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Tangential intracortical pathways and the development of iso-orientation bands in cat striate cortex

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Evidence is accumulating that tangential connections are a prominent feature of cortical organization. In the mammalian visual cortex, these connections appear to be related to columnar systems and theoretical considerations have suggested that they contribute to the development of regularly spaced orientation columns. We tested this assumption and examined whether early cortical lesions affected the formation and layout of the pattern of iso-orientation bands. In the striate cortex of 2-week-old kittens, tangential fiber paths were disrupted unilaterally along the representation of the horizontal meridian either by cuts, or by suction or by implanting pieces of Teflon. At this age, tangential fibers are still growing, orientation selectivity is only poorly developed and 2-deoxyglucose mapping does not yet reveal orientation columns. When the kittens were 7–10 weeks old, the organization of orientation bands was studied with the 2-deoxyglucose technique in flat-mounts of both visual cortices. In addition, kittens from the same litters as the experimental animals, having received the same lesions at the same postnatal day were subjected to neuroanatomical experiments to assess the effect of the lesion on the tangential fibers. These investigations revealed that a small fraction of tangential fibers had grown across the lesion when no mechanical barrier was implanted while disruption of tangential connections was complete in cases who had received Teflon implants. Apart from minor irregularities that were confined to the vicinity of the lesion, the 2-deoxyglucose experiments showed no differences in the pattern of orientation bands between the lesioned and intact hemispheres. In both, the bands extended throughout all cortical layers and their main trajectories were orthogonal to the representation of the vertical meridian. We conclude that at least from two weeks of age onwards, intracortical tangential connections are not necessary for the development of the regular pattern of iso-orientation bands in the striate cortex of cats.

INTRODUCTION

In the past, investigations of the cerebral cortex focused on the vertical 'columnar' organization. This feature has been investigated in particular detail in the primary visual cortex. In their pioneering studies, Hubel and Wiesel[22] established that neurons with similar orientation preference are clustered together. Cells recorded along electrode penetrations perpendicular to the cortical layers were found to respond to the same stimulus orientation, whereas a gradual shift of orientation preference was observed in tangential penetrations[1,2,24,26]. Since the development of the 2-deoxyglucose (2-DG) method by Sokoloff et al.[56] it has become possible to map the topographical arrangement of the orientation bands more comprehensively than with electrophysiological techniques (*cat*:[3,4,34,50,53,59]; *monkey*:[25]; *tree shrew*:[26,27,55]). In cat area 17, visual stimulation with stripes of a single orientation produced highly ordered patterns of parallel bands of increased 2-DG uptake that were often perpendicular to the boundaries of the area. The center-to-center spacing of these bands was in the order of 1 mm (see for example Löwel et al.[34]).

More recently another prominent feature of cortical organization has attracted considerable interest. Degeneration studies revealed that intrinsic cortical connections run for up to 6 mm parallel to the cortical surface thus spanning several functional columns[13,14,58]. An intriguing feature of these connections is that axon collaterals of individual cells are distributed in discrete clusters thus linking spatially separate groups of neurons[18,39] (for extracellular tracer injections see also[37,46,47,48]). The spacing of interconnected clusters of 0.5–1.0 mm suggesting relations with the functional columnar systems. Direct support for this comes from the evidence that tangential fibers selectively connect neurons of similar orientation preference[19,31] (but see[40,41]) and this is compatible with the suggestion that they contribute to the formation of elongated receptive fields[9,42].

Based on theoretical considerations von der Malsburg and Cowan[63] and Swindale[57] have attributed to the tangential fibers a 'shaping' role in the development of the regularly spaced orientation columns. Von der Malsburg postulated that the tangential connections serve to generate activity waves in the developing cortex. Simulation studies have shown that such 'waves' can support

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the development of columnar systems and determine details of their topographical layout such as the center-to-center spacing of adjacent columns and the trajectories of the bands[63]. The theory predicts that the trajectories of columns — if they are organized in elongated slabs — should be orthogonal to area boundaries. This seems to be the case whenever domains with similar functions become organized in bands[32,34]. We wondered, therefore, whether the disruption of tangential connections interferes with the development of the highly organized pattern of orientation bands, and if so, whether their trajectories become perpendicular also to an artificially introduced 'border'? To address these questions we severed the tangential connections during early postnatal development and later investigated the pattern of orientation bands with 2-DG autoradiography. To increase the chance of detecting changes, we disrupted the tangential fibers in area 17 along the presumed representation of the horizontal meridian (HM), where the regularity of orientation bands is maximal: here the bands are continuous over several mm, regularly spaced and parallel to the HM (see for example Fig. 7 in Löwel et al.[34]). Some results of this study have been published in abstract form[35].

MATERIALS AND METHODS

In area 17 of 2-week-old kittens (before eye opening) tangential intracortical fibers were disrupted unilaterally for several mm along the presumed representation of the HM (Figs. 1 and 2). When the kittens were 7–10 weeks old, the organization of orientation bands was studied with the 2-DG technique in flat-mounts of both visual cortices.

A total of 16 kittens were used for this project. In 9 kittens, orientation bands were analyzed with 2-DG (Table I, in the other 7 kittens, the localization and extent of the lesion as well as possible regeneration were investigated with different anatomical techniques after survival times ranging from 2 weeks to 16 months (Table II).

Surgical procedures

The kittens were premedicated with atropine sulfate (Pharma Hameln, dose 0.1 mg/kg), i.m., and anesthetized with a mixture of ketamine hydrochloride (Ketanest 50 mg/ml, Parke-Davis; dose 0.30–0.36 ml/kg) and xylazine hydrochloride (Rompun 5%, Bayer; dose 0.23–0.27 ml/kg) injected i.m. Using sterile surgical techniques, a bone flap was removed over the presumed representation of the HM (approximately AP-8) in the left hemispheres of the animals. After opening the dura, we either made a mediolateral cut through the lateral gyrus using a razor blade (kittens L14–L21) or removed a 0.5–1.0 mm wide strip of cortical tissue by suction (C1, C2, C4). In both conditions, we aimed at lesions comprising all cortical layers and extending from the lateral, resp. posterolateral to the splenial sulcus (see Fig. 2). Access to the medial bank was achieved by gentle retraction of the hemisphere. Because later histological examination of the brains provided indications for a regrowth of fibers across the lesion, we sought to establish a permanent barrier for growing axons by implanting pieces of Teflon into the lesion (T2, T3, T4, T5, T7). The implants (see Fig. 2) were 0.1 mm thick and L-shaped to follow the curvature of the lateral gyrus. They measured about 5–6 mm in their dorsoventral and 3–4 mm in their mediolateral extent. We fixed the Teflon-pieces to the pia by 'spot-welding' with tissue glue (Bucaryl, Lege Artis). The trepanation was closed by fixing the bone flap in its original position with tissue glue and dental cement (Paladur, Kulzer).

2-Deoxyglucose (2-DG) autoradiography

Five to 8 weeks after the lesion, we investigated the pattern of orientation bands by [^{14}C]2-DG autoradiography as described in detail previously[34]. Anesthesia was induced as described above and

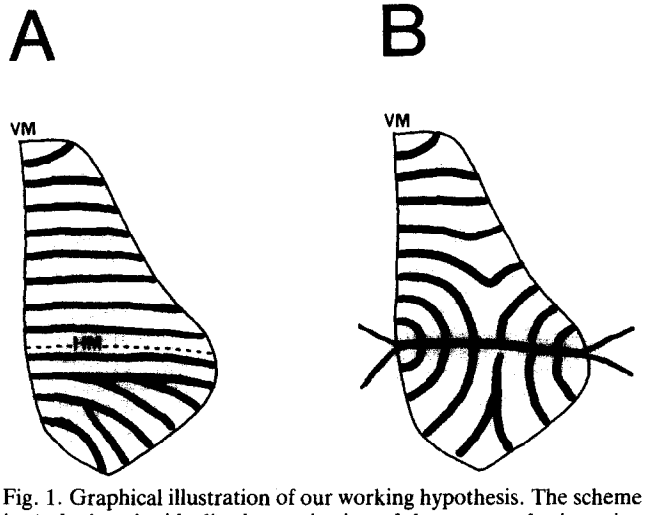

Fig. 1. Graphical illustration of our working hypothesis. The scheme in A depicts the idealized organization of the system of orientation bands in the striate cortex of normal cats. The trajectories of the bands are orthogonal to the representation of the vertical meridian (VM) and to other area boundaries and parallel to the representation of the horizontal meridian (HM). The drawing in B summarizes the expected changes after disruption of horizontal intracortical connections along the HM.

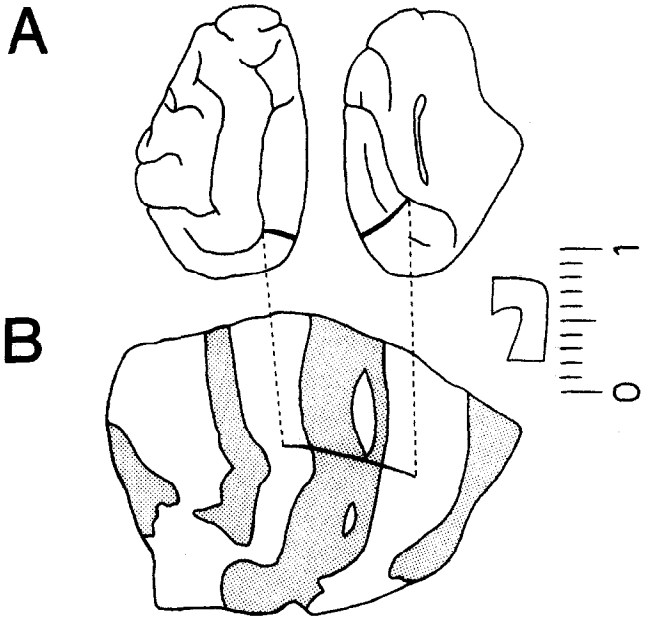

Fig. 2. Localization and extent of the lesions in a dorsal and medial view of a cat's brain (A) and in a flat-mount of the occipital lobe (B). Cortical regions exposed on the convexity of gyri are dotted, those normally hidden in the sulci are left blank (see Fig. 4 in Freeman et al.[15]). The inset to the right shows a scheme of the implants (scale 10 mm).

