(1980)], and M. R. Gross [thesis, University of Utah, Salt Lake City (1980)].
17. Since we could not open the partitions to observe actual spawning rates because of high aggression in this confined space, we used proximity to indicate preference.
18. Females could have used morphological, chemical, or behavioral cues to discriminate between the two males, as well as the observed spawning frequency. However, there were no apparent differences in the appearance or behavior of the males that had spawned and those that had not spawned.
19. T. A. Markow, M. Quaid, S. Kerr, *Nature (London)* **276**, 821 (1978).
20. We thank G. Bell, B. Chapais, M. Gross, L. Giraldeau, S. Hoffman, L. Lefebvre, N. Stacey, R. Warner, and G. Williams for discussion and comment on an earlier draft. The research was supported by a McGill University Independent Studies grant to K.N. and by an operating grant to D.L.K. from the Natural Sciences and Engineering Research Council of Canada.

28 August 1981; revised 7 December 1981

Interdigitation of Contralateral and Ipsilateral Columnar Projections to Frontal Association Cortex in Primates

Abstract. *The combined use of two anterograde axonal transport methods reveals that in the prefrontal association cortex of macaque monkeys, associational projections from the parietal lobe of one hemisphere interdigitate with callosal projections from the opposite frontal lobe, forming adjacent columns 300 to 750 micrometers wide. The finding of separate and alternating ipsilateral and contralateral inputs in the frontal association cortex opens up new possibilities for the functional analysis of this large but unexplored area of the primate brain.*

Over the past 25 years, one of the most important concepts for understanding the structure and function of the neocortex has been that of the vertical compartmentalization of its cells and connections. Such organization was suggested in electrophysiological analysis of somatosensory cortex by Mountcastle (*1*); later functional and morphological research extended this principle of organization to receptive field properties and connectivity of the primary visual and auditory as well as somatosensory areas of the cortex (*2, 3*). In these systems, vertical "columns" or "bands" related to input serving one class of sensory receptor alternate with input from another group of receptors within the same modality. More recently, it has become apparent that vertical organization of inputs is not solely a property of sensory systems but applies to association cortex as well (*4, 5*). Our previous studies using autoradiographic methods for tracing connections have shown that corticocortical projections to the prefrontal association cortex form well-defined bands or columns that in coronal section traverse the entire width of the cortex and alternate with vertical territories devoid of label. However, the input to the unlabeled spaces has so far remained unknown. By combining two anterograde tracers—one based on anterograde axonal transport of horseradish peroxidase (HRP) and the other on that of tritiated amino acids—one can label convergent projections in the same animal. Using this research strategy, we hereby provide what is, to our knowledge, the first evidence that callosal (contralateral) terminals alternate with associational (ipsilateral) terminals in selected cytoarchitectonic areas of primate association cortex. Such side-by-side registration of inputs from the two hemispheres may be relevant to the cerebral mechanisms underlying interhemispheric integration.

Our evidence is based on results obtained from four macaque monkeys (three rhesus and one fascicularis) killed 2 days after a mixture of [^{3}H]leucine and [^{3}H]proline (100 μCi/μl) was injected into the midregion of the dorsal bank of the principal sulcus (Brodmann's area 9) in the left hemisphere and acrylamide-bis gel HRP pellets (0.5 by 1.5 mm) were simultaneously implanted in the posterior rim of the intraparietal sulcus (Brodmann's area 7) of the parietal lobe in the right hemisphere (Fig. 1). In the monkey, these cortical areas project to the prefrontal cortex in the right hemisphere, via callosal and intrahemispheric routes, respectively (*4, 6*). Since the terminals of these two inputs independently exhibit a columnar mode of termination in the prefrontal cortex (*4, 7*), their arrangement within this zone could take one of several forms: (i) callosal and associational afferents may fully or partially overlap; (ii) they may interdigitate with one another, or (iii) they may have no discernible relationship to one another. To determine which of these possibilities obtained, alternate coronal sections through the prefrontal cortex of the right hemisphere were histochemically reacted with tetramethylbenzidene to reveal HRP reaction product (*8*). The intervening sections were processed by standard autoradiographic techniques. Through the use of blood vessels as fiducial marks, photographs and camera lucida drawings of the territories of terminal labeling in the adjacent 50-μm sections were overlaid to document the relationship of the labeled territories.

