Parallel visual pathways in the primate brain known as the dorsal and ventral streams receive retinal inputs mainly through the magnocellular (M) and parvocellular (P) layers of the lateral geniculate nucleus. Inputs from these layers terminate within distinct parts of layer 4C of V1 (visual area 1). Due to the complexity of M- and P-derived neural connectivity in V1 and higher visual areas, the contributions of M and P inputs to the dorsal and ventral streams remain unclear. Employing retrograde transsynaptic transport of rabies virus, we analyzed the architecture of bottom-up pathways toward ventral stream area V4 (visual area 4) and dorsal stream area MT (middle temporal area). We found that V4 receives both M and P inputs "trisynaptically" from layer 4C via layer 2/3 of V1, whereas MT receives M-dominant input "disynaptically" from layer 4C via layer 4B of V1. V4 also receives disynaptic input from the dorsal stream portion of V2 (visual area 2) (i.e., cytochrome oxidase-stained thick stripes). Moreover, both M and P inputs reach V4 trisynaptically and MT disynaptically through "short-cut" pathways that bypass layer 4C of V1. The differential patterns of multisynaptic geniculo-cortical pathways to V4 and MT imply distinct modes of information processing in the dorsal and ventral streams.

Keywords: lateral geniculate nucleus, rabies virus, transneuronal labeling, V1, V2

Introduction

Many areas of the primate cerebral cortex are devoted to processing of incoming visual information. These functionally specialized areas construct the dorsal and ventral streams, which were initially defined as parallel pathways for processing of different aspects of visual information, that is, the former stream mainly dealing with motion and depth information and the latter predominantly dealing with color and fine form information (DeYoe and Van Essen 1988; Livingstone and Hubel 1988; Zeki and Shipp 1988). In more recent years, functional differences in relation to behavioral goals, involving action guidance by the dorsal stream and object recognition by the ventral stream, have been emphasized in characterizing the parallel streams (Goodale and Milner 1992; Milner and Goodale 2008; Orban 2008; Nassi and Callaway 2009). These streams originate from the magnocellular (M), parvocellular (P), and koniocellular (K) layers at the level of the lateral geniculate nucleus (LGN; DeYoe and Van Essen 1988; Livingstone and Hubel 1988). It has also been shown that neurons participating in the M-derived pathway exhibit fast achromatic responses, whereas those participating in the P-derived pathway display relatively slow color-opponent responses (Livingstone and Hubel 1988; Merigan and Maunsell 1993). Moreover, inputs from the M and P layers are segregated, such that M-derived input terminates predominantly within layer 4C, while P-derived input terminates mainly within layer 4C/β of visual area 1 (V1) (Blasdel and Lund 1983). These electrophysiological and anatomical findings led to the belief that M and P inputs contribute separately to the dorsal and ventral streams (Livingstone and Hubel 1988).

On the other hand, evidence indicates that the segregated geniculo-cortical inputs converge at the early stage of cortical processing (Ferrera et al. 1992; Lachica et al. 1992; Sincich and Lund 1983). These electrophysiological and anatomical findings led to the belief that M and P inputs contribute separately to the dorsal and ventral streams (Livingstone and Hubel 1988). For example, Ferrera et al. (1992) examined the contributions of M and P inputs to the dorsal and ventral streams by measuring neuronal activities in the middle temporal area (MT) of the dorsal stream and visual area 4 (V4) of the ventral stream while selectively inactivating either the M or the P layers. In their study, visual responses in V4 were found to depend on both M and P inputs, in contrast to the M-dominant neuronal activity in MT.

To better understand the mode of information processing in the primate visual system, it is of crucial importance to identify the circuits that relay M and P signals to the dorsal and ventral pathways. The complexity of cortical networks makes it difficult to evaluate the extent to which M and P inputs contribute to each stream. For instance, layer 2/3 of V1 receives input from both layers 4C and 4β (Lachica et al. 1992) and sends output to V4 directly and indirectly via the cytochrome oxidase (CO)-stained thin and pale stripes of visual area 2 (V2; Sincich and Horton 2002; Ungerleider et al. 2008). This neural network thus provides both M and P signals to V4. Given the projection from layer 2/3 of V1 to the CO-stained thick stripes of V2 (Sincich and Horton 2002), the possibility remains that layer 2/3 neurons relaying M signals project directly to the thick stripes alone but not to V4. To investigate with precision the involvement of M and P inputs in the dorsal and ventral streams, multisynaptic network tracing is required.

Recently, it has been demonstrated that rabies virus labels neurons retrogradely across synapses in time-dependent fashion (Ugolini 1995; Kelly and Strick 2000). Transneuronal tracing with rabies virus has revealed the existence of multiple pathways that carry P signals to MT, in addition to the major M-dominant input through layer 4C of V1 (Nassi and Callaway 2006; NASSI et al. 2006). Using retrograde transsynaptic transport of rabies virus, we analyzed the contributions of M and P inputs to V4 by comparing the architecture of bottom-up pathways toward V4 and MT.

Materials and Methods

Experimental Animals and Surgical Procedures

Nine adult Japanese monkeys (Macaca fuscata) of either sex weighing 4-8 kg were used in this study (see Table 1). The experimental
protocol was approved by the Animal Care and Use Committee of the Tokyo Metropolitan Institute for Neuroscience (Fuchu, Tokyo, Japan), and all experiments were conducted according to the Tokyo Metropolitan Institute for Neuroscience Guidelines for the Care and Use of Animals (2000). A head holder and a recording chamber were implanted under aseptic conditions. The monkeys were first sedated with an intramuscular injection of ketamine hydrochloride (5 mg/kg) and then anesthetized with an intravenous injection of sodium pentobarbital (20 mg/kg). Under anesthesia, the head was fixed in a stereotaxic apparatus, and a head holder was placed on the skull with anchor screws and dental acrylic resin. After recovery and training periods of several weeks, the monkeys were anesthetized with an intramuscular injection of ketamine hydrochloride (5–10 mg/kg) and xyazine hydrochloride (0.5–1 mg/kg) and seated in a monkey chair with the head fixed in a stereotaxic frame attached to the chair. A recording chamber was placed after a craniotomy, guided by the stereotaxic coordinates of MT or V4.

**Fixation Task and Electrophysiological Mapping**

To evaluate the retinotopic positions of injection sites precisely, the topographic organizations of MT and V4 were mapped beforehand while the monkeys performed a fixation task. The monkey faced a 17-inch monitor (FP71V; BenQ) placed 40 cm away in a dark room. A monitoring system with an infrared camera (XC-EI50; Sony) was used to monitor the monkey’s eye position. The monkey was required to fixate within a 0.5-degree-diameter circle for 3000–5000 ms. A drop of juice was given as a reward for correct task performance at 500-ms intertrial intervals.