TABLE I

Conditions of the 2-DG experiments

Cat	*Lesion*			*2-DG*			
	Age (days)	*Weight (g)*	*Type*	*Age (weeks)*	*Weight (g)*	*Dosage (μCi/kg)*	*Orientation of visual stimuli**
L14	13	290	cut	9.5	780	119	0°
L16	13	290	cut	10	840	137	0°
L17	13	280	cut	8	750	120	0°
L18	14	330	cut	8	850	120	90°
C1	13	305	suction	7.5	670	119	0°
C2	13	280	suction	8	670	119	90°
T2	14	310	Teflon	8	630	110	90°
T4	14	320	Teflon	8	580	101	0°
T7	13	315	Teflon	7	710	121	45°

*0° = horizontal, 90° = vertical, 45° = oblique.

after tracheal intubation and cannulation of a femoral vein was maintained with a mixture of 70% N_2O/30% O_2. The animal's head was fixed in a stereotaxic frame by means of a metal bar cemented to the skull and a muscle relaxant (hexcarbacholinbromide, dose 10 mg in 45 ml of Ringer, 3 ml/kg per hour) was applied to prevent eye movements. After having received an intravenous injection of 2-deoxy-D-[U-^{14}C]glucose (Amersham, spec. act. 310 mCi/mmol; dose 101–137 μCi/kg, see Table I), the animals were exposed to moving patterns consisting of horizontal (L14, L16, L17, C1, T4), vertical (L18, C2, T2) or oblique (T7) black and white stripes. After 45 min of visual stimulation, the animals were given a lethal dose of Nembutal injected intravenously. The occipital poles of the brain were removed and the visual cortices flat-mounted prior to freezing the tissue on dry ice (technique described in detail in Freeman et al.[15]; see also Löwel et al.[34]). Subsequently, 26 μm thick serial cryostat sections were cut parallel to the cortical surface. The sections were mounted on glass slides, immediately dried on a hot plate, and then exposed to X-ray film (Agfa Mamoray, M4 or T3). To allow for a comparison between the two hemispheres, sections with corresponding serial numbers were exposed on the same film sheet. Optimal exposure time was assessed separately for each experiment and ranged from 3 to 5 weeks.

Anatomical investigation of the lesion

Nissl. To determine the localization and extent of the lesions, selected flat-mount sections of all experimental (2-DG) animals were stained with 1% Cresyl violet after their autoradiographic exposure. The brain of cat L19 (same litter as cat L18) was cut in the horizontal plane and Nissl-stained 2 weeks after the lesion.

Bodian. Seven months after the lesion, the distribution of nerve fibers was analyzed in the visual cortex of cat L15 by the Bodian silver impregnation technique[8]. This method stains neurofibrils (of the central and peripheral nervous system) which appear brownish-black to violet in the histological sections.

In vivo tract tracing with Rhodamine beads and Diamidino Yellow. To determine whether axons of cortical cells are still able to interconnect regions on either side of the lesion we injected a cocktail of Rhodamine-filled latex microspheres (RhB;[28]) and Diamidino Yellow-dihydrochloride (DY;[30]) 1 mm anterior to the lesion in the left visual cortex of cat L21. This animal was anesthetized 11 months after the lesion with a mixture of ketamine hydrochloride (10 mg/kg) and xylazine hydrochloride (7.5 mg/kg), and, using a Hamilton syringe, 0.5 μl of a 2:1 mixture of DY (2%) and RhB were injected at a depth of approximately 500–750 μm. After a survival time of 4 days, the animal was sacrificed with a lethal dose of Nembutal i.p. and perfused transcardially with 4% paraformaldehyde (PA). Subsequently 50 μm thick parasagittal cryotome sections were prepared from the occipital pole. The sections were mounted on gelatinized slides, coverslipped with UV-Inert (Serva) and stored in the dark.

In vitro tract tracing with dil. After realizing that the regions on either side of the lesion were still connected by some fibers, we wanted to analyze in more detail whether these connections resulted from growth or regeneration of fibers through or around the lesion. Therefore animals lesioned according to the 3 protocols (L20, C4, T3 and T5) were sacrificed 4–16 months after the lesions, and perfused with 2% (L20) or 4% PA. After the brains had been removed, we placed small crystals (diameter 0.1–0.2 mm) of the fluorescent carbocyanine dye dil (Molecular Probes) superficially (at a depth of approximately 300–500 μm) with a glass pipette 1 mm anterior (L20, T3, T5) or posterior (C4) to the lesion (for a description of the properties of dil see Godement et al.[20]). The brains were stored in 2% PA in the dark. Four to 6 months later, we cut 50 μm thick vibratome sections (in the horizontal or sagittal plane). In all hemispheres implanted with pieces of Teflon, these pieces were removed before cutting. The sections were mounted and coverslipped with phosphate buffer, sealed with nail polish and stored in a refrigerator.

Analysis

The Nissl-stained sections were analyzed with a macroscope (Wild), and the lesions reconstructed using a camera lucida. All other sections were analyzed with a photomicroscope resp. epifluorescence microscope (Zeiss III) equipped with a Rhodamine filter set for viewing the red dil and RhB fluorescence and a FITC filter set for the yellow DY fluorescence. Photographs were taken on 15 Din film (Agfapan), resp. 27 Din film (Kodak Tri X 400 Pan).

TABLE II

Anatomical investigation of the lesion

Cat	*Lesion*			*Anatomy*	
	Age (days)	*Weight (g)*	*Type*	*Age (months)*	*Method*
L15	13	280	cut	7.5	Bodian
L19	15	280	cut	1	Nissl
L20	15	330	cut	16	dil
L21	15	290	cut	11.5	RhB, DY
C4	13	280	suction	11	dil
T3	14	300	Teflon	4	dil
T5	13	390	Teflon	7	dil

RESULTS

Comparison of the 2-deoxyglucose patterns in the lesioned and intact hemispheres

The 2-deoxyglucose (2-DG) autoradiographs showed — apart from minor irregularities in the vicinity of the lesions — basically no differences in the pattern of iso-orientation bands between experimental and control hemispheres (Figs. 3–5). This holds true for animals with all 3 types of lesions: whether horizontal intracortical

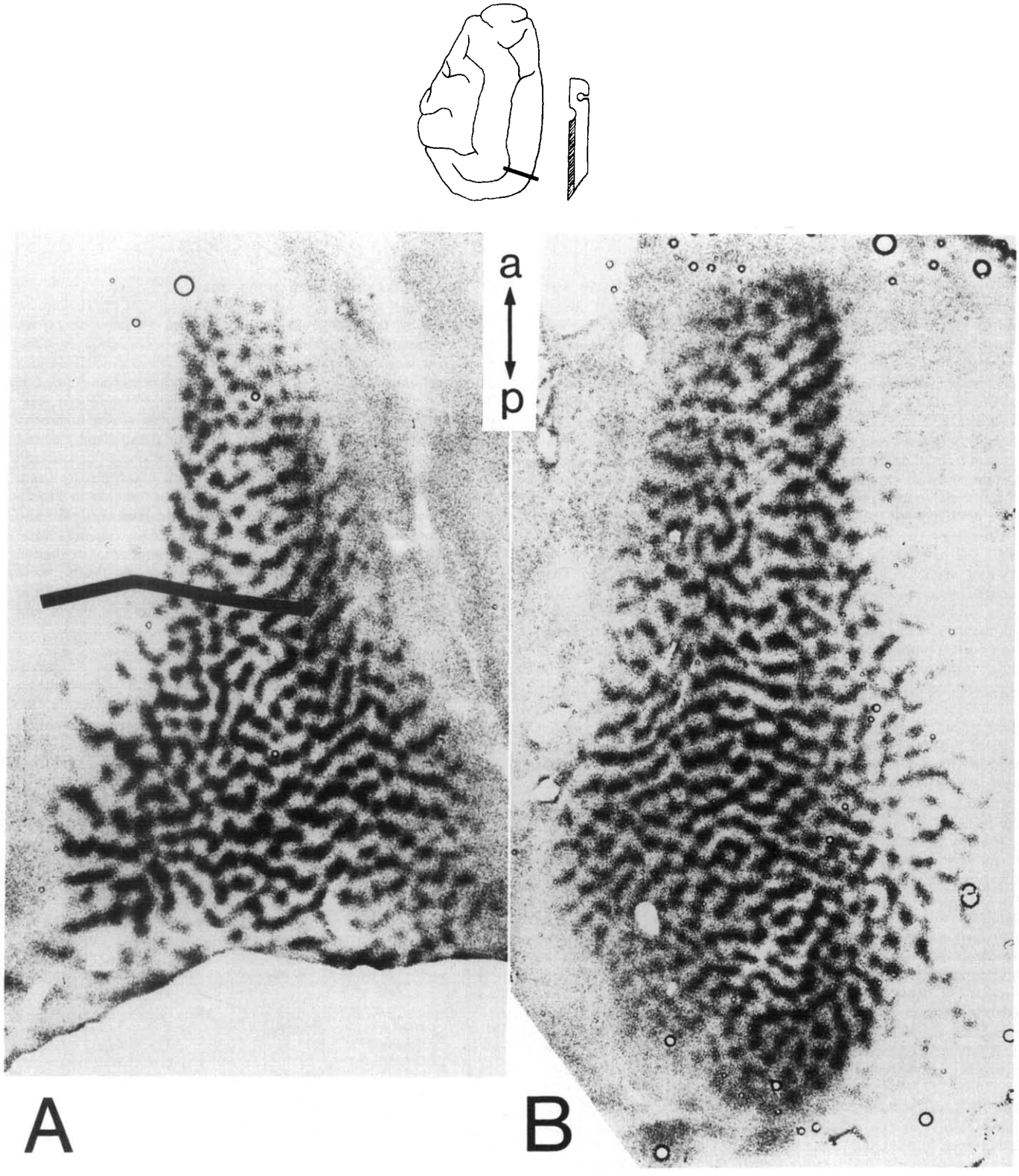

Fig. 3. A,B. (*For legend, see next page.*)