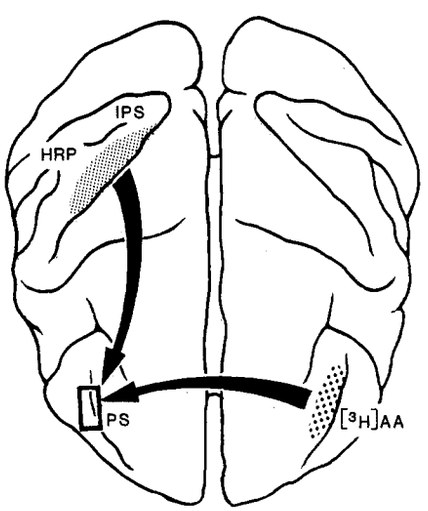

Fig. 1. Double-labeling strategy. Tritiated amino acids ([^{3}*H*]*AA*, coarse stipple) were injected into the principal sulcus (*PS*) of the left hemisphere to label callosal projections to the principal sulcus in the right hemisphere; HRP pellets (fine stipple) were implanted into the posterior bank of the intraparietal sulcus (*IPS*) in the right hemisphere to label associational projections to the principal sulcus in the right prefrontal cortex. The rectangle marks the areas of convergence of the ipsilateral and contralateral projections examined.

In accordance with previous results (*4*), analysis of the autoradiographic data revealed that labeled fibers originating from the opposite prefrontal cortex were distributed in 300- to 750-μm bands that extended across all layers of the cortex and alternated with spaces of variable width in which radioactivity was not above background (Fig. 2, A and B). For the most part, these callosal columns were located in the dorsal bank of the principal sulcus homotopic to the injection site, although some assumed a heterotopic position in the adjacent ventral bank of the same sulcus. The HRP-labeled fibers originating from the inferior parietal cortex were also distributed in roughly the same area of the prefrontal cortex; they also formed discontinuous bands alternating with spaces of variable width that contained little or no reaction product (Fig. 2, C and D). Like callosal columns, the ipsilateral associational columns spanned all cortical layers. Although the ipsilateral projections from the parietal lobe were distributed in various portions of the prefrontal cortex including the dorsal and lateral convexities, many were located in portions of the principal sulcus that contained radioactively labeled callosal terminals.

0036-8075/82/0514-0755$01.00/0

Our major finding is that callosal and associational axons generally occupied mutually exclusive columnar territories, and indeed, in areas of close convergence, columns of callosal axons alternated in short but regular sequences with those of associational input (Figs. 2 and 3). In the dorsal bank of the principal sulcus (Fig. 3), for example, one column of parietal origin (column 9) was flanked by two columns (8 and 10) of callosal origin. Likewise, in the ventral bank of the same section, parietal columns 1 and 3 alternated with prefrontal columns 2 and 4. Furthermore, reconstructions from serial sections cut through portions of the principal sulcus in two monkeys suggest that each fiber system is represented across the prefrontal cortical surface as a map of irregular stripes (5), which in restricted regions of the sulcus alternated in a manner reminiscent of alternating ocular dominance stripes in the primary visual cortex of the same species (*2, 5, 9*). A prominent difference between visual and association cortex, however, is that in the prefrontal cortex there is some overlap at the borders between cortico-cortical columns in layer IV (for example, columns 1, 2, 3, and 4 in Fig. 3); in the visual cortex, this is the layer in which the thalamic representations of left and right eyes are sharply segregated.

Two additional observations merit consideration. (i) Occasionally composite drawings or photographs contained a single column of callosal origin that extensively or even totally overlapped that of a parietal projection column (for example, columns 5 and 6 in Fig. 3). Thus, segregation of callosal and associational inputs is a rule that can be broken. (ii) Some columnar spaces adjacent to callosal and associational columns remain unlabeled (Fig. 3). Since, in addition to the two classes of cortical projections labeled in this study, the principal sulcus receives additional cortical afferents (*9*), it is possible that these unaccounted-for territories are filled by terminals from one or more of these other sources. Although further studies will be necessary to determine the relationship between various cortical connections and the columns described here, our working hypothesis is that two or more associational inputs to the prefrontal cortex are organized as columns intercalated with a unique set of callosal columns. However, since there may be a larger number of inputs than label-free spaces, it is possible that only some cortical projections interdigitate, while others overlap with those that do. The availability of double labeling methods makes it possible to test these possibilities.