Single or multiple unit recordings were performed for the mapping of MT and V4 using standard electrophysiological technique (Sawamura et al. 2006) while the monkey performed the fixation task. Typically, penetrations were made perpendicular to the dura surface with an oil hydraulic microdrive. The experimental stimuli were bars, sinusoidal gratings, and non-Cartesian (concentric-like polar and hyperbolic) gratings, as described in Maunsell and Van Essen (1983) and Gallant et al. (1996). Colors of stimuli were red, green, blue, yellow, or gray. After isolation of a spike waveform, the position and the size of the receptive field (RF) were carefully determined with a handmade mouse-controlled search program. The preferred direction and spatial frequency of neurons were also determined subjectively before subsequent quantitative tests. A direction-tuning curve was obtained with drifting sinusoidal grating stimuli in 45° or 90° steps from the optimal direction determined at the initial test. After representation of the optimal direction, cell selectivity for color and non-Cartesian gratings was separately measured. In quantitative measurements, each stimulus was presented for 750 ms with an interval of 250 ms. Stimulus sequences were randomized in every trial and repeated 3–5 times. The positions of the lunate and the superior temporal sulci were confirmed in the early part of each mapping. RF sizes, retinotopic organizations, and recording locations of neurons relative to the sulci were taken into consideration in identifying MT and V4 as well as response properties of the recorded neurons.

**Viral and Tracer Injections**

After electrophysiological identification, the challenge-virus-standard (CVS-11) strain of rabies virus or the conventional retrograde tracer wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) was injected into MT or V4 regions that represented desired eccentricities. The virus was originally obtained from the Centers for Disease Control and Prevention (Atlanta, GA, USA) and was donated by Dr Satoshi Inoue (The National Institute of Infectious Diseases, Tokyo, Japan). The rabies strain CVS-11 was the same as that introduced by Ugolini (1995) and Kelly and Strick (2000) to demonstrate specific retrograde transynaptic transport of the virus. The viral batch used in the present study was the same as in our previous study (Miyachi et al. 2005), which evaluated the multisynaptic projections from the prefrontal cortex to the primary motor cortex while calibrating the rate of viral transport with the cortico-basal ganglia and cerebro-cerebellar loop circuits. These are the neural systems that have thus far been studied, the application of which can reliably determine the order of transneuronal labeling with rabies virus (Kelly and Strick 2003, 2004; Miyachi et al. 2006; Lu et al. 2007). Thus, Miyachi et al. (2005) concluded that it takes about 2 days for first-order neuron labeling and one additional day per synapse for subsequent transneuronal labeling with our rabies strain. The titer of a viral suspension was 1.4 × 10^6 focus-forming units/mL. Monkeys were anesthetized with ketamine hydrochloride (5–10 mg/kg) and xyazine hydrochloride (0.5–1 mg/kg) before injections. To determine the sites of rabies injections precisely, neuronal activities were recorded immediately before injections with a 10-μL modified Hamilton microsyringe to which a Teflon-coated tungsten wire was attached (Tokuno et al. 1998). The wire allowed us to monitor the approximate depth at which the injection tubes penetrated the cortex and to detect neuronal activity in MT or V4. The viral suspension or a 4% solution of WGA-HRP (diluted in 0.1 M Tris-HCl buffer, pH 7.0; Toyobo) was injected by pressure through the 10-L Hamilton microsyringe, respectively. Typically, 3 penetrations were made into MT or V4 at least 1 mm apart from each other. For each penetration, 0.5–0.75 μL for rabies or 0.05–0.1 μL for WGA-HRP was deposited at 2 different depths (1–1.5 mm apart). Particularly in the case of V4 injection, loci of injection were arranged to cover more than one functional subregion within V4 at a given eccentricity (see Fellemman, Xiao, et al. 1997; Xiao et al. 1999). The locations of all V4 injections included, at least in part, the eccentricity of approximately 10° since the projection from V1 to V4 depends on the eccentricity of the injection sites (Zeki 1978; Steele et al. 1991).

**Histological Procedures and Data Analysis**

After 3–4 days of rabies injection, the monkeys were deeply anesthetized with sodium pentobarbital (50 mg/kg) and were

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**Table 1**

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Tracer</th>
<th>Injection site</th>
<th>Eccentricity (°)</th>
<th>Survival (d)</th>
<th>Injection volume (μL)</th>
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<tr>
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<td>MT</td>
<td>7–15</td>
<td>96</td>
<td>3.0</td>
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<tr>
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<td>V4</td>
<td>7–15</td>
<td>96</td>
<td>3.0</td>
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<tr>
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<td>RV</td>
<td>MT</td>
<td>15–25</td>
<td>72</td>
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</tr>
<tr>
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<td>5–10</td>
<td>72</td>
<td>4.0</td>
</tr>
<tr>
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<td>V4</td>
<td>15–40</td>
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</tr>
<tr>
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<td>RV</td>
<td>V4</td>
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<tr>
<td>MT-3c (5)</td>
<td>RV</td>
<td>MT</td>
<td>5–10</td>
<td>92</td>
<td>4.5</td>
</tr>
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</table>

Note: RV, rabies virus; WGA-HRP, wheat germ agglutinin-conjugated horseradish peroxidase. Monkeys were designated in the following fashion: [injection site] for WGA-HRP injection cases and [survival day]–[injection site]–[Alpha-numerical identification] for rabies injection cases.

