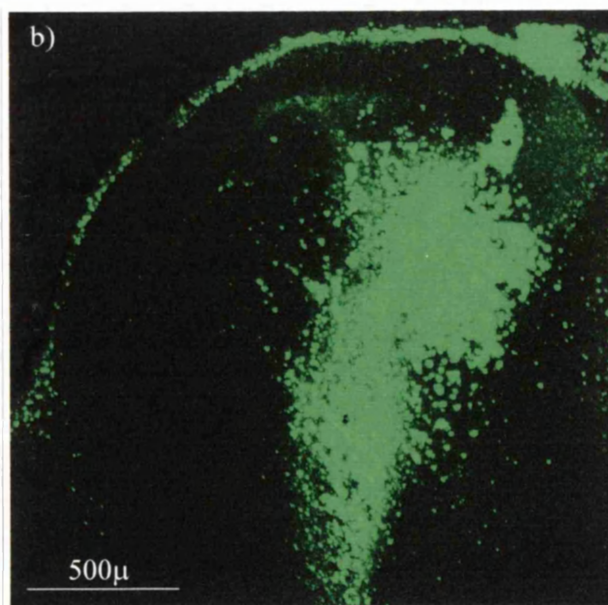


Figure III-16 b: Confocal image of the green injection site in case 98-15.



Inferior olive

Labelled cells in the inferior olive first appear at about 750 μm from the caudal end of the nucleus. At 900 μm from the caudal end of the inferior olive labelled cells are present on both sides, symmetrically in the caudal MAO, subnucleus c and subnucleus β. Further rostrally both red and green cells are present in PO and rostral MAO.

The number of green cells is much greater than the number of red cells, due to differences in size of the injection sites.

Olivary label in this case is also segregated; only the contralateral inferior olivary nuclei contain retrogradelly labelled cells.

Figure III-17: Confocal images of labelled olivary cells. Red cells in caudal MAO (a), and principal olive (b). Green cells in caudal MAO (c);rostral MAO and PO (d).

