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DIFFERENTIAL INPUT FROM THE AMYGDALOID BODY TO THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS IN THE RAT

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Differential amygdaloid afferents to anterior dorsal, anterior ventral, posterior dorsal and posterior ventral subdivisions of the ventromedial hypothalamic nucleus (VMH) were studied by means of retrograde transport of horseradish peroxidase (HRP). Injections of tracer confined to the VMH subdivisions mentioned, and enhancement of tracer uptake and transport were achieved by iontophoretic delivery of an HRP solution containing poly-L-α-ornithine. It was shown that the medial, central, basolateral, basomedial, lateroposterior and intercalated nuclei of the amygdala constitute afferent input sources to the ventromedial nucleus in a topographic pattern related to the various subdivisions of the VMH. This topographically organized amygdala–VMH projection is discussed against the background of the functional role that both amygdala and VMH play in the control of feeding, apart from various other autonomous functions that both brain centers are known to be concerned with.

It is a well-documented phenomenon that both the ventromedial hypothalamic nucleus (VMH) and the amygdaloid body of mammals function as parts of a brain substrate that controls a variety of behaviors including food and water intake, defensive, offensive and other social behaviors. Several lesion and stimulation studies, especially more recent ones, have indicated that different subdivisions of both VMH and amygdala are concerned with different functions. Knowledge on the functional differentiation of the VMH is rather limited, but several reports on lesion and electrical stimulation studies point to different roles of various anterior, posterior, as well as dorsal and ventral, parts in social and feeding behavior [6, 12, 19, 24]. Much more extensive is the literature on amygdaloid functions. In the context of this paper it suffices to conclude that, apart from various functions not to be mentioned, different aspects of social behavior [3, 17, 23] or feeding behavior [1, 2, 21, 22, 25] are mediated by different nuclei or areas of the amygdaloid complex. In general it is suggested by these authors that the various amygdaloid nuclei

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function in the selection of sensory stimuli that, via the hypothalamic projections of the amygdaloid nuclei, exert stimulating or inhibiting drives for different forms of the behaviors mentioned.

The functional differentiation of VMH and amygdala described above has received much less attention from a structural point of view. Although the cellular structure of the VMH has been described in detail [18] and subdivisions have been described based on cytological data [7], the picture is not so clear for the VMH input on a subnuclear level. Excellent autoradiographic data on amygdala–VMH projections are reported by Krettek and Price [11], which, however, reveal more about differential efferentation of the amygdala than about differential afferentation to the VMH. Retrograde transport studies on VMH afferents, on the other hand, were often hampered by the relatively large size of tracer injections that do not permit conclusions concerning afferents to subdivisions of the VMH, or lack of sensitivity of procedures employed [9, 14, 15].

In the present study we have attempted to produce HRP deposits restricted to subdivisions of the VMH by combining iontophoretic delivery methods in fine-tipped micropipettes filled with a poly-L-α-ornithine-containing HRP solution [8]. This resulted in small HRP injections, a limited diffusion area (Fig. 1) and enhancement of tracer uptake and subsequent retrograde labeling. Male albino Wistar rats (300

