Efferent projections of infralimbic and prelimbic areas of the medial prefrontal cortex in the Japanese monkey, *Macaca fuscata*

Tanemichi Chiba\(^a\)*, Tetsuro Kayahara\(^b\), Katsuma Nakano\(^b\)

\(^a\)Department of Anatomy and Neurobiology, Chiba University School of Medicine, Inohana 1-8-1, Chuo-Ku, Chiba 260-8670, Japan

\(^b\)Mie University School of Medicine, Tsu 514-8507, Japan

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**Abstract**

The infralimbic area (IL) and prelimbic area (PL) have been postulated as an autonomic motor region in the medial prefrontal cortex. The present study was conducted to reveal the projection sites of IL and PL of the monkey, *Macaca fuscata*, using biotinylated dextran amine as an anterograde tracer. IL and PL projected densely to the ventromedial caudate nucleus, the core and shell of the nucleus accumbens (Acb), parcellricular basal and magnocellular accessory basal nuclei of the amygdala, lateral preoptic area, ventromedial hypothalamic nucleus, tubero-mammillary nucleus (TM), medial part of the magnocellular and dorsal part of the parvicellular (MDpc) dorsomedial thalamic nuclei, reuniens and medial part of the medial pulvinar nucleus, and dorso-lateral part of the periaqueductal gray (PAGdl) in the mesencephalon. Moderately to weakly projected areas were the intermediate and lateral parts of the agranular insular cortex, orbital part of area 12, agranular and dysgranular part of the temporal pole cortex (TPa-g), auditory temporal cortex, lateral and medial (MS) septal nuclei, bed nucleus of the stria terminalis, diagonal band of Broca, substantia innominata, and medial preoptic area, dorsomedial, lateral, and posterior hypothalamic nuclei, magnocellular lateral basal and lateral amygdaloid nuclei, paratelenoidal, paraventricular (PV), inter-antero-medial (IAM), reticular, central medial (CeM), parafascicular (PF) and limitans nuclei of the thalamus, lateral habenular nucleus, pedunculo-pontine nucleus, dorsal part of the lateral lemniscal nucleus, ventral tegmental area (VTA), dorsal raphe, superior central nucleus, medial and lateral parabrachial nuclei (PBl) and nucleus locus coeruleus (LC). A few scattered terminals were observed in the perifornical nucleus of the hypothalamus and substantia nigra pars compacta. PL and area 24 were characterized by projections to the entorhinal (Ent) and piriform (Pir) cortex as well as to the magnocellular part of the ventral anterior thalamic nucleus (VAmc). The morphology of the terminal arborization in each nuclei was different in appearance, perhaps reflecting the synaptic interaction between the nerve terminals and postsynaptic dendrites. PL projected uniquely to Ent, Pir and VAmc and IL projected uniquely to TPa-g, MS, IAM, CeM, MDpc, PF, PBl and LC. IL projected more strongly than PL to the shell of Acb, amygdaloid nuclei, PV, TM, VTA and PAGdl. The present results support the hypothesis that IL is a major cortical autonomic motor area and PL integrates limbic and autonomic inputs in the primate. © 2001 Elsevier Science B.V. All rights reserved.