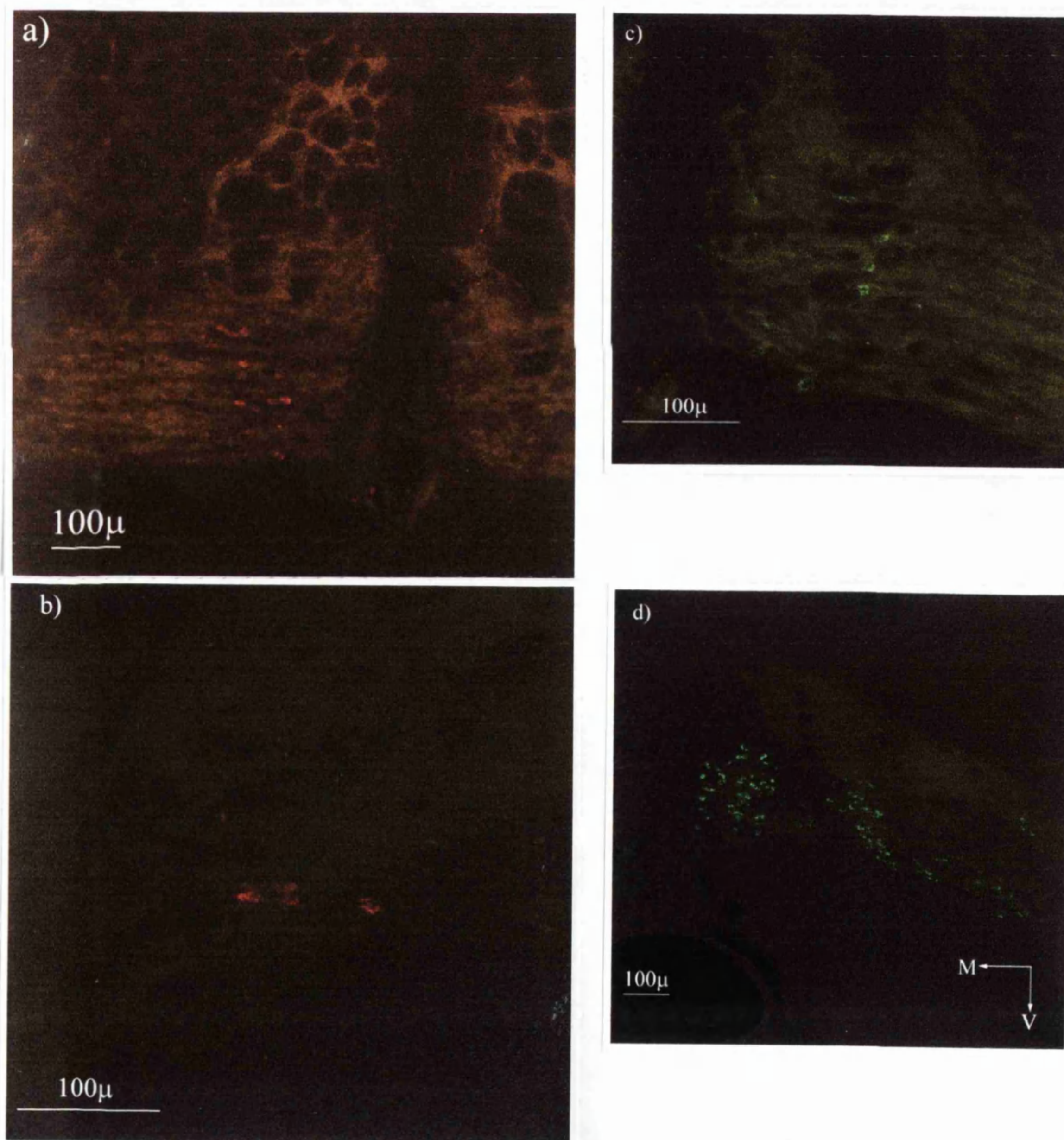
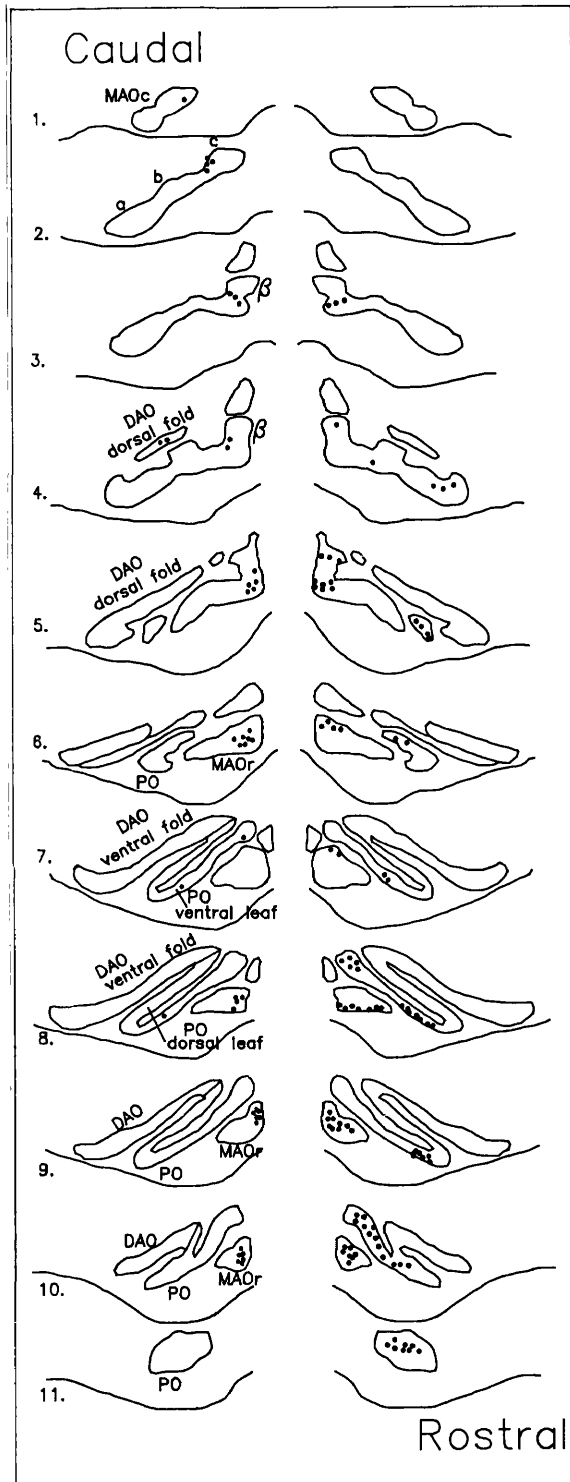


Figure III-18: A diagrammatic reconstruction of the inferior olivary nuclei and position of retrogradelly labelled cells in case 98-15.



