Dorsal Area 46 Is a Major Target of Disynaptic Projections From the Medial Temporal Lobe

Yoshihiro Hirata1,2,4,6, Shigehiro Miyachi1,3,5, Ken-ichi Inoue1,2, Taihei Ninomiya1,2, Daisuke Takahara1,2, Eiji Hoshi4,7 and Masahiko Takada1,2,5

1Department of System Neuroscience, Tokyo Metropolitan Institute for Neuroscience, Tokyo Metropolitan Organization for Medical Research, Fuchu, Tokyo, Japan 2Systems Neuroscience Section 3Cognitive Neuroscience Section, Primate Research Institute, Kyoto University, Inuyama, Aichi 484-8506, Japan 4Brain Science Institute, Tamagawa University, Machida, Tokyo 194-8610, Japan 5Japan Science and Technology Agency, CREST, Chiyoda-ku, Tokyo 102-0075, Japan 6Current address: Photonic Bioimaging Section, Research Center for Cooperative Projects, Hokkaido University Graduate School of Medicine, Sapporo 060-8638, Japan and 7Current address: Frontal Lobe Function Project, Tokyo Metropolitan Institute of Medical Science, Tokyo 156-8506, Japan

Address correspondence to Shigehiro Miyachi. Email: miyachi.shigehiro.8e@kyoto-u.ac.jp

The medial temporal lobe (MTL) is responsible for various mnemonic functions, such as association/conjunction memory. The lateral prefrontal cortex (LPFC) also plays crucial roles in mnemonic functions and memory-based cognitive behaviors, for example, decision-making. Therefore, it is considered that the MTL and LPFC connect with each other and cooperate for the control of cognitive behaviors. However, there exist very weak, if any, direct inputs from the MTL to the LPFC. Employing retrograde transsynaptic transport of rabies virus, we investigated the organization of disynaptic bottom-up pathways connecting the MTL and the inferotemporal cortex to the LPFC in macaques. Three days after rabies injections into dorsal area 46, a large number of labeled neurons were observed in the MTL, such as the hippocampal formation (including the entorhinal cortex), the perirhinal cortex, and the parahippocampal cortex. In contrast, a majority of the labeled neurons were located in the inferotemporal cortex following rabies injections into ventral area 46 and lateral area 12. Rabies injections into lateral area 9/area 8B labeled only a small number of neurons in the MTL and the inferotemporal cortex. The present results indicate that, among the LPFC, dorsal area 46 is the main target of disynaptic inputs from the MTL.

Keywords: area 46, medial temporal lobe, memory, prefrontal cortex, rabies virus, retrograde transneuronal labeling

Introduction
Since severe memory deficit in the case of the patient H.M. was reported (Scoville and Milner 1957), potential roles of the medial temporal lobe (MTL), consisting of the hippocampal formation (including the entorhinal cortex), the perirhinal cortex, and the parahippocampal cortex, in mnemonic functions have been explored in many experimental studies. Early works described that large lesions of the hippocampus and the adjacent cortical areas in monkeys deteriorated the performance of memory-related behavioral tasks, such as a delayed non-matching-to-sample task (for review, see Zola-Morgan and Squire 1993; see also Alvarez et al. 1994). In recent years, the importance of the MTL in the generation of association/conjunction memory rather than simple visual memory has been emphasized increasingly (Naya et al. 2003; Alvarado and Bachevalier 2005; Lavenex et al. 2006; Olson et al. 2006).

The lateral prefrontal cortex (LPFC) is known to play essential roles in a variety of mnemonic functions, such as working memory (for reviews, see Fuster 2008; Tanji and Hoshi 2008). The LPFC has been implicated not only in the short-term retention or retrieval of memory per se, but also in the selection or integration of different aspects of memory contents and the consequent process of cognitive behaviors, such as decision-making based on memory (Bauer and Fuster 1976; Mishkin and Manning 1978; Petrides 1991a, 1991b; Funahashi et al. 1993; Miller et al. 1996; Rao et al. 1997; Hoshi and Tanji 2004; Cadoret and Petrides 2007; Warden and Miller 2007; Buckley et al. 2009).