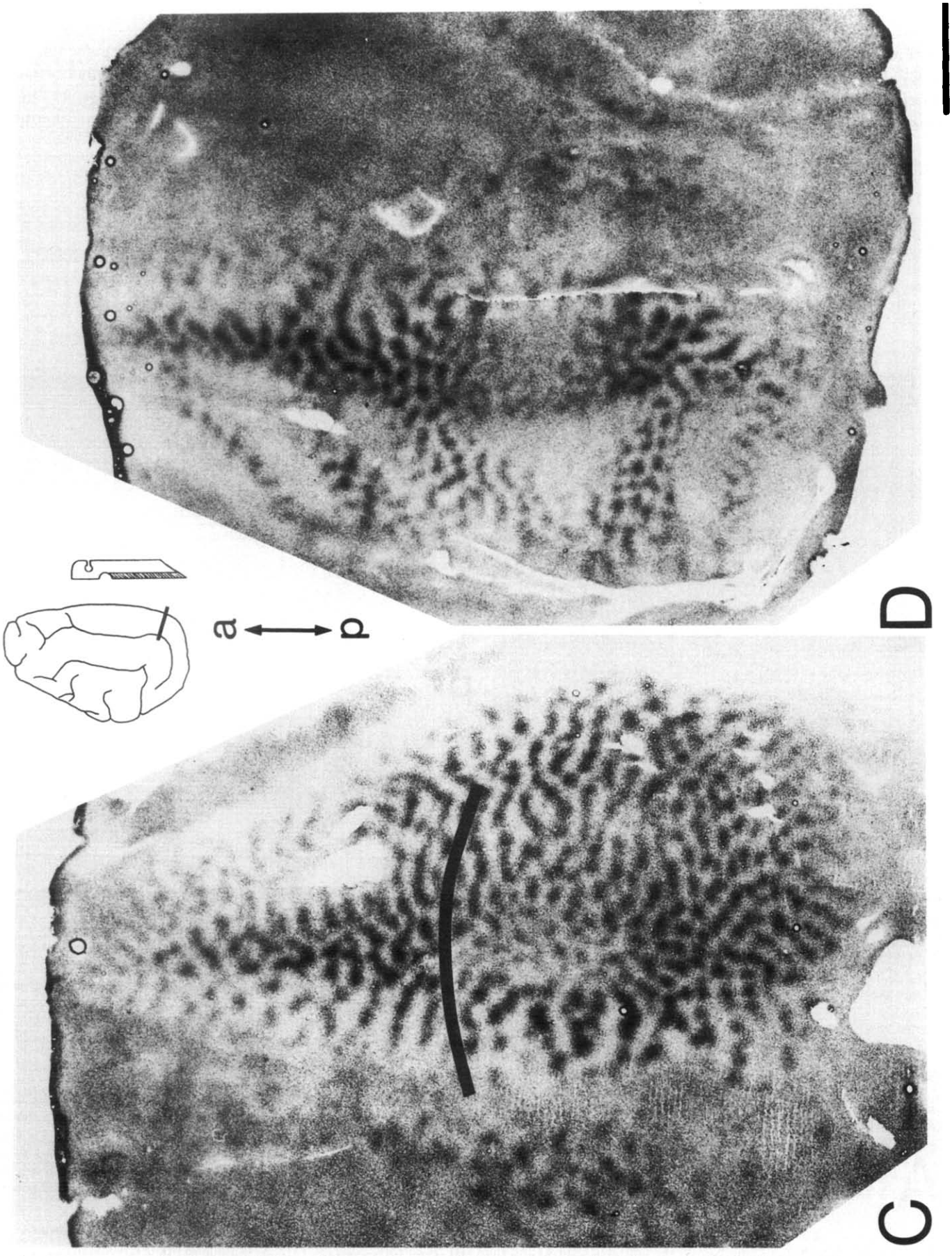

Fig. 3. Autoradiographs of sections from flat-mounted visual cortices in the left (A,C) and right (B,D) hemispheres of two cats (A,B: L18 and C,D: L16) with a mediolateral cut in areas 17 and 18. In A and C, horizontal fibers have been disrupted by a scalpel cut. The localization and extent of the lesions were determined on Nissl-stained sections and are indicated by the black lines. Visual stimulation of the cats consisted of vertical (L18), resp. horizontal (L16) stripes. In the left visual hemifield of cat L16, the stimulation was restricted to 5 horizontal sectors at elevations of +30°, +10°, 0°, −10°, −30° (width of the sectors: 2°, 4° and 6° at 0°, 10° and 30°). The 2-DG pattern in the right visual cortex of cat L16 (D) is restricted to regions corresponding to the respective elevations (see maps in Löwel et al.[34]; Tusa et al.[62]). Scale bar 5 mm. Abbreviations: a, anterior; p, posterior.

fibers were disrupted by cutting, sucking or implanting pieces of Teflon. In all visual cortices, the regions of increased radioactivity extended throughout all cortical layers and the main trajectories of the iso-orientation bands were essentially orthogonal to the representation of the vertical meridian (VM). This is especially obvious in the left hemispheres of cats C1 and T7 (Figs. 4A and 5A): radioactively labeled orientation bands run parallel

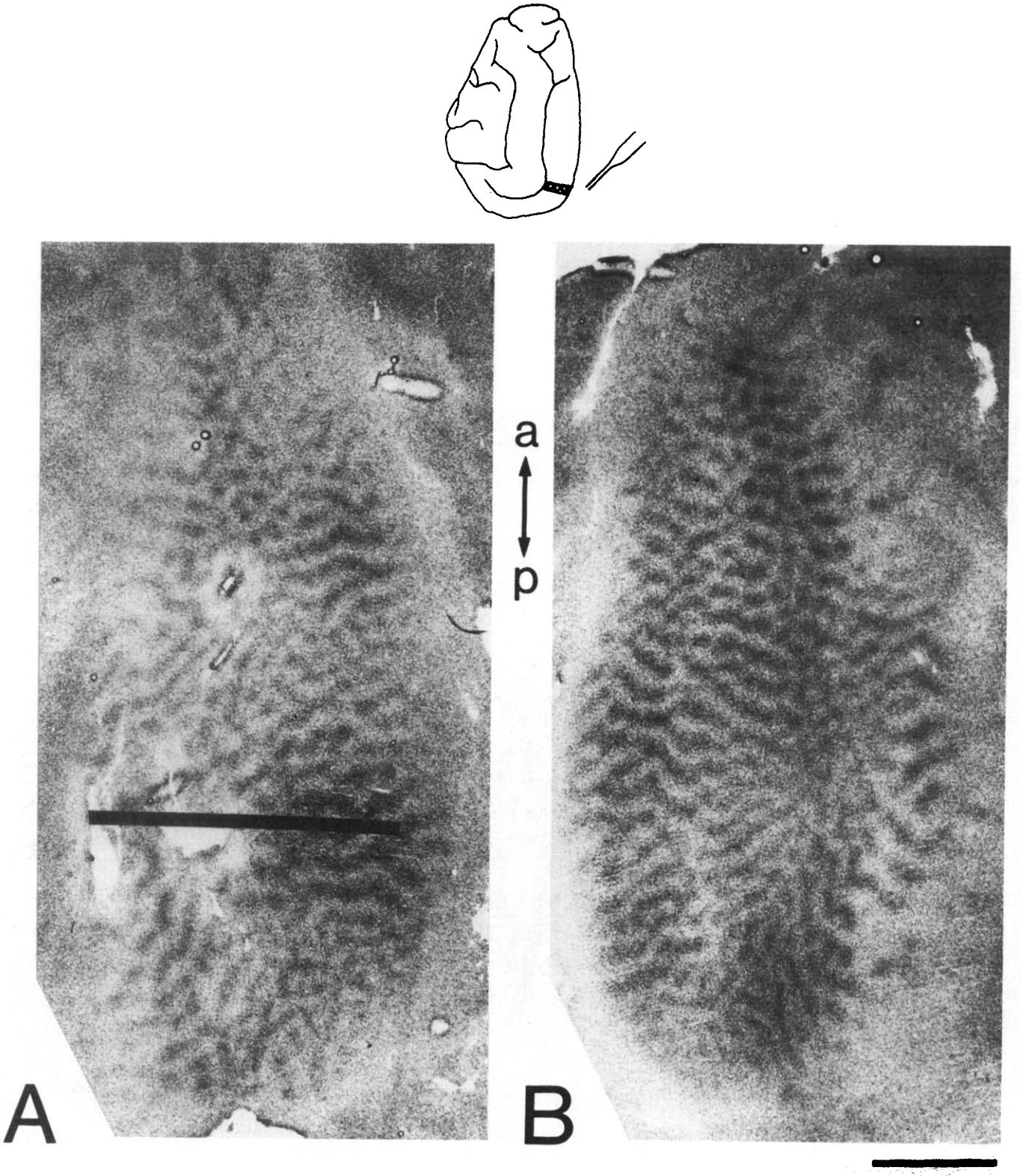

Fig. 4. Autoradiographs of sections from the unfolded left (A) and right (B) visual cortex of cat C1. In A, horizontal fibers have been disrupted by suction. The cat was visually stimulated with horizontal stripes. Note the similarity of the pattern of orientation bands in both hemispheres. Scale bar 5 mm. Abbreviations as in Fig. 3.

