SUBCORTICAL CONNECTIONS OF THE PREFRONTAL CORTEX IN DOGS: AFFERENTS TO THE MEDIAL CORTEX

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Abstract. Afferent subcortical connections to the medial prefrontal cortex (PFC) in the dog were investigated using the horseradish peroxidase retrograde transport method. The dorsal and ventral regions of the medial PFC receive different projections both from the medio-dorsal and the ventral thalamic nuclei. The dorsomedial PFC receives projection from a dorsolateral region of the "parvocellular" MD subdivision, while the ventromedial PFC— from the medial, "magnocellular" MD subdivision. The area precruciata medialis (XM) is involved in significant MD projection and should be included in the "prefrontal" not the "premotor" cortex. The ventral thalamic nuclei project mainly to the dorsomedial but not the ventromedial cortex. The distribution of this projection was correlated with antero-posterior cortical localization of injections. The main criterion for distinguishing "prefrontal" and "motor" cortex remains its projections either from MD or from VL nuclei. Only the ventromedial prefrontal cortex receives projection from anterior thalamic nuclei. The caudal region of the ventromedial prefrontal cortex seems to receive richer projection from "non-specific" thalamic nuclei as well as from the amygdaloid complex.

INTRODUCTION

The present results are a continuation of the study on afferent connection to PFC in dog's brain. The previous paper presented affe-
rents to the lateral and dorsal surfaces of PFC (11, 12), whereas the present report demonstrates afferents to the subfields of PFC localized in the medial aspect of hemisphere. The heterogeneity of this PFC region is clearly marked in both morphological and functional organization (1, 2, 5, 8–10, 17, 21, 22, 23). The morphological data concerning cytoarchitectonic (1) and myeloarchitectonic studies of PFC in the dog (13, 14), supported later through cell degeneration techniques (17), showed differences between its anterior, dorsocaudal and ventral regions. The dorsocaudal region of PFC, named by Adrianov “area precoronalis” and by Kreiner “area precruciata”, suggested its relation to the pre-motor cortex, whereas the ventral region was related to the limbic cortex. Only the anterior part of the medial surface, area frontalis II, III and IV according to Adrianov and area pregenualis I, II and III according to Kreiner, were included in the prefrontal cortex.

On the basis of the retrograde degeneration studies, however the ablation of XM area caused cell atrophy in the dorsal part of MD (20). Thus, the problem of the boundary between the prefrontal and premotor areas of the frontal cortex remains open. The results of ablation experiments support the main division of the medial PFC region. The ablation of the anterior region (area pregenualis—PG, according to Kreiner’s division) caused impairment in performance of Pavlovian differentiation tasks, producing a disinhibitory syndrome (3). On the other hand, the PFC dorsomedial region (XM, area precruciata medialis) was found to be involved in the performance of motor differentiation tasks, and the removal of this region impaired those tasks with directional cues and directional response (4, 15, 19). Relatively little is known about the function of the most ventral region of the medial PFC, probably because of the difficulties in using ablation techniques in chronic animals.

The aim of the present study was to complete the scheme of the topography of subcortical projections to the medial PFC and to analyze the extent to which the morphological and functional parcelation of this region is in agreement with the projection differentiation.

**METHOD**

Young dogs, 6–12 mo of both sexes were used. Unilateral injections, with 30% solution of the horseradish peroxidase in saline (HRP, Sigma VI) were made in distinct areas of the medial PFC surface, according to Kreiner’s division. In each limited area 3–4 injections were made with a Hamilton syringe, using a single injection consisting
of 0,3–0,5 μl of enzyme solution at a depth of 2 mm from the cortical surface, over 5 to 10 min. The subsequent procedure was described by Kosmal and Dąbrowska (12).

RESULTS

The localization and size of HRP injections in particular dogs (Fig. 1). The injections were localized in three main regions of the medial PFC (three groups of animals), according to Kreiner's division (Fig. 2) which has usually been employed in behavioral experiments using ablation techniques in dogs.

I. Anterior injections were located in area pregenualis (PG I, II and III). In some cases, the particular injections flowed together (Fig. 1, II).
dogs M1, M3), whereas others remained separate (Fig. 1, dogs M2, M4).

II. Dorsal injections were placed in different subfields: area precruciata medialis (XM, dogs M5, M6), at the border of precruciata medialis and posterior area (XM-XP, dog M7), and most caudally the large injection involved the area precruciata posterior, medialis as well as some extent of the area centralis (XP + XM, XC, dog M8).