At present, we can only speculate about the functional significance of separate but interpolated cortico-cortical projections to the frontal association cortex. In the visual system, where more is known about the physiology, apposition of left- and right-eye representation within the cortex serves to bring the disparate visual fields of the two eyes into register for subsequent binocular integration of the visual world (*2*). One may speculate that adjacent ipsilateral and contralateral bands in the prefrontal cortex represent another type of "hypercolumn" that similarly may have functional significance. It is well established that the principal sulcal cortex is essential for behavior requiring the integration of spatial and temporal information (*10*). The discovery of alternating associational and callosal columns in the prefrontal cortex opens new possibilities for physiological analysis of this major area of association cortex in primates. Further, while we have demonstrated such alternation only in a restricted portion of the prefrontal cortex, interdigitation of ipsilateral and contralateral fiber systems

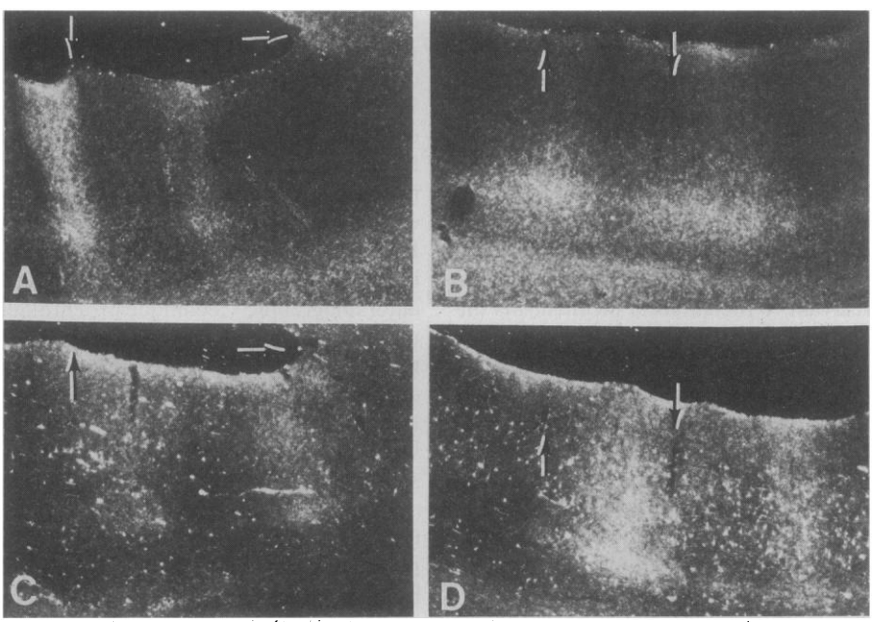

Fig. 2. (A and C) Darkfield illumination photographs of adjacent sections cut through the principal sulcus displaying columnar distribution of callosal axons labeled by axonal transport of [^{3}H]leucine and [^{3}H]proline (A) and anterogradely HRP-labeled fiber columns originating in the intraparietal sulcus of the same hemisphere (C). (B and D) Adjacent sections showing another portion of the principal sulcus with callosal (B) and associational (D) terminations. Arrows indicate blood vessels or landmarks used for lining up the adjacent sections.

