LAMINAR ORGANIZATION OF EFFERENT CONNECTIONS OF THE PREFRONTAL CORTEX IN THE DOG

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Abstract. The method of retrograde HRP transport was used to study the laminar organization of cortico-subcortical and cortico-cortical connections of the dog's prefrontal cortex (PFC). The neurons of deep cortical layers VI and V gave rise to cortico-subcortical connections, while cortico-cortical connections originated from layers II–VI. Among them, the main source of intracortical connections was layer III. Differences in the distribution of labeled cells in sublayers IIIa and IIIb seemed to depend on the length of connections, e.g., local and distal as well as ipsi- and contralateral connections. The neurons which are a source of both cortico-subcortical and cortico-cortical connections were located more superficially in the medial PFC fields. Such distribution of neurons seems to be related to the differences of cytoarchitectonic structure of the medial and lateral PFC surface. Most frequently the projective neurons were of a pyramidal type, although some fusiform and stellate types were occasionally found.

INTRODUCTION

The prefrontal cortex (PFC) in dog's brain involves a relatively large surface of the anterior part of the frontal lobe in comparison with the same region in other subprimates. However, compared with primates, its cytoarchitectonic features suggest a more primitive structure (18, 35). Dog's PFC reveals an indistinct separation from the neighboring motor...
cortex, lower distinctness of PFC layers and lack of granular layer IV. Apart from this, it shows a rather irregular arrangement of radial fibers and a large amount of improperly oriented pyramidal cells (1, 2).

Previous cytoarchitectonic (1) and myeloarchitectonic (23) studies as well as investigations of connections (19, 20, 21) of the dog's PFC indicate differences between its lateral and medial surfaces. Generally, the cytoarchitectonic feature which constitutes a difference between these cortical areas is smaller thickness of the medial PFC (1.4 mm) than of the lateral one (2 mm). According to Adrianov (1) the smaller thickness of the medial cortex is due to poor development of layers V and VI, while upper cortical layers preserve normal thickness. Especially layer III is prominent and split into two sublayers IIIa and IIIb through the whole PFC.

In relation to the above mentioned cytoarchitectonic peculiarities of dog's PFC cortex, it was interesting to establish the participation of particular layers in the formation of corticofugal connections. The method of retrograde HRP transport applied in our experiments permitted to distinguish the source of both cortico-subcortical and cortico-cortical projection and the differences in laminar distribution of labeled cells on the medial and lateral PFC surface.

METHOD

For determining the source of cortico-subcortical connections, 11 animals received unilateral injections of 0.25–0.4 μl of 30% HRP solution (Sigma, Typ VI) in saline. In dog S1 the injection involved a large territory of the thalamus; in dogs S2–S5, the magnocellular division of MD; in dogs S6–S7, the parvocellular division of MD; in dogs S8–S16, the whole MD; dog S11 was given an injection into VM nucleus of the thalamus. All injections were made with 5 μl Hamilton microsyringe mounted in a stereotaxic apparatus. The needle was inserted vertically into the thalamus via the ipsilateral cortex, using coordinates of a stereotaxic atlas (25).

The cortico-cortical projection was examined in 17 dogs, on the basis of unilateral HRP injections into different fields of the prefrontal region (dogs D1–D15), as well as into the sylvian cortex (dogs D16, D17). Each of these cortical areas received multiple injections with a total amount of 1–1.5 μl of HRP, at a depth of 2 mm from the cortical surface. All animals survived for 48 h, then they were deeply anesthetized and perfused with McEvans solution, followed by a mixture of 1% paraformaldehyde and 2.5% glutaraldehyde in 0.05 M phosphate buffer at pH 7.4. After removal from the skull, the brains were left in the mixture of
fixative with 30% sucrose for 48 h and then cut immediately at 40 µm on a freezing microtome.

In order to reveal the reactive HRP, every 10th section was incubated for 20 min in a medium containing benzidine dihydrochloride or tetramethyl benzidine according to the procedures of Mesulam (28, 29). Then the sections were mounted on glass slides (coated with alunigelatin) and lightly counterstained with cresyl violet or neutral red. Labeled cells were observed in the light microscope and localized in the cortical layer using the atlas of Adrianov, O. S. and Mering, T. A. (1).

