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AFFERENT PROJECTIONS FROM THE BRAINSTEM TO THE AREA HYPOTHALAMICA DORSALIS: A HORSERADISH PEROXIDASE STUDY IN THE CAT

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INTRODUCTION

In mammals, several studies have been reported on the input and output relations of the area hypothalamica dorsalis (aHd). Thus, in the rat, using an anterograde autoradiographic method, Loewy *et al.* (11) have shown a projection from the nucleus reticularis gigantocellularis to the dorsal hypothalamic area, whereas efferent projections from the aHd reach the nucleus raphe magnus (8). On the other hand, in a prosimian primate, Dietrichs and Haines (6) have demonstrated a reciprocal projection between the aHd and the cerebellum

In the cat, several studies using anatomical techniques have revealed that the aHd sends projections to the encephalic regions such as the prefrontal cortex and cortical area 20 (1), the thalamic mediodorsal nucleus (15) and the periaqueductal gray substance (12). In addition, it has been demonstrated that the aHd receives afferents from both subthalamic (10) and hypothalamic regions. In the latter, the ventromedial and anterior medial hypothalamus which are mediating affective defence (7) send input to the aHd. In conclusion, in the cat a complete anatomical study on the afferents to the aHd, has not been carried out. However, Berezovskii *et al.* (3) performed a horseradish peroxidase (HRP) study in the cat about the brainstem input to the hypothalamic locomotor region. They observed in some cases that the enzyme spread to the aHd, but no HRP-injection was confined strictly to the aHd.

Thus, in this work, we have used the HRP retrograde tracing method to study the ascending projections to the aHd from the brainstem in the cat. The involvement of this hypothalamic area in several functional domains is also suggested.

MATERIAL AND METHODS

Seven male adult cats weighting 2-3 kg were used for the present study. The animals were anaesthetized intraperitoneally with ketamine (50 mg/kg) and positioned in a stereotaxic frame. Unilateral injections of horseradish peroxidase (HRP) (Sigma type VI) were made into the aHd by using the coordinates A 10.5, L 0.5, H -3.0, following the stereotaxic atlas of Jasper and Ajmone-Marsan (9). By means of a Hamilton syringe 0.03 μl of 30% aqueous solution of HRP was injected. The needle was left in place 15-20 minutes after the injections. The animals were allowed to survive for 36-48 h. Thereafter, they were deeply reanaesthetized, heparinized and perfused through the ascending aorta with 500 ml of 0.9% saline followed by 2 l of a 1% paraformaldehyde - 1.25% glutaraldehyde fixative in 0.1 M phosphate buffer (pH 7.4), and 1 l of the same buffer containing 10% sucrose. Later, the brains were removed from the skulls and then placed in a solution of 10% sucrose in phosphate buffer at 4°C for 24 h. The brains were cut on a freezing microtome. Transversal frozen sections of 60 µm were collected in phosphate buffer and treated by the tetramethylbenzidine procedure (13). Then, the sections were dehydrated, cleared in xylene, coverslipped and viewed with bright illumination. Finally, the localization of labeled neurons was mapped according the stereotaxic atlas of Snider and Niemer (18). The same atlas was used for the terminology of the brainstem nuclei.

Abbreviations

aHd	: area hypothalamica dorsalis
Cu	: Nucleus cuneatus
Fil	: Nucleus filiformis
FR	: Formatio reticularis
FRM	: Formatio reticularis mesencephali
Fx	: Fornix
GC	: Substantia grisea centralis
Gra	: Nucleus gracilis
Hp	: Hypothalamus posterior
IP	: Nucleus interpeduncularis
NHvm	: Nucleus hypothalamus ventromedialis
NR	: Nucleus ruber
NRG	: Nucleus reticularis gigantocellularis
RM	: Nucleus raphe magnus
SN	: Substantia nigra
TMT	: Tractus mammillo-thalamicus
vl	: Nucleus vestibularis lateralis
vm	: Nucleus vestibularis medialis.

RESULTS

The results of the present investigation show the ascending projections to the aHd from the brainstem of the cat (Fig. 1A-E). Inputs to this hypothalamic region mainly arise from nuclei of the midbrain and medulla oblongata, since from the pons we have only found one nucleus, the raphe magnus, which sends a weakly projection to the studied area. The largest contingent of afferents to the aHd comes from the midbrain nuclei substantia grisea centralis (GC) and formatio reticularis mesencephali (FRM) and from the medulla oblongata nuclei vestibularis medialis and inferior. A summary of the brainstem afferents to the aHd is shown in Table I, while the localization of the HRP injection place and the largest diffusion region is shown in Fig. 2A-D.