**Theme:** Other systems of the CNS

**Abbreviations:** ABmc, accessory basal amygdaloid nucleus, magnocellular part; Acb, accumbens nucleus; BLmc, basolateral amygdaloid nucleus, magnocellular part; BLpc, basolateral amygdaloid nucleus, parvicellular part; BST, bed nucleus of stria terminalis; CeM, central medial thalamic nucleus; CDvm, caudate nucleus, ventromedial part; DB, nucleus of diagonal band; DMH, dorsomedial nucleus of hypothalamus; DR, dorsal raphe nucleus; Ent, entorhinal cortex; Hbl, lateral habenular nucleus; Iai, agranular insular cortex, intermediate part; Ial, agranular insular cortex, lateral part; Iam, agranular insular cortex, medial part; IAM, interanteromedial nucleus of thalamus; Iapm, agranular insular cortex, posteromedial part; L, lateral amygdaloid nucleus; LC, nucleus of locus coeruleus; LH, lateral hypothalamic nucleus; Lim, limitans nucleus of thalamus; LLd, dorsal nucleus of lateral lemniscus; LS, lateral septal nucleus; MDmc, dorsomedial nucleus of thalamus, magnocellular part; MDpc, dorsomedial nucleus of thalamus, parvicellular part; MS, medial septal nucleus; NCS, superior central nucleus; NTS, nucleus of tractus solitarius; PAG, periaqueductal gray; PAGdl, periaqueductal gray, dorso-lateral part; PBl, lateral parabrachial nucleus; PMd, medial parabrachial nucleus; PF, parafascicular nucleus of thalamus; PFC, prefrontal cortex; PHi, perifascicular nucleus of hypothalamus; PIR, posterior hypothalamic nucleus; PX, piriform cortex; PM, medial pulvinar nucleus; POK, lateral preoptic nucleus; POM, medial preoptic nucleus; PPp, pedunculo-pontine nucleus; PT, pretectal nucleus of thalamus; PV, paraventricular nucleus of thalamus; PVH, paraventricular nucleus of hypothalamus; R, reticular nucleus of thalamus; Re, reunions nucleus of thalamus; RPC, reticular parvicellular nucleus; SI, substantia innominata; SNc, substantia nigra, pars compacta; TA, temporal auditory cortical area; TM, tubero-mammillary nucleus of hypothalamus; TP, temporopolar cortex; TPa-g, temporopolar cortex, agranular area; TPdg, temporopolar cortex, dysgranular area; VAmc, ventral anterior nucleus of thalamus, magnocellular part; VMH, ventromedial nucleus of hypothalamus; VTA, ventral tegmental area; ZI, zona incerta; 12o, area 12o of prefrontal cortex; 12l, area 12l of prefrontal cortex

*Corresponding author. Tel.: +81-43-226-2022; fax: +81-43-226-2025.

E-mail address: tiba@med.m.chiba-u.ac.jp (T. Chiba).
1. Introduction

The medial and ventral parts of the frontal lobe of the monkey can modulate autonomic parameters [21,41,59] and the role of the ventromedial frontal lobe in autonomic function is that individuals with lesions of this area are unable to generate autonomic responses to emotional stimuli. Remarkably, they are also impaired in making judgments about the consequences of their actions in social situations, despite possessing the knowledge necessary to make the correct decision [9,18,19]. Damasio and his co-workers [19] have suggested that these two deficits are related and that the sociopath-behavior of these patients is due to their inability to generate ‘somatic markers’ that tag behavioral options as desirable or not [10]. The frontal lobe damage would lead to loss of affective responsiveness and foresight arising from interoceptive agnosia [32]. Damasio [18] extended this concept to explain the ‘acquired sociopathy’ of patients with bilateral orbitofrontal damage.

The viscerosensory and visceromotor areas in the frontal lobe are suggested to be localized in the agranular insular, infralimbic and prelimbic cortex (IL and PL). The viscerosensory inputs reach specific areas within the agranular insula in primates as in rodents [14]. Thus, IL and PL have been postulated to be an autonomic motor area in the medial prefrontal cortex (PFC), as efferent and afferent connections of IL in the rat were examined by several workers and IL and PL were found to be reciprocally connected with most central autonomic nuclei as far as the spinal cord in the rat [5,4,27,54].

As recent studies revealed, the cytoarchitectonic map and histochemical characteristics of the monkey PFC including IL and PL [14], we designed the present study so as to reveal the projection sites of the medial PFC of the monkey, Macaca fuscata, using biotinylated dextran amine (BDA) as an anterograde tracer. The injection sites covered PL, IL and the adjacent medial PFC including area 14, and area 24b. We concentrated on the projection pattern of IL compared with PL in this study. Part of the present studies were presented as preliminary reports on several occasions [17,38,39]. During the preparation of this manuscript, two important results of projection studies of the medial prefrontal cortex of macaque monkeys, Macaca fascicularis and Macaca nemestrina, were reported [3,43]. The major results were similar to ours except for a few different findings and the present results were carefully compared and discussed with those of their studies. We used BDA as a marker useful for precisely identifying the injection sites and also followed very thin axons of the autonomic neurons in the central nervous system for a considerable distance.