Thus, the MTL and LPFC would possibly cooperate in cognitive behaviors related to memory. For this cooperative process, both top-down and bottom-up projections linking the LPFC and the MTL would be necessary. As for the top-down projections from the LPFC to the MTL, areas TF and TH of the parahippocampal cortex receive inputs directly from various regions of the LPFC, and area 36 of the perirhinal cortex receives direct input mainly from the ventral part of the LPFC (Suzuki and Amaral 1994). On the other hand, there exist very weak, if any, direct bottom-up projections from the MTL to the LPFC. According to previous retrograde-labeling studies (Barbas and Mesulam 1985; Petrides and Pandya 1999, 2001; Muñoz and Insauti 2005), only a few or virtually no neurons were labeled in the MTL after conventional tracer injections into LPFC regions. This was confirmed by an anterograde tract-tracing study showing that projection fibers arising from the parahippocampal cortex (areas TF, TFM, and TH) and the perirhinal cortex (area 36) terminated within the LPFC very sparsely (Lavenex et al. 2002). Therefore, for the achievement of memory-based cognitive functions, bottom-up inputs from the MTL to the LPFC must be mediated by multisynaptic neuronal connections. To examine possible multisynaptic projections from the MTL to the LPFC, retrograde transsynaptic transport of rabies virus was employed in the present work. We analyzed the distribution patterns of the second-order (disynaptically connected) neuron labeling in the MTL after viral injections into different sectors of the LPFC in macaque monkeys: the dorsal and ventral parts of area 46 (area 46d and area 46v, respectively), the lateral part of area 9 and area 8B (areas 9L/8B), and the lateral part of area 12 (area 12L).

Materials and Methods

Experimental Animals
Eleven macaque (five rhesus and six Japanese) monkeys of either sex weighing 6.0–12.0 kg were used in this study (Table 1). The monkeys were kept in individual cages placed inside a special safety cabinet. Food and water were available ad libitum in each cage. 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 in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Prefrontal Injections of Rabies Virus and Conventional Tracers

The monkeys were immobilized with ketamine hydrochloride (5 mg/kg, im), deeply anesthetized with sodium pentobarbital (20 mg/kg, iv), and then positioned in a stereotaxic apparatus. A craniotomy was made over the frontal lobe under an aseptic condition. Before rabies injections, a rectangular plastic chamber was fixed over the frontal lobe and the dura mater was kept intact. After the injections, a plastic lid was tightly screwed to the chamber. For conventional tracer injections, the dura mater was cut, and the surface of the brain was exposed. After the injections, the dura mater was sutured, a bone flap was fixed with dental acrylic resin, and then the skin was sutured. In seven monkeys, the challenge virus standard (CVS-11) strain of rabies virus (Kelly and Strick 2000, 2004; Miyachi et al. 2005) was injected into one of the four sectors of the LPFC (Table 1): area 46d (partly including area 9/46d of Petrides and Pandya [Petrides and Pandya 1999]), area 46v (partly including area 9/46v of Petrides and Pandya [Petrides and Pandya 1999]), areas 9/8B (i.e. the dorsolateral convexity including the caudal part of area 9l and the rostral part of area 8B), and area 12l (partly including area 45A). These prefrontal sectors were identified visually in accordance with previous reports (Petrides and Pandya 1999, 2001b; see also Walker 1940). Detailed descriptions of the injection sites are made in the Results section. In each monkey, a total of 7–10 µL of a viral suspension (1.4 × 10⁸ focus-forming units/µL) was injected over 4–10 penetrations (1 or 2 µL per penetration) at least 1 mm apart from each other. In the remaining four monkeys, one of the following conventional tracers was injected into a given LPFC sector (Table 1): Diamidino yellow (DY; Sigma, St Louis, MI, USA; 3% suspension; 1 µL×2 penetrations), Fast blue (FB; Sigma; 5% suspension; 1 µL×3 penetrations), and wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP; Sigma; 4% solution; 0.1 µL×3 penetrations). The injections of rabies virus and the conventional tracers were performed slowly over 10 min through a 1 or 10-µL Hamilton microsyringe, and the injection needle was kept in place for another 10 min. For the penetration of the injection needle along the principal sulcus (i.e. area 46d and area 46v injections), the injection sites were placed 3 and 5-mm deep from the cortical surface (Supplementary Material Fig. 1). An analgesic (diclofenac sodium; 6.25 mg, suppository) was given immediately after surgery. Antibiotic (ceftazidine; 20 mg/kg, iv) was administered for 5 days including the day of surgery.