RESULTS

The location of HRP injections. For establishing the origin of prefronto-subcortical projections the HRP injections were localized in the thalamic nuclei, which are strongly related to PFC. Previous results have shown that these are mainly the mediodorsal (MD) and ventromedial (VM) nuclei (19, 20, 21). In the present material one injection involved a large territory of the thalamus (dog S1), whereas the others involved small parts of magnocellular division of MD (dogs S2–S4), parvocellular division of MD (dogs S5–S7), or both of them (dogs S8–S10). One injection was placed in VM nucleus (dog S11).

Laminar organization of the ipsi- and contralateral cortico-cortical connections was examined on the basis of application of small injections into the lateral (Fig. 1 D1–D5), dorsal (Fig. 1 D6–D10), and medial (Fig. 1 D11–D15) PFC surface. In all these injections local connections between

Fig. 1. Localization of cortical injections. D1–D10 injections into the lateral and dorsal PFC surface; D16, into the sylvian gyrus; D17, into the sylvian gyrus with small ventral part of the posterior ectosylvian gyrus; D11–15 into the medial PFC surface.
the neighboring PFC fields were established. Moreover, distal connections originating in the sylvian gyrus were also observed. To define whether both types of connections originate in the same cortical PFC layer, additional injections to the sylvian gyrus were given (Fig. 1 D16, D17).

**Laminar organization of cortico-subcortical projection**

Table 1 illustrates the distribution of labeled cells in the different layers of PFC. The projection to subcortical structures arises mainly from the whole width of layer VI and, in a smaller degree, from the deep part of layer V. The labeled cells are aggregated in a uniform horizontal band which involves both above mentioned layers (Fig. 2). In none of the cases of cortico-subcortical projection the division into separate bands of cells, was observed. The majority of neurons labeled in layers V and VI were pyramidal cells of different sizes. The large

<table>
<thead>
<tr>
<th>Dogs</th>
<th>Localization of labeled cells in PFC</th>
<th>Cortical layers containing the labeled cells*</th>
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<tbody>
<tr>
<td>S1</td>
<td>PFC-l, PFC-m</td>
<td>VI, V</td>
</tr>
<tr>
<td>S2</td>
<td>PFC-l, PFC-v, PFC-m</td>
<td>VI</td>
</tr>
<tr>
<td>S3</td>
<td>PFC-m</td>
<td>VI, V</td>
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<tr>
<td>S4</td>
<td>PFC-l, PFC-v, PFC-m</td>
<td>VI, V</td>
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<tr>
<td>S5, S6, S7</td>
<td>PFC-d, PFC-m</td>
<td>VI</td>
</tr>
<tr>
<td>S8</td>
<td>PFC-m</td>
<td>VI, V</td>
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<tr>
<td>S9</td>
<td>PFC-v, PFC-m</td>
<td>VI, V</td>
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<tr>
<td>S10</td>
<td>PFC-l, PFC-m</td>
<td>VI, V</td>
</tr>
<tr>
<td>S11</td>
<td>PFC-l, PFC-d, PFC-m</td>
<td>VI, V, V</td>
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</tbody>
</table>

PFC — prefrontal cortex; l, d, v, m — lateral, dorsal, ventral and medial surface respectively.

* The order of layers in the Table is related to the dominance of labeled cells in particular layers.
Fig. 2. Microphotograph of HRP labeled cells arranged in uniform horizontal band involving layers VI and Vb of the medial PFC surface which give rise to cortico-subcortical projections, in dog Sg. 200X.
Fig. 3. Laminar distribution of HRP labeled cells giving rise to cortico-subcortical connections. Camera Lucida drawing of the lateral PFC field in dog S_{lb}.