to the cortical lesions even in their immediate vicinity. The autoradiographs appear as if the cortices were cut after the 2-DG experiments. This is less obvious for the pattern of orientation bands in the visual cortex of cat L18 (Fig. 3A). In this animal, the lesion is very far anterior and the 2-DG pattern in its vicinity is not as regular as in more posterior regions. However, the pattern is similarly irregular in the anterior part of area

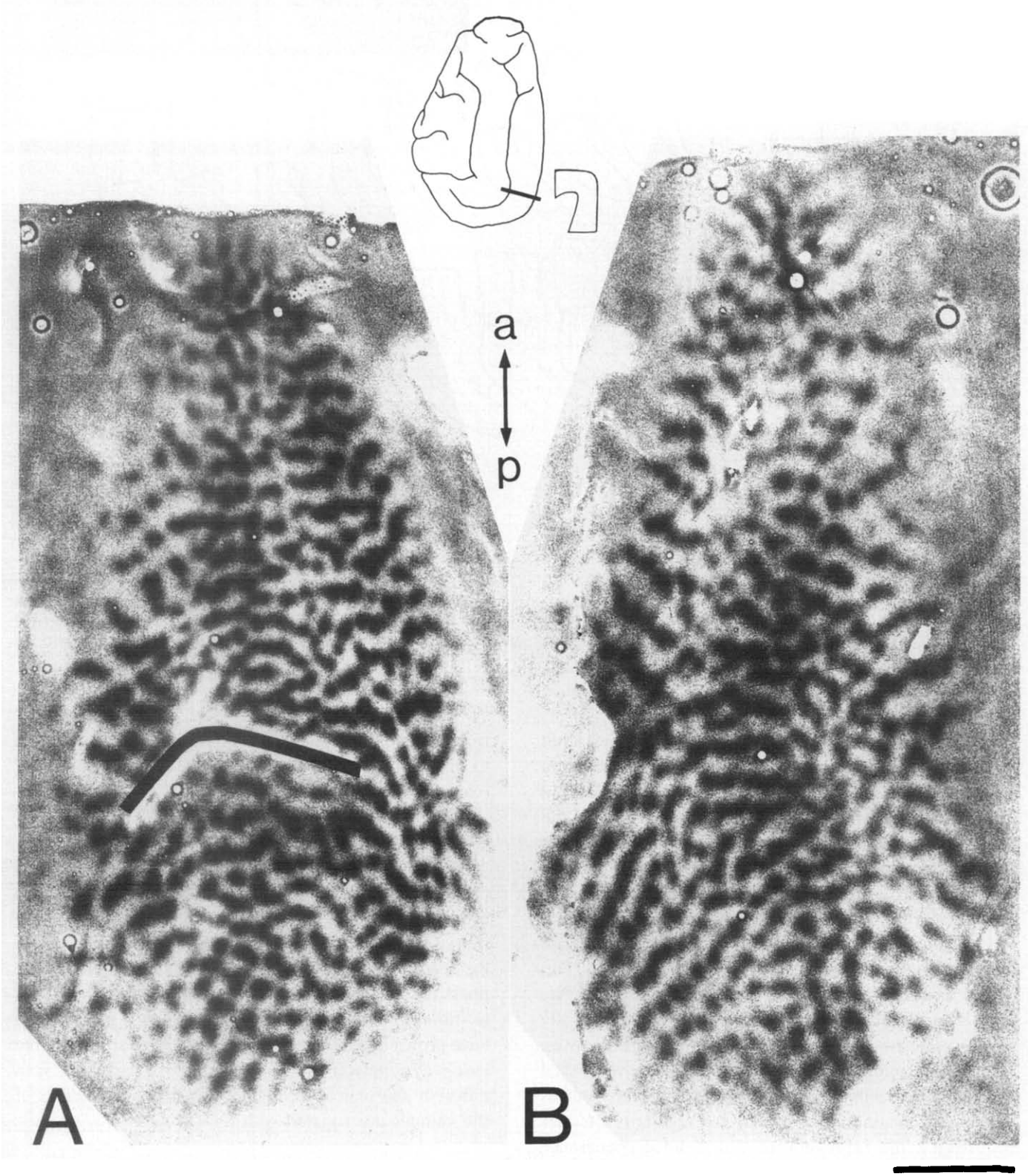

Fig. 5. Autoradiographs from sections of unfolded left (A) and right (B) visual cortex of cat T7. The animal had pieces of Teflon implanted in its left hemisphere. The cat was visually stimulated with oblique stripes. Note the similarity of the patterns in ipsi- and contralateral hemisphere and that the orientation bands in A run parallel to the lesion. Scale bar 5 mm. Abbreviations as in Fig. 3.

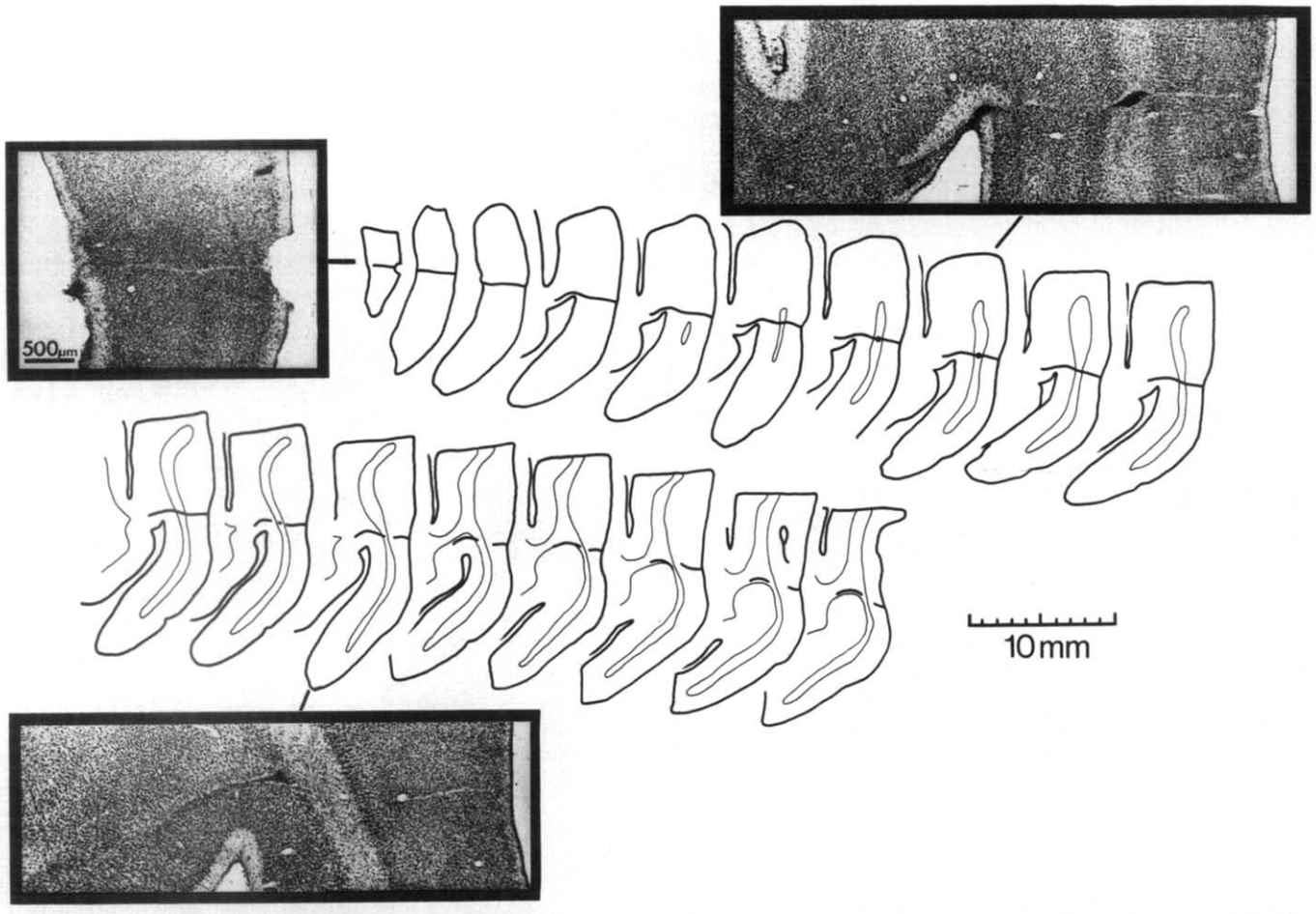

Fig. 6. Reconstruction of a cortical lesion made with a scalpel cut in the left hemisphere of cat L19 (same litter as cat L18). The localization and extent of the lesion was determined on Nissl-stained horizontal sections 2.5 weeks after the operation. The distance between the illustrated camera lucida drawings is 150 µm. Insets show selected original sections at a depth of 400 µm, 1600 µm and 2350 µm. The lesion spans the entire lateral gyrus.