1. *Input from the midbrain to the aHd.* — The main input source from the brainstem to the aHd arises from the GC (Fig. 1A, B). A large number of HRP-

labeled perikarya were found bilaterally in such midbrain structure, although we have observed more labeled neurons in the ipsilateral than in the contralateral side (Fig. 3A). In the FRM, the labeled somata were also found bilaterally, although their number was moderate (Fig. 1A, B). Finally, a weak input reachs the aHd ipsilaterally from the nucleus ruber and substantia nigra and contralaterally from the nucleus interpeduncularis. In the three regions a lower number of HRP-marked cells were observed (Fig. 1A, B).

2. *Projection of the pons to the aHd.* — The projection of the pons to the aHd is scarce. Thus, we have only observed HRP-marked perikarya in the nucleus raphe magnus, which sends a scarce contralateral input to the hypothalamic area studied in this work (Fig. 1D).

3. *Projections of the medulla oblongata structures to the aHd.* — We have observed in the nuclei of the medulla oblongata a moderate or low density of labeled cells. In particular, two vestibular nuclei, the nucleus vestibularis medialis and the nucleus vestibularis inferior, both with a moderate number of HRP-labeled neurons, send inputs to the aHd, the former bilaterally (Fig. 1D) and the latter ipsilaterally (not shown).

On the other hand, a modest number of cell bodies were observed bilaterally in the following structures: formation reticularis, nucleus reticularis gigantocellularis and nucleus cuneatus (Fig. 1C-E). Moreover, in the nucleus gracilis (ipsilaterally) (Fig. 3B) and in the nucleus vestibularis lateralis (contralaterally), we have found a scarce number of HRP-labeled neurons (Fig. 1C-E).

DISCUSSION

In the cat, the aHd extends from A 10.0 to A 11.5 according to the stereotaxic atlas of the diencephalon by Jasper and Ajmone-Marsan (9). In the present study we used only experiments in which the HRP-injections were confined strictly to the aHd and did not extend into the surrounding areas (Fig. 2).

According to the present data, the aHd receives inputs from nuclei which have been involved in many functional domains, suggesting different functional roles for the aHd.

In particular, our findings on the bilateral input to the aHd from the nucleus reticularis gigantocellularis are in agreement with the results obtained by Loewy *et al.* (11), in the rat. On the other hand, although our study has been centered mainly on the brainstem afferents to the aHd, we were unable to observe labeled neurons in the dentate and the interposite nuclei of the cat. This finding differs from the results obtained by Dietrichs and Haines (6) in *Galago crassicaudatus*, in which cerebello-hypothalamic fibers were followed ventrally and medially into the dorsal hypothalamic area. Moreover, we have demonstrated a rich input to the aHd arising from both ipsilateral and controlateral sides of the GC, while

