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The local domain for divergence of subcortical afferents to the striate and extrastriate visual cortex in the common marmoset *(Callithrix jacchus)*: a multiple labelling study

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Summary. In the common marmoset *(Callithrix jacchus)*, the cortical projection from the pulvinar and other diencephalic structures into the striate and prestriate cortex was investigated with various fluorescent retrograde tracers. Single cortical injections as well as multiple injections at distances of 1–2 mm with one tracer into an extended but coherent cortical region were applied. Fields with multiple injections were placed so that they touched each other (minimal distances 2 to 3 mm). Retrogradely labelled cells in the LGN and/or the pulvinar were arranged in coherent columns, volumes or slabs, but cell volumes resulting from neighbouring cortical injections overlapped at their border (for details of the thalamo-cortical topography see the companion paper Dick et al. (1991)). Double labelled cells (dl) were only found in the zones of overlap of the cell volumes labelled by the respective tracers. The relative number of dl-cells in these overlap zones was $6.2 \pm 3.1\%$. The dl-frequency was the same in the various nuclei of the pulvinar and the LGN. In the main layers of LGN, dl-cells were found only in the overlap zone of two injection fields into area 17, but a few dl-cells were found in interlaminar cells after injections into area 17 and 18. Maximal cortical distances between injection fields which produced dl in the pulvinar, were 3 to exceptionally 4 mm but dl was highest at injection distances ≤ 2.5 mm and decreased sharply at wider distances. Such overlap zones were concerned with identical or overlapping regions of visual field representation in the cortex and probably also in the pulvinar. Although in individual experiments up to four different tracers were injected into different striate/prestriate regions, often embracing the same visual field representation, individual cells in the pulvinar showed dl from maximally only two tracers injected into neighbouring cortical regions. We conclude that dl in the posterior thalamic projection nuclei is determined essentially by cortical distance and thus reflects the local domain of branching of thalamo-cortical afferents. Pruning of such branches during development may further restrict bifurcating axons to identical visual field representations, but representation of identical visual field regions in different visual areas is not, per se, a sufficient condition for dl. It is not found if such regions are further apart from each other than the typical local domain of 2–3 mm, exceptionally up to 4 mm in one experiment after injections into area 17 and MT. Dl in the intralaminar nucleus CeL ($5.0 \pm 4.6\%$), the claustrum ($5.4 \pm 3.6\%$) and in the amygdala ($5.7 \pm 1.9\%$) was of the same order as in the pulvinar and LGN. In the hypothalamus around 10% and in the Nucleus basalis Meynert 15.8% of the cells labelled by visual cortical injections were double labelled. In all these extrathalamic regions dl was also restricted to overlap zones, but overlap of labelled fields in these nuclei was much wider and included the whole striate/prestriate cortex except for some topographical separation of striate and prestriate projection zones in the claustrum. Only in the Nucl. basalis Meynert and the hypothalamus some cells were labelled by three tracers.

Key words: Visual cortex – Lateral geniculate body – Pulvinar – Fluorescent dyes – Double labelling – Callithrix jacchus

Introduction

The thalamo-cortical projections to the striate and extrastriate visual cortex are topographically and topologically organized in that each cortical point receives input from a restricted thalamic zone and neighbouring cortical points from neighbouring thalamic zones. This is amply documented for the projections from the lateral geniculate nucleus (LGN) to area 17, but has also been shown for the afferents from the nucleus lateralis pos-

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terior/pulvinar complex to the extrastriate visual cortex in the cat (Jones 1985; Graybiel and Berson 1980; Raczkowski and Rosenquist 1983; Creutzfeldt 1985) and in primates (Benevento and Rezak 1976; Burton and Jones 1976; Raczkowski and Diamond 1980; Tanaka et al. 1990; Dick et al. 1991).

Thalamic projection zones to nearby cortical regions show a considerable overlap especially in the pulvinar and to a lesser extent also in the LGN. In addition, studies in cats and monkeys have shown that thalamic neurons projecting into the same retinotopic region of different visual areas may show divergence of individual thalamo-cortical axons so that they can be labelled by different retrograde tracers from distant cortical regions if these represent indentical parts of the visual field (Bullier et al. 1984, 1987; Kennedy and Bullier 1985; Lysakowski 1988; Birnbacher and Albus 1987). The proportion of such double labelled (dl) neurons may vary quantitatively to some extent between primates and cats, and also between different areas, but qualitatively the finding appears to be general. From this it was concluded, that cortical areas with the same retinotopic representation receive convergent input from distant thalamic regions. This, however, could violate the topological principle of topographical organization if such corresponding retinotopic regions were far apart from each other in the thalamus or cortex. The observations of Bullier et al. (1984), Bullier and Kennedy (1987) and Lysakowski et al. (1988) that after small "point" injections into the cortex double labelled neurons were seen only if the labelled thalamic zones were overlapping also indicates that the thalamic projection zones to different cortical areas with retinotopic correspondence are overlapping, but does not, per se, exclude a break of the topological rule. Only if it can be shown that cortical distance is the determining factor for dl and that identical retino-topic positions of an injection are not a sufficient condition, double projections to various cortical areas do not violate the topological principle. It would indicate instead that thalamic input zones and cortical visual areas with identical retino-topic representation both ly close together.

In order to test this we investigated the thalamic projection to the various visual areas of the striate and extrastriate cortex in the common marmoset (Callithrix jacchus) with several tracers. With each tracer, relatively large cortical areas were labelled by several small injections located close to each other. This allowed to label larger thalamic projection zones and to see how such large zones are related to each other. The topographical organization of this projection has been described and analyzed in a companion paper (Dick et al. 1991). We analyze here the location of dl-cells in relation to the topographical projection zones to the various visual areas. Our results clearly demonstrate that dl is restricted essentially to overlapping cortico-thalamic zones as defined by the domain of local branching of thalamo-cortical afferents. Divergent projections to identical retinotopic representations in the multiple visual areas are restricted to this local domain. They therefore do not violate the topological principle of thalamo-cortical organization but are the consequence of it.

Methods

This report is based on an investigation of 6 hemispheres in 4 adult marmosets *(Callithrix jacchus)*, who were raised in our colony. The animals weighed between 280 and 450 g. Under sodium pentobarbital anaesthesia (30–45 mg/kg i.p.), and after appropriate surgical exposure of the cortex, several tracer substances were injected into each hemisphere. Each tracer was injected into several adjoining points at distances of 1 mm, so that 2–4 mm long stripes or 2×3–4 mm large areas of cortex were injected by the same tracer. Such injection fields were aimed at only one cortical area (17, 18 or 19) or subarea (e.g. medial vs. dorsal area 19) (for the definition of cortical areas see Fig. 1). The tracers used for this study were fast blue, diamidino yellow (Keizer et al. 1983; Aschoff and Holländer 1982), propidium-iodide (Kuypers et al. 1979; Aschoff and Holländer 1982), fluorogold (Schmued et al. 1986) and rhodamine latex (Katz et al. 1984). Tracers were delivered through a glass-pipette, mounted on a Hamilton-syringe of 1 µl. All injections were done in small steps at 10 min. intervalls. The tip of the glass-pipette was placed approximately 1 mm under the brain surface.