Pontine nuclei

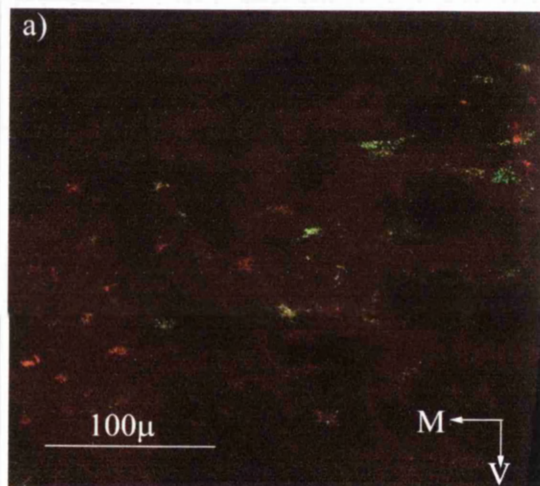
Retrogradelly labelled cells of both colours are present throughout the rostrocaudal extent of the pontine nuclei. Here, like in the inferior olive, the number of green cells outweighs the number of red cells, due to differences in injection site sizes.

At first glance labelled cells at caudal levels of pons appear to be positioned along the ventral border of the pontine nuclei. They are found in the ventral, medial and lateral pontine nuclei. Further rostrally, other nuclei contain labelled cells as well: ventral peduncular nucleus, its lateral and central portions, dorsal peduncular nucleus and medial nucleus.

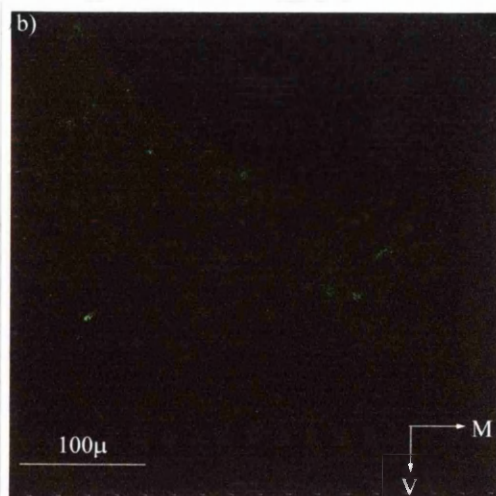
Lots of labelled cells are also found in the NRTP.

Figure III-19: Confocal images of labelled cells in the:

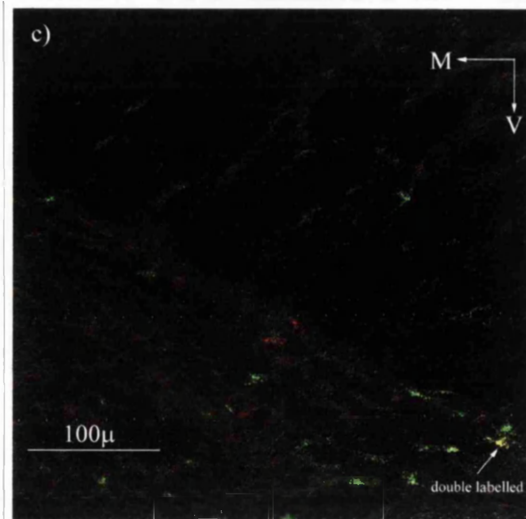
a) dorsal peduncular nucleus, left side



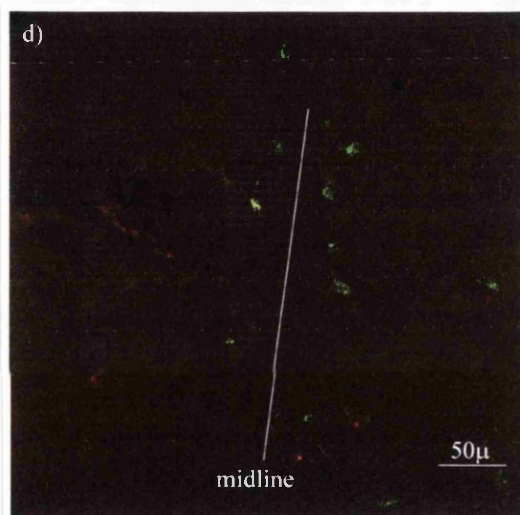
b) ventral peduncular lateral nucleus, right side