17 of the control hemisphere (Fig. 3B). This suggests that the irregularity of the pattern in the vicinity of the lesion reflects less ordered arrangement of orientation bands in anterior regions of area 17 rather than an effect of the lesion. Taken together these experiments demonstrate that there are no basic differences in the 2-DG patterns between control and experimental hemispheres.

Confirming previous results, the pattern of iso-orientation bands is not dependent on stimulus orientation: visual stimulation of the retinae with either vertical (Fig. 3A,B) or horizontal (Figs. 3C,D and 4) or oblique (Fig. 5A,B) black and white stripes produced essentially similar patterns of increased 2-DG uptake. Comparison of different animals revealed substantial interindividual variability in details of the columnar arrangement such as the length of continuous bands, the regularity of their trajectories, the expression of their beaded appearance and their average spacing. However the patterns in the two hemispheres of the same animal were always rather similar with respect to these variables. Thus, the patterns from the two hemispheres can be recognized as belonging to the same animal because of their similar texture (compare Fig. 4 to Fig. 5).

Anatomical investigation of the lesion

The absence of any obvious differences in the 2-DG patterns between experimental and control hemispheres raised the question whether the lesions effectively disrupted the connections between regions on either side of the lesion. Two possibilities need to be considered: first, fibers descending to white matter and re-entering cortex (U-fibers) may have been spared. Second, axons could have grown de novo or regenerated across or around the lesion. To answer these questions we analyzed with different anatomical techniques the brains of siblings of the animals investigated with 2-DG.

Reconstruction of the lesion with Nissl-staining

To analyze the localization and extent of the lesion, the left visual cortex of cat L19 (same litter as cat L18) was

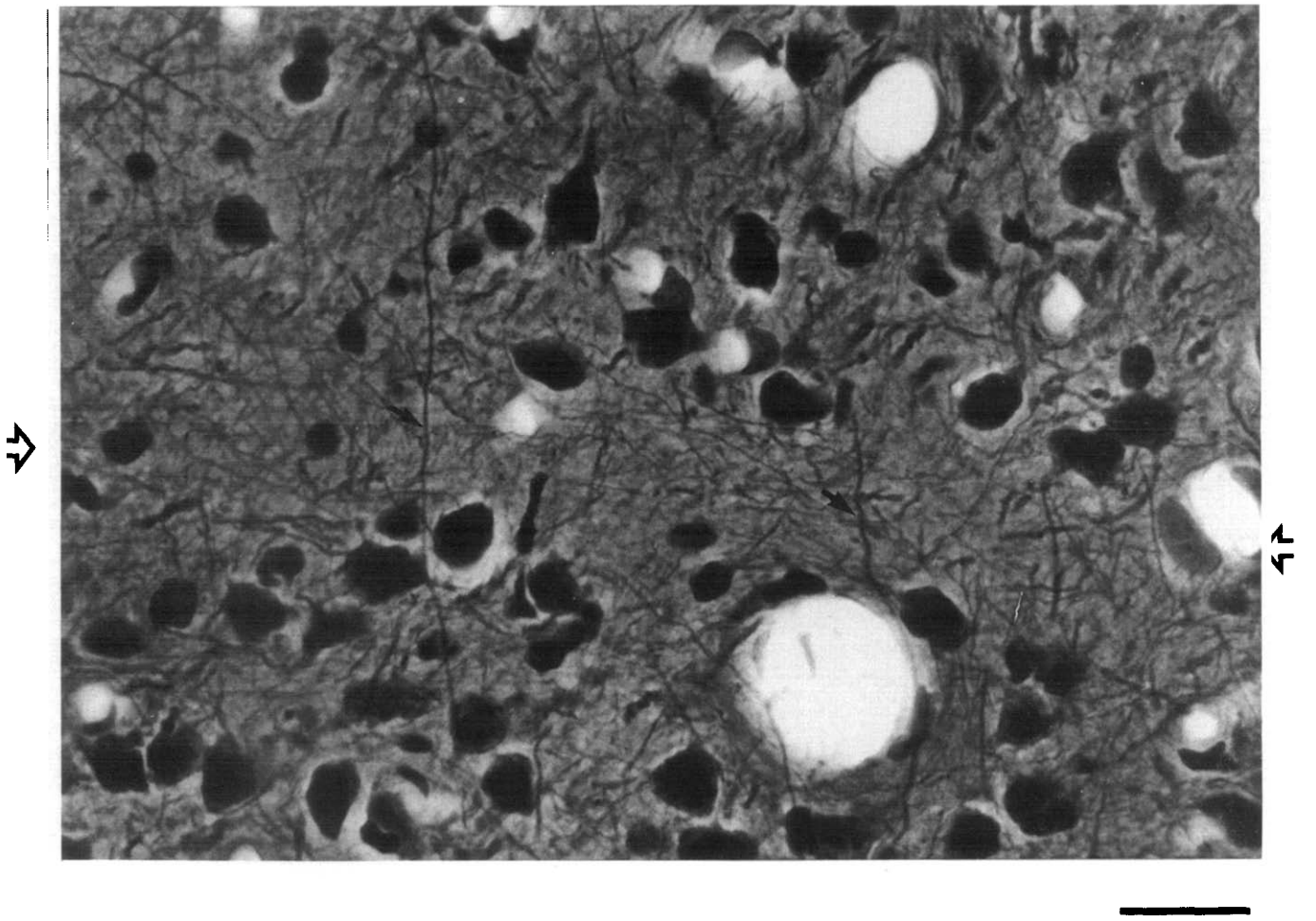

Fig. 7. Horizontal section through the left visual cortex of cat L15 (same litter as cat L16) in the region of the lesion (open arrows) made by a scalpel cut. The section was stained according to the Bodian silver impregnation technique. The scar runs from left to right through the picture. In the vicinity of the scar most fibers run horizontally, that is parallel to the scar. However occasionally fibers cross the scar (see for example those labeled with black arrows).

cut in the horizontal plane and Nissl-stained 2.5 weeks after the initial operation (Fig. 6). The analysis of the serial sections demonstrates a scar extending through the entire width of the lateral gyrus. The scar is still visible at a depth of more than 3 mm in the medial bank (splenial gyrus) and also includes white matter. The lesion thus spans the entire extension of area 17 and — most laterally — even affects parts of area 18. Interestingly the lesion is more readily detectable in infragranular compared to supragranular layers. No pronounced glial reaction could be observed.

Silver impregnation of nerve fibers (after Bodian)

Seven months after the lesion, we investigated the distribution of nerve fibers in the brain of cat L15 (same litter as cat L16) by the Bodian silver impregnation technique. Close to the lesion, most axons run parallel to the scar. However, occasionally fibers were observed which cross the lesion (Fig. 7). This growth of axons occurred more often in supragranular layers in which — similar to results of cat L19 — the lesion is more difficult to detect. This result indicates that either newly growing or regenerating fibers managed to cross the lesion.

In vivo tract tracing with Rhodamine-beads (RhB) and Diamidino Yellow (DY)

Eleven months after the initial operation, cat L21 received an injection of a mixture of RhB and DY 1 mm anterior to the lesion in the left hemisphere. After 4 days of survival, histological examination revealed several retrogradely labeled neurons 0.5 mm posterior to the lesion (Fig. 8). Both DY-labeled neurons and neurons filled with RhB were discernible. These retrogradely labeled neurons suggest that the axons which crossed the lesion are at least in part derived from cortical neurons.