*Numbers in parentheses correspond to the number attached to each of the injection sites and the labeling extent shown in Figures 18, C and 2A.
transcardially perfused with phosphate-buffered saline (PBS; 0.1 M, pH 7.4), followed by a mixture of 10% formalin and 15% saturated picric acid in phosphate buffer (PB; 0.1 M, pH 7.4). The brains were removed from the skull, postfixed in the same fresh fixative overnight, and saturated with 30% sucrose. Four days after WGA-HRP injection, the monkeys were deeply anesthetized with sodium pentobarbital (50 mg/kg) and transcardially perfused with PBS (0.1 M, pH 7.4), 8% formalin in PB (0.1 M, pH 7.4), and 10% sucrose in PB (0.1 M, pH 7.4). The brains were removed from the skull and saturated with 30% sucrose. Coronal or parasagittal sections were cut serially at 60 μm thick on a freezing microtome. In the cases of WGA-HRP injection, a series of every sixth section was reacted according to a tetramethylbenzidine-ammonium molybdate protocol (LeVay and Voigt 1990). To visualize layers and internal compartments of V1 such as layer 2/3 blobs and interblobs, adjacent sections were stained for CO by incubating at 4°C for 2-8 h in a solution of 30 mg cytochrome C, 4 g sucrose, and 40 mg diaminobenzidine (DAB) per 100 mL of Tris-HCl buffer, pH 7.6. In cases of rabies injection, a series of every sixth section was immunohistochemically stained for rabies virus with the standard avidin-biotin peroxidase complex (ABC) method as described elsewhere (Miyachi et al. 2005). Briefly, the sections were immersed in 1% skim milk for 1 h and incubated overnight at 4°C with rabbit anti-rabies virus antibody (diluted at 1:10 000; Inoue et al. 2003) in 0.1 M PBS containing 0.1% Triton X-100 and 1% normal goat serum. The sections were then incubated for 2 h in the same fresh medium containing biotinylated goat anti-rabbit IgG antibody (diluted at 1:200; Vector Laboratories) and reacted with the ABC Elite kit (Vector Laboratories) for 1.5 h. For visualization of the antigen, the sections were reacted in 0.05 M Tris-HCl buffer containing 0.04% DAB, 0.04% nickel chloride, and 0.002% hydrogen peroxide. These sections were counterstained with Neutral Red. A series of adjacent sections was Nissl- or myelin-stained to visualize laminar or areal borders, respectively. Another series of sections was double-stained for CO and rabies virus to observe the distribution of neuronal labeling in relation to V1 compartments, such as blobs in layer 2/3.

To confirm that rabies-labeled neurons primarily consist of M and P cells, sections including LGN were double-stained using rabbit anti-rabies virus antibody and mouse anti-parvalbumin or mouse anti-calbindin antibody (diluted at 1:2000; Swant). Labeled cells were revealed by the fluorescent secondary Cy3 antibody (diluted at 1:1000; Swant). To analyze the distribution of rabies-labeled neurons in V2, sections of monkey V4-3a and V4-3b (3 mm thick on a freezing microtome. In the cases of WGA-HRP injection, a series of every sixth injection cases with longer survival periods (monkeys V4-4a and V4-4b) since this labeling ensured transsynaptic transport of rabies virus. Similarly, regions that had labeled neurons in layer 4A of V1 were analyzed in the cases of V4 injection with shorter survival periods (monkeys V4-3a and V4-3b) since this layer does not project directly to V4. After plotting of labeled neurons, the Matlab (Math Works) algorithm was used to calculate the number and percentage of neurons in each layer.

Safety Issues
A few days after the mapping of MT or V4, the monkeys were moved to individual cages in a special laboratory (biosafety level 2) designed for in vivo virus experiments. Viral injections and perfusions were performed in the laboratory. To avoid accidental infection with the virus, all investigators received immunization beforehand and wore protective clothes during experimental sessions in the laboratory. Equipment was disinfected with 70% ethanol after each experimental session, and waste was autoclaved before disposal.

Results
Injection Sites
Injections of rabies virus and WGA-HRP were performed into MT and V4 after their electrophysiological identification (Fig. 1 and Table 1). Three monkeys received rabies injections into MT (monkeys MT-3a, MT-3b, and MT-3c; Fig. 1B) and were killed at the 3-day survival time. Four monkeys received rabies injections into V4 (Fig. 1C) and were allowed to survive for 3 days (monkeys V4-3a and V4-3b) or 4 days (monkeys V4-4a and V4-4b). Two monkeys received WGA-HRP injections into MT (monkey MT; Fig. 1B) or V4 (monkey V4; Fig. 1C) and were killed at the 4-day survival time. The positions of the injection sites were determined according to the results of electrophysiological mapping. RFs around the injection sites for monkeys V4-4b and MT-3c are shown in Figure 1EF, respectively. In monkey V4-4b, the RFs were in the lower right visual quadrant at an eccentricity of approximately 5°–10° (Fig. 1E). In monkey MT-3c, the RFs were in the lower right visual quadrant with a bias toward the horizontal meridian at an eccentricity of approximately 10°–20° (Fig. 1F). The approximate eccentricity of each injection site is summarized in Table 1. A series of sections were myelin stained to confirm that the locations of the tracer injections were confined to MT (densely myelinated region in the posterior bank of the superior temporal sulcus; Fig. 1D) or V4 (region in the prelunate gyrus, lateral to lightly myelinated V4; see Ungerleider and Desimone 1986; Ungerleider et al. 2008).

In the case of rabies injections, patterns of retrograde labeling in V1 and LGN were well matched retinotopically with the positions of RFs at the injection sites (Figs 1E,F and 2). In monkey V4-4b (Figs 1E and 2A, green shading), labeled neurons in V1 were found mainly in the dorsal surface of the occipital lobe and partially spread into the calcarine fissure. This location corresponded to the lower visual quadrant at an eccentricity of approximately 5°–10°. In monkey MT-3c (Figs 1F and 2A, blue shading), neuronal labeling in V1 was found primarily in the calcarine fissure, which represented the visual field along the horizontal meridian with a bias toward the lower visual field at an eccentricity of approximately 10°–25°. In LGN, transneuronally labeled neurons were distributed in both the M and P layers 3 or 4 days after the rabies injections into MT or V4, respectively. The retinotopic locations of neuronal labeling in the M and P layers were also closely matched with those of the injection sites and V1 neuron labeling in all cases. In monkey V4-4a, labeled neurons in V1 were found primarily in the posterior part of the calcarine fissure (Fig. 2A, red shading). Neuronal labeling in LGN in the same case was observed at the corresponding retinotopic locations (Fig. 2B, red shading). The close retinotopic correspondence between the injection sites and neuronal labeling in V1 and LGN indicates that the rabies injections were made properly into MT and V4 to label V1 and LGN neurons connected in transsynaptic fashion. In the case of WGA-HRP injections,
neuronal labeling in V1 appeared mainly on the dorsal surface of the occipital lobe and spread into the calcarine fissure for monkey MT, representing the upper visual quadrant along the horizontal meridian at an eccentricity of approximately 7°–15°. In monkey V4, labeled neurons in V1 were located close to those in monkey V4-4b, with a bias toward the anteromedial part, representing the lower visual quadrant at an eccentricity of approximately 7°–15°.

