Histol Histopath (1993) 8: 167-171

Histology and Histopathology

Distribution of cortico-visual neurons projecting to the pons in the cat. A retrograde labelling study with rhodamine latex microspheres

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Summary. Injections of the low diffusion retrograde tracer rhodamine latex microspheres were made in the pontine grey matter of the cat in order to study the cortical convergence to the pons. We found a different distribution of cells in the convex surface of the brain hemisphere making ventral or dorsolateral injections. In the first case, cells were grouped in the top of the gyri. In the second case, cells were more frequent in the bottom of the sulci. Our results show a possible retinotopic organization of this projection.

Key words: Visual cortico-pontine grey matter projection. Rhodamine latex microspheres, Retrograde labelling

Introduction

The cortico-visual pontine grey matter pathway is involved in many important processes such as control of ocular position, the following of objects, etc. This is because it is integrated in the cortico-ponto-cerebellar circuit. Numerous studies have been carried out using degeneration or peroxidase transport techniques.

The first description of the advantages of the rhodamine latex microspheres (RLM) was provided by Katz et al. in 1984. It involves small acrylic spheres (0.02-0.2 μm in diameter) which, when injected into the nervous tissue, diffuse very little, even over long periods of time, giving a very well delimited injection site. The transport time is very short, and generally speaking, after twelve hours it is already possible to detect a clear labelling. This marker is neither phototoxic nor cytotoxic. Once retrogradely tranported, the spheres are confined to the cytoplasm and adjacent parts of dendrites and axon (Katz, 1987). It is thought that they are trapped by the damaged fibres as a result of the injection, although it is also possible that they are uptaken by synaptic clefts through an endocytosis process (Gonatas et al., 1972). The microspheres are probably transported inside vesicles, as their negative charge would cause them to be transported anterogradely rather than retrogradely (Adams and Bray, 1983). It appears that the uptake process requires the spheres to undergo a carboxylation process (Cornwal and Phillipson, 1988). The transport capacity of the RLM depends on their size; those measuring 100 nm or more in diameter remain at the injection site or along the axon; however, those that reach the cellular soma, included in lysosomes, are always the smaller spheres (Holländer et al., 1989). This is because of the relatively small size of the extracellular space (in the case of broken ends) or of the synaptic clefts (in the case of synaptic capture), both of which act as «filters» for diffusion of the larger globules (Cornwald and Phillipson, 1988). The RLM may also be used as a tracer visible under an electron microscope, employing potassium permanganate for negative contrast (Egensperger and Holländer, 1988). One particular interesting application is the simultaneous implementation with Golgi's technique, which may constitute an alternative to the intracellular injection in the entire animal (Cactsicas et al., 1986). Katz and Iarovici (1990) have recently introduced to neurobiological research a new tracer in which the acrylic spheres emit a green light when illuminated with the appropiate filter set. The use of fluorescent dyes may be of special interest in double labelling experiments.

Our aim was to study the visual corticopontine projection by means of the transport of RLM, presenting the advantages and disadvantages of its utilization.

Materials and methods

We used 5 adult cats of both sexes, weighing between 1,700 and 2,500 grs. The animals were anaesthetized with a mixture of Ketamine chlorhydrate (Ketolar®) (0.2 ml/kg of a solution 50 mg/ml) and tiazine chlorhydrate (Rompun®) (0.06 ml/kg of a solution 23.32 mg/ml) and then placed in a stereotaxic frame. 0.5 μl of RLM (Luma Fluor, Inc.) were injected into the ventral and

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dorsolateral part of the rostral half of the pontine grey matter using a needle guided by stereotaxic coordinates (Reinoso Suarez, 1961).

After a survival time of approximately 48 hours the animals were deeply anaesthetized before intracardialy perfusion with 2 litres of 4% paraformaldehyde in standard phosphate buffer (0.1M, pH 7.4) at 4°C following a wash with saline serum at room temperature. After removing the entire brain, two sections were made, one containing the injection site and the other corresponding to the ipsilateral and contralateral visual cortex. By means of a vibratome (Campdem Instruments, Ltd.) we obtained 200 µm thick slides which were conserved in standard phosphate buffer at 4°C, remaining in optimal conditions for 7-10 days.

Photographs were taken with high sensitive film (TRI-X 400 ASA, Kodak®) by means of a Zeiss Photomicroscope II equipped with epiillumination and the right filter set to visualize the rhodamine beads (Zeiss BP 546, FT 580 and LP 590). Slice shapes were made with camera lucida (Wild) and stored in a computer (Macintosh II) for ulterior 3-D reconstruction.

Results

In this investigation we have considered two typical pontine grey matter injections: ventral and dorsolateral. It was possible to establish the differences in the distribution of the cells found in both cases thanks to the minimal diffusion of this dye once injected. Owing to the small quantity of dye that was injected and the long extension of the cortico-pontine projection, the number of cells found in each case is not very high. Due to its limited diffusion, this number is less than with other markers, for example peroxidase.