Survival periods were 3 days for rabies/WGA-HRP injections, and 23 days for DY/FB injections (Table 1). According to the previous studies using rabies virus as transneuronal tracer (Kelly and Strick 2000; Miyachi et al. 2005, 2006; Lu et al. 2007; Ninomiya et al. 2011), 3-day survival periods are optimal to observe the second-order labeling in the cerebral cortex, as well as in the cortico-basal ganglia and cortico-cerebellar systems. Note that the viral batch used in the present study was the same as in our previous studies (Miyachi et al. 2005, 2006; Lu et al. 2007; Hashimoto et al. 2010; Ninomiya et al. 2011; Saga et al. 2011). After these survival periods, the monkeys were anesthetized deeply with sodium pentobarbital (50 mg/kg, iv) and then underwent perfusion-fixation. For the individuals with rabies injections, a mixture of 10% formalin and 15% saturated picric acid in 0.1 M phosphate buffer was used as fixative. The brains were postfixed in the same fixative for 1 day. For the individuals with DY/FB injections, 10% formalin was used. Monkeys with WGA-HRP injections were perfused with 8% formalin, followed by 10% sucrose. Subsequently, all the brains were stored in 0.1 M phosphate-buffered saline containing 30% sucrose at 4°C.

Histological Procedures

After saturation with 30% sucrose, the brains were cut serially into 60-µm thick coronal sections on a freezing microtome. For the visualization of injected and transported rabies virus, every sixth section was immunohistochemically stained with a monospecific rabbit anti-serum (Inoue et al. 2003) according to the procedures described elsewhere (Miyachi et al. 2005). A series of the adjacent sections (60 µm apart) were Nissl-stained with 1% Cresyl violet. For the visualization of injected and transported WGA-HRP, a series of sections were reacted with tetramethylbenzidine (TMB) and incubated for 30 min in a 3% solution of ammonium molydate to stabilize the TMB reaction product. The sections were counterstained with 1% Neutral red and observed under a light microscope. A series of sections with DY/FB injections were observed under a fluorescent microscope.

Data Analyses

Locations of labeled neurons were plotted with camera lucida on tracings of representative coronal sections (720 µm apart) through the inferior/medial temporal cortex. To examine the distribution and density of labeled neurons in the inferior/medial temporal cortex, we made unfolded maps (two-dimensional density maps) according to the procedures as described elsewhere (Hatanaka et al. 2003). In the present case, all unfolded lines were aligned rostrocaudally at the fundus of the superior temporal sulcus (STS). Counts of labeled neurons in individual regions of the MTL and the adjoining inferotemporal cortex (Fig. 1) were performed in every 12th section (720 µm apart) by visual inspection under a light microscope.

Safety Issues

The entire experiments were carried out in a special primate laboratory (biosafety level 2) designated for in vivo viral experiments. Throughout the experiments, the monkeys were kept in individual cages, which were placed inside a special safety cabinet. To avoid accidental infection with the virus, all investigators received immunization beforehand and wore protective clothing during the experimental sessions. Equipment was disinfected with 80% (v/v) ethanol after each experimental session, and wastes were autoclaved prior to disposal.

Results

Retrograde Neuron Labeling in MTL After Prefrontal Injections of Rabies Virus and Conventional Tracers

As summarized in Table 1, rabies virus was injected separately into the following four sectors of the LPFC: area 46d (partly including area 9/46d), area 46v (partly including area 9/46v), areas 9/8B (the dorsolateral convexity), and area 12l (including the rostral part of area 45A). In each case, the rabies injections were made over multiple penetrations. The location of the injection is indicated on the surface map (inset of Figs 2–4, see Supplementary Material Figs 2 and 3). The extent of the
rabies injection site was estimated based on the previous finding that the initial spread of the viral suspension is well confined within 1 mm from the needle track (Kelly and Strick 2003; Miyachi et al. 2005; Hashimoto et al. 2010).

