Distribution of retinofugal and corticofugal axon terminals in the superior colliculus of squirrel monkey

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The distribution of terminal fields of retinocollicular fibers was studied in squirrel monkeys with the autoradiographic technique. The terminals were aggregated into patches which were separated by intervening gaps. The ipsilateral patches were particularly distinct. The patches as well as the gaps ranged in size from 50 to 200 μm. In the most posterior aspect of the contralateral superior colliculus, the gaps were absent, and the terminals formed an uninterrupted sheet. The corresponding portion of the ipsilateral colliculus had no retinal input, in agreement with the concept that this region most likely represented the temporal crescent of the visual field. In the most anterior portion of the superior colliculus where the fovea is known to be represented, the ipsilateral and contralateral projections were sparse but, nevertheless, discernible. There was a partial laminar segregation of terminals. The majority of the terminal fields in the contralateral colliculus was located in the most dorsal tier of the stratum griseum superficiale, whereas the majority of the ipsilateral input was slightly deeper in the same stratum. The distribution of corticocollicular fibers was studied by the autoradiographic technique. The fibers from areas 17 and 18 terminated predominantly in the dorsal portion of the stratum griseum superficiale. Area 19, in contrast, projected to the ventral portion of the stratum griseum superficiale. Thus the terminal fields of axons from the retina, area 17 and area 18, overlap in the superior colliculus, whereas axons arising from area 19 terminate in another substratum.

Key words: squirrel monkey, retinocollicular fibers, corticocollicular fibers, autoradiography
Figs. 1A and 1B. Diagrams depicting the distribution of transported label (black dots) in the superior colliculi following injection of tritiated proline into the left eye. Each parasagittal section is identified by its number. Hatch marks symbolize the brachium of the superior colliculus. Note the absence of silver grains at the representation of the optic disc (OD) in the contralateral superior colliculus and at the monocular temporal crescent representation in the posterior portion of the ipsilateral superior colliculus (portions between arrows in sections 263 to 323). The pretectum and its input are not indicated. A, Ipsilateral superior colliculus. B, Contralateral superior colliculus.
the colliculus but overlap in others. A separation of the two inputs seems to be more pronounced in cat than in monkey. Serial reconstructions have shown that in cat, the ipsilateral retinocollicular fibers terminate in a series of longitudinally oriented, undulating bands which may fit gaps or holes under the main contralateral layer of input. The bands are reminiscent of the stripes representing the geniculate input of the two retinas in layer IV of area 17, although there are marked differences between the inputs to the colliculus and those to area 17.²

Until now, only one brief description has been available on the terminal field distribution of retinocollicular fibers in squirrel monkey, a New World primate.⁶ The present, more extensive investigation discloses a termination pattern similar to those in cat, rhesus monkey, and chimpanzee. Additional information concerning the terminal distribution of axons originating from visual cortical areas 17, 18, and 19 is also presented, thus allowing an assessment of the question whether the terminal fields of these three cortico-collicular systems overlap with each other and with the retinocollicular terminal fields.

Fig. 2. Diagram showing the distribution of transported label (black dots) in the superior colliculi following injection of tritiated proline into the left eye. The most anterior level is at the top. Hatching symbolizes the brachium of the contralateral superior colliculus. Note the absence of silver grains in the most posterior portion of the ipsilateral colliculus except for one isolated patch (section 279). Arrowhead points to a small deeper tier of silver grains (section 269). The pretectum and its input are not indicated.
Fig. 3. Distribution of transported label in the foveal representation of the contralateral superior colliculus after a monocular injection of tritiated proline. Photomicrograph shows a portion of transverse section 220 (inset) which is adjacent to section 219 of Fig. 2. SGS, Stratum griseum superficiale; SZ, stratum zonale. (Cresyl violet acetate stain; X325.)

Fig. 4. Photomicrograph displaying black patches of silver grains with indistinct and narrow intervening gaps (white arrowheads) in the contralateral colliculus. The patches show a smooth border closely under the pia, whereas their ventral border is more irregular. Posterior portion of parasagittal section 204 (inset), which is adjacent to section 205 of Fig. 1. V. Blood vessel. (Cresyl violet acetate stain; X130.)