2. Materials and methods

Ten adult Japanese monkeys, Macaca fuscata, of both sexes and 3.5–5.5 kg body weight were used in the present study. All animal protocols were reviewed and approved by the Animal Studies Committee of Mie University. The animals were anesthetized with intramuscular injection of ketamine hydrochloride (10 mg/kg) and then with pentobarbital (25 mg/kg). The animals were fixed on stereotaxic apparatus, and 0.1–1.0 μ1×1–3 times of 5% biotinylated dextran amine (BDA, 10 000 MW, Molecular Probes Inc.) in physiological saline was injected through a glass micropipette (inner diameter of the tip was 30–50 μm) using a pneumatic picopump, Model PV800, under an operation microscope. A period of 14–21 days later, the animals were anesthetized again and fixed by perfusion with 1 l/kg body weight of 8% formalin (3.2% formaldehyde), 0.2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and 1000 ml of 10% sucrose in phosphate buffer. After the perfusion, the brain was removed and placed in the 25% sucrose in phosphate buffer at 4°C for a few days. Serial coronal sections were then cut at 50 μm thickness with a freezing microtome. The serial sections were classified into three groups and processed as follows. (1) The sections were rinsed with 0.1 M phosphate buffered-saline (PBS) and incubated in a solution composed of 15–17 μl of streptavidine, 1 ml of 2.5% Triton X-100, and 4 ml of 0.1 M PBS overnight. After rinsing five times with PBS, the sections were incubated for 3–5 h with dianimobenzidine solution composed of 50 ml 0.05 M Tris–HCl buffer (pH 7.6), 10 mg dianimobenzidine and 125 mg nickel ammonium, and added with 80–100 μl of 0.3% H2O2. (2) The sections were processed as in (1), but nickel ammonium was omitted from the final reaction solution and they were observed under-dark field optics. (3) The sections were rinsed with the phosphate buffer, processed for Nissl staining, and observed by microscope and used for reference to identify nuclear orientation. A total of ten animals were used for the present study and seven of them were used for the following data analysis. Animal number, sex, body weight, and BDA injection method of each animal are shown in the following list.
Animal | Sex | Body weight (kg) | Injection method of BDA |
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M686 | Female | 4.7 | Injected 3 times in adjacent sites with 1.0, 1.0 and 1.0 μl while withdrawing the micropipette. |
M707 | Female | 4.5 | Injected 2 times, 1.0 ml in one site and 0.8+0.2 μl in the adjacent site. |
M719 | Female | 3.9 | Injected 3 times in three adjacent sites, 0.6+0.6 μl, 0.5 and 0.6 μl. |
M723 | Female | 6.8 | Injected 2 times in the same site with 0.6+0.6 μl. |
M738 | Female | 3.0 | Injected 2 times in two adjacent sites, 0.3+0.3 and 0.3+0.25 μl. |
M746 | Male | 4.0 | Injected 2 times in the same site with 0.2+0.25 μl and 0.3 μl in the adjacent site. |
M776 | Female | 2.9 | Injected 3 times in the same site with 0.1+0.1+1.15 μl. |

3. Results

3.1. Injection sites

From a series of BDA injections into the medial prefrontal cortex of monkeys, seven cases were selected for the present analyses of the projection pattern of the medial prefrontal cortex including the anterior cingulate cortex (area 24b), IL (area 25) and PL (area 32). Injection sites were identified by the distribution of pyramidal cells labeled by the uptake of injected BDA, reconstructed from the serial frontal sections and were projected to the saggital plane of the medial prefrontal cortex as illustrated in Fig. 1A and B. The injection area of each animal was determined by referring to the atlas of the medial prefrontal plane by Carmichael and Price [14] to identify the approximate cortical area. The injection sites of cases M686 and M707 corresponded approximately to area 25 and caudal area 32. Those of case M723 corresponded to rostral area 32 and caudal area 10m and case M746 to rostral area 32. The injection sites of case M719 and M776 corresponded to area 24b and case M738 to the middle of area 10m. The cortical area was identified by referring to the cytoarchitectonic and immunohistochemical map of the rhesus monkey by Carmichael and Price [14]. Cases M707 and M686 were used for analysis of the projection sites of area 25 and M723 and M746 for that of the projection sites of area 32. The results of cases M719, M738 and M776 were used as control injections for areas 24b, 10m and 24a respectively.

