class I *spoIIIE* mutants but lost in class II mutants, in which no SpoIIIE-like protein accumulates.

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15. Strain SG38 (Spo$^+$) and isogenic strains carrying the *spoIIIE36* mutation with or without the *spoIIG55* mutation were induced to sporulate (*35*). Samples taken 3.5 hours later were fixed, embedded, sectioned, and prepared for transmission electron microscopy as described (*10, 28*).
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17. Mutations of the *spoIIIE* gene have little effect on the expression of σ^E-dependent genes in the early stages of mother cell development. However, during the later stages of mother cell development, transcription is directed by the σ^K form of RNA polymerase, the activity of which is coupled to gene expression in the prespore (*36*). Because prespore gene expression is blocked in *spoIIIE* mutants, the later stages of mother cell development are also impaired (*2, 3, 36*).
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23. The weak fluorescence sometimes associated with the mother cell compartment of both wild-type and *spoIIIE* mutant cells containing the *gpr'-'lacZ* fusion may indicate that σ^F activity is not completely localized to the prespore immediately after septation. Although this ambiguity complicates the interpretation of the results, it nevertheless does not affect the conclusion that the distribution of σ^F activity in sporulating cells of the *spoIIIE36* mutant is similar to that of wild-type cells.
24. PI was used to visualize DNA in preference to 4,6-diamidino-2-phenylindole (DAPI) because its absorbance and emission spectra are distinct from FDG and because it stains unfixed cells.
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27. To construct a deletion of the *spoIIIE* gene, plasmids containing cloned segments from the flanking regions of the *spoIIIE* gene (*37*) were linearized by digestion at restriction sites just inside the beginning and end of the coding region of the gene. Plasmid pSG204 was cleaved with *Bam HI*, which cleaves in a polylinker adjacent to the *Pvu II* site after base 1184 in the published sequence [(*37*), corrected in (*38*)]. Plasmid pSG209 was cleaved with *Hind III*, at base 3039 in the published sequence. The two digested plasmids were ligated to plasmid pSG122 (*39*), which had been digested with Bam HI and Hind III to release the selectable *aphA-3* gene encoding kanamycin resistance. The ligation products were transformed into Spo$^+$ *B. subtilis* strain 168 (*trpC2*), with selection for kanamycin resistance (5 μg/ml). Southern blot analysis confirmed that the *spoIIIE* gene was replaced by the *aphA-3* insertion.
28. L. J. Wu and J. Errington, unpublished results.
29. A 58-kD segment of SpoIIIE (residues 158 to 691) was overproduced in *Escherichia coli* as a COOH-terminal fusion to glutathione-*S*-transferase (GST), with the use of the pGEX system (*40*). The gel-purified fusion protein was used to raise polyclonal antiserum (*41*) (R. Allmansberger and J. Errington, unpublished results).
30. DNA sequence analysis revealed that *spoIIIE82* is a nonsense mutation close to the COOH-terminal coding end of the gene (codon 697 of 787) (*28, 38*).
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A Neurochemically Distinct Third Channel in the Macaque Dorsal Lateral Geniculate Nucleus

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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 parvicellular layers of the dorsal lateral geniculate nucleus. We have identified a third population of geniculocortical 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 geniculocortical 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 (*1*), that include the magnocellular and parvicellular layers in the dorsal lateral geniculate nucleus (LGN) of the thalamus (*2, 3*). Projections from M and P reach the primary visual cortex (V1), where the P channel is said to split into two streams, one that is selective for stimulus color and is represented by the cytochrome oxidase–rich puffs (or blobs) in the superficial layers, and another that is selective for stimulus form and is located in the surrounding cytochrome oxidase–poor interpuffs (*4*). From those points, cortical connections that are segregated to greater or lesser degrees reach the many extrastriate visual areas (*5*). A central issue in this division of labor in the macaque visual system is the origin of visual input to the puffs. Although in other primate species

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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 I or layers I to III of V1 were included (Fig. 2, A and B), most retrogradely labeled neurons were kinase-immunoreactive (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