![Diagram of the right hemisphere of the dog with denotation of the myeloarchitectonic areas according to Kreiner division: G, area genualis; PG, area pregenualis; POL, area polaris; PR, area prorea; SG, area subgenualis; SC, area subcallosa; SPR, area subprorea; XC, area precruciata centralis; XM area precruciata medialis; XP, area precruciata posterior.]

III. Ventral injections involved the following areas: pregenualis II (PG II), subgenualis (SG), subproreus (SPR), and genualis (G); Fig. 1, dogs M9–M13. These injections were located less precisely due to the difficulties of operating in the depth of the longitudinal fissure.

Following such injections the labeled cells were observed most frequently in the ipsilateral thalamic nuclei and occasionally in the extrathalamic structures.

Projection from the mediodorsal thalamic nucleus. Injections into the medial PFC surface led to the labeling of cells in the mediodorsal nucleus (MD), but the distribution and number of these cells differed to a high degree in particular groups of injections.

I. Anterior injection. The distribution of labeled cells showed a uniform pattern in four dogs of this group. Dog M1 was selected to demonstrate these results (Fig. 3). The labeled cells in MD were concentrated in the mediodorsal part of nucleus along the entire antero-posterior extent of the nucleus. Only single cells were found outside of this area, in the ventral half of the nucleus (Fig. 3 a–d). In relation to the cytoarchitectonic division of MD, this group of cells seems to occupy its both subdivisions, "magnocellular" and "parvocellular". Similar to the previous results, the neurons with heavy and light concentration of HRP reaction product were observed (12). However, following injections into the area pregenualis, irrespective of weak or intensive labeling, two types of neurons were observed in the dorsal part of MD (Fig. 4a), which differed significantly in their morphological peculiarities. The large neurons, often intensively labeled, possess a few strong and arborized dendrites. In contrast, the small neurons
Fig. 3. Dog M1. The distribution of HRP labeled cells following injections into pregenual area, in frontal sections (a–f) through the thalamus. One dots indicate the position of approximately 1-3 labeled cells.

always lightly labeled, are fusiform in shape and have few, poorly visible dendrites. It should be mentioned that the first type of neurons was often recognized in the medial subdivision of MD, whereas the
small ones appeared in the dorsal part of the nucleus, exclusively following the large injections into the area pregenulis.

II. The dorsal injections into the area precruciata were placed in the antero-posterior order, in various distances from the "premotor" cortex. The distribution of the labeled cells in MD differed in each case. For presentation of the results, three dogs are shown: with injection in the anterior part of XM (Fig. 5, dog M5), in the posterior part of XM together with a small extent of XP (Fig. 6, M7) and with large injection involving preferentially XP and XC with a small extent of XM (Fig. 7, dog M8). In dog M5 the labeled cells were found within the dorsal part of MD, more numerous in the caudal region of the nucleus (Fig. 5e). In dog M7 the labeled cells occupied the dorsal part of the nucleus, similar to M5, but the cells seemed to be localized more laterally (Fig. 6 c–e). In dog M8, irrespective of large injection, were found fairly less numerous scattered cells in MD. Some of cells were situated in the most lateral, "paralamellar" part of the nucleus (Fig. 7c–e). Thus, parallel to the antero-posterior localization of the injections, cell groupings seemed to change their position from medial to lateral borders of the nucleus. Moreover, it should be stressed that XM area of the medial PFC receives significant afferents from MD, similar to the area pregenualis, and on this basis, the XM area could be considered as belonging to the prefrontal cortex.

III. Ventral injections into the area pregenualis II, the gyrus subproreus, the area subgenualis (Fig. 1 dogs M9–M11) and additionally into the area genualis (dogs M12, M13) revealed significant differences in the distribution of labeled cells. Two dogs were used for presentation of the results: M9 with anteroventral injection and M12 with posterior injection. In dog M9 the labeled cells occupied the entire extent of the medial "magnocellular" part of MD nucleus, but they were more numerous in the anterior half of the nucleus (Fig. 8b, c). Small injections in dogs M10 and M11 completed the topographical scheme of this projection. Thus, the subproreal gyrus receives projection from the most ventral part of the medial subdivision of MD nucleus, the subgenual area from its intermediate part and area pregenualis from the dorsal part.

In contrast with the above results, injections into genual area led to the labeling of only a few cells in the MD nucleus and demonstrated that this region does not receive any significant projection from this nucleus (Fig. 9).