c) ventral peduncular central nucleus, left side



d) medial nucleus, bilaterally



e) ventral nucleus, right side

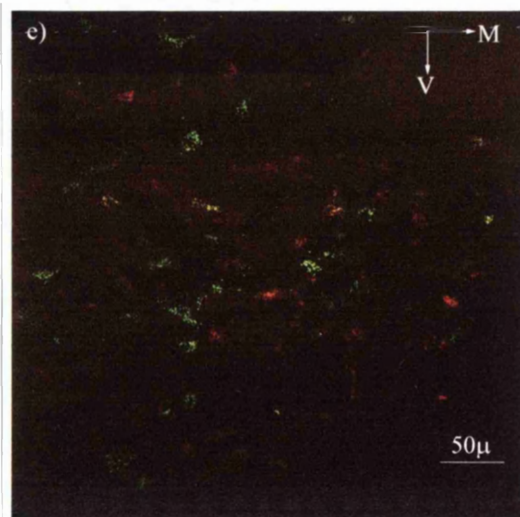
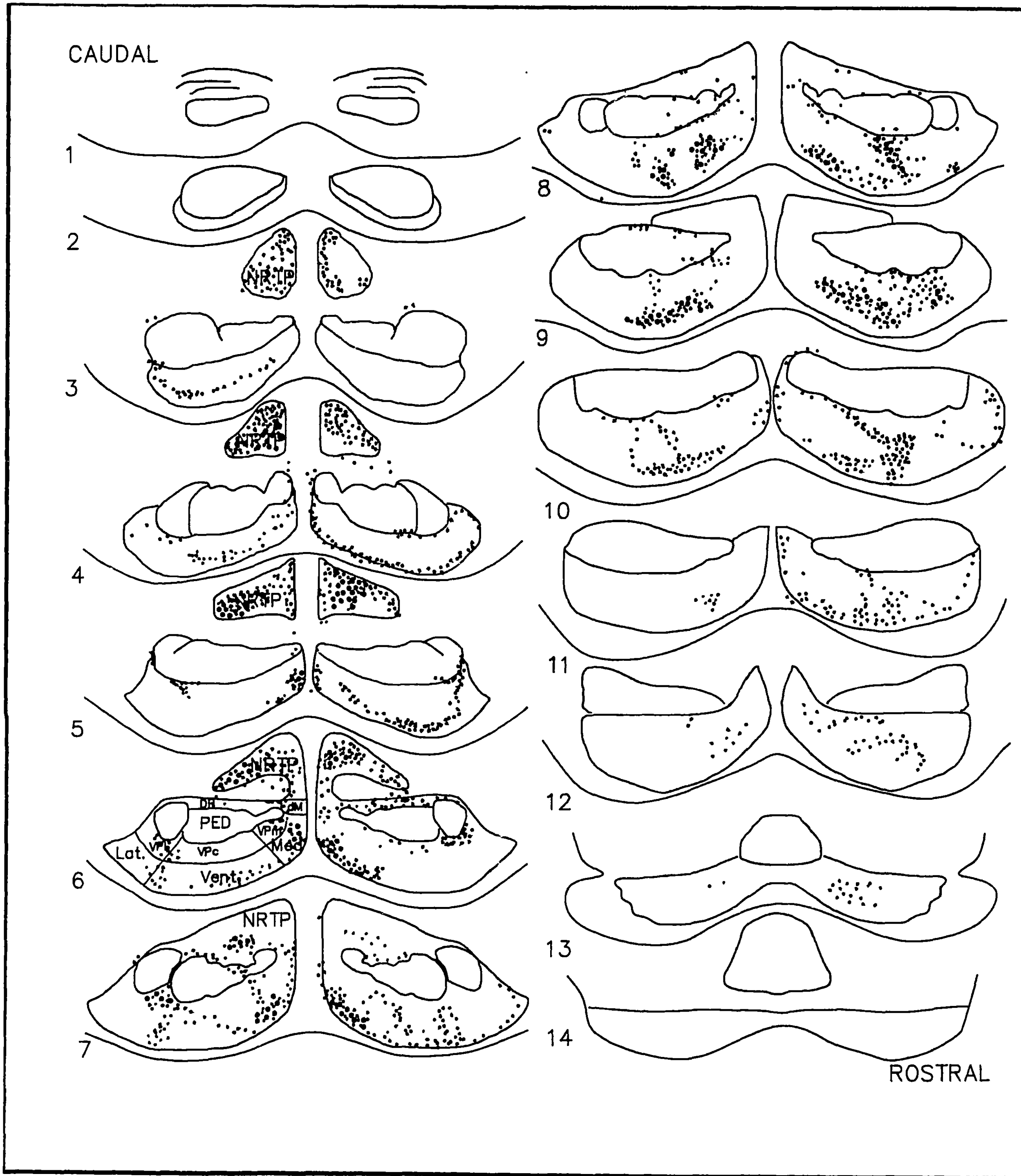


Figure III-20: Diagram illustrating position of retrogradelly labelled cells in the pontine nuclei, case 98-15.