An abstract of the findings has been presented elsewhere. 7

Materials and methods

For the analysis of retinocollicular terminal fields, seven adult squirrel monkeys (Saimiri) were anesthetized with pentobarbital (Nembutal; Abbott Laboratories, North Chicago, Ill.) and received a unilateral intraocular injection 0.1 to 1 mCi (20 to 50 μl) of tritiated proline (New England Nuclear). After survival times of 1 to 5 days, the squirrel monkeys were reanesthetized and perfused with Ringer's solution followed by 10% formalin. The brains were stored in the perfusate for 2 weeks and then blocked in a stereotaxic instrument. The brain blocks were cut on a freezing microtome at 40 μm in either the transverse or the parasagittal plane. Every section of four brains was processed for autoradiography; only every third section of the other three brains was used. The sections were coated with diluted (1:1) Kodak NTB-2 track emulsion, stored from 4 to 12 weeks in light-tight boxes at 2°C, and then developed at 18°C with D-19. Most sections were stained for somata with cresyl violet acetate.

In order to study corticoocollicular fibers, the following experiments were designed. Right area
17 of two adult squirrel monkeys received multiple injections of tritiated proline, totaling 180 and 240 μCi in 1.8 and 2.4 μl, respectively. Right area 18 of three other squirrel monkeys received a single 30 μCi (0.3 μl) injection of tritiated proline. Three additional squirrel monkeys were injected in right area 19 with a single dose of 30 μCi (0.3 μl) of tritiated proline. Survival times ranged from 1 to 3 days. The brains were cut in the transverse plane, and the occipital lobes in the horizontal plane. Every third section was processed for autoradiography. The injection sites in area 17, 18, and 19 were verified histologically according to previously established criteria. The cortical injections were restricted to the respective representations of the central visual field.

No single type of graphic presentation is adequate to demonstrate the complex distribution pattern of retinocollicular terminals, and various investigators have sought to solve this problem differently. In the present study, representative sections were drawn from bright-field photographs of the superior colliculi. Frequent checks of the respective sections under the microscope assured a high degree of accuracy of the drawings.

Results

Retinocollicular projection. The distribution of labeled retinofugal axon terminals in the superficial layers of the superior colliculus was similar in all animals. The description that follows is based primarily on Saimiri 901 and 902, each of which received an injection of 0.9 mCi of tritiated proline and survived for 3 days.

Contralateral superior colliculus. The largest number of labeled retinocollicular terminals occurred in the contralateral superior colliculus. Their distribution was not uniform but displayed a rather complicated pattern which varied mediolaterally and anteroposteriorly across the colliculus and dorsoventrally with respect to the depth below the pia. The concentration of labeled retinofugal terminals was lowest in the anterior portion of the superior colliculus where the central visual field is represented (Figs. 1B to 3). The silver grains exhibited a tendency toward clustering. More posteriorly, the clustering of label was more pronounced, and distinct intervening gaps occurred. In some places, the concentration of silver grains in these clusters became so dense that they appeared as almost solidly black patches. The gaps were not entirely devoid of silver grains, since they showed labeling densities above background level. The clusters or patches of labeled material measured about 50 to 200 μm in width and were spaced approximately 50 to 200 μm apart. A notable exception was one large gap which most likely represented the contralateral optic disc because of its location; it measured approximately 300 μm in width (OD in Fig. 1B, section 185). In the

Fig. 5. Autoradiograph exhibiting several distinct ipsilateral patches of silver grains isolated from each other by intervening gaps (white arrowheads). One patch (p) is closer to the collicular surface than the others. Posterior portion of parasagittal section 322 (inset), which is adjacent to section 323 of Fig. 1. (Cresyl violet acetate stain; X130.)
Figs. 6 through 10. For legend see facing page.
posterior portion of the colliculus the gaps between the dense patches became narrower and indistinct; they could be resolved only at higher magnification (Fig. 4). Eventually, the gaps disappeared completely, so that the silver grains formed an uninterrupted sheet. This broad band of densely packed silver grains presumably represented the temporal crescent of the visual field, as has been shown in the owl monkey, another New World primate. 15

Most contralateral terminals were concentrated in the dorsal portion of the stratum griseum superficiale, with some invasion into the stratum zonale (Figs. 3 and 4). Labeling in the deeper parts of the stratum griseum superficiale was minimal. In the stratum opticum the label seemed to be confined to axons of passage, although termination of retinofugal fibers in this stratum could not be excluded. The dorsal border of the dense silver grain aggregation was close to the pia in the anteromedial portion of the colliculus, whereas its position in the lateroposterior portion of the colliculus was more varied, sometimes extending as much as 50 μm below the pia. The dorsal border of this tier of label was smooth and regular, whereas the ventral boundary was scalloped due to irregular fingerlike extensions of label reaching deeper into the stratum griseum superficiale. In the medial aspect of section 269 of Fig. 2, a small accumulation of silver grains was located at a deeper level; this was the only indication of a second tier of contralateral label. When measured at an angle of 90 degrees to the surface, the thickness of heavily labeled tissue increased from 25 μm in the anterior portion to about 250 μm in the posterior portion of the colliculus.

