The primate visual system is often divided into two channels, designated M and P, whose signals are relayed to the cerebral cortex by neurons in the magnocellular and parvcellular layers of the Macaque dorsal lateral geniculate nucleus. We have identified a third population of geniculo-cortical neurons in the dorsal lateral geniculate nucleus of macaques, which is immunoreactive for the α subunit of type II calmodulin–dependent protein kinase. This large third population occupies interlaminar regions (intercalated layers) ventral to each principal layer. Retrograde labeling of kinase-immunoreactive cells from the primary visual cortex shows that they provide the geniculo-cortical input to cytochrome oxidase–rich puffs in layers II and III.

The primate visual system is commonly viewed as an amalgam of two functional channels, designated M and P (I), that include the magnocellular and parvcellular layers of the Macaque dorsal lateral geniculate nucleus. We have identified a third population of geniculo-cortical neurons in the dorsal lateral geniculate nucleus of macaques, which is immunoreactive for the α subunit of type II calmodulin–dependent protein kinase. This large third population occupies interlaminar regions (intercalated layers) ventral to each principal layer. Retrograde labeling of kinase-immunoreactive cells from the primary visual cortex shows that they provide the geniculo-cortical input to cytochrome oxidase–rich puffs in layers II and III.

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neurons in distinct small-celled layers or in interlaminar regions are the source of geniculocortical terminations to puff neurons (6, 7), in macaques the color-opponent properties of most puff neurons (8) and the apparently small size of the interlaminar cell population (9) have led to the conclusion that input from P neurons predominates in the puffs of Old World primates (10). Here we viewed the geniculate population projecting to the puffs by combining immunoreactivity for the α subunit of type II calmodulin–dependent (CaM II) protein kinase (11) with retrograde transport of fluorescent markers from the superficial layers of V1. The results showed that the direct geniculate input to the puffs arises from a large population of cells that are analogous to cells of a third, koniocellular or K channel in other primates, which display heterogeneous receptive properties and long response latencies (W cells).

A section through the macaque LGN, stained with thionin to show all neurons, gave the impression that very few neurons exist outside the six principal layers (Fig. 1A). The regions between the principal layers are often referred to as interlaminar zones and are frequently described as being relatively cell-free. However, in a section through the LGN that was immunostained for CaM II kinase (12), a large population of neurons was found intercalated between the principal layers and below layer 1 (Fig. 1B). The neurons were numerous ventral to principal layers 1, 2, and 3; they were variable ventral to layer 4; and they were much more sparsely distributed ventral to layers 5 and 6. Following the nomenclature of Fitzpatrick and colleagues (6), we refer to these layers collectively as the intercalated layers.

Neurons immunoreactive for CaM II kinase also formed thin immunostained bridges that ran through each principal layer (Fig. 1, B and C). Other immunostained cells were scattered diffusely through layers 5 and 6 and in the white matter dorsal to layer 6. Most neurons immunostained with CaM II–kinase had small somata (8 to 10 µm in diameter) that were substantially smaller than those of the neurons of the magnocellular and parvicellular layers. They gave rise to several immunostained processes (Fig. 1D) that formed dense plexuses in each of the intercalated layers (Fig. 1E) and in the cell bridges between them (Fig. 1C).

When deposits of Fast Blue or rhodamine dextran were made so that layer 1 or layers I to III of V1 were included (Fig. 2, A and B), most retrogradely labeled neurons were kinase-immunoreactive to puff neurons (13) (Fig. 2, C through F). Analysis of 16 deposits made in six monkeys showed that as long as the V1 deposit remained above layer IVA, only very few non-immunostained parvicellular neurons were retrogradely labeled (a total of 14 such cells out of several hundred labeled by the superficial deposits). All others were CaM II kinase–immunostained intercalated neurons. They occupied the intercalated layers and the cell bridges between two intercalated layers and included neurons scattered through layers 5 and 6 and through the overlying white matter. When

![Fig. 1](https://via.placeholder.com/150)

**Fig. 1.** (A) Frontal section through macaque LGN stained with thionin. Dense collections of neurons are evident in principal layers 1 through 6 and in an isolated cluster (double arrowheads), designated the S lamina, which represents a displaced part of layer 2 (6). Many of the stained nuclei ventral to layers 5 and 6 are those of neuroglial cells. (B) Frontal section immunostained for CaM II kinase, showing the large population of immunostained neurons. Preliminary stereological analyses indicate that the immunostained neurons are as numerous as the unstained neurons in the two magnocellular layers. (C) Photomicrograph of kinase-immunostained neurons ventral to layers 4, 5, and 6, and in cell bridges (arrow) within layers 4 and 5. (D and E) Photomicrographs showing the morphological features of individual kinase-immunostained neurons (D) and of the plexuses formed by their processes (E). Scale bar: 200 µm in (A) and (B); 40 µm in (C); and 10 µm in (D) and (E).