Figure 1. Locations of injection sites in macaque MT and V4. The injection sites in MT and V4 are summarized in panels B and C, respectively. Each panel represents a flattened or enlarged part, which is taken from the dashed (B) or dotted (C) area, respectively, in the lateral view of the macaque brain shown in A. (B) Injection sites of WGA-HRP and rabies virus in MT, as shown on a 2-dimensional flattened map (Van Essen and Maunsell 1980). The solid lines indicate the lips of the sulci, and the dotted line indicates the myeloarchitectural boundary of MT. Each injection site is colored and numbered according to Table 1. (D) Coronal section stained for myelin in a representative case (monkey MT-3c). The injection needle track (red arrow) is seen within MT, which is identified by dense myelination (white arrows). (E) RFs of 3 neurons recorded at the injection sites in case 9 (monkey V4-4b). Each rectangle denotes the approximate size and position of RF in the visual field. Numbers indicate eccentricities. (F) RFs of 3 neurons recorded at the injection sites in case 5 (monkey MT-3c). Arrows indicate the direction preference of each neuron. Other conventions are as in E. Scale bars, 3 mm. D, dorsal; HM, horizontal meridian; IOS, inferior occipital sulcus; IPS, intraparietal sulcus; L, lateral; LS, lateral sulcus; LuS, lunate sulcus; P, posterior; STS, superior temporal sulcus; VM, vertical meridian.

Figure 2. Retinotopic distribution of rabies-labeled neurons in V1 and LGN. (A) Locations of retrogradely labeled neurons in V1 after rabies injections. Data in the MT-injection case are taken from case 5 (monkey MT-3c; blue) and those in the V4-injection case from cases 8 (monkey V4-4a; red) and 9 (monkey V4-4b; green). The images, taken from a representative brain, demonstrate a dorsal view of the posterior end of the brain (left) and a medial view of the calcarine fissure, which is partially cut (right). The vertical (squares) and horizontal (circles) meridians are marked. Azimuths 1°, 5°, 10°, 20°, and 40° are depicted, along with iso-azimuth contours (black lines). Upper V1 (+) and lower V1 (−) quadrants of the visual field are noted. The retinotopic summary is adapted from Lyon and Kaas (2002) and Nassi et al. (2006) and based on retinotopic maps of Weller and Kaas (1983) and Van Essen et al. (1984). Scale bar, 10 mm. (B) Locations of rabies-labeled neurons in LGN from cases 5 (blue), 8 (red), and 9 (green) shown in representative parasagittal (top) and coronal (bottom) sections. The retinotopy of LGN is based on Malpeli and Baker (1975) and Erwin et al. (1999). The M and P layers are outlined by orange lines. Scale bar, 1 mm. A, anterior; Cal, calcarine fissure; D, dorsal; IPS, intraparietal sulcus; LuS, lunate sulcus; M, medial; OTS, occipitotemporal sulcus; POS, parietooccipital sulcus; STS, superior temporal sulcus.
Laminar Distribution of Retrogradely Labeled Neurons in V1 after WGA-HRP Injections into MT and V4

Prior to analyses of multisynaptic pathways from V1 to MT and V4, we first examined direct monosynaptic connections between these areas by injecting WGA-HRP into MT and V4 (Fig. 3). We counted labeled neurons in 5 sections in the MT-injection case and in 6 sections in the V4-injection case to cover the entire labeled regions in V1. In the case of MT injection, retrogradely labeled neurons were seen in higher and lower visual areas, such as V1, V2, visual area 3 (V3), V4, MST, and FST. Neuronal labeling in V1 occurred predominantly in layer 4B (435 labels) and, to a lesser extent, in layer 6 (91 labels). In the case of V4 injection, labeled neurons were located in many of the visual areas, including V1, V2, V3, MT, TEO, and inferior temporal cortex. In V1, the vast majority of labeled neurons appeared in layer 2/3 (272 labels), and only a few neurons were labeled in layer 4A (9 labels). The laminar distributions in V1 were consistent with previous data on anatomical connections between V1 and MT or V4 (Maunsell and Van Essen 1983; Shipp and Zeki 1989a; Ungerleider et al. 2008). To elucidate the architecture of multisynaptic pathways to MT and V4, transneuronal labeling in V1 after rabies injections was then compared with the patterns of monosynaptic labeling with WGA-HRP.

Laminar Distribution of Retrogradely Labeled Neurons in V1 after Rabies Injections into MT and V4

The pattern of distribution of rabies labeling on day 3 after the MT injection was initially tested to confirm consistency with recent findings on rabies labeling in the same visual system (Nassi and Callaway 2006; Nassi et al. 2006). On day 3 postinjection, the specific batch of rabies strain used in the present study had time to be transported across one synapse and infect neurons disynaptic to the injection site (see Materials and Methods). Neuronal labeling was observed in all visual areas and layers that have been reported to have monosynaptic or disynaptic connections with MT (Maunsell and Van Essen 1983; Shipp and Zeki 1989a; Nassi and Callaway 2006; Nassi et al. 2006). As our major interest was focused on the multisynaptic pathways from V1, we initially analyzed the laminar distribution of labeled neurons in V1. A total of 17 sections were analyzed, 6 each from monkeys MT-3a and MT-3b and 5 from monkey MT-3c. Neuronal labeling was seen primarily in layers 4B and 6, each of which is known to be monosynaptically connected to MT (Maunsell and Van Essen 1983), and, in addition, in layer 4C and then layer 2/3 (Fig. 4). Because layer 4C neurons do not send their axons outside V1, the existence of labeled neurons in layer 4C was believed to result from transsynaptic transport of rabies virus. Thus, neuronal labeling in layer 4C indicates the occurrence of transneuronal labeling at the 3-day survival period, consistent with the previous findings of Callaway and colleagues (Nassi and Callaway 2006; Nassi et al. 2006). This confirmed that the second-order neuron labeling appeared on day 3 after the rabies injection into electrophysiologically identified MT.

On day 3 after the rabies injection into V4, neuronal labeling was found in all visual areas known to possess monosynaptic connections with V4, including V1, V2, and V3 (Ungerleider

Figure 3. Laminar distribution of labeled neurons in V1 after WGA-HRP injections into MT and V4. (A and B) Dark-field photomicrographs of coronal sections of V1 (A) showing the laminar distributions of labeled neurons after WGA-HRP injections into MT (upper, monkey MT) and V4 (lower, monkey V4) and adjacent CO-stained sections (B). In the MT-injection case, labeled neurons appear predominantly in layers 4B and 6. In the V4-injection case, neuronal labeling occurs mainly in layer 2/3. Cortical layers are indicated on the left side of each panel. Scale bar, 100 μm. (C) Percentage of labeled neurons in each layer of V1 after WGA-HRP injections into MT (upper) and V4 (lower). Layer 4C was divided into 2 sublayers, 4Cα and 4Cβ, based on Nissl-stained sections. The numbers of total labeled neurons in the MT- and V4-injection cases are 538 and 283, respectively.
rabies injection into V4, averaged values of labeled neurons. The numbers of total labeled neurons in specified by symbols indicated on the upper right of each panel. Bar graphs represent are 9625, 6322, and 15131, respectively.