Three days after the rabies injections, the distribution of retrogradely labeled second-order (disynaptically-connected) neurons was examined in the MTL and the adjacent inferotemporal cortex. In the present study, the nomenclature of the MTL was adopted according to the parcellation of Amaral et al. (Amaral et al. 1983) and Suzuki and Amaral (Suzuki and Amaral 2003) (Fig. 1). The MTL consists of the hippocampal formation, the perirhinal cortex, and the parahippocampal cortex. Following Amaral et al. (Amaral et al. 1987), the hippocampus, the subiculum, and the entorhinal cortex are included as the hippocampal formation. The parahippocampal cortex is classified primarily into areas TH, TFm, and TFi. The perirhinal cortex includes areas 35 and 36. The inferotemporal cortex was divided into area TE (i.e. the inferotemporal convexity) and the lower bank of the STS (Saleem et al. 2000). The distribution patterns of labeled neurons in these

![Figure 1](https://example.com/figure1.png)
Figure 2. (A) The distribution of labeled neurons in the inferior/medial temporal cortex 3 days after rabies injections into area 46d of monkey Be. Eight representative coronal sections are arranged rostrocaudally in (a)–(h). Shown in the lateral view of the brain (inset) are the approximate rostrocaudal levels of the sections (a–h) and the injection sites (specified by the gray area). Surface-view reconstructions showing the distribution pattern of labeled neurons are also indicated. Cell counts were done in coronal sections 720 µm apart. The size of each bin is 1 mm. Four different sizes of filled circles represent the ranges of labeled neuron number. Arrows (a–h) point to the approximate rostrocaudal levels of the coronal sections (a–h) depicted on the left. Other conventions are as in Figure 1B. (B) The distribution of labeled neurons in the inferior/medial temporal cortex after DY injections into area 46d. Five representative coronal sections are arranged rostrocaudally in (a)–(e). In (A) and (B), all abbreviations are as in Figure 1.
MTL regions largely differed by the injection sites in the LPFC. We judged that the rabies labeling in each case reached second-order neurons based on the extent to which neuronal labeling via the thalamus appeared within the basal ganglia: the labeled neurons were seen in the internal segment of the globus pallidus and the substantia nigra pars reticulata, but not in the external segment of the globus pallidus or the striatum (see also Materials and Methods for the rate of transsynaptic transport of the virus). To confirm the localization of first-order (monosynaptically connected) neurons, conventional tracers (DY, FB, and WGA-HRP) were injected into the four LPFC sectors, as shown in Table 1.

Distribution of Neuronal Labeling From Area 46d
In two monkeys (monkeys Be and Aa), rabies injections were performed into the dorsal bank and lip of the principal sulcus, corresponding to area 46d (partly involving area 9/46d of Petrides and Pandya [Petrides and Pandya 1999]). In

Figure 3. (A) The distribution of labeled neurons in the inferior/medial temporal cortex 3 days after rabies injections into area 46v (monkey Zo). All conventions are as in Figure 2A. (B) The distribution of labeled neurons in the inferior/medial temporal cortex after FB injections into area 46v. All conventions are as in Figure 2B. In (A) and (B), all abbreviations are as in Figure 1.
monkey Be, the caudalmost level of multiple injections was 2-mm rostral to the rostral end of the superior limb of the arcuate sulcus, and the injection sites extended 4-mm rostral therefrom. The site of the rabies injections involved both the depth and the lip of the dorsal bank of the principal sulcus (Fig. 2, Supplementary Material Figs 1 and 2A). In addition to dense labeling around the injection site in the dorsal bank of the sulcus, many labeled neurons were seen in the ventral bank of the sulcus. This corresponds well with the fact that area 46d (and area 9/46d) receives strong projections directly from area 46v (Petrides and Pandya 1999). In two monkeys (Fig. 2A for monkey Be; see also Supplementary Material Fig. 2A for monkey Aa), retrogradely labeled neurons were widely distributed over the temporal cortex at the 3-day post-injection period. Dense neuronal labeling occurred in the MTL, predominantly in the entorhinal cortex of the hippocampal formation and the parahippocampal cortex (areas TFI, TFm, and TH), and, to a lesser extent, in the perirhinal cortex.
The hippocampus proper and the subiculum also contained labeled neurons. In the hippocampus proper, labeled neurons were seen mostly in the CA1 region. Additional labeling of temporal cortical neurons was found in the inferotemporal cortex, especially in the lower bank of the STS. Furthermore, a large number of labeled neurons were observed in the upper bank and fundus of the STS (Fig. 2A) and the posterior cingulate cortex, retrosplenial cortex, and medial parietal cortex (Supplementary Material Fig. 3). Each of which has been reported to connect directly with both the LPFC and the MTL (Seltzer and Pandya 1994; Petrides and Pandya 1999, 2001; Kobayashi and Amaral 2003, 2007). Many labeled neurons were found in the amygdala, in particular abundance in the basal nucleus (Fig. 2A).