The cells labelled in both cases were located at a height corresponding to the cortical layer V (Fig. 1). The soma showed a mainly pyramidal shape. We were unable to observe cells in the contralateral visual cortex. Below are the results of these small volume injections which allow better differentiation of projection to these two pontine portions.

Ventral injections (Fig. 2)

These affected the ventral nucleus and adjacent parts of the medial, paramedial, peduncular and lateral nuclei. The number of cells marked per slice was very small (approximately 10-15 cells). The cells marked by retrograde transport were located: 1) in the medial visual cortex a few cells were found from slices corresponding to level P 2.2 (2.2 mm behind the interaural line) (Tusa et al., 1981), originally situated in the parasplenialis gyrus and at the end of the sulcus splenialis, and subsequently forming a continuous band embracing both zones. These cells are always very apparent in the medial bank of the sulcus splenialis. Towards rostral, approximately at level A 0.8, the number of cells was highest, decreasing progressively in both the gyrus parasplenialis and at the end of the sulcus splenialis, but remaining steady in the ventral bank of this sulcus. 2) in the lateral part of the visual cortex a very low number of cells appeared from caudal levels (P 5.2) in the sulcus suprasylvius, with some cells to be found in the ventral or lateral bank of the sulcus lateralis. It was not possible in either of these cases to identify a clearly defined topography of the projection. It was only in rostral levels that cells were more noticeably grouped together. From this point, cells could be seen in the gyrus ectosilvius. Near rostral levels they were found in the lateral or medial bank of the sulcus suprasylvius, above all in the highest part of the bank, but more rostrally, more cells were found in the bottom of the sulcus. From caudal slices we found cells labelled in the gyrus suprasilvius, in its highest part and descending the ventral or lateral

Fig. 1. Microphotography of an RLM-labelled neuron projecting to the pons. Fluorescent beads are located in the cellular soma. x 200

banks of sulcus lateralis and medial or dorsal part of sulcus suprasylvius. A few cells sometimes appeared in the gyrus lateralis.

Dorsolateral injections (Fig. 3)

When the RLM injections were more lateral (and therefore more dorsal, given the convexity of the pons) the areas most affected were the dorsolateral nucleus and adjacent parts of the peduncular, lateral and paramedian nuclei, as well as the ventral section of the reticularis tegmenti pontis. Even fewer cells were found than in the case of ventral injections, which demonstrates the weak projection from the dorsolateral portion of the pons to the visual cortex. We also found that in the medial part of the visual cortex some cells appeared in the high part of the gyrus parasplenialis, up to a level corresponding approximately to P 1.2. The number of labelled cells in the gyrus increase progressively towards the rostral and the cells began to appear in the end of the sulcus splenialis. In more rostral sections the projection from gyrus parasplenialis is more evident. In the lateral part of the visual cortex we found a few isolated cells, without any clear pattern in the more caudal levels, but nearly always in or around the suprasylvius and lateralis sulci. From the interaural line to rostral, the arrangement of cells was quite clear, embracing the bottom of these sulci. Towards rostral, cells were seen in the gyrus suprasylvius but fewer than in ventral injections. We also found fewer cells in the gyrus ectosylvius. The dorsal injections differed further from the ventral injections in that the former left a large number of cells visible in the