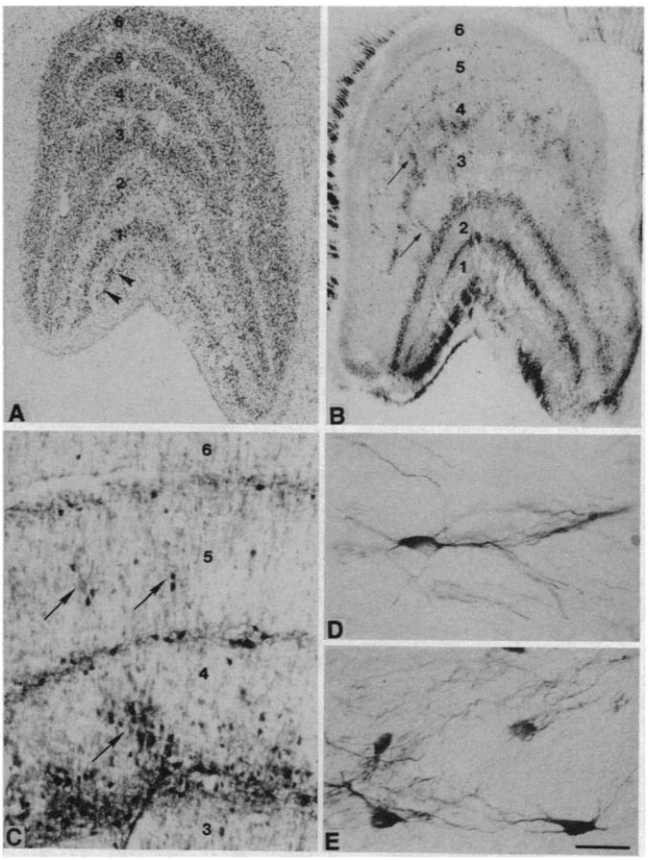

Fig. 1. (**A**) Frontal section through macaque LGN stained with thionin. Dense collections of neurons are evident in principal layers 1 through 6 and in an isolated cluster (double arrowheads), designated the S lamina, which represents a displaced part of layer 2 (6). Many of the stained nuclei ventral 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 (arrows) 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).

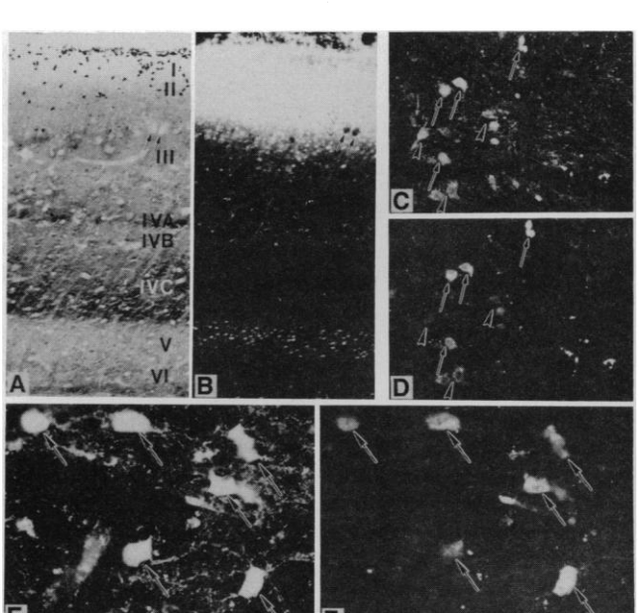

Fig. 2. (**A** and **B**) Photomicrographs of frontal section through V1, containing part of a Fast Blue deposit (B) and histochemically stained for cytochrome oxidase (A). Arrows indicate the same blood vessel profiles in the two micrographs. The deposit includes layers I and II and the superficial 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).

a deposit included layer IVA, many neurons in the parvicellular layers were retrogradely labeled.

The pattern of labeling from deposits in layers I to III of V1 appeared as an interrupted line of projection, with clusters of labeled, kinase-positive neurons lining up along a radial line from the intercalated layer ventral to principal layer 1 to cells overlying layer 6 (Fig. 3, C and D). In contrast, deposits restricted to layer I of V1 produced retrograde labeling only of the intercalated neurons in and ventral to layers 5 and 6 and in the white matter above layer 6 (Fig. 3, A and B). These findings suggest that the geniculocortical projection to layer I of V1 arises from dorsally located intercalated neurons, whereas the projection to layers II and III arises from the intercalated neurons in the ventral part of the LGN.

Geniculocortical terminations above layer IVA and beneath layer I of the macaque V1 are restricted to periodic patches that line up precisely with the cytochrome oxidase–rich puffs in these layers (*14*). Because the puffs contain a physiologically distinct collection of neurons, many of which display color-opponent properties (*8*), their neurons have been most frequently classified as members of the P channel (*4, 8*). Our data indicate that the puffs in the macaque V1 are innervated by neurons in the intercalated layers of the LGN and, through that geniculocortical 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-recipient and P-recipient 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 the 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 decimated 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.

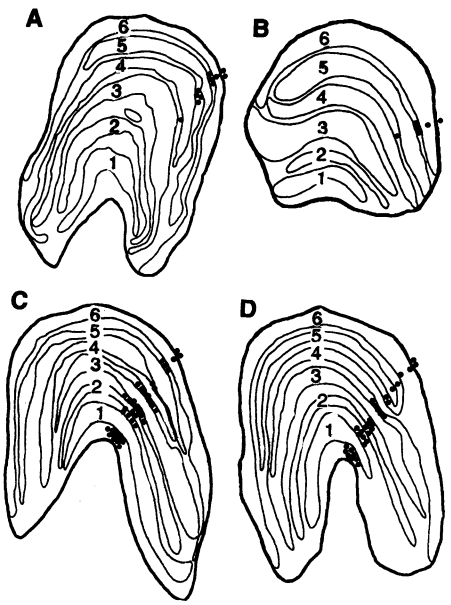

Fig. 3. Drawings of sections through LGNs from two monkeys, each ipsilateral to two deposits of tracer (one Fast Blue and one rhodamine dextran) in V1, either restricted to layer I (**A** and **B**) or including layers I through III (**C** and **D**). Neurons retrogradely labeled and immunocytochemically stained for CaM II kinase are indicated by dots.

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13. Six macaques (five *M. mulatta* and one *M. fascicularis*) 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. G. Jones, *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 tracers that was restricted to layer I; longer deposit times produced diffusion 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 immunoglobulins. Fluorescein (indicating CaM II kinase) and either rhodamine dextran or Fast Blue (retrograde transport) were viewed and photographed in the same fields with a Zeiss MPS microscope equipped with appropriate fluorescent filter cubes.
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