After completion of all injections, wound closure and i.m. injection of an antibiotic (Albiotic (R), 20–30 mg/kg), the animals survived 10 to 14 days in their home cage and their social environment. They were then again anaesthetized with an overdose of sodium pentobarbital and killed with transcardial perfusion of a 3% saline solution to which 0.1 ml/l liquemin was added, followed by a solution of 3% paraformaldehyde in 0.1 M phosphatebuffer and then by a buffered solution of 5% sucrose.

The brain was removed and cut in two blocks, then immediately mounted on a freezing microtome and cut into 52 or 60 µm frontal or parasagital sections. The slices were mounted on glass-slides with gelatine and were allowed to dry for several hours. These were stored at 4 °C in darkness. Under these conditions fluorescence stayed stable for at least two months. Fluorescent cells were made visible by a Zeiss fluorescence microscope with exciting wavelengths of 365 nm (for fluorogold, diamidino yellow and fast blue, propidium-iodide) and 564 nm (for rhodamine and propidium-iodide). Photomicrographs with double exposure were possible. Single and double-labelled cells were plotted with an X–Y plotter connected to the microdrive of the microscope-table.

For allocation of the injection sites to the various cytoarchitectonic areas and for location of labelled cells in the cortex and diencephalon, every fourth section was saved for immediate myelin (haemotoxylin-Woelcke) or acetylcholine-esterase staining (after Storm-Mathisen 1970). After fluorescence microscopy and plotting, alternate slides were counterstained with Kresylviolett or impregnated with a silverstain (after Gallyas 1979). A control series of the marmoset brain with alternate cell and fibre stains was also available. Cytoarchitectonic areas were determined on the basis of this material and guided by the cytoarchitectural mapping of the marmoset visual cortex by Spatz (1977) (see Fig. 1C). For further detail, see Dick et al. (1991). Abbreviations of thalamic nuclei are the same as those listed in this companion paper.

Results

In each hemisphere, we injected 3–5 different tracers. Each tracer was injected into 2–10 points separated by 1–1.5 mm and arranged in rows or rectangular fields. Arrays of injections with one tracer were adjacent to but not overlapping with the injection array with another tracer. Each array was aimed at a certain area or subarea of the striate and prestriate cortex. The exact assignment to one cytoarchitectonic area was later checked histologically. The survey sketches of the cortex in the following figures indicate the location of the injection arrays projected on a semischematic areal map of the occipital

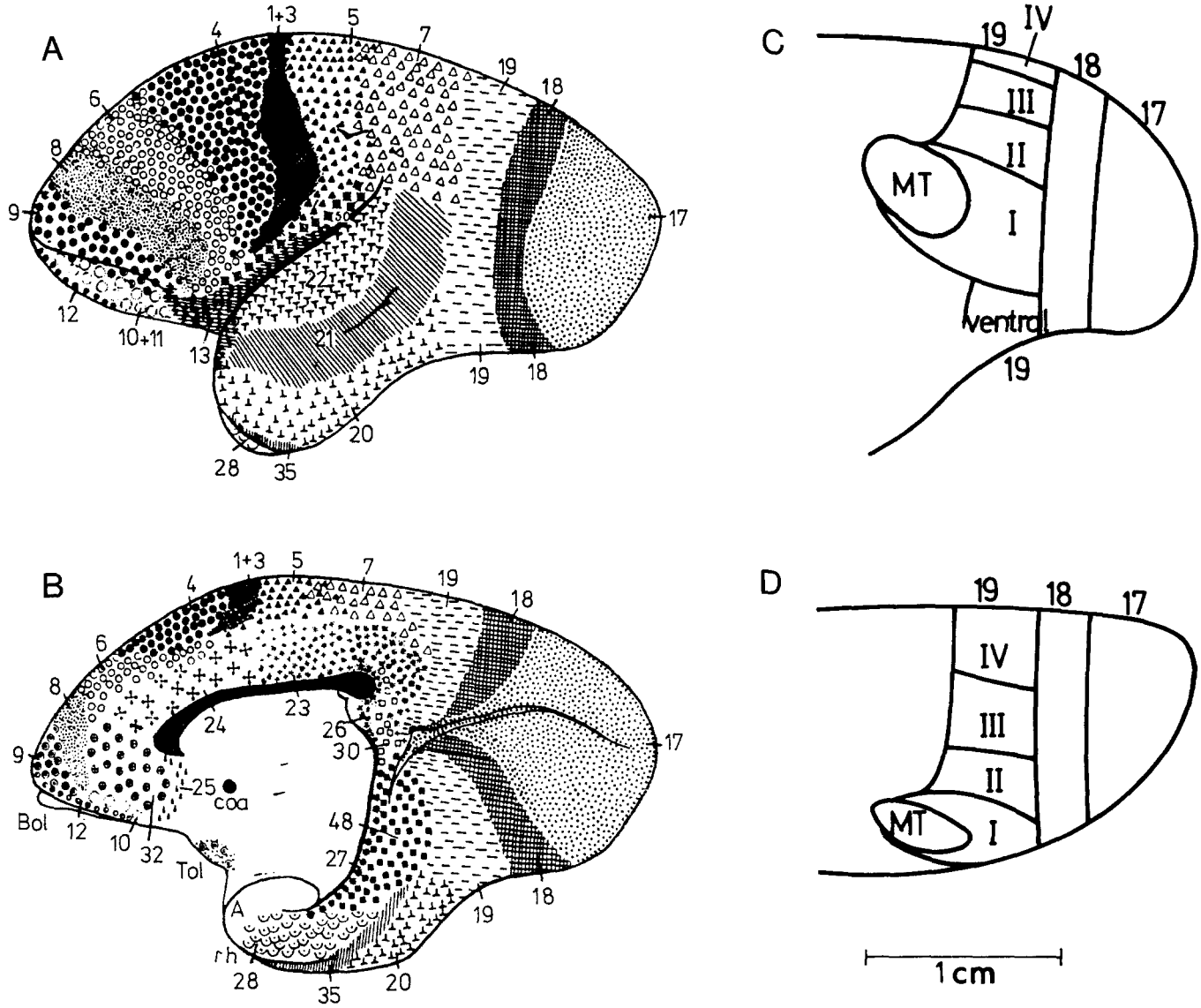
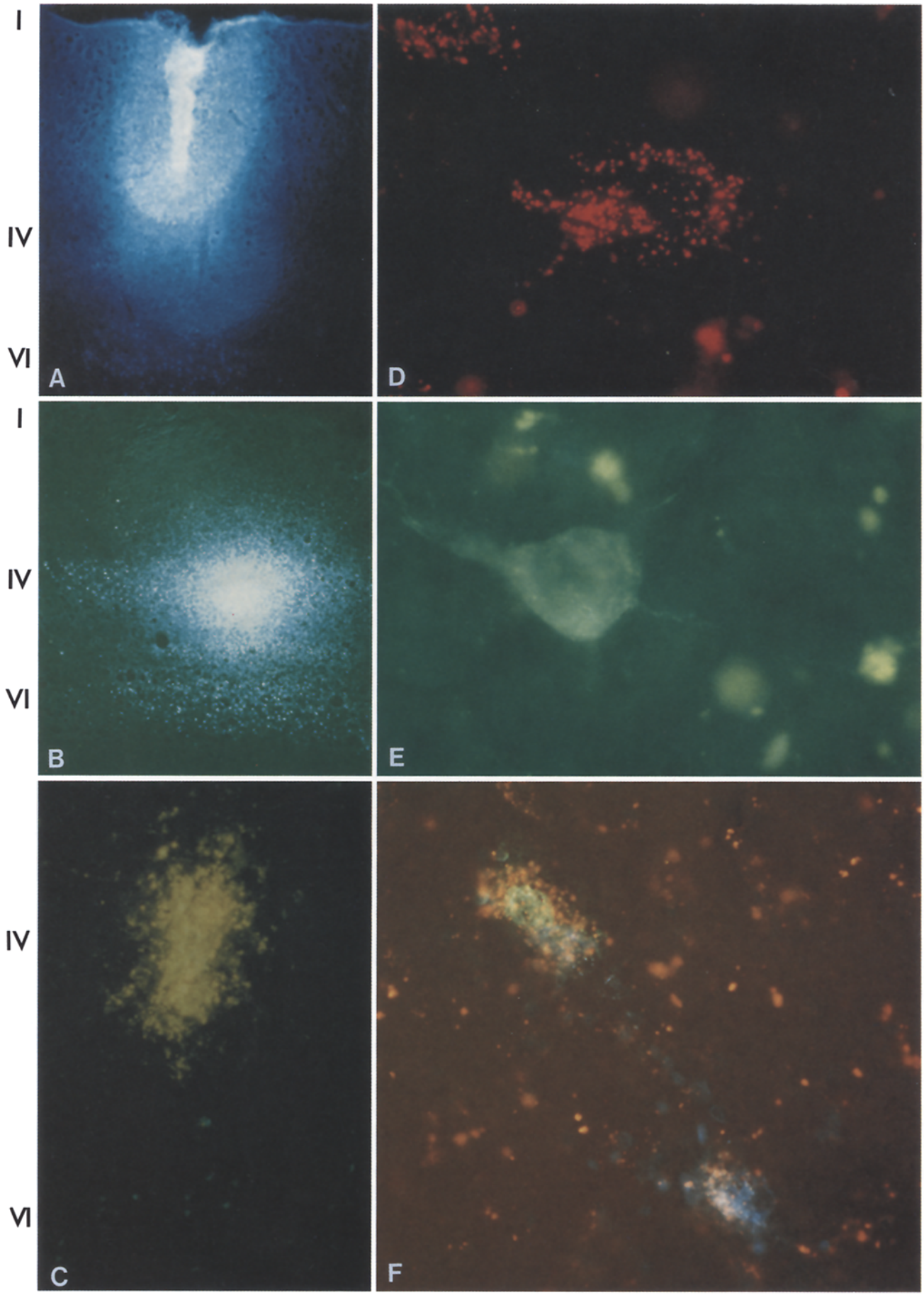