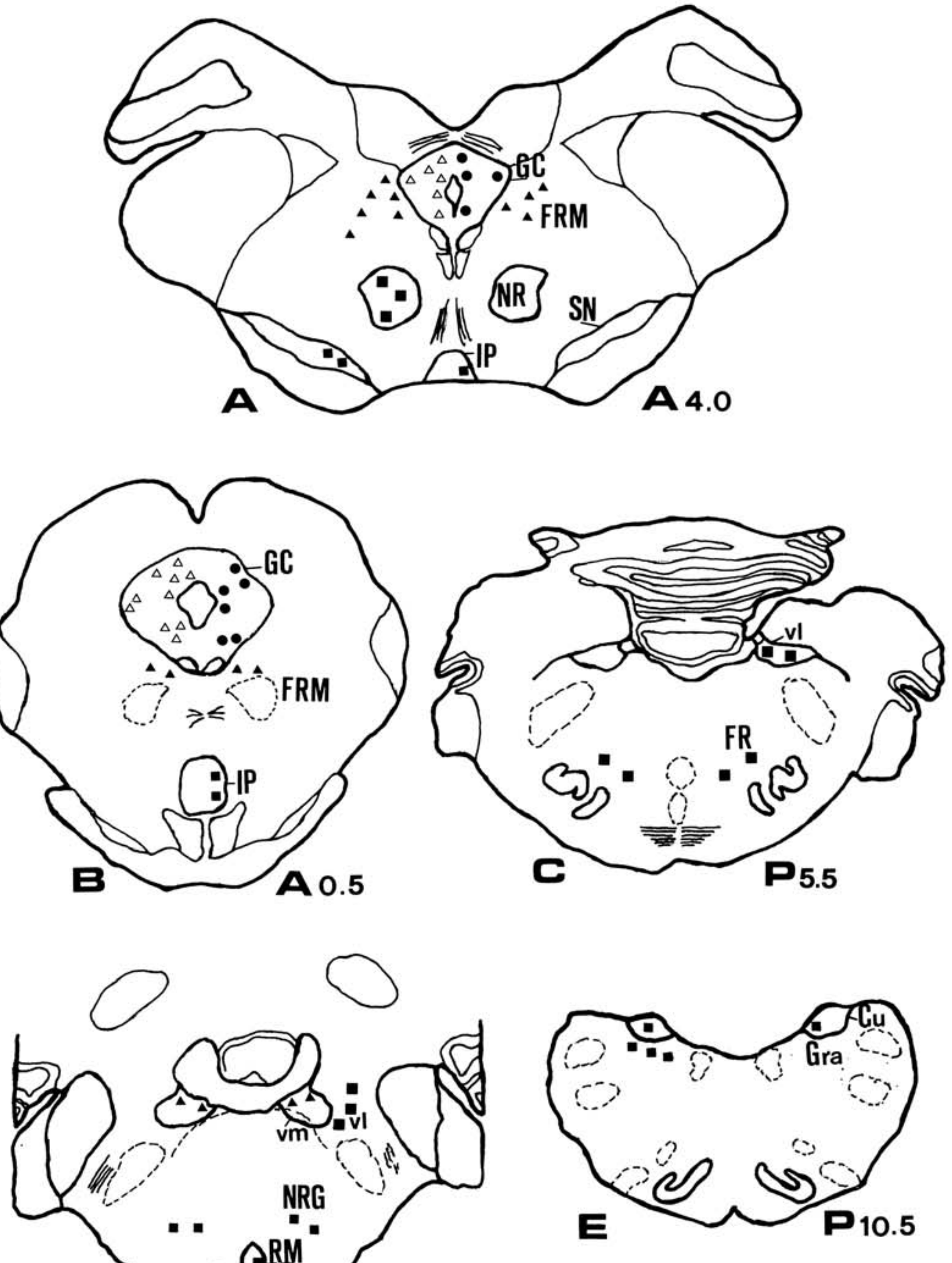

Fig. 1. – *Distribution in the cat brainstem of HRP-positive cell bodies from A 4.0 to P 10.5.*

The left side of each section represents the ipsilateral side, whereas the contralateral one is on the right. The symbols indicate the density of cell bodies (△ very high density, > 20 cell bodies; ● high density, 10-20 cell bodies; ▲ middle density, 6-10 cell bodies; ■ lower density, 1-5 cell bodies). The anterior (A) or posterior (P) level of each section is indicated in the lower right side.

Table 1. — *Summary of the area hypothalamica dorsalis inputs.* — The relative density of labeled neurons is indicated by the following symbols: + + + +, very high; + + +, high; + +, moderate; +, low.

Region of labeled cells	*Ipsilateral*	*Contralateral*
MIDBRAIN		
Substantia grisea centralis	+ + + +	+ + +
Nucleus ruber	+	
Formatio reticularis mesencephali	+ +	+ +
Nucleus interpeduncularis		+
Substantia nigra	+	
PONS		
Raphe magnus		+
MEDULLA OBLONGATA		
Formatio reticularis	+	+
Nucleus reticularis gigantocellularis	+	+
Nucleus cuneatus	+	+
Nucleus gracilis	+	
Nucleus vestibularis lateralis		+
Nucleus vestibularis medialis	+ +	+ +
Nucleus vestibularis inferior	+ +	

scarce afferents to the aHd originated from the nucleus raphe magnus. These observations should be related to previous findings showing projections from the aHd to both GC (12) and nucleus raphe magnus (8). Thus, all these data suggest the existence of a remarkably close anatomical relationship between the aHd and the GC and between the former structure and the nucleus raphe magnus.