Fig. 4. Laminar distribution of HRP labeled cells giving rise to cortico-subcortical connections. Camera Lucida drawing of the medial PFC field in dog S_{s}.
Fig. 5. Microphotograph of HRP labeled neurons arranged in separate horizontal bands localized in layers II, IIIa, IIIb and Va, Vb of the paraorbital PFC area in dog D1, which give rise to local cortico-cortical connections. 200×.
Fig. 6. Laminar distribution of HRP labeled cells giving rise to cortico-cortical connections. Camera Lucida drawing of the lateral PFC field in dog $D_3$.

Fig. 7. Laminar distribution of HRP labeled cells giving rise to cortico-cortical connections. Camera Lucida drawing of the medial PFC field in dog $D_{13}$. 
### Table II

Distribution of HRP labeled cells in the prefrontal cortical layers. Ipsilateral local (dogs D1–D15) and distal (dogs D16, D17) connections.

| Surface of prefrontal cortex | D1  | D2  | D3  | D4  | D5  | D6  | D7  | D8  | D9  | D10 | D11 | D12 | D13 | D14 | D15 | D16 | D17 |
|------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| PFC-l                        | IIIa+b V | IIIb | IIIb | IIIa+b V | IIIa+b V | IIIa | IIIb | IIIb | IIIa+b V | IIIa+b V | IIIb | IIIb | IIIa | IIIb | IIIa | IIIb |
| PFC-d                        | II  | II  | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V |
| PFC-v                        | IIIb | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V | IIIa+b V |
| PFC-m                        | IIIb | IIIa | IIIa | IIIb | IIIb | IIIa | IIIa | IIIa | IIIa | IIIa | IIIa | IIIa | IIIa | IIIb | IIIa | IIIb | IIIb | IIIb |

The order of layers in the Table is related to the dominance of labeled cells in particular layers.
pyramidal cells were always more intensively labeled, but numerous small and weakly labeled pyramids were also observed. Besides the pyramidal type, layer VI contained also single labeled fusiform neurons. In the case of intensive axonal transport, when the neurons were labeled in great numbers, they were uniformly distributed through the whole width of layer VI. On the contrary, if the cells were labeled in small number they were always scattered in separate groups involving 3–6 neurons. After injections to magno- and parvocellular parts of MD as well as an injection to VM, the considerable difference in the laminar distribution of labeled cells was not observed. However, comparing the distribution of labeled cells on the medial and lateral surface, some slight differences occurred (Fig. 3, 4). On the medial wall of the hemisphere where the total width of the cortical layers is smaller, the labeled cells, apart from layer VI, were localized more frequently in the inner part of layer V (Fig. 4). Particularly, it was seen in the case of injection to the VM, when the numerous labeled cells in layer V were found (see Table I, S11).

Laminar organization of cortico-cortical projection

Table II and III illustrate the laminar distribution of labeled cells of cortico-cortical projections following injections to PFC fields located on the lateral (PFC-1) and medial (PFC-m) surface of the hemisphere as well as in the sylvian gyrus. The HRP positive cells were observed in the prefrontal cortical fields outside the injection place, ipsilaterally and in some cases contralaterally, according to previously established topography of local connections (own unpublished data). Additionally,

<table>
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<th>Surface of prefrontal cortex</th>
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<tr>
<td></td>
<td>D1</td>
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<tr>
<td>PFC-1</td>
<td>IIIb</td>
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<td>V</td>
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<td></td>
<td>II</td>
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<td>PFC-m</td>
<td>IIIb</td>
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The order of layers in the Table is related to the dominance of labeled cells in particular layers.
in all cases of PFC injections, the labeled cells were found in the cortex of the sylvian gyrus.

The cortico-cortical projection originates principally in layer III and, to a smaller degree, in layers V and II. Some single neurons were found also in layer VI. In consequence of intensive HRP transport great amount of cells arranged in the horizontal bands were labeled (Fig. 5). The neurons of outer bands were localized in layers IIIa, IIIb and II, the inner band involved the cells of layer V. In some cases only one continuous band of labeled cells appeared in one of sublayer of layer III, while in other layers the cells were scattered in small groups (3–6 cells). On the basis of such distribution we estimate the predominance of layer participation in the projection. The neurons giving rise to cortico-cortical connections were predominantly typical medium to large pyramids, whereas small pyramids, oval in shape, were observed rarely. The latter type of cells existed more often on the medial surface of PFC. Occasionally, we found (mainly in the cingular gyrus) neurons with radial arrangement of dendrites. Some specific features of the laminar distribution of labeled cells in particular cases seem to be depending on length of connections e.g., local and distal as well as ipsi- or contralateral. However, in spite of their length certain differences of cell localization can be observed on the medial and lateral PFC surface (Table II, D₁₁–D₁₄).