Fig. 1. A, B Cytoarchitectonic map of the cortex of the marmoset, *Callithrix jacchus* (Brodmann 1909). **C, D** Topographical subdivisions of the visual cortex of the marmoset after Spatz (1977). **A, C** Lateral, **B** medial, and **C** dorsal view

cortex of the marmoset as proposed by Spatz (1977) (Fig. 1C, D). For visual field representation we used the detailled maps elaborated for the owl monkey (Allmann and Kaas 1975), and the approximate retino-topic subdivision for the marmoset of Spatz (1977). In two monkeys, we also recorded multiunit activity from several points and could confirm in essence the findings of Allmann and Kaas (1975), i.e. an increase of receptive field size when advancing the electrode forward from area 17 to 19, and restriction of receptive fields to the fovea and the parafoveal 10° in the dorso-lateral cortex.

Individual injection sites (60–100 µl) were less than 1 mm in diameter and restricted to the grey matter. Examples are shown in Fig. 2A–C for a fast-blue, a diamidino-yellow and a rhodamin-latex injection, respectively. Neighbouring injections were well separated from each other. (Only fluoro-gold injections tended to be slightly larger and could touch each other). Cells labelled by one or 2–3 tracers could be clearly recognized in the fluorescence microscope wherever present (see Fig. 2D–F). We have found cells labelled with 2 tracers in all diencephalic structures in which labelled cells were found at all, i.e. in the lateral geniculate nucleus (LGN), the pulvinar, the nucleus basalis Meynert, the claustrum, the amygdala and the lateral hypothalamus. Triple labelled cells (see Fig. 2F) were only found in the Nucleus basalis Meynert and the hypothalamus. In all regions double

Fig. 2A–F. Examples of cortical injection sites (**A–C**) and of retrogradely labelled cells (**D–F**). **A** Injection of fast blue, **B** of diamidino-yellow, and **C** of rhodamin latex. **D** Pulvinar cell retrogradely labelled with rhodamin latex. **E** Cortical pyramidal cell retrogradely labelled with fluorogold. **F** Multiple labelling of two cells in the *Nucleus basalis Meynert*. Lower left: Triple labelling with rhodamin latex, fast blue and diamidino-yellow, the latter being restricted to the nucleus. Upper right: Double labelling with fast blue and rhodamin latex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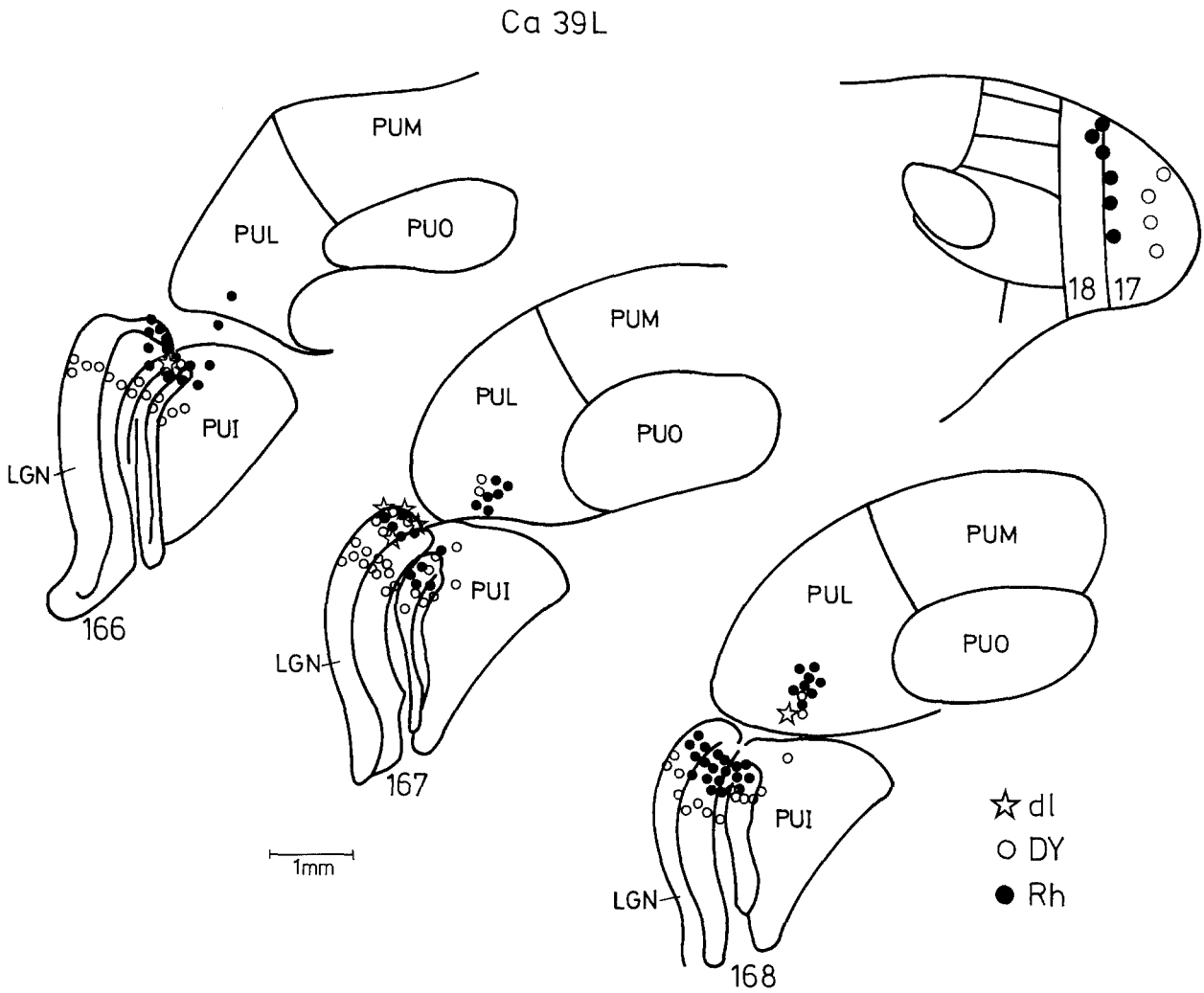