In view of the different afferent projections to the aHd in the cat, it is likely to assume that this area is involved in several functions. Thus, the large input to the aHd from the GC suggests the involvement of the hypothalamic area in visceral (17) and/or sexual (16) behaviour. Moreover, the afferents to the aHd from the GC, nucleus raphe magnus and nucleus reticularis gigantocellularis which have been implicated by physiological findings in pain mechanisms (2, 5, 19), suggest a possible role of the aHd in nociceptive behaviour. In addition, the dorsal column nuclei (gracilis and cuneatus) also reach the aHd suggesting that the aHd could be involved in somatosensory mechanisms (4).

A final comment concerns the demonstration that in the cat motor related structures, such as the nucleus ruber from the midbrain, and the nuclei vestibularis lateralis, medialis and inferior from the medulla oblongata, send afferents to the aHd. Thus, the location of HRP-labeled neurons in such nuclei suggests the involvement of the aHd in motor mechanisms. Finally, the input to the aHd from the FRM suggests the involvement of the former structure in mechanisms controlling the sleep-waking cycle, since the aHd might represent a link of the ascending activating circuits originated in the FRM (14).

In summary, the present data show that the largest contingent of afferents to

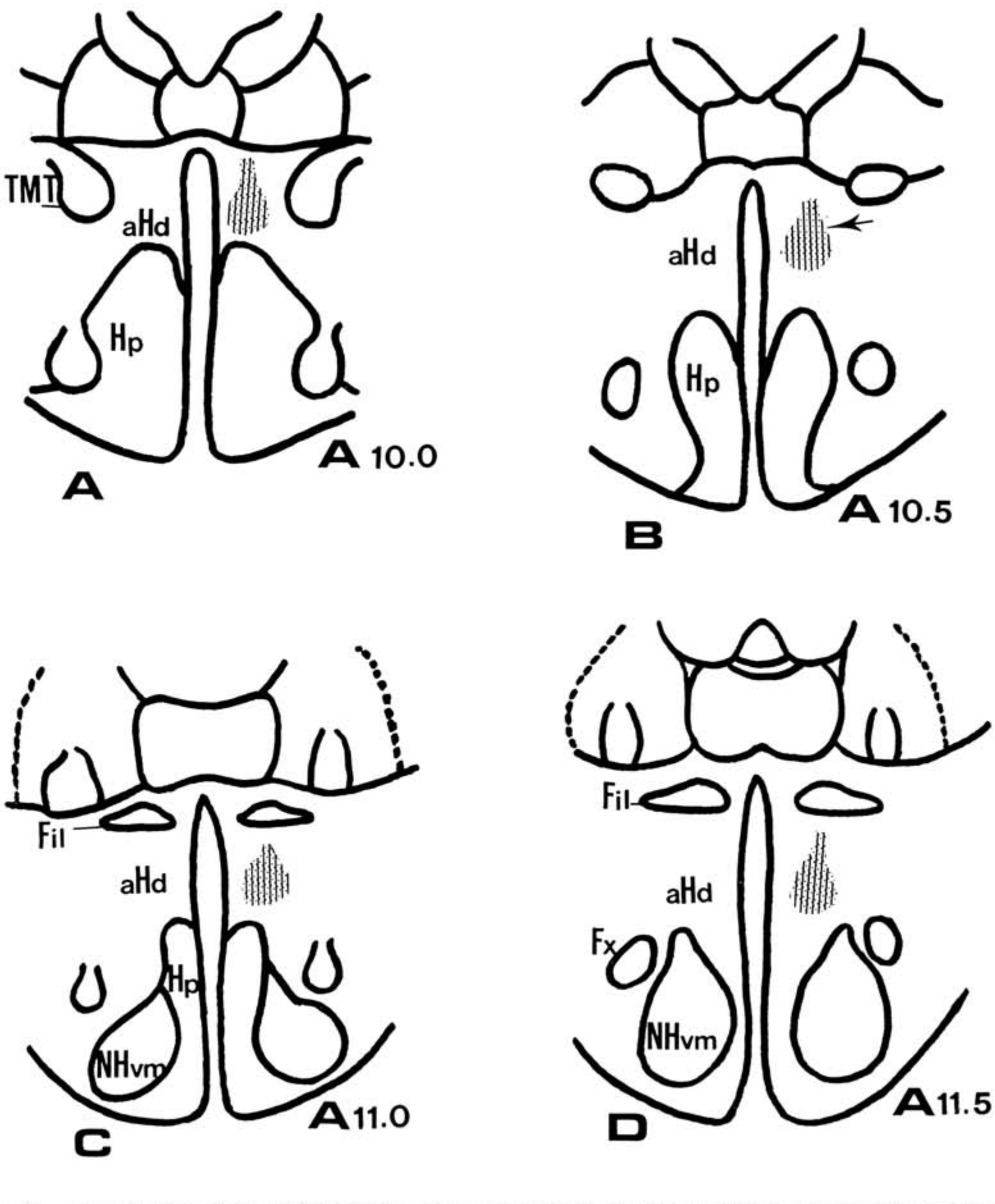

Fig. 2. – *Localization of the HRP injection place (arrow) and the largest diffusion region (shaded areas) observed in the seven experiments from A 10.0 to A 11.5.*