3.2. Case M686

DAB was injected into three adjacent locations of area 25 in this case as illustrated in Fig. 1. The distribution of projection nerve terminals was illustrated in Fig. 2. The densest network of nerve terminals with many varicosities was observed in the ventro-medial caudate nucleus (CDvm), core and shell of the nucleus accumbens (Acb), magnocellular accessory basal and parvicellular lateral basal (ABmc, BLpc) nuclei of the amygdala, and the magnocellular part of the mediodorsal (MDmc) nuclei of the thalamus. Moderately to weakly projected areas were the lateral septum (LS), bed nucleus of the stria terminalis (BST), diagonal band of Broca (DB), substantia innominata (SI), dorsomedial, ventro-medial, lateral and posterior hypothalamic nuclei (DMH, VMH, LH, PH).

Fig. 1. Schema demonstrating the location of injection sites of BDA in seven monkeys used in the present analysis. (A) Injection sites of seven monkeys were shown reconstructed from serial prefrontal sections and projected to the parasagittal plane. (B) The injection sites of BDA in the present study were superimposed on the map of the cytoarchitectonic cortical area of the prefrontal cortex of Carmichael and Price [14].
Fig. 2. A series of frontal sections of M686 demonstrating the distribution of nerve terminals labeled by BDA.

tubero-mammillary nucleus (TM), magnocellular part of basolateral and lateral amygdaloid nuclei (BLmc, L), paratenial, paraventricular, reticular, inter-antero-medial, central medial, reunience, parvicellular part of mediodorsal, medial part of the medial pulvinar and limitans nuclei of the thalamus (PT, PV, R, IAM, CeM, Re, MDpc, PM,
between the nerve terminals and postsynaptic dendrites may have been responsible. Typical photomicrographs of such terminals were observed in the shell and core of Acb (Fig. 4B), BL and AB amygdaloid nuclei (Fig. 4A), TM hypothalamic nucleus (Fig. 4B), MDmc (Fig. 4C) and PM thalamic nuclei (Fig. 4C) and TP (Fig. 4A).

3.4. Case M746

The injection site in this case was localized in the rostral part of area 32 as shown in Fig. 1. Moderately to weakly labeled networks of nerve terminals were observed in Iai, 12o of the frontal cortex, TA of the temporal cortex, core and shell of Acb, DB, LS, CDvm, SI, as well as Ent and Pir cortex. The nerve terminals were also seen in PT, PV, R, Re, MDmc, PM and Lim of the thalamus, POL, DMH, VMH, LH, PH and TM of the hypothalamus, Hbl, PPN, LLd, VTA, DR, NCS and PAGdl of the midbrain (Fig. 5).

3.5. Case M723

DAB was injected into the rostral part of area 32 and caudal part of area 10m as illustrated in Fig. 1. Moderately to weakly labeled nerve terminals with varicosities were distributed in Iai, 12o and the medial part of the agranular insular cortex (Ial), orbital part of area 14 and 13 of the frontal cortex, TA, of the agranular insular cortex, TPdg and TA of the temporopolar cortex, the core of Acb, DB, BST, LS, CDvm, SI, as well as Ent and Pir in the frontal cortex, ABmc, BLmc, BLpc and L of the hypothalamus, Hbl, VTA, DR, NCS, and PAGdl of the midbrain (Fig. 6).

3.6. Case M719

DAB was injected almost exclusively in area 24b in this case as illustrated in Fig. 1. Medium to weakly labeled networks of nerve terminals were observed in Iai, Ial and areas 14 and 13 of the frontal cortex, TA of the temporopolar cortex, core and shell of Acb, CDvm, DB, BST, LS, MS, SI and Pir in the cortex, ABmc, BLmc and BLpc of the amygdala, PV, R, IAM, CeM, Re, VAmc, MDmc, MDpc, Lim of the thalamus, POL, POM, DMH, VMH, LH, PH, TM, and PH of the hypothalamus, Hbl, VTA, SNc, DR, NCS, PAGdl, PBl and LC of the midbrain (Fig. 7).

3.7. Case M776

DAB was injected in the caudal part of area 24b and a small part of area 25a in this case as depicted in Fig. 1. A strongly labeled nerve terminal network was observed in Lim, ventral tegmental area (VTA, A10), substantia nigra pars compacta (SNc), dorsolateral part of the periaqueductal gray (PAGdl) and nucleus locus coeruleus (LC).