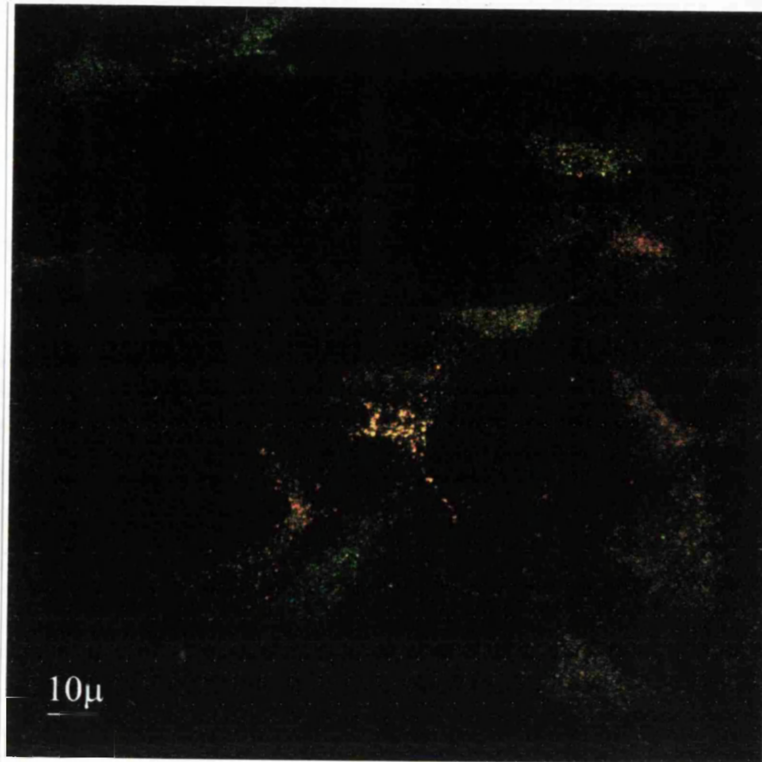
From the confocal images it is readily seen that a number of cells are double labelled, suggesting that these cells provide mossy fibre input to both cerebellar hemispheres. Even with classical fluorescent microscopy it was apparent that a number of double labelled cells in the population of ponto-cerebellar neurones was larger in this case than in experiments with vermal injections. At nearly all sections there were double labelled cells present, as shown in the Table III-3. Counts in this case show that cerebellar lobule Crus I receives about 70% of its pontine mossy fibre terminals from the contralateral side.

Table III-3: Results of the cell counts in case 98-15

	Right side of the pons			Left side of the pons		
Section at level....	red	green	double	red	green	double
3	2	33	0	7	63	0
4	82	50	10	32	132	0
5	76	54	16	32	171	17
6	94	58	12	40	133	10
7	69	73	15	23	129	8
8	83	55	12	36	140	12
9	45	29	12	13	103	11
Total	451	352	79	183	871	58

Although more double labelled cells were seen in this case, they represent only about 8% of the total of the retrogradely labelled pontocerebellar cells.
They were distributed in different pontine nuclei as well as NRTP.

Figure III-21: High magnification image of double labelled cells in the NRTP in case 98-15.



DISCUSSION

The results of this study showed that the cerebellar hemivermis and hemisphere receive their pontine mossy fibre input largely from the contralateral side of the pontine nuclei. The contribution from the ipsilateral pons varies between about 20% in case of vermal injections to 30% in case of hemispheric injections. There are reports in the literature of bilateral pontocerebellar mossy fibre afferents, but to my knowledge this is the first quantitative data on the relative contributions from ipsi- and contralateral side.