Consistent with data obtained in previous studies (Barbas and Mesulam 1985; Petrides and Pandya 1999), DY injections into area 46d resulted in neuronal labeling in the upper bank of the STS (Fig. 2B) and the posterior cingulate cortex and medial parietal cortex (data not shown). A few neurons were labeled in the lateral bank of the intraparietal sulcus (IPS). Only a few neurons were labeled in the perirhinal cortex, and other MTL regions were virtually devoid of labeled neurons (Fig. 2B). No labeled neurons were seen in the inferotemporal cortex including the inferotemporal convexity (area TE) and the lower bank of the STS.

**Distribution of Neuronal Labeling From Area 46v**

In two monkeys (monkeys Zo and Ju), rabies injections were performed into the ventral bank and lip of the principal sulcus, corresponding to area 46v (partly involving area 9/46v of Petrides and Pandya [Petrides and Pandya 1999]). In monkey Zo, the injection sites were placed most caudally at the level of the rostral end of the superior limb of the arcuate
sulcus and most rostrally at the level 7-mm rostral therefrom (Fig. 3A). The site of the rabies injections involved both the depth and the lip of the ventral bank of the principal sulcus (Fig. 3, Supplementary Material Figs. 1 and 2B). In the temporal lobe, 3 days after the injections, many labeled neurons were seen as in the case of the area 46d injections (Fig. 3A for monkey Zo; see also Supplementary Material Fig. 2B for monkey Ju). However, the distribution patterns in the area 46d versus area 46v injection cases were quite different from each other. After the area 46v injections, a large number of labeled neurons were located around the STS, especially in the lower bank. Additionally, a small number of labeled neurons were distributed in the inferotemporal convexity (area TE). Moreover, some labeled neurons were observed in the MTL. Most of them were distributed in the entorhinal cortex of the hippocampal formation, the perirhinal cortex, and area TFl of the parahippocampal cortex, though the number of the labeled neurons was much smaller than in the case of the area 46d injections (Figs. 3A and 5B). No neurons were labeled in either the hippocampus proper or the subiculum. Only a few labeled neurons were found in the posterior cingulate cortex and the retrosplenial cortex, whereas a large number of neurons were labeled in the lateral bank of the IPS and adjacent inferior parietal convexity (Supplementary Material Fig. 3B). In the amygdala, the basal nucleus contained a dense cluster of labeled neurons (Fig. 3A).

In favor of previous findings (Barbas and Mesulam 1985), FB injections into area 46v produced neuronal labeling in the fundus and upper bank of the STS (Fig. 3B). On the other hand, the inferotemporal cortex was devoid of labeled neurons. Virtually no labeled neurons were found in the MTL, except that a small number of labeled neurons were scattered in the perirhinal cortex and area TFl of the parahippocampal cortex (Fig. 3B). In the parietal cortex, neuronal labeling was found in the lateral bank of the IPS (mainly the rostral part) and the adjacent convexity (see also Petrides and Pandya 2001).

Distribution of Neuronal Labeling From Areas 9l/8B

In two monkeys (Ke and Ja), the rabies injection sites were located medial to the suprangular principal dimple, and included the caudal part of area 9l and the rostral part of area 8B of Petrides and Pandya (Petrides and Pandya 1999). The injection site was placed near the border with area 9/46d, which is characterized by relatively well-developed layer IV (Supplementary Material Fig. 1). Three days after rabies injections, a large number of labeled neurons were located in the upper bank of the STS (Fig. 4A for monkey Ke; see also Supplementary Material Fig. 2C for monkey Ja). These labeled neurons were distributed in a columnar fashion with the higher density in more caudal portions. Only a few labeled neurons were observed in area TE. Neuronal labeling was further seen in the MTL regions, such as the entorhinal cortex and parahippocampal areas (areas TFl, TFm, and TH). However, the number of these labeled MTL neurons was much smaller than in the case of the area 46d injections (Figs. 4A and 5C). No labeled neurons were found in either the hippocampus proper or the subiculum. Like the case of area 46d injections, many neurons were labeled in the posterior cingulate cortex, retrosplenial cortex, and medial parietal cortex, though the dense neuronal labeling was limited at the most ventral part (Supplementary Material Fig. 3C). In addition, labeled neurons were found in the most posterior part of the lateral bank of the IPS and the inferior parietal convexity. Considerable neuronal labeling was seen in the basal nucleus of the amygdala (Fig. 4A).