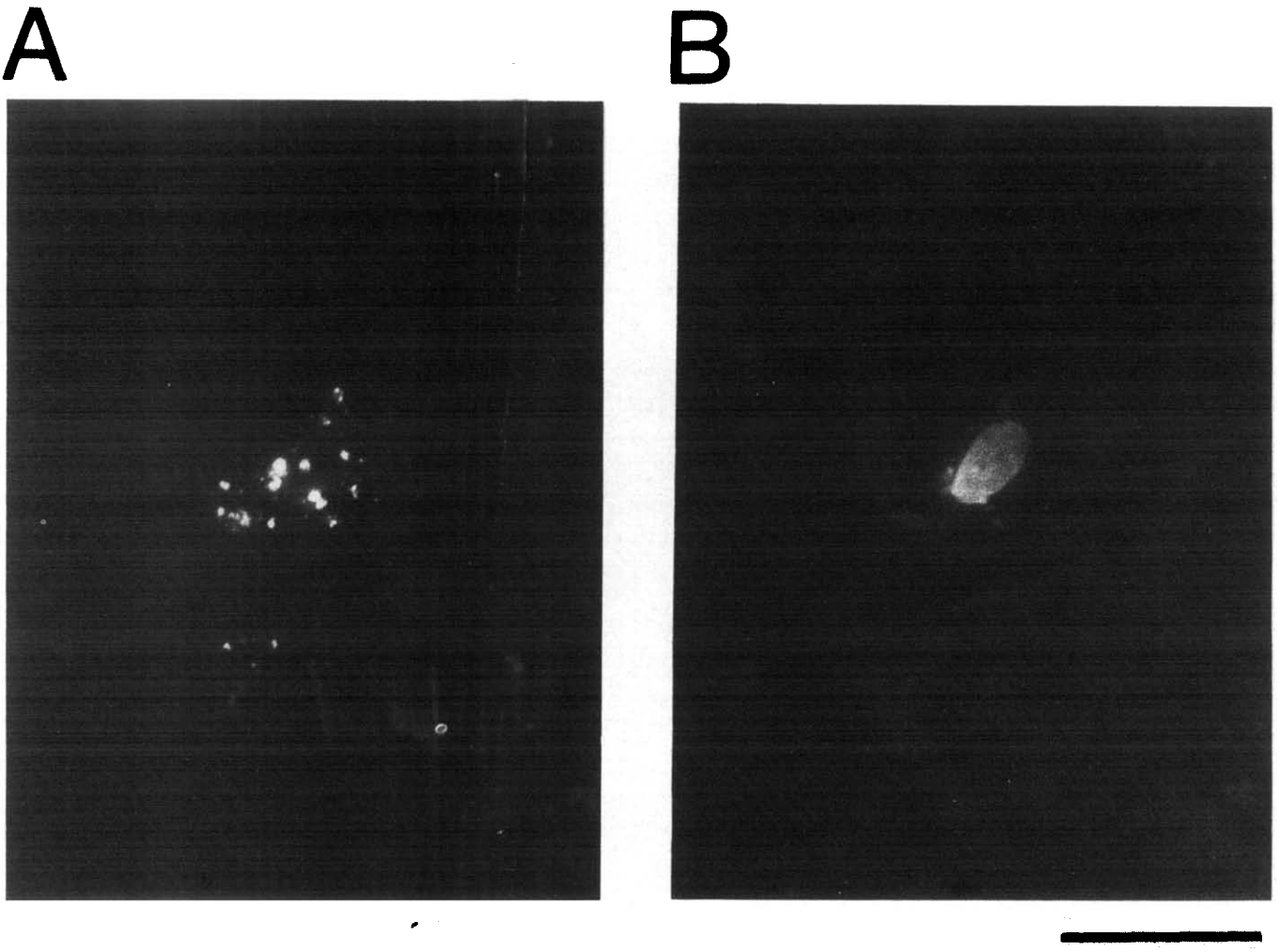

Fig. 8. Retrogradely (double-)labeled neuron in the left visual cortex of cat L21 located 0.5 mm posterior of the lesion (scalpel cut). Eleven months after the lesion, a mixture of Rhodamine-beads (RhB) and Diamidino Yellow (DY) was injected 1 mm anterior to the lesion. A: the Rhodamine-filled latex microspheres in the cell body of the neuron light up with excitation at 510–560 nm. B: at 400–440 nm the DY in the nucleus of the neuron is visible. Scale bar 20 μm.

In vitro tract tracing with dil

In cat L20, we placed a crystal of the fluorescent carbocyanine dye dil anterior to the lesion. As shown in Fig. 9A the lesion causes an abrupt decrease of fluorescence indicating effective disruption of tangential connections. Inspection at higher magnification revealed that in the immediate vicinity of the lesion many axons make sharp turns (B) or grow in loops (D). However some fibers undoubtedly cross the lesion (C). In addition, labeled neurons are occasionally visible beyond the scar (E). In the vicinity of the dye depot and anterior to the lesion, the label shows a characteristic patchy distribution (F). Higher magnification shows that the patches consist of labeled axon terminals and neurons (G). In cat C4, a crystal of dil was placed posterior to the lesion (Fig. 10A). Again, most of the fluorescence stops at the scar but a few fibers can be seen to cross the lesion (B and D). Beyond the scar, the axonal trajectories are less regular (C) than on the side of the 'injection' where most fibers have a straight course (horizontal, vertical or oblique). A similar observation has recently been reported by Schnell and Schwab[49]: in the cortico-spinal tract of rats, regenerating fibers grew in an irregular way while unlesioned fibers were straight.

These results demonstrate that axons of some cortical cells were able to grow through the lesion. This somewhat unexpected finding prompted us to implant mechanical barriers (pieces of Teflon) into the visual cortices of our experimental animals.

To control for the effectiveness of this procedure we applied crystals of dil on one side of the lesion in cat T5. The analysis of the histological material revealed that labeled axons and neurons were entirely restricted to cortical areas on the side of the 'injection' (Fig. 11). No fluorescence could be observed beyond the implant. Thus, the implantation of pieces of Teflon reliably disrupted any horizontal interactions across the lesions.

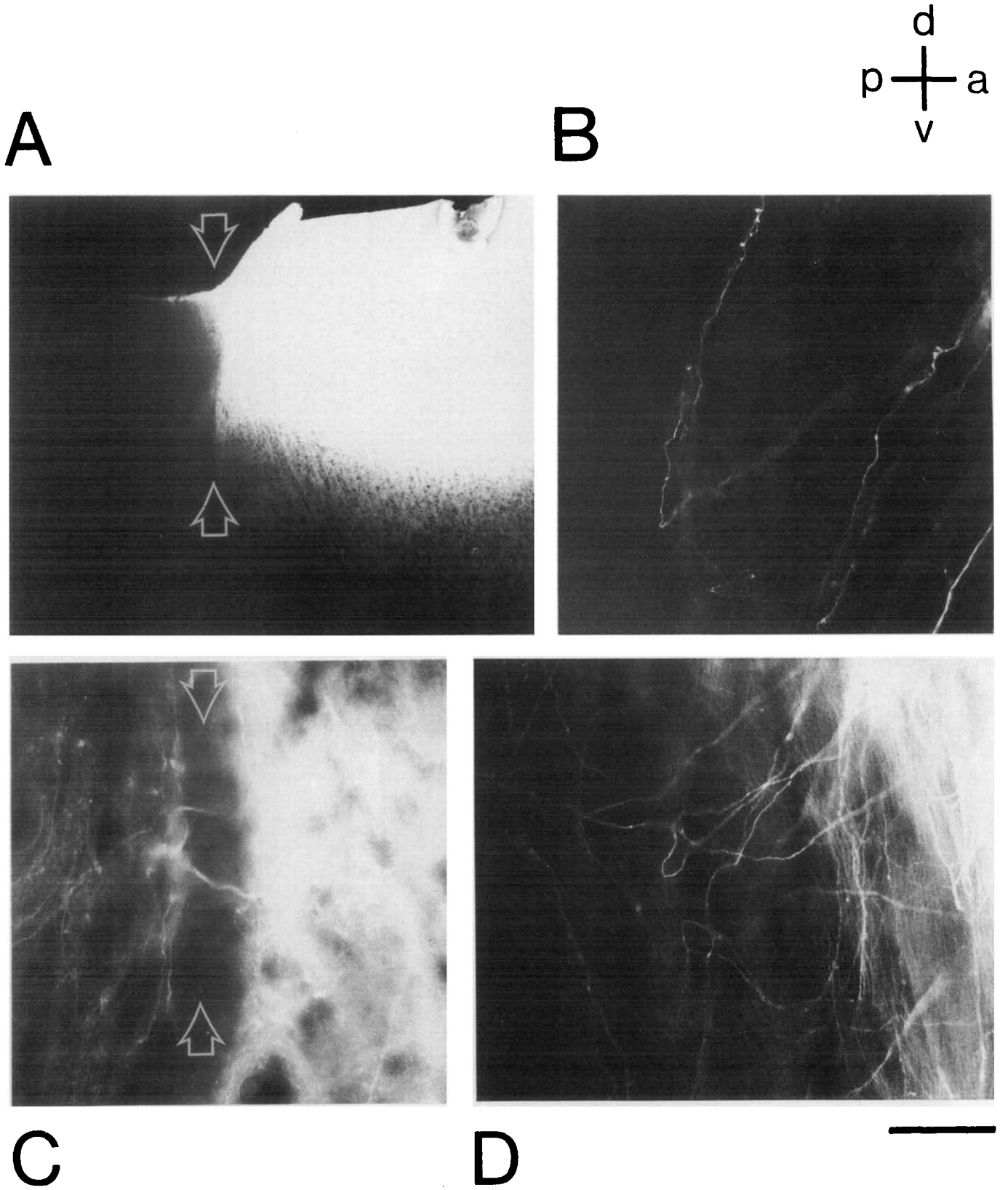

Fig. 9. In vitro tract tracing with dil 15.5 months after a scalpel cut lesion in area 17 of cat L20. Parasagittal section of the left hemisphere. A: location of the dil crystal 1 mm anterior to the lesion (white arrows). B and D: closely anterior to the lesion, fibers make sharp turns (B) or grow in loops (D). C: fibers crossing the scar (white arrows). Scale bar 500 μm (A), 50 μm (B, C and D). E: retrogradely labeled neuron about 0.5 mm beyond the cut. F: patchy pattern of fluorescence surrounding the dye crystal. The section plane is approximately tangential to the cortical surface in the lower half of the photograph. The curved line across the middle is the joint between the two parts of the photomontage. G: three labeled neurons in a patch (detail of F) located at a distance of 2.5 mm from the crystal. Scale bar 50 μm (E), 500 μm (F), 90 μm (G). Abbreviations as in Fig. 3 and d, dorsal; v, ventral.