Projection from the ventral thalamic nuclei. Injections into the particular subfields of the medial PFC revealed a great difference in projections from ventral nuclei to its dorsal and ventral region.
Fig. 4. Microphotograph of HRP labeled cells in the: a, dorsal part of the mediodorsal thalamic nucleus following injection into pregenual area. Two types of neurons, large projective and small of a "reticular" type are seen; b, ventromedial thalamic nucleus following injection into pregenual area; c, paraventricular nucleus; d, parataenial nucleus, and e, parafascicular nucleus, following injection into genual and subgenual area.
Fig. 5. Dog M5. The distribution of HRP labeled cells following injection into anterior part of XM, in frontal sections (a–f) through the thalamus. Denotation as in Fig. 3.
Fig. 6. Dog M7. The distribution of HRP labeled cells following injection into posterior part of XM and anterior XP, in frontal sections (a–f) through the thalamus. Denotation as in Fig. 3.
Fig. 7. Dog M8. The distribution of HRP labeled cells following large injection into caudal part of the precruciate area, in frontal sections (a-f) through the thalamus. Denotation as in Fig. 3.
Fig. 8. Dog M9. The distribution of HRP labeled cells following large injection into anteroventral region of medial PFC, in frontal sections (a-f) through the thalamus. Denotation as in Fig. 3.
Fig. 9. Dog M12. The distribution of HRP labeled cells following injection into genual and subgenual area, in frontal sections (a–f) through the thalamus. Denotation as in Fig. 3.
I. Anterior injections into the area pregenualis led to the labeling of a moderate number of cells in the ventralis medialis (VM) and the ventralis anterior (VA) nuclei, (Figs. 3a–c and 4b). There were not any significant differences in the distribution of labeled cells when injections involved both dorsal (PG I) and ventral (PG II) subfields of area pregenualis together (Fig. 1, dogs M1, M2), or involving exclusively its dorsal subfield (Fig. 1, dogs M3, M4). Probably this entire region receives a uniform projection from VA and VM thalamic nuclei.

II. The dorsal injections demonstrated some relations between the labeling of the cells in the particular ventral nuclei, and their antero-posterior localization. Following injection into the anterior part of XM, the distribution of labeled cells was similar to that after injections into PG (Fig. 5a–c), because the labeled cells were observed in VM and VA. Injection into the posterior part of XM led to the labeling of cells mainly in VA and only of single cells in VM (Fig. 6a–c). However following the injection which also involved the area precruciata posterior, the labeled cells appeared in four ventral nuclei VA, VM, VPM and VL (Fig. 7a–d). This result supported once more that the principal criterion for determining the extent of “motor” cortex on the basis of afferent projection may be the projection from VL.

III. The ventral injections produced labeling of cells, mainly in VA, only a few labeled cells were found as well in VU (Figs. 8a, b, and 9a).

Projection from the lateral thalamic nucleus. The projection from this nucleus to the whole region of the medial PFC seems to be very weak. In dogs with relatively large injections, only single labeled cells were found in the lateral posterior nucleus (LP). The localization of these cells was the same as in the previous material (11, 12), in the medial part of the nucleus, at the caudal level of MD and posteriorly to it (Figs. 3, 5 and 6e–f). Only in dog M8 the labeled cells were more numerous, but the injection was the largest one and involved not only the prefrontal cortex (Fig. 7e–f).

Projection from the anterior thalamic nuclei. In this material, the injections into the medial PFC led also to the labeling of cells in the anterior thalamic nuclei. The labeled cells were found in dogs M7, M9, M10 and M12. It may be concluded that in dogs M7 and M12 the labeling of the cells seems to be due to the spreading of the enzyme into the cingular gyrus (Fig. 1). However, in two other cases, M9 and M10, the possibility of enzyme diffusion into this gyrus may be excluded. Thus, the ventral region of the medial PFC also receives some projection from the anterior thalamic nuclei. In last two cases, the labeled cells were observed in Ad, Av and Am (Fig. 8a–b).
Projection from the midline and intralaminar thalamic nuclei. Following all large injections into subfields of the medial PFC the labeled cells were observed in the midline and intralaminar nuclei. A correlation between the extent of injection and the appearance of the labeled cells in these nuclei seemed to be evident. On the other hand irrespective of the extent of the injections, the ventrocaudal part of the medial PFC seems to receive a more rich projection from these nuclei.

I. The anterior injections led to the labeling of single cells in three nuclei: parataenial, paraventricular and central medial (Fig. 3a, c, d).

II. For the dorsal injections, in addition to nuclei mentioned above, labeled cells in nucleus reuniens and centrum medianum were observed. In centrum medianum the labeled cells were found in two cases, following the injection into XM (Fig. 5e) and after large injection into XM and XP (Fig. 7e).