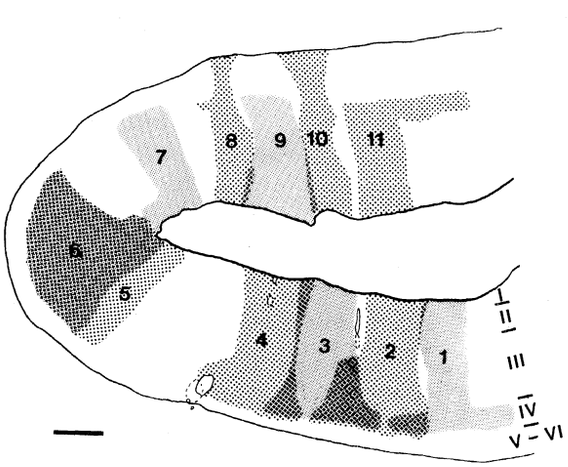

Fig. 3. Composite diagram of two adjacent 50-μm coronal sections cut through the convergence zone in the principal sulcus indicated by the rectangle in Fig. 1. Autoradiographically labeled collosal fiber columns (1, 3, 6, 7, and 9) are indicated by coarse stipple; associational fiber columns (2, 4, 5, 8, 10, and 11) originating in the parietal cortex, labeled by anterograde transport of HRP, are shown in fine stipple. Blood vessels in the section reacted with HRP are shown by dashed lines; those from the autoradiogram are outlined by continuous lines. Several small blood vessels penetrating layers I and II orthogonally were used to align the adjacent sections. The larger round vessel shown in layer V-VI passes obliquely through this plane of section and thus was not aligned. Bar, 0.5 mm.

may be a major principle of organization in other regions of the primate cortex as well.

PATRICIA S. GOLDMAN-RAKIC
MICHAEL L. SCHWARTZ
Yale University School of Medicine, New Haven, Connecticut 06510

References and Notes

1. V. B. Mountcastle, *J. Neurophysiol.* **20**, 408 (1957).
2. D. H. Hubel and T. N. Wiesel, *Proc. R. Soc. London Ser. B* **198**, 1, (1977).
3. E. G. Jones, H. Burton, R. Porter, *Science* **190**, 572 (1975); M. Wong-Riley, *Brain Res.* **162**, 201 (1979); T. J. Imig and J. F. Brugge, *J. Comp. Neurol.* **182**, 637 (1978); T. J. Imig and R. A. Reale, *ibid.* **192**, 293 (1980); E. Kosar and P. J. Hand, *ibid.* **198**, 515 (1981); M. Sur, J. T. Wall, J. H. Kaas, *Science* **212**, 1059 (1981).
4. P. S. Goldman and W. H. J. Nauta, *Brain Res.* **122**, 393 (1977).
5. P. S. Goldman-Rakic, in *The Organization of Cerebral Cortex*, F. O. Schmitt, F. G. Worden, S. G. Dennis, G. Adelman, Eds. (MIT Press, Cambridge, Mass., 1981), pp. 69–97.
6. D. N. Pandya and H. G. J. M. Kuypers, *Brain Res.* **13**, 13 (1969); S. Jacobson and J. Q. Trojanowski, *ibid.* **132**, 209 (1977); E. G. Jones and T. P. S. Powell, *Brain* **93**, 793 (1970).
7. G. R. Leichnetz, *Neurosci. Lett.* **18**, 119 (1980).
8. M. M. Mesulam, *J. Histochem. Cytochem.* **26**, 106 (1978).
9. In terms of comparison with the ocular dominance system, the terminal fields of callosal and associational projections labeled in the present and similar studies represent only a small fraction of their total representations, whereas virtually the entire system of ocular dominance stripes can be labeled by injections into the eye.
10. N. Butters, D. Pandya, D. Stein, J. Rosen, *Acta Neurobiol. Exp.* **32**, 305 (1972); P. S. Goldman and H. E. Rosvold, *Exp. Neurol.* **27**, 291 (1970); ———, B. Vest, T. W. Galkin, *J. Comp. Physiol. Psychol.* **77**, 212 (1971).
11. Supported by PHS grants NS 16666 and MH 00298.

4 November 1981

Lipofuscin: Resolution of Discrepant Fluorescence Data

Abstract. *Lipofuscin granules (age pigments) emit yellow light under ultraviolet excitation in the fluorescence microscope. The reported blue emission maximum of extracts of lipofuscin-laden cells may result from instrumental bias. The major fluorescent components that accumulate with age in these lysosomal residual bodies of human retinal pigment epithelium are yellow-emitting fluorophores. Different age-related fluorophores, which do emit blue light, are derived from other intracellular sources. A reevaluation of the connection between blue-emitting lipid peroxidation products and the age-related lipofuscin granules of classical pathology is necessary.*

Postmitotic cells of various aging tissues accumulate heterogeneous nondegradable lipophilic materials in lysosomal residual bodies called lipofuscin granules. The residues are believed to be damaged cell membrane components that have been autophagized or phagocytized during the life of the cell (*1*). In situ, lipofuscin granules fluoresce upon excitation with ultraviolet light (*2*). The identity of the fluorophores is not established, however, in part because fluorescence microscopists have consistently reported the color of lipofuscin fluorescent emission to be yellow or red (*3*), whereas extracts of lipofuscin-rich cells have been reported to exhibit a blue fluoresence (*4*, *5*).