Fig. 3. Retrogradely labelled cells in the pulvinar and LGN after injection of diamidino-yellow (DY) into area 17 (open circles) and Rhodamin Latex (Rh) into the 17/18 border (closed circles). Double labelled cells (dl) are indicated by asterics. In this and the following figures, the representation of labelled cells is semischematic and only represents the relative density of labelled cells in the regions indicated but not their absolute number. Injection sites are drawn into the areal map of Spatz (1977; see Fig. 1C, D). Coronal sections with schematic indications of nuclear borders. For abbrevations of names of nuclei, see Table in Dick et al. (1991). The numbers below each section indicate the slice number

labelled cells were only found in zones where cell populations labelled by one or the other tracer overlapped.

Double labelled (dl-) cells in different diencephalic structures

Lateral geniculate nucleus (LGN). Cells in the main layers (I–VI) were only labelled by injections into area 17, but a few scattered cells in the interlaminar layers could be labelled from various injection sites in areas 17–19. Double labelling in the main layers was found in one hemisphere in which two injection rows of different tracers were located in area 17, one near the 17/18 border and one approximately 1.5 mm posterior (see Fig. 3). These injections labelled cells in LGN as well as in PuL. As is typical for the geniculo-cortical projection to area 17, individual injections labelled cones of cells stretching through all layers. Depending on the injection size, such cones may merge to some extent. In Fig. 3 we have plotted the labelled cells in the LGN and the lateral and inferior pulvinar resulting from the anterior (rhodamine latex) and the posterior (diamidino yellow) row. These were, in our hands, the tracers with the best confined injection sites and the least diffusion. The labelling zones from both injection areas overlapped in the dorsal segment of the LGN including both, the magno- and the parvocellular layers (see sections 166 and 167 in Fig. 3). Cells double labelled (dl) by both tracers (stars) were only present in this overlap zone, but no dl cells were found in the non-overlapping zones of the LGN.

In the interlaminar zones of the LGN, dl-cells were rare and we found only some in one case from injections into area 17 and 18 (see Fig. 4, section 180). Although interlaminar cells and a few scattered cells in the main layers were labelled from most extrastriate injections, no cells were double-labelled by injections into other extra-

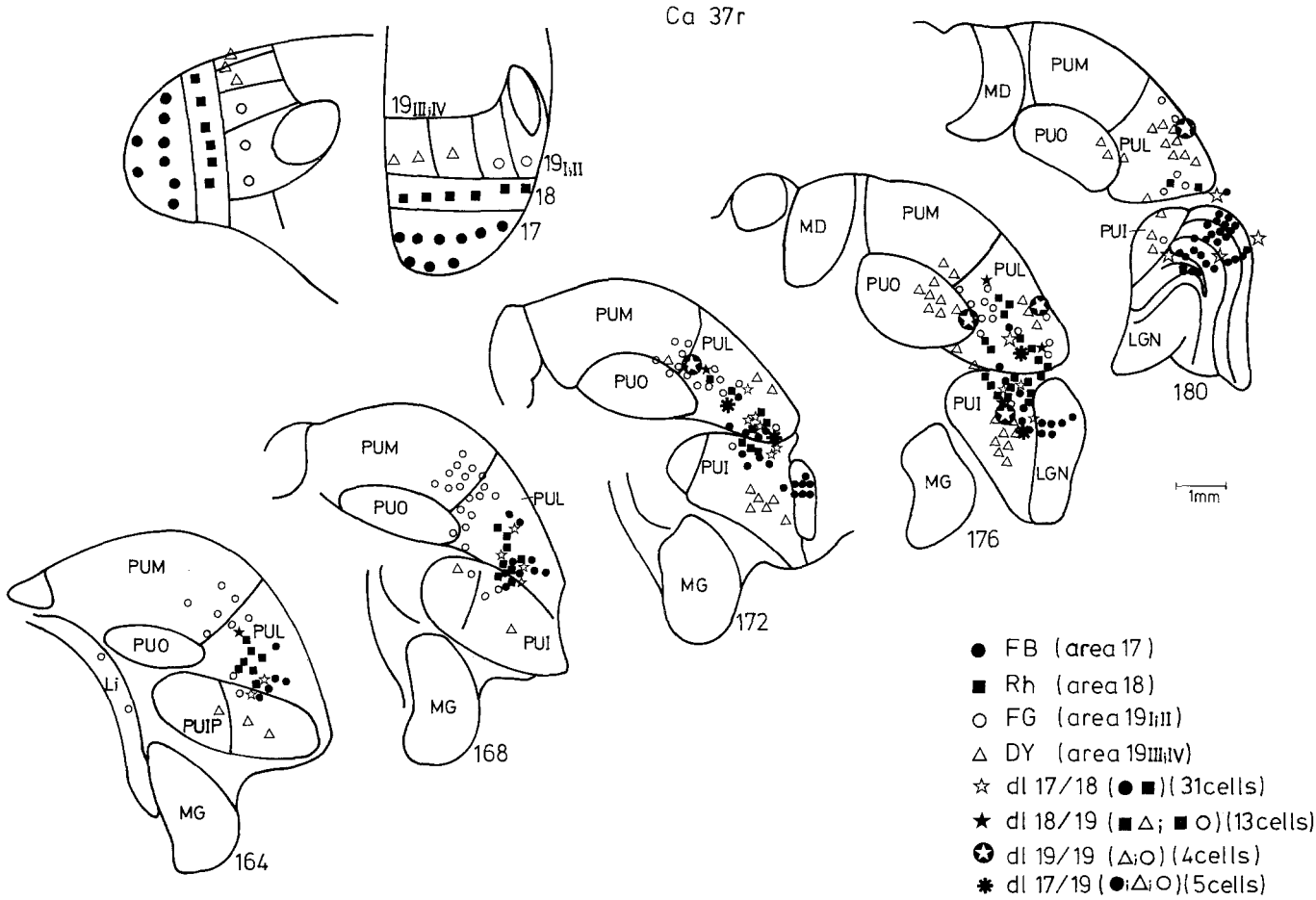

Fig. 4. Retrogradely labelled cells in LGN and pulvinar after injection of fast blue (FB, closed circles) into area 17, rhodamin latex (Rh, closed squares) into area 18, fluorogold (FG, open circles) into area 19 I, II and diamidino yellow (DY, open triangles) into area 19 III, IV. Cells which are double labelled with the different combinations of fluorescent tracers are marked by different asterics as indicated. The numbers of dL-cells in the different combinations found in the whole experiment are given in parenthesis for each dL-combination. Coronal sections

striate areas and area 17. This is in agreement with the lack of interlaminar dl-cells after injections into areae V1 and V4 in the macaque (Lysakowski et al. 1988).