III. The ventral injections led to the labeling of numerous cells in many nuclei. The number of cells was evidently higher in dogs with large injections. For example in dog M9 the labeled cells were found in the parataenial, paraventricular, central medial, paracentral, reuniens and parafascicular nuclei (Fig. 8a–c, f). However in dog M10, with relatively small injections although involving ventral region of medial PFC, the labeled cells were observed more frequently than in cases with anterior and dorsal injections. The phenomenon of the labeling of cells in these nuclei was especially striking in dog M12, in which the injection involved the genual area, particularly in comparison with the insignificant labeling of neurons in MD (Fig. 9a–d, f).

Figure 4c–e shows three types of neurons which were labeled in dog M12 in the parataenial, paraventricular and parafascicular nuclei. It is interesting to note the differences in their morphological features. It should be mentioned that the type of labeled neurons in the paraventricular nucleus could be compared with labeled small neurons in the dorsal part of MD observed following the injections into the area pregenualis (Fig. 4a, c).

In the parafascicular nucleus, a small number of labeled cells was observed in all three groups of injections. The localization of these cells was similar to the one presented previously in the lateral part and less frequently, in the medial part of nucleus (Figs. 3 and 5–8f).

Projection from extrathalamic structures. The injections into the medial PFC labeled cells in some extrathalamic structures. However, the labeling of these cells in particular structures was observed only in some dogs, probably due to the extent of injections.
I. Injections into the area pregenualis led to the labeling of single cells in the nucleus of the diagonal band in the supramamillary and in the lateral mamillary nuclei, the perifornical region, the lateral hypothalamus occasionally, the ventral tegmental area (Fig. 3) as well as the medial raphe nucleus.

II. Injections into the precruciatal region labeled cells in the lateral hypothalamus, supramamillary and lateral mamillary nuclei, the perifornical region, the ventral tegmental area and the locus coeruleus.

III. Following the ventral injections the most numerous labeled cells were found in the amygdala, particularly in dogs M9 and M12. In dog M12 numerous labeled cells were localized in the caudal region of the basal magnocellular nucleus. Some single cells were found also in the amygdaloïd anterior area. Moreover, the large injections labeled cells in the claustrum, the ventral tegmental area, the dorsal raphe nucleus and the locus coeruleus.

DISCUSSION

On the basis of the results presented, it was shown that the medial surface of PFC receives differentiated projection from thalamic and extrathalamic structures. The majority of structures that send projections to the medial PFC area are the same as those projecting into the lateral and dorsal PFC (11, 12). However, the thalamic projection that arises the mediodorsal, ventral and additionally, the anterior nuclei showed a specific topography, different in its distribution to the dorsal and ventral regions of medial PFC.

The localization of HRP injections in this material in the dorsal region of the medial PFC, from the anterior to the posterior direction, toward the “premotor” cortex, demonstrated that both subfields area pregenualis (PG) and area precruciata medialis (XM) receive afferents from the dorsal part of the MD nucleus with a slight shift in the localization of labeled neurons to the lateral border of the nucleus. However, in the most lateral “paralamellar” parts of the nucleus they were not numerous. Thus the area precruciata medialis is situated in the region of afferent projection from MD and in consequence it should be included into the region of “prefrontal” not “premotor” cortex. These results agree with earlier finding obtained by using the retrograde degeneration method (20). Moreover, injections into PG and XM led to the labeling of cells in two ventral nuclei VM and VA. Labeled cells in VL as well as single cells in VPM were visible only when injections involved the XP subfield. This finding is consistent with previously described results of injections, into caudal part of the proreal gyrus and XC area (13). According to
the present results projection from VA and VM involving the whole
dorsal (dorsomedial and dorsolateral) region of PFC is not a proper
criterion for dividing the region into “prefrontal” and “motor” cortices.
The supposition that XM subfield should be regarded as belonging to
the “prefrontal” cortex seems to be in accordance with electrophysio-
logical data in dogs. The experiments demonstrated that electrical
stimulation of frontal cortical regions, localized rostrally in close prox-
imity to the cruciate sulcus, does not produce any movements of the
extremities or the trunk, and was not included in the “motor” or
“supplementary motor” areas (6). Cytoarchitectonic data showed in dogs
(1) that the precruciata area possesses neither large piramidal cells in
layer V, like the postcruciata area, nor granular cells in layer IV,
like the entire prefrontal cortex. Therefore, there could not be
any basis for distinguishing these cortices as well. The overlapping
projections from the mediodorsal and ventral (VM, VA) nuclei are con-
sistent with results from other species (2, 5) and seem to be important
for the functional meaning of this cortical region.