The local cortico-cortical connections of the lateral PFC fields, originated mainly from sublayer IIIb (Fig. 6). But in the medial PFC fields, the source of such connections were neurons localized more superficially. The main band of these neurons was placed in sublayer IIIa often involving also layer II (Fig. 7).

The distal cortico-cortical connections, which can be illustrated by cases of the ipsilateral, reciprocal PFC-sylvian gyrus connections, arose most often from sublayer IIIb. The participation of other cortical layers in the creation of such connections is different (Table II, D₁₆, D₁₇).

Following of the contralateral HRP injections the bulk of labeled cells was seen in sublayer IIIb. Less frequently, the labeled cells were also noted in layers V and IIIa. Seldom, the interhemispheric afferent neurons to PFC were located in superficial layer II as well as in deep layer VI (Table III). The deeper location of these neurons, principally in layers IIIb and V, seems to be a specific feature of the callosal connections.

DISCUSSION

It has been found that in dog's PFC, the cortico-subcortical projection originated from deep cortical layers VI and V, which is in agreement with earlier findings concerning various cortical regions in other mam-
mals. The data are mostly related to efferent connections of the visual, auditory, motor and somatosensory cortical projection regions. These results suggest the existence of separate neuron populations in the mentioned two layers, which give rise to efferent projections to particular subcortical structures.

In cat's visual cortex the cells sending their axons to the colliculus superior lie in layer V, whereas neurons projecting to LGN are located in layer VI (7, 8). In the monkey the cortico-LGN projection undergoes further differentiation. The HRP injections to the parvo- and magnocellular LGN revealed the labeled cells localized in the upper and lower part of the cortical layer VI, respectively (26). Similarly, the neurons of cat's auditory cortex sending their axons to MGN or colliculus inferior were aggregated in two bands situated in the outer part of layer V and in layer VI. These two bands of labeled neurons were separated by the inner rim of cells of layer V, which seem not to participate in these projections (17).

The efferent subcortical projections from the motor- and somatosensory cortex in the rat, cat and monkey, originate from layers V and VI (6, 10). In the monkey, a separate population of neurons projecting from these cortical regions to various subcortical structures was also detected. The cortico-striate neurons were placed in the outer part of layer V, the cortico-spinal ones in its inner part, and the cortico-rubral neurons were located between them. Moreover, in the deep part of layer V, a few cortico-thalamic neurons were found, but the majority of them were situated in layer VI (13, 14).

Little is known about the layer organization of efferent connections of cortical association regions. Jacobson and Trojanowski (12) and Siegel et al. (33) examining PFC-MD connections in the rat, suggested that their parent cells were located in layers V and VI. The results of our experiments, performed on the dog's prefrontal cortex, confirmed the above mentioned data. In the dog the neurons of layers V and VI were the source of cortico-thalamic projection. Certain differences in the participation of neurons of layers V and VI in efferent projection seem to be related to different cytoarchitectonic structure of the medial and lateral PFC. On the medial surface, where the total thickness of the cortex in comparison with the lateral surface is smaller labeled neurons appear more often in layer V. It was seen most clearly in the distribution of labeled cells after the injection to VM.