Pulvinar. All injection sites in area 17, 18, 19 and MT labelled cells in the pulvinar but within the pulvinar a separation of labelling fields could be clearly recognized. This topography is described in detail in the accompanying paper (Dick et al. 1991). In short, more rostral cortical injection sites resulted in more medial labelling fields in the dorsal pulvinar, while more posterior cortical injections labelled more lateral segments. Labelling zones were arranged predominantly as shells staggered on top of each other, which stretched across nuclear borders, leaving free a gap between the latero-medial and inferior pulvinar which is occupied by the occipito-tectal tract. Thalamic zones projecting to areas 17, 18 and 19 were wrapped around each other with the zone projecting to area 18 as a core region. There was clear overlap between neighbouring labelling fields, but thalamic projection zones to distant cortical injection fields were clearly separated.

Dl-cells were restricted to the zones of overlap between labelling fields and to the tracers which labelled neurons in the respective fields. This is obvious when looking at the semischematic plots of Figs. 4 and 5. In the experiment of Fig. 4, with injections into area 17, 18, medial and lateral area 19, dl-cells were found in the lateral and inferior pulvinar in places where labelling zones from injections in area 17 and 18, 17 and 19, 18 and 19, 19 lateral and 19 medial overlapped, respectively. In addition, a few cells in the interlaminar layers of the LGN were labelled by the 17 and 18 injection (see above). In Fig. 5, with injections into areas 17, medial and lateral 18 and into 19, dl-cells were restricted essentially to a lamina which constitutes the contact and overlap zones of the labelling fields resulting from injections into areas 17, 18 and 19, respectively. Cells labelled by more than 2 tracers were not found.

The number of dL-cells is written in parenthesis after the respective symbols in figs. 4 and 5. It can be noted that the number is largest where neighbouring injection fields had a long common border as e.g. the dl-cells following injections into areas 18 and 19, but that it was small if such labelling fields had only a narrow common border such as the two injection fields into the medial and

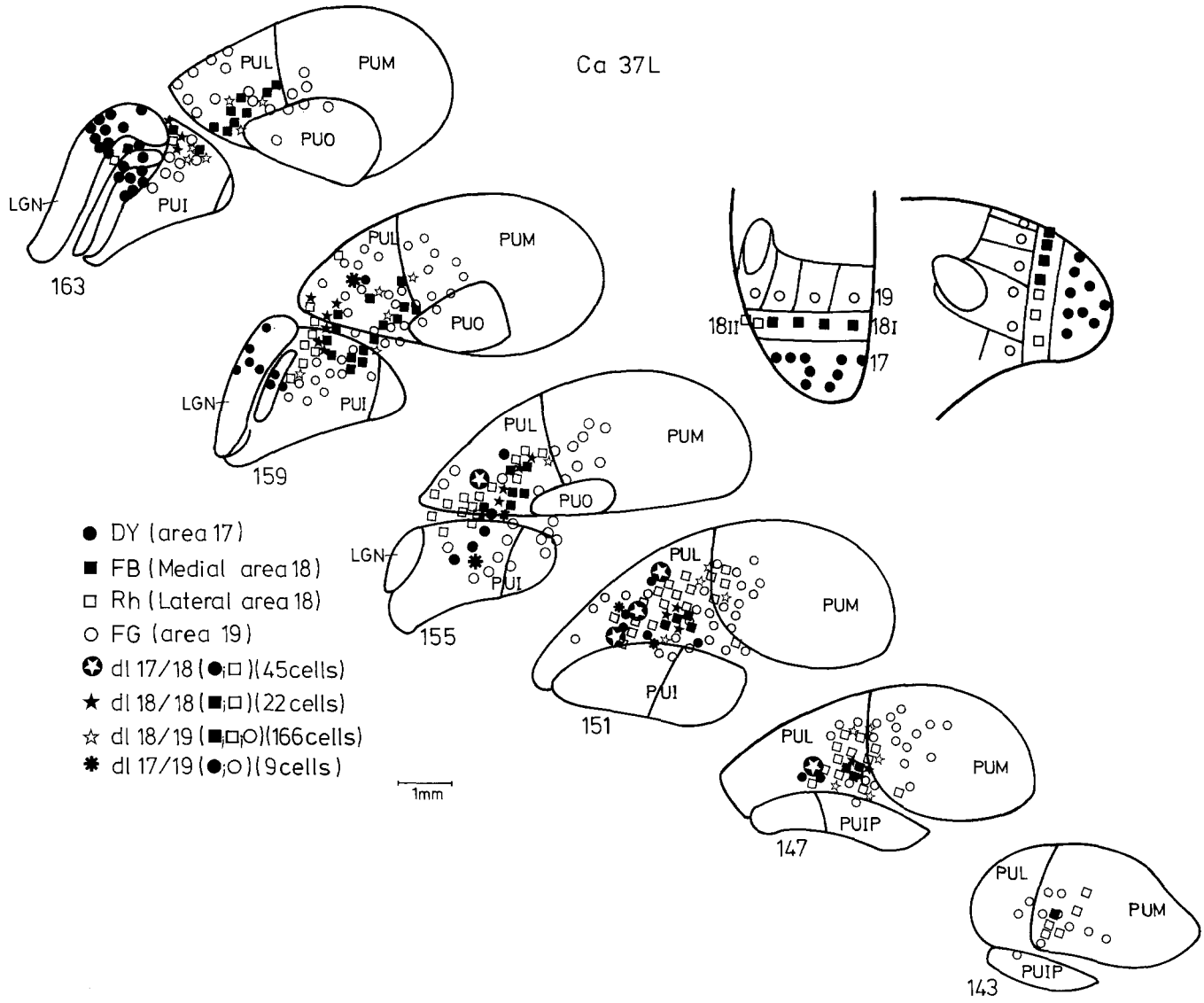

Fig. 5. Cells in the Pulvinar and LGN labelled by injections of DY (closed circles) into area 17, FB (closed squares) into the dorso-medial area 18 (18 I), Rh (open squares) into the lateral area 18 (18 II), and FG (open circles) into area 19. Cells which were double labelled by two tracers in the different combinations are marked by different asterics as indicated. The total number of dl-cells found in the whole pulvinar with the various tracer combinations is written behind the respective symbols. Coronal sections

lateral area 19 in Fig. 4 and medial and lateral 18 in Fig. 5 (see below). Only few dl-cells resulted from the injections into areas 17 and 19 (5 cells in the experiment of Fig. 4 and 9 cells in that of Fig. 5), although it can be assumed that the area 19 injections also included regions where the same part of the visual field is represented as by some of the area 17 injections (see Discussion).

The restriction of dl to the overlap zones of two labelling fields is further demonstrated in Fig. 6. Here, we counted the number of labelled cells along a latero-medial stripe in one section of the experiment of Fig. 5. The broken line shows the number of cells labelled by fast blue injected into area 18 and the dotted line that of cells labelled by fluoro-gold injected into area 19 (injection fields are shown in Fig. 5). The continuous curve represents the number of cells labelled by both tracers.