Figure 4. Laminar distribution of labeled neurons in V1 after rabies injections into MT and V4. (A, C, F) Representative coronal sections showing the laminar distributions of rabies-labeled neurons (black). Three days after rabies injection into MT (A, monkey MT-3c), labeled neurons are found primarily in layers 4B, and 4Cα, and, to a lesser extent, in layers 2/3 and 6. Only a few neurons are labeled in layers 4A, 4Cβ, and 5. The insets show higher-magnification images of labeled granular neurons in layers 2/3 and 4Cα. Three days after rabies injection into V4 (C, monkey V4-3b), rabies-labeled neurons are found predominantly in layer 2/3, with a low but substantial number in layer 4A. Only a few or almost no neurons are labeled in other layers. Four days after rabies injection into V4 (F, monkey V4-4a), labeled neurons appear in all layers. The inset shows a higher-magnification image of granular neurons labeled in layers 2/3 and 4Cβ. Cortical layers are indicated on the left side of each panel. Scale bar, 100 μm. (B, D, E) Percentage of rabies-labeled neurons in each layer of V1 3 days after rabies injection into MT (B), 3 days after rabies injection into V4 (D), and 4 days after rabies injection into V4 (F). The percentage of labeled neurons in 2 or 3 cases is specified by symbols indicated on the upper right of each panel. Bar graphs represent averaged values of labeled neurons. The numbers of total labeled neurons in B, D, and F are 9625, 6322, and 15131, respectively.

et al. 2008). Within these areas, labeled neurons were located not only in the layers that have been reported to project monosynaptically to V4 but also in other layers probably due to transneuronal labeling. We counted labeled neurons in a total of 11 sections, 6 from monkey V4-3a and 5 from monkey V4-3b. In V1, the laminar distribution of labeled neurons was quite different from that in the case of MT injection (Fig. 4C). Neuronal labeling was seen predominantly in layer 2/3, the only layer known to project directly to V4 (Nakamura et al. 1993; Ninomiya et al. 1997). Furthermore, the layer 2/3 labeling exhibited 2 distinct patterns. One was characterized by a clear bias toward the CO-stained blob regions, while the other exhibited no bias toward the blob or interblob regions (Supplementary Fig. 1). Some labeled neurons were also observed in layer 4A. In the regions of blob-biased labeling, labeled neurons in layer 4A were basically found beneath the blobs. Although this neuronal labeling in layer 4A was presumably due to transsynaptic transport of rabies virus, only a few labeled neurons appeared in layer 4C of V1 at the 3-day survival period.

To test how long the survival period must be to detect neuronal labeling in layer 4C of V1, 2 monkeys were allowed to survive for 4 days after the rabies injection into V4. Many labeled neurons were distributed over all layers (Fig. 4E), indicating that further transneuronal labeling must have occurred during one additional survival day. We counted labeled neurons in a total of 13 sections, 6 from monkey V4-4a and 7 from monkey V4-4b. Again, the labeled neurons in the upper layer exhibited the blob-biased or blob-unbiased pattern (Supplementary Fig. 1). This differential pattern of neuronal labeling in V1 probably depends on the difference in injection sites in relation to the internal compartments of V4 reported in previous studies (DeYoe and Van Essen 1985; Zeki and Shipp 1989; Nakamura et al. 1993; DeYoe et al. 1994; Pellegman, Xiao, et al. 1997). Of most importance was that retrograde labeling of V1 neurons that appeared in layer 4C 4 days after the rabies injection into V4.

The laminar distribution of neuronal labeling in V1 was analyzed quantitatively by cell counts in each layer. In the case of MT injections (Fig. 4B), labeled neurons were located primarily in layers 4B (40.8%) and 4Cα (29.8%) at the 3-day survival period. Substantial labeling was also observed in layers 6 (17.6%) and 2/3 (7.8%). As described above, the labeled neurons in layers 4Cα and 2/3 likely consisted of neurons that are transsynaptically connected to MT. In the case of V4 injections, rabies-labeled neurons were found mostly in layer 2/3 (81.5%) and, in addition, layer 4A (12.0%) on day 3 after rabies injection (Fig. 4D). Labeling of only a few neurons was seen in other layers, including layer 4C. At the 4-day survival period (Fig. 4F), populations of labeled neurons were also detected in layers 4Cα (4.2%) and 4Cβ (6.3%) of V1. This suggests that at least a portion of neurons in layer 4C are connected trisynaptically to V4. Thus, it should be emphasized here that neuronal labeling in layer 4Cα of V1 emerged as second-order neuron labeling from MT, whereas neuronal labeling in layers 4Cβ and 4Cβ of V1 emerged as third-order neuron labeling from V4.

Retrogradely Labeled Neurons in LGN after Rabies Injections into MT and V4

Neurons in LGN were strongly labeled after rabies injections into MT and V4, as illustrated in Figure 2B. To assess the contributions of the M, P, and K layers, the layer specificity of labeled neurons in LGN was analyzed in each case. Cell counts were performed in a series of 26–31 sections through LGN. On
day 3 after the rabies injection into MT, many labeled neurons were observed in the M and P layers of LGN (Supplementary Fig. 2). The timing and distribution of neuronal labeling in LGN corresponded well to those reported by Nassi et al. (2006). On day 3 after the rabies injection into V4, however, only very rarely were neurons labeled in LGN (Fig. 5D). With this length of survival, neuronal labeling was seen only in the K layers of LGN, which might reflect the K-dominant direct projection from LGN to V4 (Yukie and Iwai 1981). On day 4 after the rabies injection into V4, neuronal labeling was apparent in the M and P layers of LGN (Fig. 5A–C). When the number of labeled neurons was counted in each layer of LGN, many of the labeled neurons were found in the M and P layers (Fig. 5D). However, a substantial proportion of labeled neurons were observed in the K layers (22.9%, 331/1447 neurons; Fig. 5D) compared with the case of MT injections (7.0%, 82/1177 neurons; Supplementary Fig. 2D).

