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Projections of the Basal Ganglia to the Zona Incerta of the Dog Diencephalon

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Retrograde axonal transport of horseradish peroxidase was used to show that the projections of the globus pallidus, entopeduncular nucleus, substantia nigra, and pedunculopontine tegmental nucleus in dogs are directed to all segments of the zone incerta. The experiments reported here identified no topical features in the organization of these projections in dogs, as application of marker to different areas of the zona incerta yielded similar distributions of labeled neurons in the basal ganglia. No striatal projections to the zone incerta were found.

KEY WORDS: diencephalon, zona incerta, basal ganglia, dogs.

The basal ganglia (BG) constitute a large forebrain structure, the major components of which are the striatum (caudate nucleus and nucleus accumbens, putamen) and the pallidum (globus pallidus, entopeduncular nucleus, ventral pallidum) [8]; other structures have more recently been regarded as components of the basal ganglia – the substantia nigra and the pedunculopontine tegmental nucleus (PPN), which connect them to underlying brain centers [4, 9].

The existence of a functional connection between the zone incerta (ZI) and the BG is evident on the basis of both physiological data [4, 5, 7] and clinical observations [2], though there are as yet no detailed data on the organization of the projections of each of the BG structures to the ZI. It should be noted that the interactions between BG structures and the ZI, which are characterized by cytoarchitectonic and neurochemical heterogeneity [7, 8], have not been evaluated in dogs, which represent an important system for behavioral studies. The aim of the present work was to study the afferent connections of the ZI with the BG.

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MATERIALS AND METHODS

Studies were performed on adult mongrel dogs in accord with the "Regulations for Studies using Experimental Animals" (Decree No. 755, August 12, 1977, Ministry of Health of the USSR). Surgery and perfusion were performed under i.v. anesthesia with propofol (B. Braun Melzungen AG, Germany, 2.5 mg/kg) after preliminary i.m. Rometar (4 mg/kg). Further procedures were performed in sterile conditions using stereotaxic coordinates from an atlas [3]; 0.05–0.08 μl of 40% aqueous horseradish peroxidase solution (Sigma, type VI) was injected into various zones of the ZI: the rostral (Fr 16.0–15.5), intermediate (Fr 15.0–13.5), and caudal (Fr 13.0–11.0). Frontal levels (Fr) of histological brain sections were determined and the ZI was identified using an atlas [3] and our own data obtained from studies of the topography and cytoarchitectonics of this nucleus [1]. Brains were perfused after 48 h. This procedure and subsequent processing of brains were performed as described by Mesulam [6] using tetramethylbenzene for histochemical detection of horseradish peroxidase in PPN neurons.

The distribution of labeled neurons in BG structures after administration of marker into the ZI with a syringe was analyzed in seven animals. The control group consisted of two dogs subjected to the same procedure but without

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TABLE 1. Distribution of Retrograde Labeled Neurons in the Basal Ganglia after Injection of Marker into the Zona Incerta (ZI) of the Dog Diencephalon

Animal No.	Location of marker injection zones in different parts of the ZI	Frontal level as per atlas [3]	Labeled neurons in the basal ganglia						
			SNi		PPN			GP	ENT
			SNc	SNr	PPNd lat	PPND med	PPNc		
1	ZI (r)	16–15.5	+	+	+	0	0	+	+
2	ZI (r, m)	16.5–13.5	+++	+	++	+	+	+++	++
3	ZI (m)	14.5	++	+	++	+	0	++	+
4	ZI (m)	14.5–14	++	+	++	+	+	++	+
5	ZI (m)	14.5–14	++	+	+	+	0	++	+
6	ZI (m, c)	14.5–13	+++	+	+	+	+	+++	++
7	ZI (c)	13.0–11.5	+++	+	+	+	+	++	+

Notes. Numbers of marker-labeled neurons (N) per frontal section: 0 = no labeled neurons seen; + = 1–3 N; ++ = 4–10 N; +++ = >10 N. Basal ganglia: ENT = entopeduncular nucleus; GP = globus pallidus; PPN = pedunculopontine tegmental nucleus; PPNd = diffuse zone of PPN; lat and med = lateral and medial parts of PPNd; PPNc = compact zone of PPN; SNi = substantia nigra; SNc and SNr = compact and reticular zones of SNi; c = posterior part of ZI; m = intermediate part of ZI; r = anterior part of ZI.

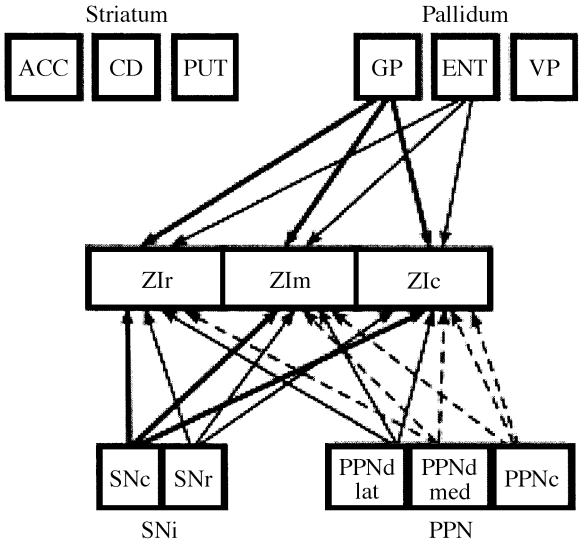

Fig. 1. Organization of the projections of the basal ganglia to the zone incerta (ZI) of the dog diencephalon. Arrows show projections to the ZI: thick arrows show projections from large numbers of neurons (corresponding to +++ in Table 1); thin arrows show projections from small numbers of neurons (corresponding to ++ in Table 1); dotted arrows show projections from occasional neurons (corresponding to + in Table 1). No projections to the ZI from the nucleus accumbens (ACC), caudate nucleus (CD), putamen (PUT), or ventral pallidum (VP) were found. For further details see Table 1.

marker injection. No labeled cells were found in BG structures in control animals.