Like double labelling in the LGN is found only in the contact zone of two area 17-injections (see section A), double labelled cells were found in the pulvinar also only in the contact zones of two projection fields resulting from injections of different tracers into one prestriate area or subarea. An example for two area 19 injections is shown in Fig. 4, where the tracers fluoro-gold (lateral area 19) and diamidino yellow (medial area 19) were used. The contact and overlap zone in the pulvinar of the labelling fields resulting from the two area 19 injections (open circles and open triangles) was very restricted and, consequently, only 4 dl-cells were found. In the experiment of Fig. 5, the lateral part of area 18 was injected with fast blue and the dorso-medial part with rhodamin latex. The zone of overlap and dl in the pulvinar from the two area 18 injections was much larger than in the preceding experiment and was arranged like a cone stretching from rostral to caudal over about 2.0 mm through the lateral and inferior pulvinar. Dl-cells in this extended contact zone was therefore quite large.

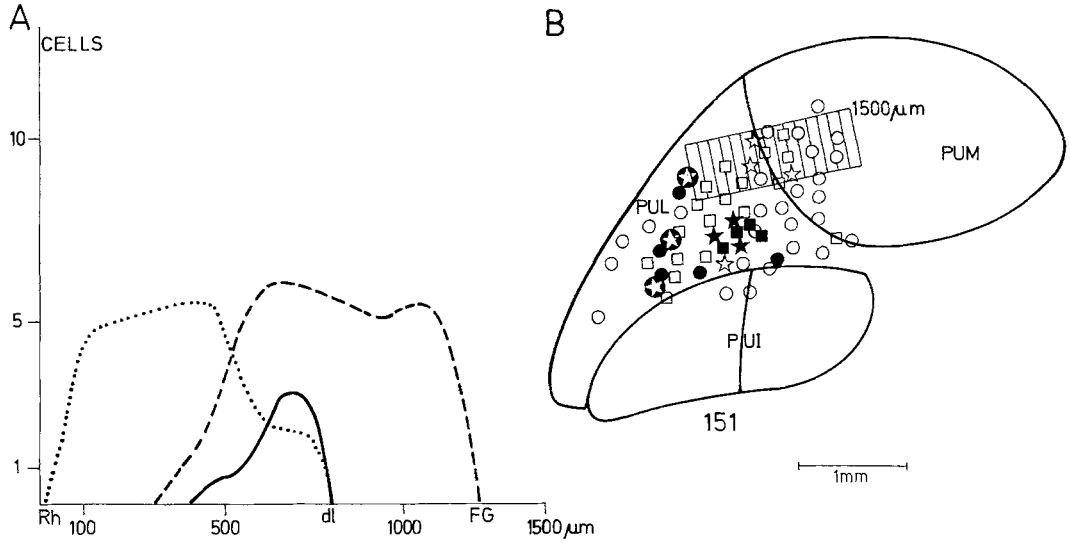

Fig. 6A, B. Same hemisphere as Fig. 5. **A** Numbers of cells in slice 151 labelled by Rh (injection into lateral area 19, open squares, dotted line), and by FG (area 19, open circles, broken line) and cells double labelled by both dyes (open stars, continuous curve). Abscissa: Location of the cells in the window drawn into slice 151 in **B**, from lateral (left) to medial (right). Ordinate: Total number of cells in each stripe of the window. Note that dl-cells are restricted to the overlap zone

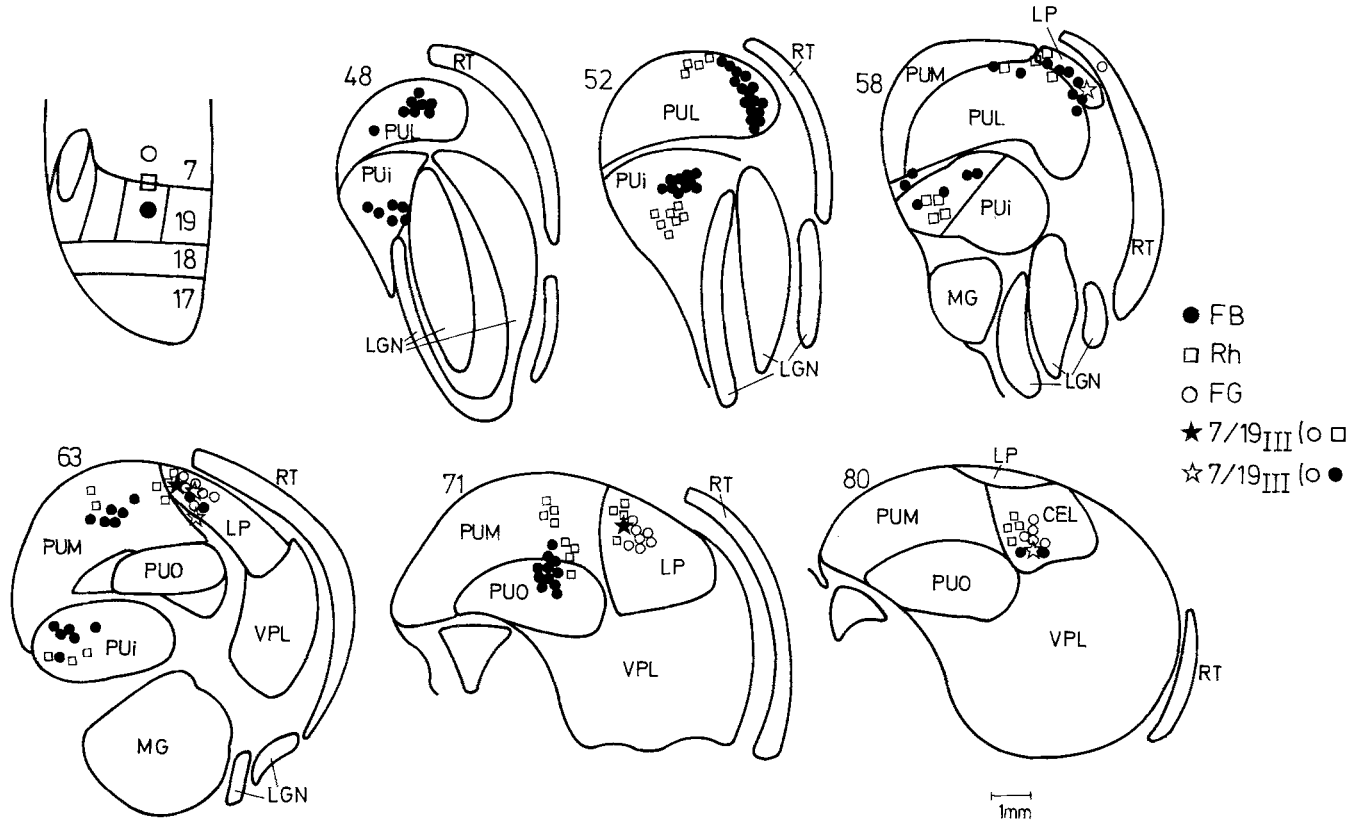

Fig. 7. Cells in pulvinar, LP and CEL after injection ot FB (closed circles) into area 19 III, of FG (open circles) into area 7 and of rhodamin latex into the border region of these two areas (open squares). Double labelling indicated by different asterics, according to the combination of dyes as indicated. Parasagittal sections

The border between the two area 18 injections was near the representation of the horizontal meridian and we may therefore reasonably conclude that the contact zone between both injection fields and the zone of double labelling in the pulvinar also represented the horizontal meridian (see Discussion).