Ipsilateral superior colliculus. The overall silver grain density in this colliculus was considerably lower in comparison to the contralateral colliculus. The foveal projection site14 in the most anterior region of the ipsilateral colliculus exhibited sparse but distinct label in all the animals (Figs. 1A and 2). Posterior to this level, the silver grains accumulated to form conspicuously dense patches separated by gaps of low grain density. Here the pattern of patches and gaps was more pronounced than in the contralateral colliculus. The range in sizes of patches and gaps was comparable to that of the contralateral colliculus (50 to 200 μm), although in the ipsilateral colliculus the sizes were more often found at the large end of the range. It was not possible to identify unequivocally the representation of the optic disc, since several isolated patches of grains occupied its presumed location. However, none of these patches exhibited the dense accumulation of silver grains extending from immediately below the pial surface to deep in the stratum griseum superficiale, which has been described as characteristic for the ipsilateral optic disc representation in cat13, 2 and macaque.3, 4 A large strip paralleling the posterior curvature of the ipsilateral colliculus was devoid of label. This strip most likely represented the temporal crescent of the visual field and received only a contralat-

Fig. 6. Lateral view of squirrel monkey brain and the delineation of areas 17, 18, and 19. Crosses indicate locations of proline injections.

Fig. 7. Summary diagram depicting the collicular distribution of terminals of axons that originate in the retina, areas 17, 18, and 19. SGS, Stratum griseum superficiale; SO, stratum opticum; SZ, stratum zonale.

Fig. 8. Distribution of silver grains in the ipsilateral superior colliculus after injection of Tritiated proline in area 17. SGS, Stratum griseum superficiale; SZ, stratum zonale. (Cresyl violet acetate stain; ×280.)

Fig. 9. Distribution of silver grains in the ipsilateral superior colliculus after injection of Tritiated proline in area 18. SGS, Stratum griseum superficiale; SZ, stratum zonale. (Cresyl violet acetate stain; ×280.)

Fig. 10. Distribution of silver grains in the ipsilateral superior colliculus after injection of Tritiated proline in area 19. SGS, Stratum griseum superficiale; SZ, stratum zonale. (Cresyl violet acetate stain; ×280.)
eral retinal projection (Figs. 1 and 2). Because of the curved shape of the colliculus, the monocular segment can be seen in only a few transversally cut sections (Fig. 2, section 279).

By a comparison of the ipsilateral to the contralateral colliculus, it could be seen clearly that the label occurred at deeper levels in the ipsilateral superficial gray and more superficially in the contralateral gray. The dorsal border of most patches on the ipsilateral side was found between 30 and 50 µm below the pial surface. Some patches, however, occurred directly beneath the pia (Fig. 5). The dorsal contours of silver grain accumulations immediately beneath the pial surface were fairly smooth, whereas deeper patches exhibited more irregular dorsal border configurations. The ventral border of many patches was scalloped, tapering out into fingerlike extensions.

Corticocollicular projections. Histological examination of the cortical injection sites revealed that in each case, the tritiated proline had labeled a column of cells from the pia to the white matter. The injections of radioactive isotope were limited to the central representation of the visual field in areas 17, 18, and 19 (Fig. 6). The projections from all three cortical areas were to the anterior third of the ipsilateral superior colliculus, which is the collicular region representing the central visual field. Thus it might be surmised from these limited experiments that the corticocollicular projections are retinotopically organized. It was interesting to note that single injections resulted in a single, well-circumscribed field of evenly distributed silver granules in the colliculus. This was in marked contrast to the multiple vertical, columnar-like arrangements of silver granules found in the areas of corticocortical projections.\(^\text{16, 17}\)

After injections of tritiated proline into area 17, label was found to extend from directly beneath the pia to approximately the dorsal half of the stratum griseum superficiale (Figs. 7 and 8). A few silver grains were located over the more ventral portions of the stratum griseum superficiale, and some were seen in the stratum opticum. After an injection of tritiated proline into area 18, the peak grain density was also observed over the dorsal portion of the stratum griseum superficiale, with some invasion into the stratum zonale, ventral stratum griseum superficiale, and stratum opticum (Figs. 7 and 9). An injection into area 19 resulted in a different laminar termination pattern of corticocollicular axon terminals. The highest grain density was found in the ventral half of the stratum griseum superficiale (Figs. 7 and 10), with a small amount of label reaching more dorsal aspects of this stratum. Silver grains above background level also occurred over the stratum opticum. In no instance was label observed over the contralateral superior colliculus after injections in cortical visual areas.