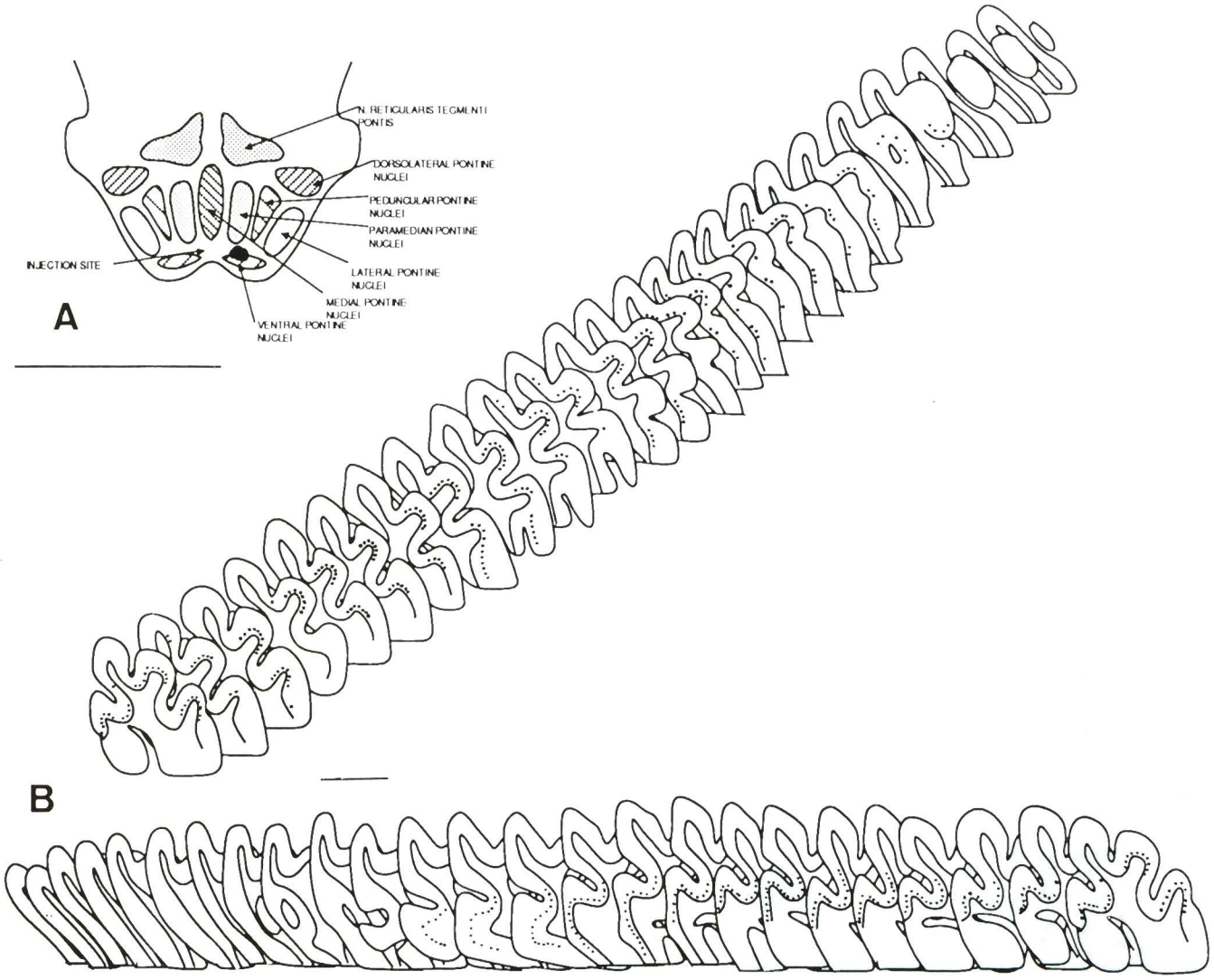

Fig. 2. a. Schematic drawing of the injection site in the ventral part of the pons. Calibration bar= 10 mm. **b.** 3-D schematic reconstruction of the convex surface (above) and the interhemispheric surface (below) of the right brain hemisphere showing the visual cells projecting to the pons. Schemes represent alternative slices 250 μm thick. Calibration bar= 10 mm.

end of the sulcus suprasylvius, whilst with the latter type of injection cells were practically non-existent in this particular area.

Discussion

Methodological considerations

The use of RLM makes it possible to perform studies of distribution via retrograde labelling and to combine this with later techniques such as intracellular iontophoretic injection (Pérez-Samartín et al., 1990). This is also possible with other fluorescent dyes. We found that Fluoro gold or Fast blue almost faded after 1 min illumination, while RLM resisted more than 30 min without visible fading. Another advantage of RLM over other tracers, including peroxidase, is that it diffuses very little from the injection site, which permits investigation of more specific regions. Besides, RLM makes it possible to perform long term research because there is no metabolization of the beads for a long time (Katz et al., 1984).

Unfortunately, the RLM are limited in their distribution along the soma and adjacent parts of dendrites and axon, evidencing a more incomplete morphology than that provided by Fluoro gold, Fast blue or peroxidase. We found that another disadvantage of the RLM was their labelling in organic solvents like alcohol or xylene. For this reason, dehydration and clearing was avoided.