3.3. Case M707

DAB was injected into area 25 and the caudal part of area 32 as seen in Fig. 1. Projection sites of the case M707 were schematically demonstrated in Fig. 3. The densest network of nerve terminals with many varicosities was observed in the CDvm, core and shell of Acb, BLpc and ABmc, lateral preoptic area (POL), VMH, TM, medial part of MDmc and dorsal part of MDpc, Re and PM of the thalamus, PAGdl, pedunculo-pontine nucleus (PPN) and dorsal lateral lemniscal nucleus (LLd).

Moderately to weekly projected areas were the lateral part of the agranular insular cortex (Ial), orbital part of area 12 (12o), agranular and dysgranular part of the temporal pole cortex (TPa-g, TPdg), auditory temporal cortex (TA), medial septal nucleus (MS), BST, DB, SI, medial preoptic area (POM), DMH, LH, PH of the hypothalamus, Blmc, L of the amygdala, and magnocellular part of the ventral-anterior (VAmc), parafascicular (PF) and PT, PV, R, IAM, CeM and Lim of the thalamus, lateral habenular nucleus (Hbl), VTA, dorsal raphe (DR), superior central nucleus (NCS), medial and lateral parabrachial nuclei (PBm and PBl) and LC.

Only a few scattered terminals were observed in the perifornical nucleus of the hypothalamus (PH) and SNc. The morphology of the terminal arborization in each nuclei was different in appearance for which synaptic interaction

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**Fig. 2. (continued)***
Fig. 3. A series of frontal sections of M707 showing the distribution of nerve terminals labeled by BDA. Note that retrogradely labeled nerve cells are also observed in this particular case (indicated by triangles).
CDvm, core and shell of Acb and rostral cingulate cortex. The distribution of labeled terminals was more intense in the core than the shell of Acb. Moderately labeled terminals were seen in the Iai, Ial, and 12o of the prefrontal cortex, and TPa-p segment of the temporo-polar cortex.

Medium density of nerve terminals was seen in DB, LS, MS, SI and PV, MDmc, PF, PM, IAM and CeM of the thalamus. Medium to weak labeling was seen in POL, POM, DMH, VMH, LH, PH, and TM of the hypothalamus, as well as ABmc, BLmc, BLpc in the amygdala. Medium intensity of labeling was also seen in DR, NCS and PAGdl of the midbrain.

3.8. Case M738

The injection site in this case was small and localized in area 10m as shown in Fig. 1. Distribution of labeled nerve terminals was very limited, being mainly found in the
Fig. 4. Representative photomicrographs of nerve terminals and cells labeled by biotinylated dextran amine injected into the medial prefrontal cortex. A network of fine axons and small varicosities is seen in accessory basal (AB) and basolateral (BL) nuclei of amygdala (upper panel, M686). A column-like distribution of labeled nerve terminals and some pyramidal cells in the layer V is observed in the temporo-polar cortex (TP). The surface of the cortex is directed to the left (lower panel, M707). Scale bar=50 μm in AB and BL; 200 μm in TP. A dense network of fine nerve terminals and varicosities is seen in both core and shell of the nucleus accumbens (upper panel, Acb M686). A dense network of axon terminals and varicosities is observed in the tuberomammillary nucleus of the hypothalamus (lower panel, TM M707). Scale bar=100 μm in Acb and 20 μm in TM. A dense network of fine nerve terminals and axon varicosities is seen in the magnocellular part of the dorsomedial nucleus (MD) and medial part of the medial pulvinar nucleus (PM) of the thalamus (lower panel, M707). Scale bar=50 μm.
prefrontal cortex as Iai, Iam, IL and 12o as well as in TA of the temporo-polar cortex. Weakly labeled nerve terminals were scattered in the core and shell of Acb, CDvm, LS, IAM and PF of the thalamus and POL of the hypothalamus.

3.9. A summary of the efferent projections of the IL and PL from the seven cases examined in the present study

In the present study, the projection sites of areas 25 and 32 could be clearly differentiated by comparing the
observations of M686 and M707 versus M746 and M723. Projections to TPa-g, MS, IAM, CeM, MDpc, PF, PBl, and LC were observed from area 25 but not from the area 32. Further, projections to TPdg, TA, CDvm, DB, BST, LS, SI, core and shell of Acb, ABmc, BLmc, BLpc, PT, PV, R, Re, MDmc, PM, Lim, VMH, LH, PH, TM, VTA, and PAGdl were stronger from area 25 than 32. On the other hand, projections to Ent, Pir and VAmc were observed from area 32 but not from area 25. Projection sites of area 24 seemed to be similar but much weaker than that of area 25.