Discussion

The terminations of ipsilateral and contralateral retinocollicular fibers in squirrel monkey exhibit a rather complex pattern. The synaptic fields of the fibers vary in size, depth, and density, depending on the location within the colliculi. Both the ipsilateral and the contralateral terminal fields are located in the dorsal part of the stratum griseum superficiale. The majority of contralateral fibers terminate in the most dorsal tier of this stratum, whereas the greatest density of ipsilateral terminals occurs slightly more ventrally. It should be stressed, however, that ipsilateral and contralateral retinocollicular axon terminals may not be strictly segregated but, rather, that their highest concentrations occur in separate tiers. Since there is a dorsoventral concentration gradient, the contralateral input tapers out ventrally in the tier principally occupied by the greatest density of ipsilateral input. The ipsilateral patches taper out ventrally but also dorsally in the tier principally occupied by the greatest concentration of contralateral fiber terminals. Occasionally, even an entire dense ipsilateral patch is located in the tier principally occupied by the contralateral fields (compare Figs. 4 and 5). Thus there is only a partial laminar segregation and considerable overlap of ipsilateral and contralateral retinal inputs to the superior colliculus. This
general laminar organization pattern of retinofugal terminals has also been described in cat,\textsuperscript{1} 2, 13 several prosimians (for references see ref. 18), macaque,\textsuperscript{3} 4 and chimpanzee.\textsuperscript{5}

Attempts have been made to match contralateral gaps with ipsilateral patches and vice versa. By marking the ipsilateral and contralateral retinofugal fibers with two different methods in the same specimen, Pol- lack and Hickey\textsuperscript{4} demonstrated a certain degree of segregation between the termination sites of these fibers in macaque. Since we used only one method in squirrel monkey, we cannot speak in detail to the issue of complementarity of contralateral gaps and ipsilateral patches. The only area of strict segregation of fibers from both retinas that was clearly revealed in the present study was the monocular crescent of the temporal visual field in the posterior colliculus. Furthermore, although there was a distinctly label-free optic disc representation site in the contralateral colliculus, in no instance was there a conspicuous label-dense patch of comparable size or location on the ipsilateral side. This latter example may be taken as an indication of how difficult it is to demonstrate ocular complementarity in the colliculus of squirrel monkey. The partial laminar overlap of terminals from both eyes in the binocular part of the colliculus, as demonstrated by our results, seems to be in agreement with the results of physiologic recording experiments. Most collicular neurons in the binocular portion can be driven equally well by both eyes; there is, however, a tendency toward clustering of cells in the same ocular dominance group within vertical electrode penetrations.\textsuperscript{3} 19

Fig. 7 summarizes the collicular synaptic fields of axons arising in the retina and in areas 17, 18, and 19 of the cerebral cortex. The majority of axon terminals from the first three sources overlap in the stratum zonale and about the dorsal half of the stratum griseum superficiale. Significantly, the projection from area 19 is not involved in the overlap, but the terminals are concentrated in the lower half of the stratum griseum superficiale. These results confirm a recent study on some of these connections in owl monkey\textsuperscript{20} and are similar to those obtained in cat.\textsuperscript{21}

The present finding of overlapping projections from the retina and areas 17 and 18 does not necessarily imply convergence of these fiber systems onto collicular neurons. The afferents from the retina and area 17 seem to project to separate collicular cell pools in macaque, since cooling or ablation of area 17 has little or no effect on the cells of the superficial layers whereas it disrupts visual responses in deeper layers.\textsuperscript{22} A physiologic study in squirrel monkey\textsuperscript{23} revealed that collicular cells driven by striate cortex stimulation were more deeply located than the terminal fields of retinofugal fibers of the present study. Together, the physiologic and the present anatomic results suggest that although the terminal fields of fibers originating in the retina and area 17 overlap in the stratum zonale and in the dorsal portion of the stratum griseum superficiale, the recipient cells reside in different collicular layers; cells with retinal input seem to be located superficially in the colliculus, whereas cells receiving input from area 17 are located more deeply and have their recipient dendrites in superficial layers. No study has yet elucidated whether inputs from areas 17, 18, and 19 converge upon the same collicular cells in monkey; however, in cat it was shown that there is substantial convergence of topographically organized monosynaptic excitatory input from these three cortical areas onto single neurons in the stratum griseum superficiale.\textsuperscript{24}

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