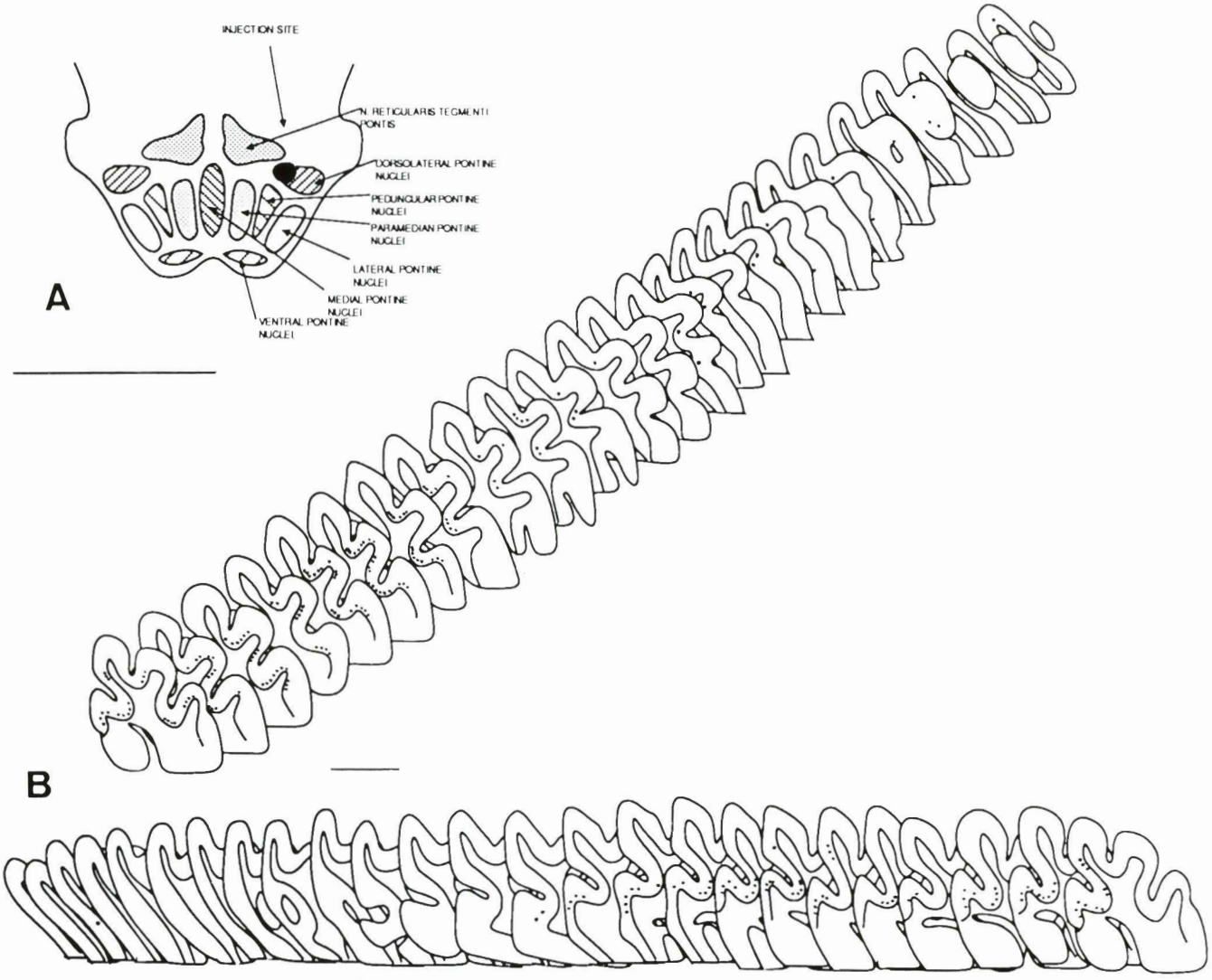

Fig. 3. a. Schematic drawing of the injection site in the dorsolateral part of the pons. Calibration bar= 10 mm. **b.** 3-D schematic reconstruction of the convex surface (above) and the interhemispheric surface (below) of the right brain hemisphere showing the visual cells projecting to the pons. Schemes represent alternative slices 250 μm thick. Calibration bar= 10 mm.

Retrograde labelling

The RLM have enabled us to carry out a study of the convergence of the cortical visual areas into the basilar pontine grey matter of the cat. The relative differences recorded in comparison with other authors may be attributed to the use of a minimal diffusion dye. This could alter the results in the case of cortical areas with a very diffused projection in the pons or a projection located at a point far away from the injection site. Most of these differences between our results and previous studies are probably based on the use of high diffusion dyes such a horseradish peroxidase, which provides a global knowledge about a pathway, but no selective information. On the other hand, contralateral or lateral nuclei contamination is more probable than with RLM.

There are not a lot of studies of cortical convergence to the pons. However, some aspects can be pointed out. The distribution we found by means of ventral injections is very similar to peroxidase (Bjaalie and Brodal, 1983, Bjaalie, 1985, 1986, 1989) and corresponds to a very constant pattern of labelling. Cells can be seen mainly in the upper part of the external surface gyri. This distribution corresponds, as a general rule, to the location of the central visual fields of the different cortical visual areas. With dorsolateral injections the pattern changes and the cells can be seen in more peripheral visual regions of the external surface too.

According to these results, we think there is a certain level of convergence whose pattern seems to adopt a retinotopic distribution. Further analysis should be done with low diffusion dyes in order to study in more detail this projection.

Acknowledgements. We are indebted to Dr. J.F. Madrid and Dr. F. Ortega for their valuable criticism of this work. We also thank M. Bidaurrazaga and Z. Alvarez for technical assistance.

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Accepted September 2, 1992