To confirm that neuronal labeling in LGN consists primarily of M and P cells, double immunostaining for rabies and parvalbumin, a marker for M and P cells, was performed in each case (Jones and Hendry 1989; Supplementary Fig. 3). Many double-labeled neurons were found in the M (72.2% for MT, 71.3% for V4) and P (67.5% for MT, 66.4% for V4) layers, with many fewer neurons double-labeled for rabies and parvalbumin in the K layers (15.0% for MT, 14.6% for V4). Additionally, the proportion of rabies-labeled K cells was assessed by double immunostaining for the K cell marker calbindin (Jones and Hendry 1989; Supplementary Fig. 4). In contrast to the parvalbumin-staining case, double-labeled neurons were observed predominantly in the K layers (73.3% for MT, 74.5% for V4), while many fewer neurons were double-labeled for rabies and calbindin in the M (10.1% for MT, 7.0% for V4) and P (12.8% for MT, 7.6% for V4) layers. These results suggest that V4, as well as MT, receives strong inputs from both the M and the P layers of LGN, with at least one more synaptic relay than MT.

**Retrogradely Labeled Neurons in CO-Stained Thick Stripes of V2 after Rabies Injection into V4**

It has been shown that CO histochemistry reveals a repetitive pattern of pale-thin-pale-thick stripes in V2 (Tootell et al. 1983). The thick stripes of V2 send major outputs to V3 and MT (Shipp and Zeki 1989b) and belong to the dorsal stream. Recent anatomical studies suggest that the thick stripes receive input from the interblobs in layer 2/3 of V1 (Sincich and Horton 2002; Sincich et al. 2010), which relay both M and P inputs via layers 4Cα and 4Cβ (Yabuta and Callaway 1998). As V4 receives inputs from V3 and MT (Ungerleider et al. 2008), the thick stripes can provide M and P inputs to V4. Furthermore, the existence of interconnections between the 3 stripes indicates the possibility that the thick stripes relay M and P signals indirectly to V4 by way of the thin and pale stripes (Levitt et al. 1994). To examine whether V4 receives multisynaptic input from the thick stripes of V2, we analyzed the distribution of neuronal labeling in V2 3 days after the rabies injection into V4. Although the CO stripes are best visualized by CO histochemistry of unfolded and flattened tissue, the sections were cut coronally to examine the laminar distributions of labeled neurons in V1 precisely. Instead of CO histochemistry, the thick stripes of V2 were identified based on Cat-301 immunoreactivity (Olavarria and Van Essen 1997).

**Figure 5.** Retrogradely labeled neurons in LGN after rabies injection into V4. (A) Representative coronal section of LGN stained for rabies virus (black) and CO (brown) 4 days after rabies injection into V4 (monkey V4-4b). The blue and red lines indicate the M (magnocellular) and P (parvocellular) layers, respectively. Labeled neurons are observed in all layers of LGN. Scale bar: 500 μm. D, dorsal; M, medial. (B, C) Higher-magnification images of P and M cells, taken from the rectangular areas in A. Scale bar, 100 μm. (D) Number of rabies-labeled neurons in each layer of LGN for monkeys V4-3a (filled circles), V4-3b (open circles), V4-4a (filled triangles), and V4-4b (open triangles). Bar graphs show averaged values of labeled neurons in the 4 cases with survival times of 3 or 4 days: black, total; blue, M layers; red, P layers; green, K layers.

Figure 6A demonstrates a representative section double-stained for rabies and Cat-301 (monkey V4-3b). A large number of rabies-labeled neurons were observed in Cat-301-negative zones, corresponding presumably to the thin and pale stripes of V2. In addition, rabies-labeled neurons emerged in Cat-301-
positive zones that probably represent the thick stripes. The vast majority of labels in Cat-301-positive zones were positioned out of layer 4. A total of 9 coronal sections (5 from monkey V4-3b and 4 from monkey V4-3a) just posterior to the lunate sulcus were analyzed since the 3 stripes of V2 in this region could be seen periodically as in Figure 6A, making it easier to evaluate the distribution of neuronal labeling in the V2 stripes. We observed labeled neurons within at least 1 Cat-301-positive zone in 8 of the 9 coronal sections.

Many rabies- and Cat-301-labeled neurons were plotted along layer 4 to evaluate possible input from the thick stripes of V2 to V4 in monkeys V4-3b (Fig. 6B, upper panel) and V4-3a (Fig. 6B, lower panel). Three thick stripes are included in each panel. Some peaks of rabies-labeled neurons were observed between the Cat-301-positive zones, which may reflect strongly labeled thin or pale stripes. The Cat-301-positive zones contained a substantial number of rabies-labeled neurons, suggesting the existence of multisynaptic input from the thick stripes of V2 to V4.

Discussion
In the present study, we employed retrograde transsynaptic transport of rabies virus to analyze the architecture of geniculo-cortical pathways toward ventral stream area V4 and dorsal stream area MT of macaques. When layer 4C labeling in V1 was compared, the major M-input layer 4Cα was labeled 3 days after rabies injection into MT (second-order labeling), whereas the major P-input layer 4Cβ as well as layer 4Cα were labeled 4 days after the injection into V4 (third-order labeling). At the same time, the MT injection produced transneuronal labeling in LGN as second-order neurons, while the V4 injection yielded LGN neuron labeling as third-order neurons. The labeled neurons were distributed in both the M and the P layers. Furthermore, neuronal labeling in the dorsal stream portion of V2 (i.e., the CO-stained thick stripes) appeared on day 3 after the V4 injection.

Pattern and Order of Transneuronal Labeling in V1
In the present study, we used rabies virus to explore multisynaptic pathways from LGN and V1 to MT and V4. For this purpose, it is crucial to determine in what order transneuronal labeling occurs. We analyzed the pattern of transneuronal labeling in V1 3 days after rabies injection into MT. At this survival period, neuronal labeling in layers 2/3 and 4Cα was observed in addition to that in the direct projection layers 4B and 6. The laminar distribution of V1 labeling was identical to that reported in the study of Nassi et al. (2006) with rabies labeling and therefore indicates that the 3-day survival period after rabies virus permits second-order neuron labeling through disynaptic transport of the virus. At the same survival period after V4 injection, layers 2/3 and 4A contained labeled neurons. Although a minor projection from layer 4A to V4 cannot be omitted (Yukie and Iwai 1985; Nakamura et al. 1993; present results), neuronal labeling in this layer is believed to be due primarily to the second-order labeling from V4. At the 4-day survival period, neuronal labeling from V4 emerged in all V1 layers, suggesting the occurrence of further transneuronal labeling. Taking the rate of rabies transport into account (Miyachi et al. 2005), the labeled neurons must involve third-order neurons. Moreover, the patterns of distribution of neuronal labeling in V1 and LGN were well matched retinotopically with the injection sites. This provides strong evidence that neuronal labeling in V1 and LGN is ascribable to retrograde transneuronal labeling from MT and V4.