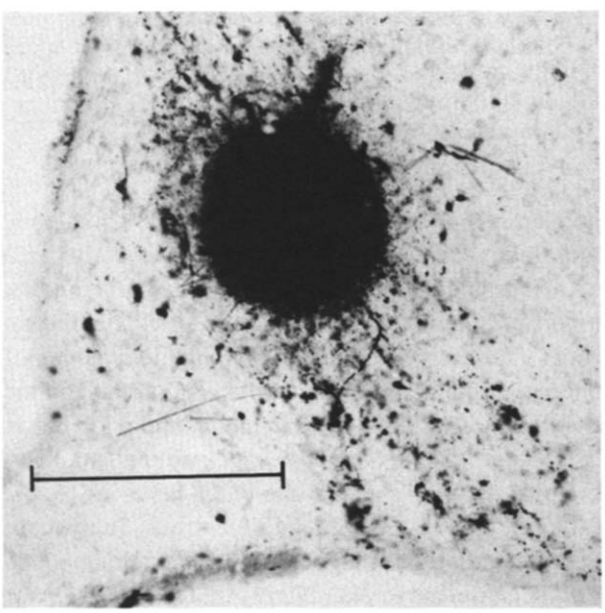

Fig. 1. Photomicrograph of an HRP injection in the ventromedial hypothalamic nucleus stained according to Mesulam's TMB procedure. The tracer, that was injected iontophoretically with a solution containing 0.3% poly-L-α-ornithine, is limited to subnuclear levels and does not show an extensive diffusion area in spite of the sensitive staining procedure employed. Scale bar = 250 μm.

g) were anesthetized with sodium pentobarbital and placed in a Narishige stereotaxic apparatus. Glass micropipettes filled with a 20% HRP (Sigma VI) in saline solution containing 0.3% poly-L-α-ornithine (Sigma), with tip diameters between 10 and 15 μm, were placed in the medial hypothalamic area according to König and Klippel coordinates [10]. An interrupted rectangular, positive DC current of 2–3 μA was applied to the pipette by means of a constant current source (Nihon Kohden Co.) during 7.5 min total on-time (cycle 7 sec on/7 sec off). After a 24 h survial period brains were fixed by perfusion with a buffered 0.5% paraformaldehyde, 1.5% glutaraldehyde, 4% sucrose solution, stored overnight at 4°C in buffered 30% sucrose, and cut at 40 μm sections on a cryostat microtome. Every second section was reacted for HRP according to Mesulam's TMB method [16] or the benzidine-dihydrochloride procedure of De Olmos and Heimer [5], and quickly counterstained with neutral red. From 18 successful injections we selected 8 cases with tracer deposits confined to subdivisions of the VMH, which we divided into 4 groups: anterior dorsal, anterior ventral, posterior dorsal and posterior ventral.

The present results (Fig. 2) demonstrate that the VMH as a whole receives afferents mainly from the medial amygdaloid nucleus (am), but also from the central (ac), the basolateral (abl), the basomedial (abm) and the lateroposterior (alp) nuclei and from the area around the intercalated mass (mi). There is, however, a clear organization of these amygdaloid afferents related to the location of the tracer injection within the VMH (Fig. 2). Anterior am cells (level A 5.66) appear to send projections to dorsal VMH divisions only. Lateroposterior (alp) and basomedial (abm) amygdaloid cells situated around level A 4.62 maintain efferent connections limited to the anterior dorsal subdivision of the VMH, while the same appears to be true for the central amygdaloid neurons at level A 4.23. At the same level (A 4.23) in the amygdala a considerable afferent input to the anterior ventral part of the VMH was observed from cells in the intermediate cells mass between basal and medial amygdaloid nuclei. Considerable differentiation of am projections to the VMH can be observed at various levels of the amygdaloid body. In general it can be concluded that the anterior parts of the am have more extensive projections to dorsal subdivisions of the VMH, both anterior and posterior, while the more posterior parts of the am maintain more numerous efferent connections to the ventral aspects of the VMH. With respect to the most posterior pole of the medial amygdala (not illustrated in the figure), there does not seem to exist a differentiation in VMH projections. This part of the am constitutes an extensive input source to all subdivisions of the VMH.

In summary we can state that the amygdaloid body contains a variety of input sources to the ventromedial hypothalamic nucleus aimed at different targets within the VMH. A most interesting question arises whether this topographic pattern of amygdala–VMH interrelations is reflected in a functional differentiation. From a number of studies on the effects of localized amygdala lesions we may conclude that all amygdaloid input sources to the VMH as found in this study appear to be involv-

ed in taste-dependent integration processes, such as taste discrimination by the central amygdala, conditioned taste aversion by lateroanterior nucleus (ala), alp and abl [1, 13, 21], or inhibition of taste-mediated drives by the medial amygdala [21]. The obvious conclusion that such amygdaloid functions are related to the role the VMH plays in the control of food intake is supported by the rather extensive set of amygdala–VMH projections as described here and elsewhere [9, 11, 14, 15, 20]. It remains unclear, however, in what sense the topographic organization of these projections as described in this report is reflected in the various aspects of the nervous