Labelled cells in each case were found in all pontine nuclei but not necessarily at all levels through pons. Densities of the projections to different cerebellar lobules varied somewhat. From qualitative analyses of the pontine sections it appears that the densest projections to lobule VII originate mostly from cells in ventral, ventral peduncular lateral and medial nucleus. Lobule IX receives most of its projections from medial, lateral and dorsomedial pontine nuclei. Crus I appears to receive most of its projections from the ventral and medial pontine nuclei and the NRTP. However, trying to ascertain precise contribution from each nuclei to any of the cerebellar lobules is not an easy task. The borders between the nuclei are arbitrary and some errors in quantifying cells in a particular nucleus are inevitable.

Based on these data, it is clear that many retrogradelly labelled cells are found in the domain of the cortico-pontine terminal patches, but there is also a great number of cells which are outside these regions.

The great majority of pontine cells appear not to send bifurcating axons to the cerebellar cortex. The largest number of double labelled cells was only about 8% of the total of the retrogradelly labelled pontine neurones, as was shown in case of homotopical Crus I injections. It is possible, however, that the pontine axons bifurcate and terminate in much widely separated regions of the cerebellar cortex or that they simultaneously innervate regions of vermis and regions of hemisphere, but these experiments did not explore this possibility.

The smallest number of double labelled cells was found following vermal injections of lobules VII and IX. It seems reasonable to conclude that most axons send their collaterals across the midline and that a negligible population of neurones collateralise parallel to the midsagittal plane. This finding is at variance with the conclusions of Palay and Chan-Palay (1974) who found that mossy fibre collaterals tend to stay in the

parasagittal plane as they enter different cerebellar folia. This issue has been controversial. On the basis of double retrograde tracing results, Mihailoff (1983) suggested that more mossy fibres collateralise in a transverse plane. Shambes, Gibson and Welker (1978) found electrophysiological evidence for both parasagittal and transverse collateralisation of the mossy fibres. Results in this study are in agreement with those of Mihailoff's (1983), but further experiments are needed to establish whether different folia of the same cerebellar lobules receive parasagittally ramifying mossy fibres. Recent observations in monkeys would suggests quite the contrary. Thielert and Thier (1993) studied dorsolateral pontine projections to different sublobules of vermal VII using fast blue and fluorogold or cholera toxin and fast blue as a combination of retrograde tracers. They found no double labelled cells but pontine cells projecting to different sublobules were spatially close.

Rosina et al. (1980) and Rosina and Provini (1984) studied the problem of pontocerebellar collateralisation in cats, using fluorescent dyes (bisbenzimide, Evans blue and granular blue). They reported "up to 30% of double labelled cells in some microscope fields". There are no other parameters to help interpret these results, so it may be that the authors saw a lot of labelled cells in a very few microscopic fields or that they, indeed, saw up to 30% of double labelled cells. If the latter is the case then the results of my study are sharply at variance with their findings. Mihailoff (1983) addressed the problem of axonal branching in rat pontocerebellar system using different dyes, nuclear yellow and propidium iodide. The author attempted to see whether pontocerebellar axons branch more within the cerebellar hemisphere or between both hemispheres. In cases in which injections were placed in both hemispheres there were modest numbers of double labelled cells. About 90 double labelled cells were reported in cases where injections involved combination of lobules: simplex-simplex, Crus I-Crus I, Crus II-Crus II, Crus II- paramedian lobule. Slightly more (105) double labelled cells were seen in cases where the injection sites involved different lobules in the same hemisphere : simplex- Crus II and paraflocculus -Crus II.

In the present study, bilateral injection into Crus I produced the total of 137 double labelled cells, about 50% more than in Mihailoff study. However, the percentage of double labelled cells in the population of all retrogradelly labelled pontine neurones was only about 8%, which is very low. Mihailoff's study does not show the relative values but he also suggests that the number of branching axons overall is very low.

The larger number of double labelled neurones seen in my study is easily explained by the superior quality of optics available with modern confocal microscope, which makes it easy to register cells which fluoresce under both wavelengths. Also, nuclear yellow is a dye which is very toxic and produces extensive necrosis at the injection site. It is not possible to determine the actual area of uptake accurately so the injection site produced by this dye may, indeed, be significantly different from the region which fluoresces. Fluorescent latex beads, which I used, are non toxic, so all the uncertainties related to nuclear yellow do not apply here.