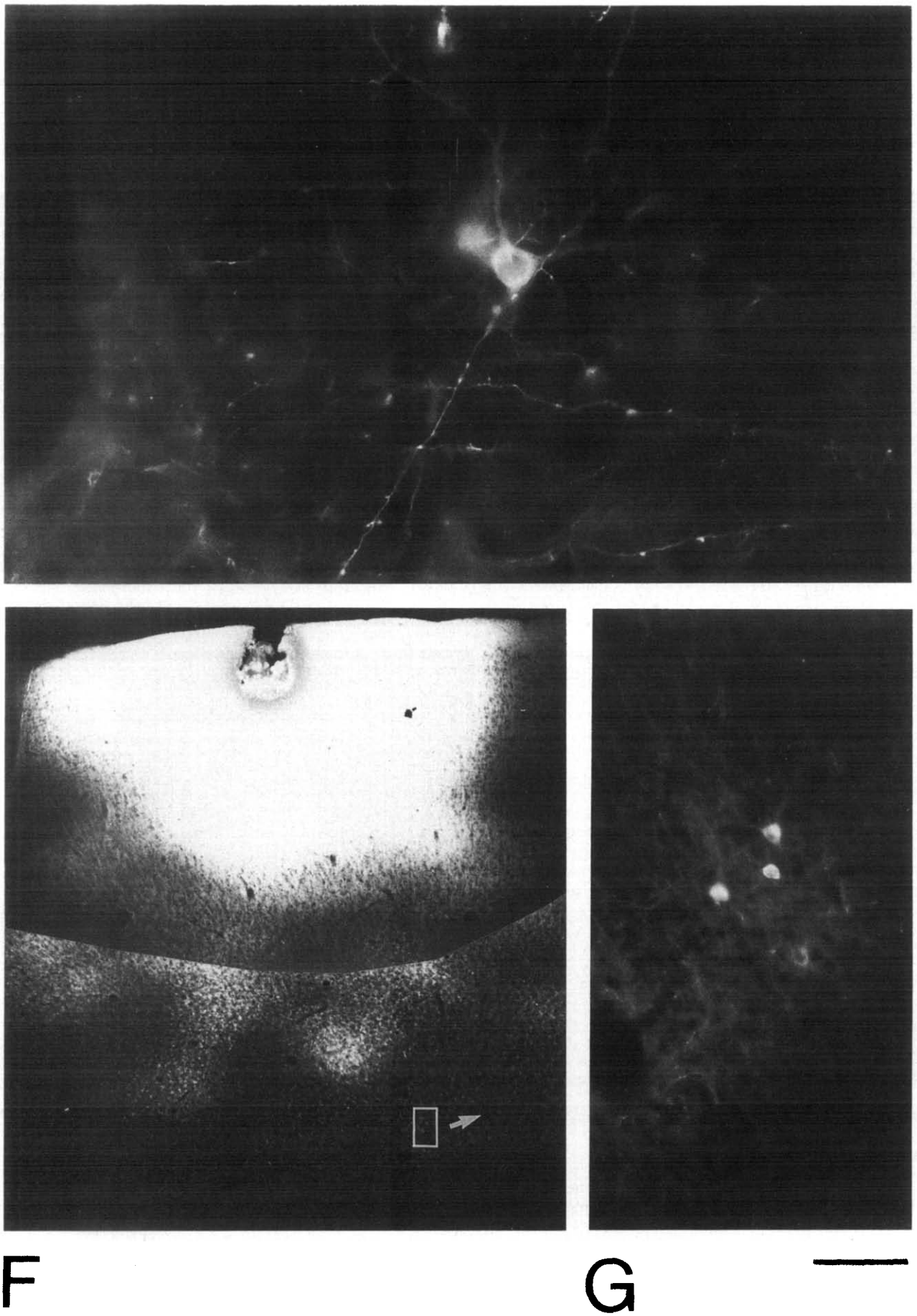

Fig. 9. E–G. (*For legend, see previous page.*)

DISCUSSION

Our results demonstrate that the disruption of horizontal intracortical connections does not disturb the development of the regular pattern of iso-orientation bands in cat striate cortex when the lesions are inflicted at two weeks of age. Apart from minor irregularities in the immediate vicinity of the lesions, there are no

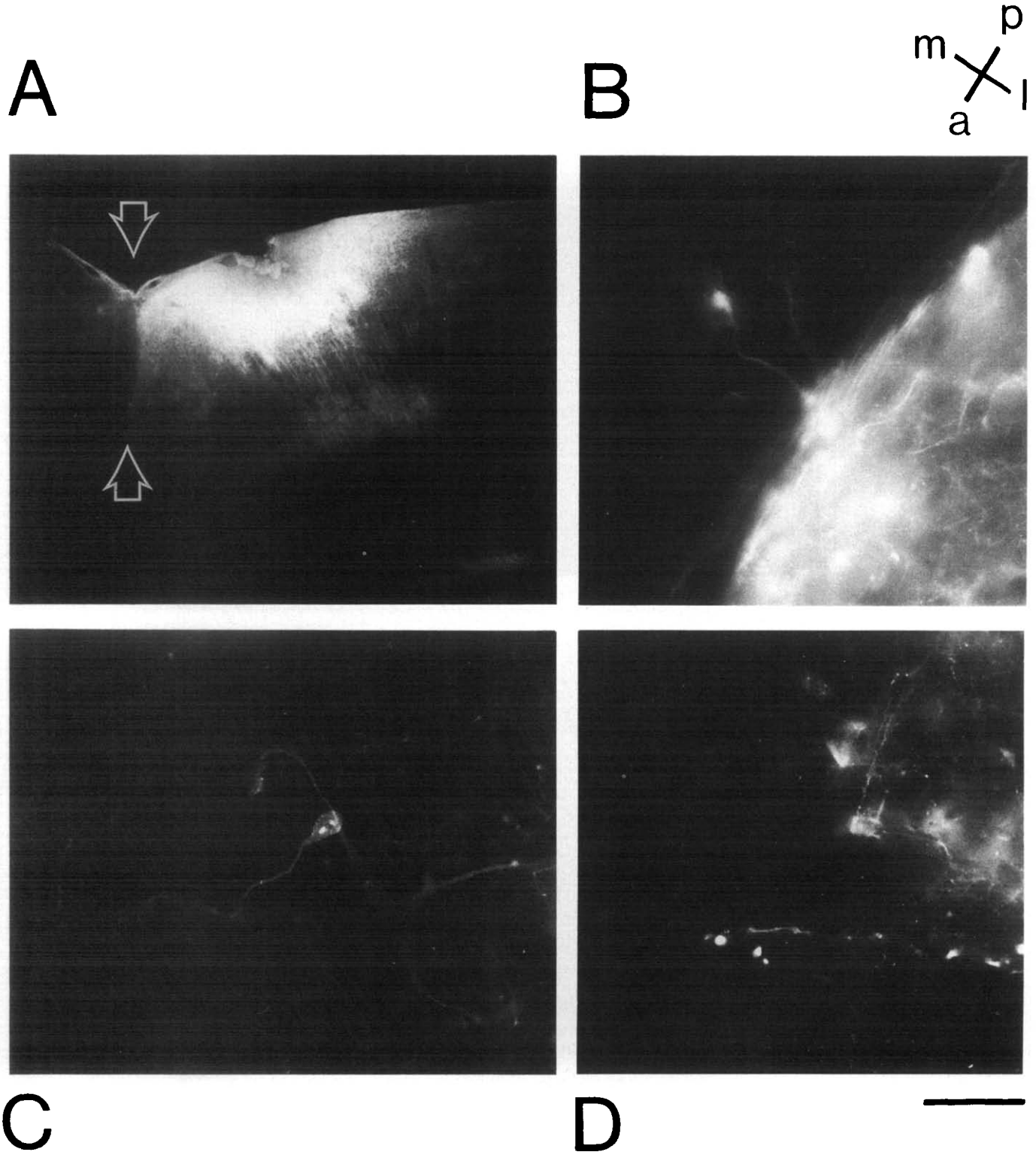

Fig. 10. In vitro tract tracing with dil, 10.5 months after a lesion by suction in area 17 of cat C4. Horizontal section of the left hemisphere. A: location of the dil crystal 0.7 mm posterior to the lesion. Note the abrupt cessation of fluorescence at the site of the lesion (white arrows) anterior to the 'dye depot'. B and D: fluorescent axons crossing the scar. C: fiber plexus beyond the lesion. Scale bar 500 μm (A), 50 μm (B and D), 30 μm (C). Abbreviations as in Fig. 3, and m, medial; l, lateral.