The ventromedial region of PFC receives projection from different
subdivision of the MD nucleus. Following the injections into the area
pregenualis II (PG), gyrus subproreus (SPR), and area subgenualis (SG)
the labeled cells occupied mainly the medial “magnocellular” part of
MD nucleus.

The difference in the distribution of the thalamic projection into
the dorsal and ventral parts of the medial PFC was marked by the
fact that only the ventromedial region seems to receive projection from
the anterior thalamic nuclei. In the material presented the labeled-cells
in the anterior nuclei were not numerous, but the existence of such
a projection to the medial prefrontal cortex is known in other species,
and seems to support the relation of this cortical region to limbic
structures (5).

Injections into the ventral region of the medial cortex demonstrated
that the number of labeled cells in MD was significantly smaller fol-
lowing the ventrocaudal injections. In parallel, the number of labeled
cells was more numerous in “nonspecific” thalamic nuclei and in the
amygdala.

Although the peroxidase technique does not permit us to recognize
the detailed structure of neuronal processes the intensive labeling al-
lowed us to observe some of their morphological peculiarities (7). How-
ever, the only type of labeled cells found in the periventricular
nucleus in this material and in the paracentral nucleus in the previously
presented material (12) seemed to correspond to the type of “reticular
neuron” described in the Golgi material (15, 18, 24). The appearance
of the same type of small, fusiform neuron in the dorsal part of MD nucleus should be stressed. Labeling of these neurons following the cortical injection excluded their classification as Golgi II type, therefore it may correspond to a "reticular" type recognized in Golgi material. They were observed in MD exclusively following the injections into the anterodorsal region of medial PFC, the ablation of which elicited the "disinhibitory" syndrome (3).

Thus, from comparing the present results of injections into the medial PFC cortex with the results of injections into the lateral and dorsal PFC cortex presented in previous material (11, 12), a general organization of subcortical projections can be drawn. The MD nucleus is a source of the most significant projections to the entire PFC region, which show a characteristic topography related to PFC subfields. The entire dorsal (dorsomedial and dorsolateral) region of PFC receive projection from the lateral, "parvocellular" MD subdivision, whereas the ventromedial region of PFC, including the subproreal gyrus, receives projection from the medial, "magnocellular" MD subdivision. However, the orbital gyrus is a transitional region which is innervated by both medial and lateral subdivisions of MD (12, 22). Still, the other region that receives projection from the most lateral "paralamellar" MD subdivision and could correspond to the monkey's "frontal eye fields" is not known (21). Single labeled cells appeared in this part of the nucleus following the injections into the posterior PFC in the vicinity of the "premotor" cortex, but it is not a massive projection. This problem needs further investigation. The second source of characteristic projections to the dorsal PFC are the ventromedial and the ventral anterior thalamic nuclei. The topography of the projection from the ventral thalamic nuclei should be regarded in relation to the whole dorsal part of the frontal cortex (2). The ventral part of PFC does not receive any significant projection from ventral nuclei, and seems to be related to limbic structures either directly or by connections from the thalamic anterior nuclei. Some structures, like the lateral thalamic nuclei, the hypothalamus, the ventral tegmental area, raphe nuclei and the locus coeruleus, send scanty and nonspecific projection to the entire PFC cortex.

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REFERENCES


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ABBREVIATIONS

AD  nucleus anterodorsalis thalami
AM  nucleus anteromedialis thalami
AV  nucleus anteroventralis thalami
Cm  nucleus centralis medialis thalami
CM  centrum medianum thalami
CL  nucleus centralis lateralis thalami
G   area genualis
HM  hypothalamus medialis
HL  hypothalamus lateralis
LA  nucleus lateralis anterior thalami
LI  nucleus lateralis intermedius thalami
LP  nucleus lateralis posterior thalami
MD  nucleus medialis dorsalis thalami
ML  nucleus mamillaris lateralis
P   nucleus pulvinaris thalami
Pc  nucleus paracentralis thalami
PG  area pregenualis
POL area polaris
PR  area prorea
PrC area precentralis
Pt  nucleus parataenialis thalami
Pv  nucleus paraventricularis thalami
R   nucleus reuniens thalami
SC  area subcallosa
SG  area subgenualis
Sm  nucleus supramamillaris
SM  nucleus submedius thalami
SPR area subprorea
VA  nucleus ventralis anterior thalami
VL  nucleus ventralis lateralis thalami
VM  nucleus ventralis medialis thalami
VPM nucleus ventralis posteromedialis thalami
VPL nucleus ventralis posterolateralis thalami
TV  area ventralis tegmentalis
XC  area precruciata centralis
XM  area precruciata medialis
XP  area precruciata posterior
ZI  zona incerta