The internal control tested in our laboratory (Miyachi et al. 2005) and the consistency of our data obtained in the MT-injection case with previous findings make it quite likely that the rate of transsynaptic transport of rabies virus was not over- or underestimated. However, the possibility cannot as yet entirely be ruled out that neuronal labeling may have occurred
later than expected at the 3- or 4-day survival period. We may have mistakenly interpreted the second-order labeling in layer 4A on day 3 or the third-order labeling in layer 4C on day 4 in the V4-injection case, given that the linkage between V1 and V4 does not appear to be as prominent as that between V1 and MT. In fact, the second-order neuron labeling in layer 4Cα may have been expected since projection neurons in layer 2/3 receive input directly from layer 4Cα (Sawatari and Callaway 2000). On the other hand, third-order neuron labeling in layer 4Cβ is reasonable since projection neurons in layer 2/3 do not receive input directly from layer 4Cβ (Sawatari and Callaway 2000). It should be emphasized that, in the case of V4 injections, neuronal labeling in layer 4C of V1 probably emerged with one more survival day than in the case of MT injections.

Architecture of Multisynaptic Pathways from V1 to V4 and MT

Based on previous anatomical data, we propose the possibility of existence of multisynaptic pathways from layer 4C of V1 to MT and V4 (Fig. 7). MT probably receives disynaptic input from layer 4Cα via layer 4B, which has been emphasized as a prominent link that provides MT with V1 input (Shipp and Zeki 1989a; Nassi and Callaway 2006). With respect to multisynaptic pathways to V4, on the other hand, all possible pathways are quite likely to go through layer 2/3 of V1. Neurons in layer 2/3 receive input from layer 4C directly or indirectly via layer 4A (Sawatari and Callaway 2000), where second-order labeling was observed in our study. Neither V3 nor MT presumably participates in multisynaptic pathways to V4. If these areas are involved in the pathways, neuronal labeling in layer 4B should appear prior to that in layer 4C of V1 because of the existence of robust projections from layer 4B to V3 and MT (Maunsell and Van Essen 1983). In addition, V2 does not appear to participate in the pathway from layer 4Cβ of V1 to V4. The CO-stained thin and pale stripes of V2 can relay V1 signals to V4 (DeYoe and Van Essen 1985; Shipp and Zeki 1985). However, Nassi and Callaway (2006) have shown that 4Cβ input may reach the thin and pale stripes of V2 trisynaptically. Thus, any trisynaptic pathways from layer 4Cβ to V4 likely go through other layers of V1. Indeed, previous studies on local circuits in V1 can explain such pathways. Neurons in layer 4Cβ restrict their layer 2/3 projections to its lower third (i.e., layer 3B; Lachica et al. 1992; Yabuta and Callaway 1998). The projections of layer 3 neurons, which receive layer 4Cβ input, are localized exclusively within V1; layer 3 neurons projecting to other visual areas do not receive input from layer 4Cβ (Sawatari and Callaway 2000). On the other hand, it is possible that neurons in layer 4Cα send output trisynaptically to V4 via the thin and pale stripes of V2, as layer 4Cα is connected disynaptically to these V2 regions (Nassi and Callaway 2006). However, this is unlikely since the second-order labeling in V1 was restricted to layers 2/3 and 4A despite the direct projection to V2 from all V1 layers but layer 4C (Sincich and Horton 2002). Taken together, the present findings suggest that neurons in layers 4Cα and 4Cβ likely send outputs to V4 trisynaptically through layers 4A and 2/3 or through layer 2/3 and its intralaminar connection within V1.

It is somewhat surprising that neuronal labeling in layer 4Cα of V1 was not observed 3 days following V4 injection. As many of the projection neurons in layer 2/3 receive input from layer 4Cα (Sawatari and Callaway 2000), a disynaptic connection from layer 4Cα to V4 via layer 2/3 might be expected. However, only a negligible level of neuronal labeling appeared in 4Cα at the 3-day survival time. This implies that layer 2/3 neurons projecting to V4 do not receive input from layer 4Cα. Alternatively, the neurons receiving 4Cα input may project to visual areas other than V4.

In the present study, we have elucidated the architecture of multisynaptic pathways from V1 to MT and V4. However, prominent pathways through V2/V3 also exist. Ponce et al. (2008) performed a cooling experiment to define the functional roles of such pathways to MT. They reported that inactivation of V2 and V3 caused disproportionate degradation of binocular disparity tuning relative to direction tuning in MT neurons. The disynaptic pathway arising from layer 4Cα via layer 4B of V1 may convey motion information to MT. In fact, Movshon and Newsome (1996) have shown that layer 4B neurons projecting to MT are strongly tuned to direction. On the other hand, neurons in the thick stripes of V2 exhibited selectivity to binocular disparity more frequently than direction selectivity (DeYoe and Van Essen 1985; Peterhans and von der Heydt 1993). Likewise, multisynaptic pathways from V1 to V4 with and without V2/V3 relay may convey different aspects of visual information.