RESULTS

Analysis of the data obtained here and presented in Table 1 and Fig. 1 shows that after injection of marker into different areas of the ZI (rostral, intermediate, and caudal), the distributions of retrograde labeled neurons in BG structures were essentially similar, albeit with some differences in numbers. All dogs, regardless of the location at which marker was injected into the ZI, labeled neurons were seen in only two structures of the pallidum: the globus pallidus and the entopeduncular nucleus (Fig. 2, *a*, *b*). Labeled neurons were also seen in the substantia nigra (see Fig. 2, *c*),

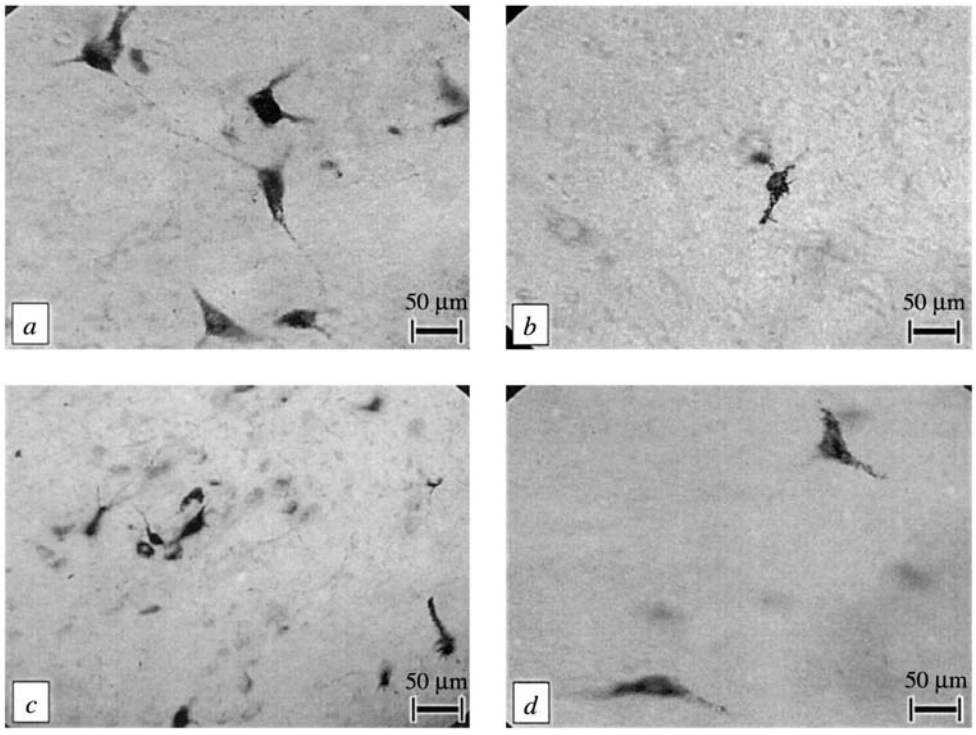

Fig. 2. Labeled neurons projecting to the zona incerta of the dog diencephalon. *a*) Globus pallidus; *b*) entopeduncular nucleus; *c*) substantia nigra; *d*) pedunculopontine tegmental nucleus. Histochemical detection of horseradish peroxidase.

the number of labeled neurons in the compact part being greater than that in the reticular part. In the PPN, labeled neurons were mainly located in the lateral part of the diffuse zone (see Fig. 2, *d*), while the compact zone and the medial part of the diffuse zone showed only occasional labeled neurons, and not in all animals. In particular, it should be noted that no labeled cells were seen in other BG structures (caudate nucleus, putamen, nucleus accumbens, ventral pallidum).

DISCUSSION

These studies showed that in dogs, neurons in the globus pallidus, entopeduncular nucleus, substantia nigra, and pedunculpontine tegmental nucleus project to all parts of the ZI. No topical characteristics were seen in the organization of these projections in dogs, as was also the case in studies of the analogous projections in rats [4, 5], the only animal in which the connections of the ZI with the BG have been studied. Regardless of the locations of the marker injection sites in the ZI, labeled neurons were mainly located in analogous structures of the BG in both species, the only difference being variations in numbers. The main source of afferent projection fibers from BG structures to the ZI of the rat brain consisted of neurons in the compact and reticular zones of the substantia nigra and the diffuse zone of the PPN. In dogs, the largest numbers of projection neurons were located in the globus pallidus and the compact zone of the substantia nigra, the smallest numbers being in the PPN, entopeduncular nucleus, and reticular zone of the substantia nigra. In rats, conversely, only insignificant numbers of neurons in the globus pallidus and entopeduncular nucleus project to the ZI. No projections from structures of the striatum and ventral pallidum to the ZI were found in either species.

It should be emphasized that there are no studies in any animal species, including dogs, addressing and describing the afferent projections of BG structures to the ZI with consideration of its morphological heterogeneity. Thus, our data on the organization of the afferent projection systems of the ZI from BG structures contribute to evolutionary morphology and aid the development of concepts of the conduction pathways for functionally diverse information in the system consisting of the largest subcortical formations – the BG. The data obtained here, along with results obtained from physiological and clinical investigations, may be useful for creating models better reflecting the mechanisms of the functioning of the BG and the structures with which they are connected.

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