Nucleus lateralis-posterior (LP). In LP, cells were labelled by injections into areas 7 and the dorso-medial part of area 19. The labelling zones resulting from these cortical injection fields were continuous with overlap at the contact zone. Dl-cells were restricted to the overlap zone (see Fig. 7).

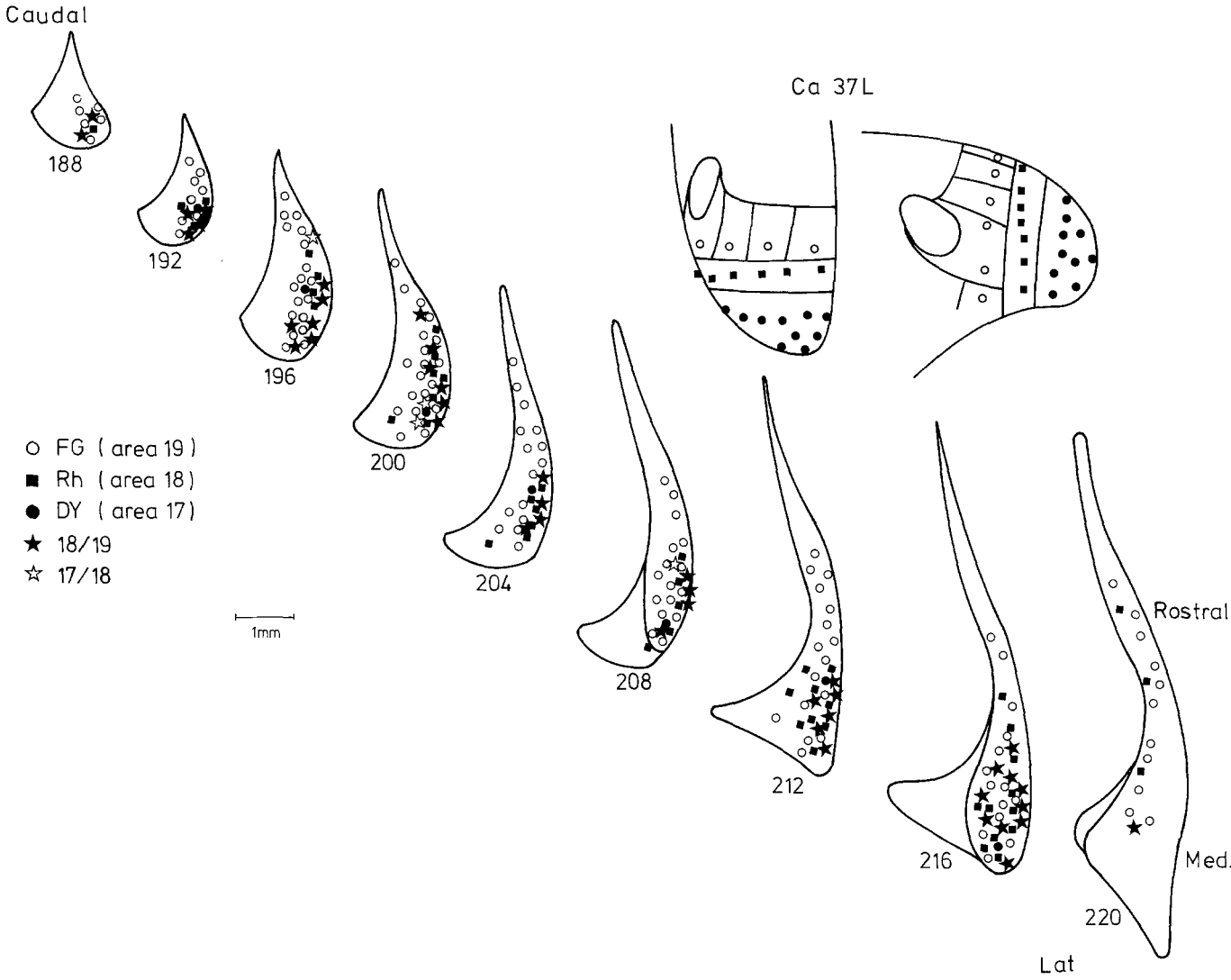

Fig. 8. Cells labelled in the claustrum after injection of DY (closed circles) into area 17, Rh (closed squares) into area 18 and FG (open circles) into area 19. Double labelled cells in the various combinations shown as different asterics. Coronal sections

Nucl. centralis lateralis and medialis. Cells in these intralaminar nuclei were labelled from all injection sites. Although a coarse topographic gradient of cells projecting to the frontal, parietal and occipital cortex has been shown to exist (Brysch et al. 1984), there was no obvious topographic organization of the intralaminar-cortical projection within the restricted region of the occipital injection sites of the present experiments. Cell populations projecting to all injection sites were mixed, though some parts of the intralaminar nuclei were completely spared, thus supporting the presence of a coarse topographic organization. Dl-cells were scattered over the whole intralaminar labelling zone, and their proportion varied in the different experiments between 1 and 14%. Interestingly, only cells double labelled from two cortical injection fields were found, but none labelled from more than two fields.

Claustrum. The claustro-cortical projection showed a coarse topographic organization with cells labelled by area 17 injections concentrated in the medio-ventral and those from area 19 in the dorso-lateral sectors (Fig. 8). Labelling zones resulting from the various injection fields were stretched as lamellae through the claustrum and overlapped extensively, especially in the ventral parts, which correspond roughly to the representation of the central parts of the visual field (cf. Le Vay and Sherk 1981). Dl-cells were found in these contact and overlap zones of the labelling fields, and were therefore concentrated in the medio-ventral segments.

Amygdala, hypothalamus and nucl. basalis Meynert. In the *amygdala,* no topographic organization of cells labelled from the different cortical injection fields could be recognized, but area 17 injections labelled cells mainly in the central and basal nuclei, while those labelled from prestriate injections were also found in the inferior, lateral and medial nuclei though with the highest concentration also in the basal and central nuclei. Dl-cells were scattered over the whole labelling zone and varied between 1 and 12% in different combinations and experiments, but also here no more than two labels were found

in one cell. In the *hypothalamus,* only few cells (10–40 in one experiment) in the lateral area were labelled. In this experiment, cells projecting to any of the striate and prestriate injection areas were mixed without any indication of a topographic organization, and the concentration of cells projecting to any single injection site was approximately the same. Dl was only found in cells labelled from area 17 and 18, but this maybe chance as the total number of labelled cells was low and combination of these two injections was more frequent than that of others.

In the *Nucleus basalis Meynert,* cells labelled from all cortical injection fields were mixed and scattered over the whole area of this extended region. The proportion of dl-cells was clearly higher than in the other structures (see below). About 1/4 to 1/3 of the dl-cells here were also labelled by a third tracer (see Fig. 2F). Such multiple labelling might be expected in view of the widespread, non-topographic projection of this region. Cortical injection sites resulting in double or triple labelling could be as far apart as 10 mm. Interestingly, only the combination of rhodamin latex, fast blue and diamidino-yellow were combined in triple labelled cells.