As previously reported (Petrides and Pandya 1999), many neurons in the upper bank of the STS were labeled after WGA-HRP injections into areas 9l/8B (data not shown). Very few, if any, labeled neurons were detected in either the inferotemporal cortex or the MTL. In addition, a small number of neurons were labeled in the posterior cingulate cortex, the retrosplenial cortex, as well as in the caudal portion of the lateral bank of the IPS.

Distribution of Neuronal Labeling From Area 12l

In one monkey (Br), rabies injections were made into the inferior convexity of the prefrontal cortex. The most part of the injection site was placed in area 12l, though some of the caudally situated needle tracks were found in area 45A, which is characterized by large pyramidal neurons in the deeper part of layer III. In this case, a tremendous number of neurons were labeled in the inferotemporal cortex: two extremely dense clusters of labeled neurons were observed rostrally and caudally in both the lower bank of the STS and the inferotemporal convexity (area TE) (Fig. 4B). When compared with the other prefrontal injections, the area 12l injections conferred very sparse neuronal labeling in the upper bank of the STS. Within the MTL, a number of labeled neurons were located in the perirhinal cortex, and only a few labeled neurons were found in the entorhinal and parahippocampal cortical areas (Figs 4B and 5D). The hippocampus proper and the subiculum were both devoid of labeled neurons. The basal nucleus of the amygdala contained many labeled neurons (Fig. 4B). Like the case of area 46v, a large number of neurons were labeled in the lateral bank of the IPS, but not in the inferior parietal convexity (Supplementary Material Fig. 3D).

As previously reported (Petrides and Pandya 2001), WGA-HRP injections into area 12l labeled a large number of neurons in the inferotemporal cortex, both the lower bank of the STS and the inferotemporal convexity (area TE) (data not shown). No labeled neurons were found in the MTL. In the parietal cortex, dense neuronal labeling was found in the lateral bank of the IPS (mainly the rostral part) and the rostral part of the inferior parietal convexity. Virtually no neuronal labeling was found in the posterior cingulate cortex, retrosplenial cortex, and the medial wall of the parietal cortex (Petrides and Pandya 2001).

Discussion

To examine the organization of multisynaptic pathways from the MTL to the LPFC, we injected rabies virus separately into four distinct sectors of the monkey LPFC: area 46d, area 46v, areas 9l/8B, and area 12l. In each injection case, the distribution pattern of retrogradely labeled neurons was analyzed in the MTL and the neighboring temporal cortical areas at the 3-day postinjection period when the second-order neuron labeling occurred. This stage of transneuronal labeling has been examined repeatedly in previous studies (Kelly and Strick 2000; Miyachi et al. 2005, 2006; Lu et al. 2007; Hashimoto et al. 2010; Ninomiya et al. 2011; Saga et al. 2011). In

Downloaded from https://academic.oup.com/cercor/article-abstract/23/12/2965/469280 by guest on 21 March 2019
In the present work, we further observed that some rabies-labeled neurons were located in the hippocampus proper (i.e. the CA1 region). Such disinaptic projections to area 46d could be mediated through the medial prefrontal and orbitofrontal areas (Barbas and Blatt 1995; Carmichael and Price 1995).