I tried to relate the injection sites in my study to particular areas of the cerebellar cortex that were described as receiving whisker input from the trigeminal nuclei and cerebral cortex (via pons). However, the injections did not involve whisker responsive areas exclusively. Firstly, for placement of injection cannulae I was guided by detailed diagrams of Shambes et al. (1978 a,b) and Joseph et al. (1978), but could not be absolutely certain that the injection sites were always centred in the whisker responsive patches. Even if that was somehow achieved, it would still be unlikely that only the tactile regions of the cerebellar cortex were involved. For instance, midvermis in all mammals (lobules VI and VII) is traditionally considered as the primary cerebellar area receiving visual and auditory inputs (Snider and Stowell, 1940). Electrophysiological studies in rats (Burne and Woodward, 1984; Azizi, Burne and Woodward, 1985) demonstrated that vermal lobule VII, indeed, receives auditory and visual inputs. The data for response properties of granule cells in rat lobule IX is lacking, apart from the studies on tactile input. However, Azizi et al.(1981) suggested that uvula also receives visual and auditory inputs, on the basis of its anatomical connections. Retrogradely labelled pontine neurones in this study are therefore likely to be a part of tactile, auditory and visual ponto-cerebellar systems.

The low counts of double labelled cells is surprising, considering the widespread nature of mossy fibre terminals that are seen following small injections of anterograde tracers into pontine nuclei (Serapide et al., 1994). However, the injection sites in this study involved only easily accessible patches of cerebellar cortex responsive to whisker manipulation. Moreover, my expectation was that collaterals of the pontocerebellar neurones would preferentially cross the midline and innervate the homotopical sites in vermis and hemispheres in order to enable bilateral coordination of body parts to affect smooth movement. Hemispheric injection sites in this study were, in fact, close to their

medial end, so the further lateral regions remain to be investigated. Another obvious question, which is not answered here, is whether whisker patches in vermis and hemisphere receive collaterals of the pontine nuclei. These issues may be addressed simultaneously in some future study, since the new confocal microscopes are capable of differentiating more than two wavelengths at the same time, and there are latex microspheres labelled with five different colours.

Conclusions

At the end of this thesis I wanted to sum up the findings and highlight questions which arose as a result of this work.

First of all, I find that the cortico-ponto-cerebellar pathway can be unravelled using rat barrel system as a model, because, as expected, it shows quite a precise pattern in its organisation. There are four pontine areas receiving projections from cortical barrel columns: ventral peduncular, dorsal peduncular, medial and ventral nuclei. Cortical cells representing large facial whiskers project to the pontine nuclei in a more restricted fashion than the cells representing smaller, rostrally placed whiskers. Larger cortical barrels project largely to the ipsilateral pontine nuclei, whereas smaller cortical barrels have bilateral projections. An obvious question is whether these contralateral projections arise form a separate population of the layer Vb cells or are they collaterals of the ipisilaterally terminating axons? If there is a separate population of cells projecting bilaterally, it would be interesting to characterise their electrophysiological properties and their anatomical connectivity with other brain structures.

Almost all the cells in layer Vb that project to the pontine nuclei send collaterals to the deep layers of the superior colliculus. There were no differences in projections from large and small cortical barrels to the superior colliculus, although the physiological recordings show augmented representation of the whiskers that cross the visual field (especially rows A and B). Likewise, cells in the dysgranular zone as well as cells in the granular zones of the SI project to the deep layers of the superior colliculus. Cortical information related to whiskers and smaller hairs on the face is, obviously, shared among the structures that have a function in movement. However, pontine nuclei, and hence the cerebellum, appear to receive projections related to the smaller hairs on the face in a different manner than the information related to the large whiskers. Superior colliculus, on the other hand, receives a collateral projection regardless of this distinction.

Pontine cells receiving information from cortical barrells project to the cerebellar cortex. In the third experimental chapter it was shown that pontine cells projecting to different regions of the cerebellar cortex can be found in the neighbouring regions of the pons, but very few cells send collaterals to different areas of the cerebellar cortex. However, it is still not known whether individual pontine cells branch extensively

within a single tactile representation patch in a cerebellar lobule or whether they send collaterals reaching further across the midline. This question can be answered in two ways. First would be to make small injections of retrograde tracers into a single electrophysiologically defined tactile patch of the cerebellar cortex and map retrogradelly filled pontine cells. Another, more demanding, approach would be to record from a whisker driven pontine cell and inject it with an anterograde tracer to reveal all of its orthograde axonal terminals.

Characterising the response properties and connections of pontine cells is the logical next step to bring us closer to understanding the role of the cerebellum in smooth execution of movement.

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