Multisynaptic Inputs from LGN to V4 and MT

In both the case of V4 and that of MT injection, neuronal labeling in the M and P layers of LGN occurred with the same timing as that in V1 layer 4C. If the rabies injection retrogradely traced major ascending projections from LGN to V1 and higher visual areas, neurons in the M and P layers must have been labeled after the appearance of neuronal inputs from layer 4C of V1 to MT and V4 (Fig. 7). MT probably receives disynaptic input from layer 4Cα via layer 4B, which has been emphasized as a prominent link that provides MT with V1 input (Shipp and Zeki 1989a; Nassi and Callaway 2006). With respect to multisynaptic pathways to V4, on the other hand, all possible pathways are quite likely to go through layer 2/3 of V1. Neurons in layer 2/3 receive input from layer 4C directly or indirectly via layer 4A (Sawatari and Callaway 2000), where second-order labeling was observed in our study. Neither V3 nor MT presumably participates in multisynaptic pathways to V4. If these areas are involved in the pathways, neuronal labeling in layer 4B should appear prior to that in layer 4C of V1 because of the existence of robust projections from layer 4B to V3 and MT (Maunsell and Van Essen 1983). In addition, V2 does not appear to participate in the pathway from layer 4Cβ of V1 to V4. The CO-stained thin and pale stripes of V2 can relay V1 signals to V4 (DeYoe and Van Essen 1985; Shipp and Zeki 1985). However, Nassi and Callaway (2006) have shown that 4Cβ input may reach the thin and pale stripes of V2 trisynaptically. Thus, any trisynaptic pathways from layer 4Cβ to V4 likely go through other layers of V1. Indeed, previous studies on local circuits in V1 can explain such pathways. Neurons in layer 4Cβ restrict their layer 2/3 projections to its lower third (i.e., layer 3B; Lachica et al. 1992; Yabuta and Callaway 1998). The projections of layer 3 neurons, which receive layer 4Cβ input, are localized exclusively within V1; layer 3 neurons projecting to other visual areas do not receive input from layer 4Cβ (Sawatari and Callaway 2000). On the other hand, it is possible that neurons in layer 4Cα send output trisynaptically to V4 via the thin and pale stripes of V2, as layer 4Cα is connected disynaptically to these V2 regions (Nassi and Callaway 2006). However, this is unlikely since the second-order labeling in V1 was restricted to layers 2/3 and 4A despite the direct projection to V2 from all V1 layers but layer 4C (Sincich and Horton 2002). Taken together, the present findings suggest that neurons in layers 4Cα and 4Cβ likely send outputs to V4 trisynaptically through layers 4A and 2/3 or through layer 2/3 and its intralaminar connection within V1.

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labeling in layer 4C. Thus, neuronal labeling in these layers can probably be attributed to transsynaptic transport of rabies virus through "short-cut" pathways. MT likely receives disynaptic input from the M and P layers via V1 layer 6, as reported by Nassi et al. (2006). The trisynaptic pathway from LGN to V4 may be mediated not only by V1 but also by other cortical areas, such as V2, V3, and MT (Fig. 8). In the present study, the second-order neuron labeling in V1 after rabies injection into V4 appeared only in layers 2/3 and 4A. Layer 4A receives a weak projection from the P layers of LGN (Blasdel and Lund 1983) and can relay P signals to V4 via layer 2/3. On the other hand, V1 is not likely to relay M signals to V4 in trisynaptic fashion since neither layer 2/3 nor layer 4A primarily receives input from the M layers. Thus, we assume that geniculo-extrastriate pathways, like the direct projection from LGN to V2, may be involved in relaying M and P signals to V4. Although LGN neurons projecting to V2 generally arise from the K layers, a small but substantial number of neurons in the M and P layers also project to V2 (Bullier and Kennedy 1983). Indeed, the assumption that the LGN-V2 projection participates in the trisynaptic pathways toward V4 is consistent with the observation that neuronal labeling in the K layers occurred within the 4-day survival period.

Previous studies (Schiller and Malpeli 1977; Girard and Bullier 1989) demonstrated that when neuronal activity in V1 was blocked by cooling, the vast majority of V2 neurons in the retinotopically corresponding region were inactivated. This observation raises a question as to the functional significance of a geniculo-extrastriate pathway. The inactivated neurons in V2 may play only a modulatory role in the extrastriate areas. However, Boyer et al. (2005) examined unconscious processing of orientation and color by inducing a scotoma in V1 with transcranial magnetic stimulation and suggested that a geniculo-extrastriate pathway could process orientation and color. Furthermore, an fMRI study by Schmid et al. (2009) showed visually driven V2 activity 1--22 months after V1 lesions. These studies suggest that geniculo-extrastriate input can activate the extrastriate areas and even process various aspects of visual information to some extent.

**Multisynaptic Input from V2 to V4**

The present results also indicate that the V2 thick stripes send output disynaptically to V4. This disynaptic pathway can be explained by the connectivity of the thick stripes with other stripes of V2 (Levitt et al. 1994). V3, and/or MT (Fig. 8). Previous anatomical studies, however, suggest that V3 and MT are not likely to contribute to neuronal labeling in the thick stripes of V2. The projection from the V2 thick stripes to V3 and MT terminates mainly within layer 4, whereas the V3 projection to V4 arises primarily from layer 2/3 and the MT projection to V4 arises predominantly from layers 2/3 and 6 (Maunsell and Van Essen 1983; Felleman, Burkhalter, et al. 1997). Therefore, signals from V3 and MT presumably reach V4 trisynaptically. We also confirmed in our own cases that the laminar patterns of the V2-MT, V3-MT, V3-V4, and MT-V4 projections were essentially the same as previously reported (Maunsell and Van Essen 1983; Ungerleider et al. 2008) and concluded that neither V3 nor MT appears to participate in disynaptic connectivity from the V2 thick stripes to V4. On the other hand, layer 2/3 in the V2 thin or pale stripes, which projects to V4 directly, receives input from the V2 thick stripes (Levitt et al. 1994). These findings suggest that V4 receives disynaptic input from the thick stripes via the thin and/or pale stripes of V2. It is possible, however, that the existence of a direct projection from the V2 thick stripes to V4 results in neuronal labeling in Cat-301-positive zones of V2 at the 3-day survival period. Although the projections from V2 to V4 arise mostly from the thin and pale stripes, some of them appear to originate from the thick stripes (Zeki and Shipp 1989; Nakamura et al. 1993). Indeed, we observed neuronal labeling in layer 4 within the Cat-301-positive zones, suggesting that retrograde transneuronal labeling from the thick stripes may occur through layer 2/3 neurons projecting to V4. However, most of the Cat-301-positive zones contained only a small number of labeled neurons in layer 4. This further supports our interpretation that V4 receives disynaptic input from the thick stripes of V2 predominantly via the thin and/or pale stripes.

**Conclusions**

The present study provides evidence that V4 receives convergent M and P inputs through multiple pathways. The trisynaptic pathway from layer 4C via layer 2/3 of V1 might contribute to relay of both M and P signals to V4. This pathway is apparently different from the M-dominant disynaptic one from layer 4C to MT. This anatomical difference can account for the involvement of both M and P inputs in visual responses of V4 neurons, in contrast to the M-dominant activity in MT.
(Ferrera et al. 1992). On the other hand, the “short-cut” pathways from LGN to MT and V4 relay both M and P signals. Furthermore, V4 receives stronger K input than MT. V4 also receives disynaptic input from the dorsal stream portion of V2, that is, the thick stripes of V2, which probably carries M input. Our findings indicate that multisynaptic geniculo-cortical pathways to MT and V4 instruct the dorsal and ventral streams in processing of distinct aspects of visual information.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

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Notes
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