Frequency of double labelled cells

Our analysis so far clearly indicates that cells in the thalamic nuclei double labelled from two cortical injection fields were found only in the region of overlap of the respective thalamic projection zones, confirming similar observations in the rhesus monkey (Bullier et al. 1984; Kennedy and Bullier 1985). In these zones of overlap, the relative number of dl-cells ranged, in all our experiments, between 3 and 14% in all specific thalamic projection nuclei, i.e. the LGN, the various nuclei of the pulvinar and in LP (mean value of all experiments $6.2 \pm 3.1\%$). In the intralaminar nuclei, the claustrum and the amygdala, the relative numbers of dl-cells showed the same range in the various experiments and about the same mean values as the specific thalamic projection nuclei ($5.9 \pm 4.6\%$ in CeL, $5.4 \pm 3.6\%$ in the claustrum, and $5.7 \pm 1.9\%$ in the amygdala). In the Nucleus basalis Meynert the relative number of dl-cells was clearly higher with $15.8 \pm 8.1\%$ (range 7–25%), and also in the lateral hypothalamic area, 10% double labelled cells were found. In evaluating these data, it must be pointed out, however, that double labelling may not only depend on anatomical but also on methodological variables. Thus, the number of cells labelled from one injection field and with one tracer may differ considerably from that of cells labelled from another area and by a different tracer because the speed of transport differs for various tracers, so that more distant regions such as e.g. the amygdala or the hypothalamus were reached more by some tracers, but only little or not at all by others (e.g. rhodamin latex, fluorogold). Therefore, the relative number of dl-cells especially in the more distant regions such as the amygdala and the hypothalamus as found in our experiments may be considered as a lower limit. On the other hand and in view of this, the similar ranges and mean values for the various structures except the Nucleus basalis Meynert and, to some extent, the hypothalamus is worth emphasizing.

Discussion

Our findings in the new world monkey *Callithrix jacchus* on the frequency of double labelled (dl) cells in the thalamus, especially in the pulvinar, following extended cortical injections into different regions and areas of the striate and prestriate cortex are consistent with earlier reports in macaques after injections into V1 and V2 (Kennedy and Bullier 1985) and areas 17, 18 and 19 in the cat (Birnbacher and Albus 1987; Bullier et al. 1984). In these earlier experiments, dl was investigated using relatively small single injections into selected cortical regions. Our experiments with large but precisely limited injection fields did not result in higher dl-rates but demonstrated that dl-neurons were restricted to regions where thalamic projection fields to circumscribed regions of the cortex touch and overlap. Cortical injection sites with overlapping or contiguous pulvinar projection fields were separated by maximally 3–4 mm. The relative frequency of dl-cells decreases with distance between injection sites. At an injection distance of 4 mm we saw a few dl cells only in two hemispheres of the same experiment after injections into area 17 and 19 (Figs. 4 and 5), but dl was absent in all other experiments with such widely separated injection fields. The local domains for high dl-incidence is ≤ 2–2.5 mm. This would indicate that branches of individual thalamo-cortical axons are essentially restricted to such a local domain of 2–2.5 mm, but that some branches may reach out to 3–4 mm. This applies to double labelling of cells in the pulvinar following injections into areas 17, 18 and the various subareas of area 19. In the geniculate-striate projection dl-cells in the LGN were found only, if injections in area 17 were separated by 2 mm or less.

Intracortical branching of afferent thalamo-cortical fibres is well documented for many cortical areas such as the primary visual, the somato- sensory or the auditory cortex where terminal branching is typically restricted to distances of 1–2 mm, with some branches reaching further as e.g. into alternating ocularity stripes in area 17 (Ferster and LeVay 1978; Gilbert and Wiesel 1983; Martin 1986; Parnavelas 1986; Redies et al. 1989; Brandner and Redies 1990). On the other hand, the thalamo-cortical mapping appears to be somewhat more precise in the geniculo-cortical than in the pulvinar-cortical projection as indicated by the larger overlap zones in the pulvinar for neighbouring injections (see Dick et al. 1991) and therefore the local domain for dl is more restricted in area 17 than in the prestriate cortex.

Correspondence of visual field position within the limited distances of the local domain of branching and dl depends on magnification factor and scatter of retinotopy within the respective regions. If injection sites into corresponding visual field representations in different visual areas were far apart from each other and if the respective thalamic projection fields were not contiguous or overlapping, no double labelling was found, such as e.g. after injections of two different tracers into the 17/18

border and into MT, respectively. Also after injections into corresponding parts of the visual field in cortical regions as far apart from each other such as the visual association areas in the temporal and occipital lobe of the rhesus monkey no dl was found (s. Bullier and Kennedy 1987). In the cat, cortical injections into corresponding parts of the visual field which produced double labelling were typically within 3–4 mm, i.e. the extended local domain (Birnbacher and Albus 1987). In our experiments, even the extended injections of one or two tracers in area 19 which covered a large region with more than one visual field representation including the central visual field produced dl-cells only in the overlap zones but not, if the respective thalamic projection zones were clearly separated. Also if such area 19 injections were combined with injection of a wide region in area 17, only few dl-cells were found in that part of the pulvinar where the projection fields into area 17 and 19 overlapped (PuL and PuI). In this region of the pulvinar the central visual field is represented and projection fields to a variety of visual areas appear to converge (see Dick et al. 1991). The relatively wide distance (4 mm) between injection sites over which, in one experiment dl was found in a few cells after area 17 and 19 injections (see above) could be due to the fact that the thalamic projection from the pulvinar into area 17 is special in as far as it terminates in layers I/II in contrast to the usual termination of thalamo-cortical fibres in layer IV and VI (Weller and Kaas 1981; see also Dick et al. 1991). The pulvinar-striate projection is clearly a supplementary projection with respect to the major geniculo-cortical input into area 17 (cf. Doty 1983).

The confinement of dl to the domain of local intracortical branching of thalamo-cortical afferents is consistent with the fact that dl is found in regions which share the same part of the visual field as long as these cortical regions are close to each other, and that not all areas in which the same region of the visual field is represented receive bifurcating branches of thalamic afferents. Physiological recordings indicate that at least two independent retinotopic representations exist in the pulvinar of old world and new world monkeys (Bender 1981; Allmann et al. 1972; Petersen et al. 1988) as well as in the LP/Pulv-complex of cats (Raczkowski and Rosenquist 1982; Mason 1978, 1981; Benedek et al. 1983). Our anatomical analysis in the companion paper even suggests that there may be more than two visual field representations in the pulvinar (Dick et al. 1991). It appears that these retinotopic maps merge in the ventro-lateral segment of the pulvinar, where the central visual field is represented. Thus, pulvinar-cortical projections into different cortical areas representing the same part of the visual field and with nearby projection fields in the pulvinar could share some thalamic neurons, while those with the same visual field representation but whose thalamic projection zones are separated and part of different retino-topic maps in the pulvinar would share no afferent fibres.

In conclusion, our results do not indicate that dl violates the topological principle. The relatively low figure of dl ($6.2 \pm 3.1\%$) even in the overlap zones of adjacent thalamo-cortical projection zones attests, instead, to the very precise projection from thalamus to cortex allowing for only little scatter or fuzzy thalamo-cortical mapping. Some functionally inappropriate connections within the wider local domain of overlap may be further pruned during development, similar to the pruning of transcallosal connections in area 17 (Innocenti et al. 1981) or the restriction of the relatively widespread and overlapping ocularity domains to clearly delineated ocularity stripes in cats and monkeys during early postnatal development (Hubel et al. 1977).

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