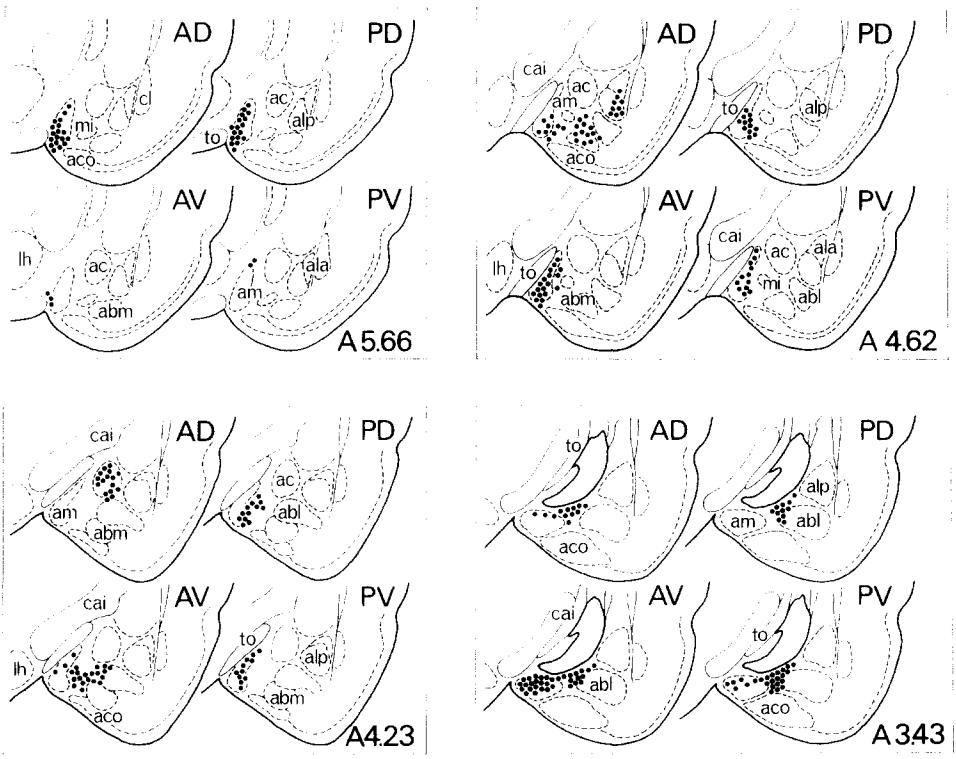

Fig. 2. The figure is composed of 4 outlined sets of transverse sections through the amygdaloid body drawn from König and Klippel's atlas [10]. Each set consists of 4 identical transverse sections at anterior–posterior levels indicated at the bottom right within each outline in millimeters anterior to the interaural line. In each of the identical sections retrogradely labeled somata are indicated by black dots, following HRP deposits in the anterior dorsal (AD), the posterior dorsal (PD), the anterior ventral (AV) and the posterior ventral (PV) subdivisions of the VMH. This presentation of results readily visualizes the differences in amygdala labeling following different tracer injections in the various subdivisions of the VMH. Abbreviations: abl, nucleus amygdaloideus basalis pars lateralis; abm, nucleus amygdaloideus basalis pars medialis; ac, nucleus amygdaloideus centralis; aco, nucleus amygdaloideus corticalis; ala, nucleus amygdaloideus lateralis pars anterior; alp, nucleus amygdaloideus lateralis pars posterior; am, nucleus amygdaloideus medialis; cai, capsula interna; lh, lateral hypothalamic area; cl, claustrum; mi, massa intercalata; to, tractus opticus.

control of a complicated process like food and water intake, which is mainly due to our scarce knowledge on the effects of partial VMH lesions. Moreover, it appears to be that the electrophysiological method to establish the nature of the brain areas under study allows a much more precise functional determination at subnucleus levels, as compared to the lesion method [4, 12, 20].

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