In summary, the projection sites of IL were classified into four major categories (Fig. 8). The first was the limbic sensory integration area including the amygdaloid, tem-
poro-polar cortex, PM and midline nuclei of the thalamus. Second was the autonomic relay nuclei such as LH, PAGdl of the mesencephalon, and parabrachial nuclei. The third was the nuclei related with feedback loop in parallel to the cortico-striato-pallido-thalamic motor loop that is concerned with the coordinated regulation of autonomic motor with behavioral outputs such as shell and core of Acb, ventral pallidum, ventral tegmental area and MD. The fourth was amine containing neuronal cell groups such as histaminergic TM, dopaminergic VTA, serotonergic DR,
and noradrenergic LC that are most likely concerned with the regulation of the synaptic activity in various target nuclei of these amine neurons.

4. Discussion

4.1. Methodological considerations

The present study using BDA as an anterograde tracer clearly demonstrated the distribution of labeled perikarya and dendrites in the injection sites of mPFC, and axons and terminal arborizations with varicosities in the projected areas. The axons of autonomic and limbic nervous systems were extremely thin and it was difficult to identify the labeled ones with a low magnification microscope, but were able to observe them at higher magnifications even if they were scattered in a few fiber bundles or a network of axon terminals. Dark-field optics also helped the observation of thin bundles and terminal axon networks. The optimal duration of survival time after the injection of BDA was presumed to be 2 or 3 weeks, although successful labeling seemed to be intimately related to the amount of uptake of tracers rather than the survival period of the animals. We could follow projection axon terminals as far as the medulla oblongata in this study, and only a few scattered nerve terminals were found in NTS. The locations of the injection sites of BDA were reconstructed from serial frontal sections, superimposed on the parasagittal plane of mPFC and distributed in areas 25, 32, 24b, 10 and 14 of mPFC as shown in Fig. 1. The areas in the mPFC were determined by referring to the map of the monkey described by Carmichael and Price [14]. The actual shape of the brain of the monkeys used in this study varied from animal to animal; also, the species used in this study was the Japanese monkey, *Macaca fuscata*, which belongs to the same class but in different subclass from *Macaca nemestrina, fascicularis* and *mulatta* used by Carmichael and Price [14]. The location maps of the areas were not as exact as those determined cytoarchitectonically or histochemically. The results, however, clearly showed the difference in projection patterns after the tracer was injected in area 25 compared to the other cases in which the marker was injected in rostral area 32, areas 10, 24b and 14.

4.2. Projection to prosencephalon

Projections from IL and PL were observed in Iai, Ial and 12o of the frontal cortex and TPa-g, TPdg and TA of TP. Iai and Ial have been determined as pressor related areas of PFC in the rat [60] and have reciprocal connections with IL and PL of mPFC [54,60]. IL projects to area 14 (*Gyrus rectus*) [37] and PL projects to IL, and areas 14, 24, 9 and 10 [42].

TPa-g, TPdg and TA in TP are also reciprocally connected with IL, PL, and areas 24, 12 and 13 in the monkey [15,42] and the distribution of nerve terminals had a column-like appearance (Fig. 4A). Auditory inputs predominate in the dorsolateral part and visual inputs
become prominent only in the ventral portion of this region. Olfactory inputs are directed mostly to the medial part of the temporal pole where extensive projections from the amygdaloid nuclei also converge. Afferents from limbic and paralimbic regions are directed mostly to the agranular and dysgranular sectors of TP [36]. The medial temporal cortex are concerned with object recognition memory and IL also has additional access to this information via reciprocal connections with the medial MDmc in rhesus monkeys [6,42]. IL, PL, and areas 13 and 24 are also connected with the temporal proisocortical area, as well as the perirhinal and entorhinal regions and
the parahippocampal cortex [6,56]. The mPFC reciprocally connects with the piriform cortex in the rat [20] and projects to the parahippocampal cortex in the monkey [53]. Thus, the temporal pole is most probably a site where sensory and limbic inputs converge, sending integrated outputs to mPFC which also sends feedback projections to TP reciprocally.