According to present results, as well as those obtained in other species, layers II–VI are the source of cortico-cortical projection regardless of cytoarchitectonic differences of various cortical regions. However, most of such connections originate in layer III. The data concerning the
participation of various cortical layers (besides layer III) in the formation of ipsilateral cortico-cortical connections, originating in both the projection and association cortex of other species, are divergent. In the cortical projection regions such connections, originating mainly in layer III, arise also in a more superficial layer (II), as well as in deeper layers (IV, V, VI). In the cat’s primary and secondary visual cortex most of the cells sending their axons to the visual association field were found in layers II and III (7), less frequently in layers IV and V (15, 16), and also in layer VI (30). On the contrary, in supragranular layers of the monkey’s visual cortex such cells were not found (31). In the cat’s auditory and somatosensory cortex the cells of intracortical projection were primarily detected in layer III (22). However, in some studies they were found also in layer V (35), layer II and IV (15, 16).

The neurons of cortico-cortical connections of the parietal, temporal and prefrontal association cortex in the cat and monkey, as in the above mentioned data, were localized mainly in layer III (27), and also in layer V (4, 24, 34), irrespectively whether their axons reach the projection or association cortical fields. The participation of other cortical layers in the formation of these connections seems to be insignificant. Only in some investigations the presence of labeled cells in layers II, IV and VI was described (3, 11). On the other hand, the results of Jacobson and Trojanowski (11) provide information about unequivocal participation of sublayers IIIa and IIIb in PFC connections in monkey. According to these data, the neurons of sublayer IIIb, primarily, and the neurons of sublayer IIIa secondarily give rise to these projections.

On the basis of our findings we too can suggest that there is a different participation of sublayer IIIa and IIIb on the medial and lateral PFC surfaces in the formation of cortico-cortical connections. It could be related to a different cytoarchitectonic structure of these PFC fields, as well as to the length of such connections. In the medial PFC fields the labeled cells were localized more superficially, in sublayer IIIa and layer II. As was mentioned above, a more superficial location of labeled cells giving rise to cortico-subcortical connections, on the medial PFC surface was also observed. Considering the distal connections of PFC with sylvian gyrus cortex, it appeared that principally sublayer IIIb was involved in the formation of these connections.

The contralateral cortico-cortical connections like to ipsilateral ones also originated primarily in layer III. The neurons which to a smaller degree give rise to this projection were found in the deep cortical layers V and VI. On the other hand, the neurons in layers II and IV were identified occasionally. Data obtained in several mammalian species for various investigated cortical regions were in accordance. Such results
were established in the cat's visual association (30) and projection cortex (9, 32) in the visual projection cortex of the rabbit (5) and the monkey (36), in the cat's (17) and monkey's (12) projections auditory cortex. Our results are in agreement with the above-mentioned reports. But it should be emphasized that in dog's PFC predominantly the neurons of sublayer IIIb give rise to callosal connections, like to distal, ipsilateral connections. The appearance of separate horizontal bands in particular layers seems to be related to the intensity of HRP transport and may be an expression of the pattern of horizontal organization of cortico-fugal connections.

In relation to cytoarchitectonic structure of the cortex in dog described in introduction, some general features concerning the cortico-subcortical and cortico-cortical connections can be suggested. In the poorly developed deep cortical layers V and VI which give rise to cortico-subcortical projections, separate populations of neurons sending their axons to various subcortical structures, could not be distinguished, contrary to other mammalian species. Nevertheless, layer III well developed and divided into sublayers, shows some differentiation in the formation of cortico-cortical projections. It should be emphasized that the different participation of particular PFC layers in forming the cortico-fugal connections concerns mainly its medial and lateral surface. In our opinion, the more superficial localization of the medial PFC neurons from which cortico-subcortical and cortico-cortical connections originate could be related to a somewhat more primitive structure of the medial cortical fields. The question whether these differences are connected with the variation of structure and function of PFC as an association type of cortex, or with a general degree of its development in dog's brain remains open.

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**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>MD</td>
<td>nucleus medialis dorsalis thalami</td>
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<td>PFC</td>
<td>prefrontal cortex</td>
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<tr>
<td>PFC l</td>
<td>lateral surface of the prefrontal cortex</td>
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<tr>
<td>PFC d</td>
<td>dorsal surface of the prefrontal cortex</td>
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<tr>
<td>PFC v</td>
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<td>VM</td>
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REFERENCES


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