![Fig. 2](https://via.placeholder.com/150)

**Fig. 2.** (A and B) Photomicrographs of frontal section through V1, containing part of a Fast Blue deposit (B) and histochemically stained for cytochrome oxidase (A). Arrows indicate the same blood vessel profiles in the two micrographs. The deposit includes layers I and II and the superficial half of layer III. Individual dots in layers IVB, V, and VI in (B) are neurons labeled by intracortical transport of Fast Blue. (C through F) Fluorescence photomicrographs of the same sections through the LGN ipsilateral to the deposit in (B), showing neurons immunostained for CaM II kinase (C and E) and retrogradely labeled with Fast Blue (D and F). Arrows indicate neurons intensely labeled for both CaM II kinase and Fast Blue, and arrowheads show neurons intensely immunostained but weakly labeled with Fast Blue. Scale bar: 300 µm in (A) and (B); 40 µm in (C) and (D); 15 µm in (E) and (F).
input, are members of a group that most closely resembles the koniocellular or K channel of other primates species (7, 15). By their geniculate input from the intercalated neurons and by the intracortical projection from M-representant and P-representant layers in V1 (16), the puffs appear to be sites in which three types of visual input converge.

The contributions of M and P systems to the physiological properties of cortical neurons or to the psychophysically measured visual capabilities of alert monkeys have been tested by the placement of lesions or deposits of pharmacological agents in magnocellular or parvicellular layers of the LGN (17). The general conclusion of these studies, in which the M or P channel was targeted selectively, has been that two channels converge early in the cortex, thereby contributing jointly to most physiological properties and to many visual functions. Our data indicate that neurons in a third channel that is anatomically and neurochemically distinct are distributed so that any lesion or injection in the LGN would eliminate part of their contribution to cortical physiology and to visual function. That contribution may include some color-opponent responses (18), although color discrimination is decapitated by parvicellular lesions (17), which leave most intercalated neurons intact. Instead, on the basis of comparative studies with other species of primates, the physiology of intercalated neurons in macaques is likely to resemble that of a population of W cells (19), with heterogeneous functional properties, large receptive fields, and long response latencies.

REFERENCES AND NOTES
2. T. N. Wiesel and D. H. Hubel [J. Neurophysiol. 29, 1115 (1966)] first reported the systematic variation in receptive field properties shown by neurons in parvocellular and magnocellular layers.
12. Experiments were done in 11 normal macaques (8 Macaca mulatta and 3 M. fasciculata). All were killed by a lethal dose of Nembutal (100 mg per kilogram of body weight, given intravenously) and perfused through the heart with 3 or 4% paraformaldehyde. Blocks of each LGN were cut frozen at a thickness of 15 or 30 μM. Most sections were processed for immunocytochemistry [S. H. C. Hendry and B. M. Kennedy, Proc. Natl. Acad. Sci. U.S.A. 83, 6380 (1986); (1985)] (Boehminger Mannheim Biochima). Special spectrally pure filters were used for fluorescent microscopy [M. A. Sesma and M. G. Ungerleider and M. Mishkin, in Visual Neuroscience (Springer-Verlag, New York, 1984), pp. 549-586, L. Krubitzer and J. Kaas, Trends Neurosci. 7, 1 (1984)].
13. Six macaques (five M. mulatta and one M. fasciculata) received deposits of the tracers Fast Blue or rhodamine dextran on the surface of one or both occipital lobes 2 weeks before killing [E. Rausell and E. C. Colby, J. Neurosci. 11, 226 (1991)]. In most cases, two deposits spaced several millimeters apart were made with different tracers in the same hemisphere. The deposits were left in place for periods as short as 2 hours and as long as 2 weeks. The short-term deposits (four deposits in three monkeys) produced a diffusion of tracer into deeper layers (12 deposits in three monkeys), and included layer IVA in the case of two deposits. Immunostaining was done by indirect immunofluorescence, with the primary antibody localized with a fluorescein-conjugated antibody to mouse immunoglobulin G (indicating CaM II kinase) and either rhodamine dextran or Fast Blue (retrograde transport) were viewed and photographed in the same fields with a Zeiss MIP microscope equipped with appropriate fluorescent filter cubes.
18. D. H. Hubel and M. S. Livingstone [J. Neurosci. 10, 2223 (1990)] suggest that type 2 cells with center-only, color-opponent receptive fields occupy regions in and around the intercalated layers of the dorsal LGN.
20. We thank M. B. Kennedy for the antibody to CaM II kinase and K. Miller for technical assistance. Supported by grant EY 06342 from NIH.
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