Retinal pigment epithelium (RPE) consists of postmitotic cells that phagocytize photoreceptor outer segment disks (*6*), the major source of the lysosomal residue in these cells. Human RPE accumulates lipofuscin with age (*7*) and is therefore a good model for our spectral studies.

To decide whether the fluorescence of lipofuscin is yellow or blue and whether the reported blue peak of extracts (*5*) could arise from unusually large solvent effects (*8*), we analyzed both intact cells and extracts in calibrated spectrofluorometers. In addition, high-performance thin-layer chromatography (HPTLC) was used to separate and detect the fluorescent materials.

The emission spectra were corrected for the overall spectral sensitivity of the instruments used. The microspectrofluorometer was a Leitz Dialux fluorescence microscope with appropriate detecting, amplification, and recording equipment (*9*). Interference filters were placed in front of the photomultiplier tube (PMT) to select specific wavelengths. For corrected spectral analysis, calibrations were made on the transmission of all components of the optical path and on the spectral sensitivity of the PMT (*10*). Extracts were measured in both the microspectrofluorometer and a spectrofluorometer (Turner model 430) whose emission monochromator and PMT unit were spectrally calibrated for subsequent correction of emission data (*11*) and whose monochromator wavelength settings were calibrated with a quinine sulfate standard.

The RPE samples were separated into two age groups, those from persons over 40 years of age and those from persons under 40 years of age. For microspectrofluorometry, sheets of RPE were dissected, placed on slides to dry and covered with immersion oil of very low fluorescence. Emission from about seven cells was analyzed. The RPE from a young person was matched with the RPE from an old person, and their respective emission spectra were measured. The spectrum of the RPE from the young donor was subtracted from that from the old donor. This difference spectrum eliminates background fluorescence (such as that from cellular flavinoids or stored retinol derivatives). In all, 11 matched pairs were analyzed, the difference spectra were calculated, and the results were pooled.

In the microscope, lipofuscin granules appeared to emit broad-band yellow light when excited with 366 nm. The age-related cellular emission showed a major peak at 540 to 640 nm (yellow-orange) and a minor peak at 460 nm (blue) (Fig. 1A). Thus, a substance accumulating with age in human RPE cells emits yellow light.

To separate the component fluorophores, we lyophilized human RPE cells (*12*) and extracted them by the Folch chloroform-methanol procedure (*13*). All solvents contained butylated hydroxytoluene (5 mg/100 ml), and tissues and HPTLC plates were stored under argon to retard peroxidation (*14*). In polar extracts, the chromatographic profile (*15*) showed a weak blue fluorescence, but only in the RPE from old donors (Fig. 2, left). However, most of the fluorescence was in the nonpolar extract.

The corrected emission spectrum of the highly fluorescent nonpolar extracts of RPE from old donors (Fig. 1B) was similar to that of the intact cells (Fig. 1A). A smaller blue emission peak was largely overshadowed by a broad peak at 540 to 580 nm. Chromatographic separation of this extract (*16*) revealed two intense blue bands that were not detected in similar extracts from young individuals (Fig. 2, middle). Several other bands common to samples from young and old donors were intensely fluorescent in the yellow and orange range of the spectrum. The RPE from young donors also contained some fluorescent granules, but quantitation of fluorophores was not attempted.

To determine whether the age-related blue-emitting fluorophores had originated in the lipofuscin granules, we fractionated the RPE homogenates from old donors (*17*) and isolated the lipofuscin granules. Analysis of nonpolar extracts of the lipofuscin granule fraction by HPTLC (*18*) revealed only yellow-emitting components, the major one being a band at the origin (Fig. 2, right).

Our study reemphasizes that the spectral characteristics of all components of

0036-8075/82/0514-0757$01.00/0