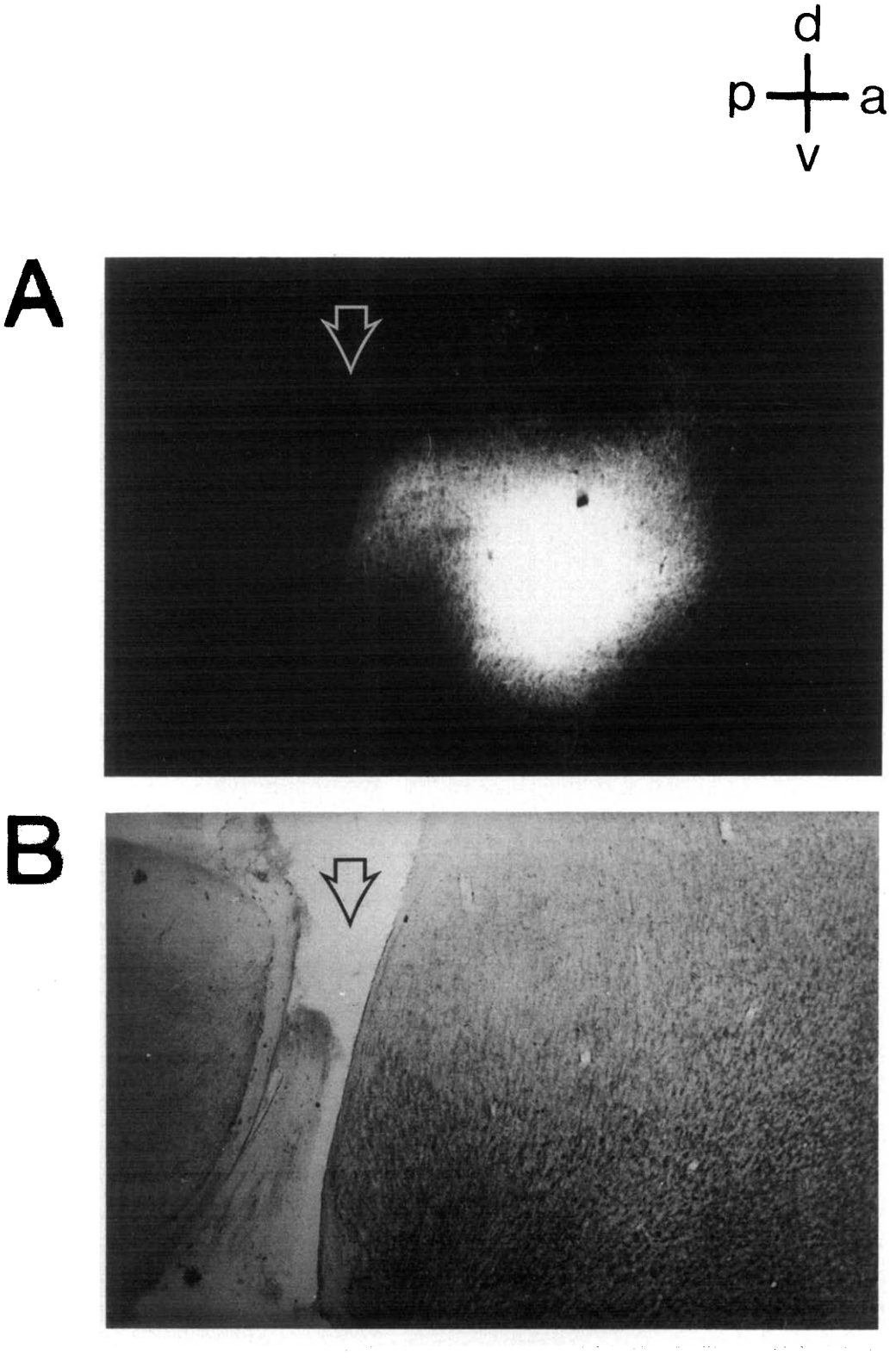

Fig. 11. In vitro tract tracing with dil 6.5 months after the implantation of a piece of Teflon in the left visual cortex of cat T5. The piece of Teflon was removed before cutting the tissue leaving a cleft in the tissue (white, resp. black arrow, see vertical cleft in B). Parasagittal section 3.5 mm lateral to the medial bank. In the middle part of the photographs, the plane of sectioning is nearly tangential to the cortical surface. A: pattern of fluorescence close to the crystal. Fluorescence does not spread beyond the lesion and no labeled cells or axons are detectable to the left of the implant. B: same section as in A photographed under normal light field conditions. Scale bar 500 μm. Abbreviations as in Fig. 9.

obvious differences in the topographical organization of orientation bands between experimental and control hemispheres. Even in close proximity of the lesions, the main trajectories of the bands were still perpendicular to the representation of the vertical meridian (VM) and hence parallel to the lesion. As the experiments with Teflon implantation indicate this preservation of a normal columnar organization cannot be attributed to spared U-fibers or de novo growth or regeneration of axons. Thus, our results suggest that -- at least from the age of two weeks onwards — horizontal intracortical connections are no longer necessary for the development of the highly regular pattern of orientation bands.

Axons crossing the lesion

Using different anatomical techniques we could demonstrate that axons of cortical neurons are able to grow across the lesion. Two possibilities have to be considered: regeneration and de novo growth. Our data do not allow us to distinguish between these possibilities.

According to Luhmann[36] and Luhmann et al.[38] horizontal fibers reach their maximal extent (10.5 mm) between the second and fifth postnatal week and subsequently get pruned back to the adult level. At the time of the lesions, horizontal fibers should be already several mm long but still growing[12]. This is compatible with the assumption that the population of axons crossing the lesions consists of newly growing axons but it does not rule out regeneration. The labeled neurons right beyond the lesion probably had axons extending already far enough to be severed which might be taken as an argument in favor of regeneration. The finding that only few axons crossed even though crossing is in principle possible, suggests that only a small fraction of cells has the ability to extend processes across the lesion. It could be either those which had their axons severed or, and we consider this more likely, those which had not yet extended processes to the region of the lesion. Our data clearly indicate that the lesion becomes a barrier for outgrowing axons since numerous fibers were seen to make sharp turns at the scar or form loops as if they were exploring new target sites. According to Reier et al.[45] CNS lesions in 2-week-old kittens do not yet produce a massive glial reaction and Berry et al.[6] describe a similar age dependence of the glial reaction in the rat. Thus, it is conceivable that because of the attenuated glial reaction there is a short interval after the lesion during which crossing is possible. Thus, fibers which happen to grow during the temporal window could cross while those arriving later already encounter a glial boundary (see also Lindsay[33]). This could explain why only so few axons crossed. Interestingly in all our experiments the lesions were especially difficult to detect in supragranular layers suggesting that these layers mature later than the deep layers. This possibility is supported by the evidence that neurons destined for layers II and III are still migrating and reach their final position only at the age of 3 weeks, i.e., about one week after our lesions[52].

Thus, there seems to be a correlation between the ability of neurons to establish connections across the lesion and the 'immaturity' of cortical tissue. Holder and Clarke[21] argue that injuries can be repaired only within a certain period of time after the end of neurogenesis. Our observation of regenerated axons suggests that the brain of 2-week-old kittens is still in this 'plastic' phase.

Relation with theoretical work

The theories on the self-organization of columnar systems by Swindale[57] and von der Malsburg and Cowan[63] assign an important role to tangential interactions. They are held responsible for the spacing of columns and the lack of tangential interactions at the boundaries of areas is thought to introduce singularities which influence the trajectories of columns if these assume the shape of bands or slabs. Tangential interactions are also a necessary prerequisite in other processes of pattern formation such as e.g. in the development of periodic patterns of shells[17] and furs[43]. In all cases, lateral interactions mediate reciprocal facilitatory and inhibitory influences; these lead to both synergistic and competitive interactions and eventually to clustering of elements with alike properties. The fact that our lesions had no effect on the spacing of columns nor on the direction of their trajectories is unexpected in view of these theoretical issues and lends itself to several interpretations: (i) Our lesions may have come too late and the columnar arrangement has been layed out already. (ii) Cortico-cortical connections from adjacent area 18 or the contralateral intact visual cortex may have compensated for the loss of a substantial fraction of connections in the sagittal plane. (iii) The formation of iso-orientation domains may not require long-range tangential interactions but actually result from local processes.

Single unit and 2-DG studies suggest that in kittens the development of orientation selectivity and of orientation columns continues beyond 2 weeks of age and is not completed before 5 weeks. A 2-DG study on the development of orientation bands[59] shows that at 21 days radioactive labeling is still restricted to layer IV. The columnar pattern is not fully expressed before 35 days of age. In line with these results, electrophysiological data demonstrate that (1) orientation-selective neurons are first detectable in layers IV and VI, (2) orientation selectivity in layers II/III and V develops later (starting with the third postnatal week[5,7,10,44]), and (3) orientation selectivity develops only *after* eye opening which occurs

at around 2 weeks[5,11,16,23,61]. Moreover, the system of orientation columns remains malleable and susceptible to experience-dependent modifications until at least 5 weeks of age[7,54].

The intracortical connections also continue to develop beyond the second postnatal week[29,36,38]. Crude clusters of horizontal connections first appear during the second postnatal week but at this developmental stage the distances spanned by tangential fibers are shorter than in the adult[29]. Subsequently, tangential fibers go through a phase of exuberant proliferation and then undergo extensive pruning in the following month. The characteristic adult pattern is expressed not before 6 weeks of age.

Thus, at the time of the lesion, neither the development of orientation columns nor that of tangential connections is completed. One would expect therefore that adaptive changes in response to the lesion should have occurred if far-reaching tangential interactions were still relevant for the organization of the system. This is supported by a report on postlesion adaptivity of the cortical barrelfield in rats. Barrels are fully developed at an age of 5 days but cortical lesions up to an age of 10 days still lead to a rearrangement[51]. The authors conclude from this that the barrelfield remains plastic at least for some days after its formation. Since we observed neither a change in the spacing nor in the trajectories of bands this suggests that even at a rather early stage of column formation tangential interactions are no longer crucial and disruptions of horizontal connections not equivalent to a 'border' singularity. Thus, if requiring tangential interactions the basic layout of the columnar system would have to be specified and firmly determined long before the columns mature functionally and before the network of tangential connections is fully developed. Alternatively, there remains the possibility that intrinsic connections continue to be important beyond this early stage and that there is sufficient redundancy in the network of the remaining cortico-cortical connections to shape the development of orientation bands. However, we consider this possibility as unlikely because the rather extensive disruption of horizontal connections should have caused at least some compensatory changes in the highly regular patterns of orientation bands. But these appeared as regular as if the lesions had been made only after the development of columns was completed (see for example Fig. 5A).

Finally there is the alternative possibility that columnar layout may be entirely determined by local interactions without any involvement of long-range horizontal connections. According to Tootell et al.[60], anisotropies in the retino-cortical mapping might influence the trajectories of ocular dominance strips in monkey V1. In both monkey and cat[34], the overall cortical magnification factor is greater along the VM as compared to the HM. In order to minimize anisotropy in the retinotopic map, the bands must run at right angles to the axis along which the visual map is expanded[32], i.e. the VM which coincides with the 17/18 border. Thus after the establishment of the retinotopic map, the columnar layout might be controlled by local rather than by global factors. Whether similar constraints could be responsible for the trajectories of iso-orientation bands remains to be determined.

In conclusion then our results indicate that either horizontal connections are not required for column formation at all or they are important only during an unexpectedly early phase of cortical development. In addition, our results demonstrate that the lack of tangential interactions at an artificially introduced border does *not* seem to change the trajectories of orientation bands as predicted by theoretical work.

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