The stereotaxic level of each section is indicated in the lower right side. In all instances the HRP injections were confined strictly to the aHd.

the aHd comes from the GC. According to the aHd sources this hypothalamic region could play a role in visceral, sexual, nociceptive, somatosensorial and motor mechanisms.

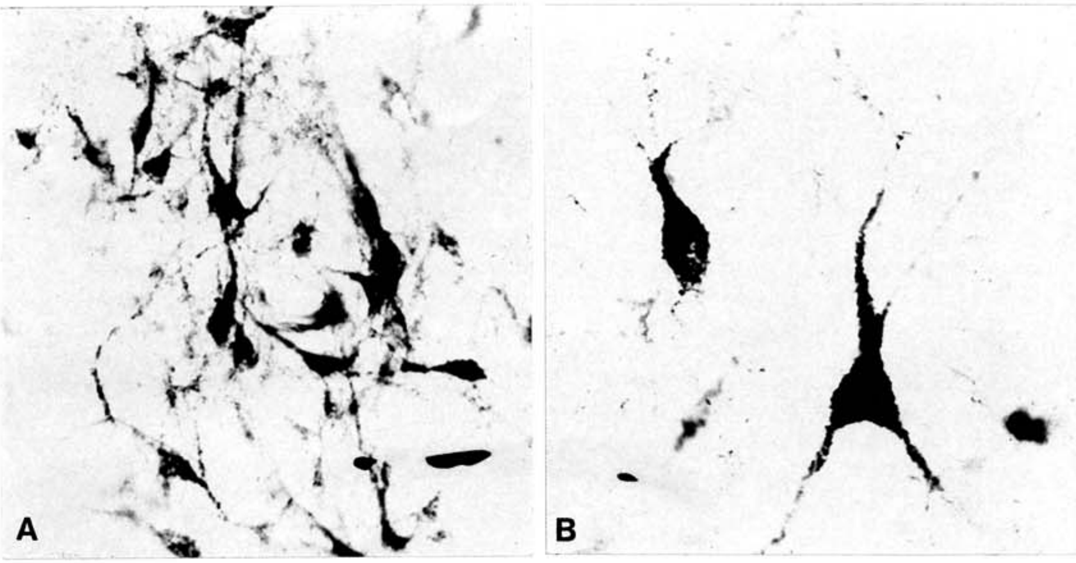

Fig. 3. - *Photomicrographs of retrogradely labeled neurons.*

A: HRP-positive neurons located in the substantia grisea centralis (×250).
B: HRP-labeled cell bodies in the nucleus gracilis (×400).

SUMMARY

Experiments using the retrograde transport of horseradish peroxidase were performed in order to identify the cells of origin the ascending projections from different brainstem regions to the area hypothalamica dorsalis (aHd) in the cat. The afferent inputs to this area originate mainly from the midbrain and medulla oblongata regions. The main afferent source of the area hypothalamica dorsalis arises from the substantia grisea centralis, where a large number of labeled cells were observed bilaterally, although more abundant on the ipsilateral side. Substantial afferents reach the aHd from the nuclei vestibularis medialis and inferior and the formatio reticularis mesencephali. A modest number of peroxidase-labeled neurons were observed in the nuclei ruber, interpeduncularis, substantia nigra, reticularis gigantocellularis, vestibularis lateralis, cuneatus and gracilis. From the pons, the nucleus raphe magnus sends a weak projection to the aHd. These anatomical data suggest that such area could be involved in visceral, sexual, nociceptive somatosensorial, sleep-waking and motor mechanisms.

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