Concerning the functions of area 46d, Petrides has proposed that the dorsal aspect of the dorsal LPFC (“mid-dorsolateral prefrontal cortex”; corresponding to the central part of area 46d and area 9I) is crucial for monitoring of multiple contents of working memory, but not for simple visual short-term memory (Petrides 1991b, 2000a; for review, see Petrides 2000b). Hasegawa et al. (Hasegawa et al. 2004) have provided electrophysiological data supporting this idea. Hoshi and Tanji (Hoshi and Tanji 2004) have further reported that neurons in the dorsal aspect of the dorsal LPFC, especially in area 46d, principally code the target to reach (left or right) or the arm to use (left or right) for reaching behavior depending on a combination of two visual cues. In addition, Fuster et al. (Fuster et al. 2000) have shown that the dorsal aspect of the dorsal LPFC is responsible for cross-modal (audio-visual) association. These findings suggest a specific role for this region in executive functions, for example decision-making, based on association/conjunction memory on multiple objects/events. On the other hand, recent emphasis has been placed on the involvement of the MTL in the generation of association/conjunction memory. According to previous electrophysiological studies (Higuchi and Miyashita 1996; Naya et al. 2005), neuronal activity in the MTL, especially in the perirhinal cortex, represents paired associates of visual objects, and such activity critically contributes to pair-coding activity in area TE. Studies in human subjects (including patients) and experimental animals also indicate the importance of the MTL for memory on associations or relations of multiple objects/events (Alvarado et al. 2002; Alvarado and Bachevalier 2005; Olson et al. 2006; Browning et al. 2010). More recently, Heuer and Bachevalier have reported that hippocampal lesions in macaques affected the monitoring of multiple contents of working memory (Heuer and Bachevalier 2011). Taken together, the MTL and the dorsal aspect of the dorsal LPFC might probably exert cooperative actions on association/conjunction memory functions and cognitive behaviors based on such memory (Browning and Gaffan 2008). Thus, it is most likely that multisynaptic pathways connecting the two cortical areas participate in this mechanism. With respect to the dorsal aspect of the dorsal LPFC, however, neither functional nor hodological differences between area 46d and area 9I have so far been identified. In a series of experiments with the mid-dorsolateral prefrontal cortex lesioned, Petrides (Petrides 2000a) described that large lesions involving area 46d conferred more drastic behavioral effects than lesions restricted to area 9I. The present results have elucidated that area 46d receives dense input disinaptically from the MTL regions, whereas area 9I does not. This implies that area 46d may be a key structure for control of memory-based cognitive behaviors, which specifically receives disinaptic input from the MTL (Fig. 6).

Our rabies injections into area 46v, areas 9I/8B, and area 12I (partly including area 45A) yielded only weak disinaptic labeling of MTL neurons. Instead, many neurons in the inferotemporal cortex, including the STS lower bank, were labeled
after the injections into area 46v and area 12l. According to prior anatomical findings (Barbas 1988; Petrides and Pandya 2001), the inferotemporal cortex, especially the STS lower bank, projects directly to the ventrolateral prefrontal cortex including area 12l, which was also verified by our conventional tracer injection experiments. On the other hand, it has been reported that area 46v receives direct projections from the fundus and upper bank of the STS, but not from the inferotemporal cortex (Barbas and Mesulam 1985; Seltzer and Pandya 1990), which was also reconfirmed in the present study. Based on previous works (Barbas and Mesulam 1985; Barbas 1988, 1993; Petrides and Pandya 2001), the ventral LPFC and the orbitofrontal cortex receive inputs directly from the inferotemporal cortex and, in turn, send outputs to area 46v. Thus, the disynaptic projections from the inferotemporal cortex to area 46v may possibly be relayed via these prefrontal areas (Fig. 6). It has been demonstrated that neurons in area 46v become active in response to the process of decision-making depending on the shape of visual stimuli (Miller et al. 1996; Hoshi et al. 2000). Together with the present data, this suggests that area 46v may receive such visual information from the inferotemporal cortex (especially the STS lower bank) through the disynaptic projection. In conclusion, our study has revealed that among the LPFC, area 46d preferentially receives strong disynaptic inputs from the MTL, whereas area 46v is dominated by the inferotemporal cortex input. The multisynaptic bottom-up pathways linking the MTL to area 46d may play pivotal roles in memory-based cognitive behaviors, for example, decision-making.

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

Funding
This work was supported by Grants-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology of Japan (20019021 to S.M., 17021050 to M.T.), and by Japan Science and Technology Agency, CREST.

Notes
We thank Ms. M. Imanishi and T. Kuroda for excellent technical support. Conflict of Interest: None declared.

References