IL projects to the lateral capsular subdivision of the central amygdaloid nucleus, corticomedial amygdaloid nuclei, medial, anterior subdivision of the cortical-periamygdaloid cortex, ventromedial subdivision of L, accessory basal amygdaloid nucleus and anterior amygdaloid area, while PL projects to the lateral capsular subdivision of the central and medial portion of BLmc and the adjacent portions of L in the rat [11,33]. Porrino et al. [46] concluded that the ventromedial region of the frontal cortex receives both direct amygdalo-cortical and indirect amygdalo-thalamo-cortical input from the amygdala in the monkey and Aggleton et al. [1] provided evidence of reciprocal projections between frontal cortex and amygdala. An intimate reciprocal connection has also been reported between mPFC and the amygdaloid nuclear complex including periamygdaloid cortex, AB, BL and L of the primate [2].

The shell of Acb received projections primarily from IL and PL of mPFC in the monkey. Projections from the ventral striatum are represented topographically in the ventral pallidum and non-topographically in the substantia nigra, pars compacta [26]. Projections to the shell predominated from IL rather than PL, and the core of Acb received more projections from PL than IL in the present study, supporting the previous suggestion that the shell is related to autonomic and limbic functions and the core to motor control; further, the core is a kind of interface between limbic, autonomic and motor behavioral outputs in response to sensory inputs [22,34,35].

4.3. Projection to diencephalon

The medial part of PM is connected reciprocally with PFC including areas 9, 12l, 10, 24, 25, 32 as well as with TP, rostral superior temporal gyrus and sulcus, amygdala and anterior cingulate cortex. Medial PM with connections with dorsomedial PFC, auditory cortical regions of the superior temporal gyrus, polymodal processing areas of the superior temporal sulcus and the amygdala might play a role in auditory, auditory-spatial, or other attentional processes [49]. Projection from mPFC to PM was strong and indicated that this area of the pulvinar is intimately related with autonomic and limbic functions in connection with integrated audio–visual information.

As mentioned above, Acb has been postulated as a site of functional interface between the limbic and motor systems [22,34,35]. The present study revealed that PL and IL of mPFC projected to VAmc which is under the control of GABA neurons in the reticular part of substantia nigra [28] and is intimately related to the motor system and projects to PFC including PL [8]. We suggest that VAmc is another site of interaction between the motor and autonomic/limbic control systems of the cortico-basal ganglia-thalamus-cortical loops [39]. Ventral precallosal area 14 and subcallosal area 25 and the ventral, subcallosal part of area 32 receive projections from the mediodorsal portion of MDmc and caudodorsal part of MD [47]. The dorsal, precallosal part of PL receives projections from the dorsal portion of MDpc. Area 24 receives additional input from the anterior medial nucleus and midline thalamic
nuclei [6]. TP projects to the ventral part of the mid-rostrocaudal level of MDmc in the monkey [50].

Öngür et al. [43,44] examined projections from areas 25, 32 and 24b to the hypothalamus in Macaque monkeys. They found heavy projections from area 25 to POL, POM, VMH, LH, PH, zona incerta (ZI) and SI. They observed moderate to weak projections from area 32 to the anterior hypothalamic area, LH, DMH, VMH and PH as well as from 24b to POM, LH and PH. The present study confirmed similar projections and observed dense nerve terminals in TM. Ventromedial PFC projects to LH and ZI and also has reciprocal connections with the amygdala and ‘hypothalamic area controlling emotional responses’, corresponding with PH and the most medial portion of LH and could be regarded as a center controlling the cardiovascular responses accompanying emotion [52]. Histaminergic neurons of TM receive inputs from PFC [23], preoptic area and septum, and project to almost all parts of the brain; they may transmit information originating from the limbic system to most parts of the brain, release histamine non-synaptically, and regulate the activities of widely divergent regions of the brain [58].

Fig. 7. A series of frontal sections of M719 showing the distribution of nerve terminals labeled by BDA.
4.4. **Projection to mesencephalon**

DA inhibits pyramidal neurons of IL and PL of mPFC that project to subcortical targets through D2 DA receptor [51], and in addition to direct inhibition of cortical projection neurons, DA inhibits pyramidal cells indirectly by augmenting GABA release from interneurons [48].

An et al. [3] reported that PFC projected densely to the periaqueductal gray (PAG) of Macaque monkeys, and that areas 25, 32 and 10m projected predominantly to the bilateral dorsolateral columns of PAG. The present results were similar, showing dense bilateral projections to PAGdl with ipsilateral predominance. PFC projects to PAG which in turn projects to intermedio-lateral nucleus of the spinal cord [52]. Layers V and VI of IL and PL project to PAGdl, a visceral motor area of midbrain in the rat [63]. The lateral and dorsolateral region of PAG is considered as a midbrain aversive system, and stimulation in this area produces intense fear with autonomic activation in man [40,61] and aversive behavioral responses with sympathetic activation in animals ([12] for reviews, [30,31]).

IL, insular cortex, BST, perifornical region, TM, dorsal hypothalamic area, VMH, PVH, PDL, M, ZI, raphe nucleus, cuneiform nucleus, Kölliker–Fuse nucleus, PBl, PBm, NTS and ventrolateral part of PAG project to corticotropin-releasing hormone-rich neurons of the pontine micturition center, known as Barrington’s nucleus in the rat [55].

4.5. **Projection to medulla and spinal cord**

Neurons in the rostral ventrolateral medulla area project to IL of the rat [62]. IL and PL projected to NTS, dorsal motor nucleus of vagus, nucleus ambiguus, and the ventrolateral medulla as well as lamina I and intermedio-
Fig. 8. A schematic diagram summarizing the results of the present study showing nuclei which received projections from the infralimbic area (IL, area 25) of the medial prefrontal cortex.

lateral nucleus of the spinal cord in the rat [27]. The vasomotor center in the mPFC of the rat corresponded to the PL by Krettek and Price [29] or Cg3 by Paxinos and Watson [45] projecting bilaterally and directly to the central autonomic area of the thoracic spinal cord through the dorsal cortico spinal tract [5].

As a control study to reveal projections to the spinal cord from IL or PL, we injected CTb into the caudal medulla oblongata of the monkey (unpublished data). We could observe retrogradely labeled neuronal perikarya in the cingulate cortex, area 14, and latero–dorsal aspect of the frontal cortex. Many labeled neurons were also found in PPN, pretectal nucleus, DR, LC, interstitial nucleus of Cajal, Darkschewitch nucleus, nucleus of Bechterew, nucleus subfascicularis, ZI, substantia nigra, central amygdaloid nucleus, PVH and LH. We injected WGA–HRP into NTS of the medulla oblongata (unpublished data) and found retrogradely labeled neurons in the cingulate cortex, opercular cortex, and supplementary motor area. Many labeled neurons were found in the hypothalamus (LH, DA, PTH, PVH), central nucleus of the amygdala, Acb, BST, ZI, DR, pretectal nucleus, LC and PBm. We could observe only a few labeled terminals in NTS after BDA injection into mPFC and, further, we could find few labeled perikarya in IL and PL after injection of retrograde neuronal tracers into the medulla oblongata, and concluded that IL and PL projected indirectly to the spinal cord in the monkey.

4.6. Structure and function of mPFC

The cytoarchitectonic and histochemical atlas of the prefrontal cortex of the monkey has clearly identified areas 25, 32 and 24 of mPFC [14]. Gabbott and Bacon [24,25] further showed histochemical characteristics in relation to calcium binding proteins, GABA and nitric oxide in mPFC and differentiated IL, PL and anterior cingulate cortices of the monkey.

The PL in the rat receives a direct input from the hippocampus, a connection that is essential for spatial memory, and Group 1 mGluRs are present on layer V neurons of PL participating in the production of long-term potentiation [57]. mPFC of the rat has been implicated as an autonomic motor area by many investigators morphologically [5,16,27,41,54] and functionally [13,41,42]. Electrophysiological stimulation of the gyrus orbitalis corresponding to area 13m, 13l, Iam, Iai, Iapm of Carmichael and Price [14], which corresponds to the agranular insular cortex of the rat [60], resulted in inhibition of respiration, rise of blood pressure and decrease in the tonus of the gastric musculature of the monkey, Macaca mulatta [7]. Although no functional studies have been performed
yet, the quite similar projection pattern of mPFC in the monkey as seen in this study to that of rodents strongly suggests that mPFC is an autonomic motor area in this species too.

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