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ASCENDING PARABRACHIO-THALAMO-STRIATAL PATHWAYS: POTENTIAL CIRCUITS FOR INTEGRATION OF GUSTATORY AND ORAL MOTOR FUNCTIONS

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Abstract—The medial parabrachial nucleus (MPB) and external part of the medial parabrachial nucleus (MPBE) relay gustatory, oral mechanosensory and other visceral information in the rat brain and reportedly project not only to the parvicellular part of the posteromedial ventral thalamic nucleus (VPMpc) but also to the ventrocaudal part of the intralaminar thalamic nuclei. Generally, the intralaminar thalamic nuclei project topographically to the caudate putamen (CPu); however, it is unclear where the ventrocaudal part of the intralaminar thalamic nuclei projects within the CPu. Thus, we visualized neural pathways from the MPB and MPBE to the CPu via the ventrocaudal part of the intralaminar thalamic nuclei using an anterograde tracer, biotinylated dextran amine, and a retrograde tracer, cholera toxin B subunit. We found that the MPB and MPBE sent a relatively stronger input to the ventrocaudal part of the intralaminar thalamic nuclei such as the oval paracentral thalamic nucleus (OPC), central medial thalamic nucleus (CM) and parafascicular thalamic nucleus (PF) and retroreuniens area (RRe) as compared to the VPMpc. In turn, these thalamic nuclei projected to the ventral part of the CPu with the topographical arrangement as follows: the OPC to the ventrocentral part of the CPu; ventrolateral part of the PF to the ventrolateral part of the CPu; and the caudal part of the CM, ventromedial part of the PF and RRe to the ventromedial part of the CPu. Further, we found that the VPMpc rather projected to the interstitial nucleus of the posterior limb of the anterior commissure than the CPu. The ventral part of the CPu is reported to be involved in jaw movement as well as food and water intake functions. Therefore, these parabrachio-thalamo-striatal pathways that we demonstrated here suggest that gustatory and oral mechanosensory information affects feeding behavior within the ventral part of the CPu.

Key words: parabrachial nucleus, thalamus, corpus striatum, taste, rat.

INTRODUCTION

The rat parabrachial nuclei lie in the dorsolateral pons surrounding the superior cerebellar peduncle (i.e., brachium conjunctivum) and are composed of multiple, morphologically distinct subnuclei (Lundy and Norgren, 2004; Saper and Stornetta, 2014). These subnuclei are known to have different input-output organization and to serve different functions such as gustation, visceral sense, nociception, thermal sense and respiration (Herbert et al., 1990; Feil and Herbert, 1995; Lundy and Norgren, 2004; Saper and Stornetta, 2014). Among the subnuclei, the medial parabrachial nucleus (MPB) and external part of the medial parabrachial nucleus (MPBE) have been reported to be main gustatory and oral mechanosensory relay sites to the insular cortex through the parvicellular part of the posteromedial ventral thalamic nucleus (VPMpc) (Halsell and Travers, 1997; Lundy and Norgren, 2004) as well as the MPB has related to non-rapid eye movement sleep in the mouse brain (Kaur et al., 2013). In addition to the VPMpc, the MPB and MPBE have been described to project to the ventrocaudal part of the intralaminar thalamic nuclei around the VPMpc (Bester et al., 1999; Shin et al., 2011).

The rat intralaminar thalamic nuclei are composed of the centrolateral thalamic nucleus (CL), paracentral thalamic nucleus (PC), central medial thalamic nucleus (CM), parafascicular thalamic nucleus (PF) and oval paracentral thalamic nucleus (OPC), and mainly project

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Abbreviations: ac, anterior commissure; acp, posterior part of the anterior commissure; ASt, amygdalostriatal transition area; BDA, biotinylated dextran amine; BLA, basolateral amygdaloid nucleus; CeA, central amygdaloid nucleus; CL, centrolateral thalamic nucleus; CM, central medial thalamic nucleus; CPu, caudate putamen; CTb, cholera toxin B subunit; fr, fasciculus retroflexus; GP, globus pallidus; IMD, intermediodorsal thalamic nucleus; IPAC, interstitial nucleus of the posterior limb of the anterior commissure; LA, lateral amygdaloid nucleus; LPBE, external part of the lateral parabrachial nucleus; LPBV, ventral part of the lateral parabrachial nucleus; MD, mediodorsal thalamic nucleus; ml, medial lemniscus; MPB, medial parabrachial nucleus; MPBE, external part of the medial parabrachial nucleus; OPC, oval paracentral thalamic nucleus; PBS, Phosphate-buffered saline; PC, paracentral thalamic nucleus; PF, parafascicular thalamic nucleus; PHA-L, *Phaseolus vulgaris* leucoagglutinin; PR, prerubral field; RRe, retroreuniens area; scp, superior cerebellar peduncle; SPF, subparafascicular thalamic nucleus; VM, ventromedial thalamic nucleus; VPM, posteromedial ventral thalamic nucleus; VPMpc, parvicellular part of the posteromedial ventral thalamic nucleus; VPpc, ventral posterior parvicellular thalamic nucleus.

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to the cerebral cortex and caudate putamen (CPu) (Groenewegen and Witter, 2004; Vertes et al., 2014). Since it has been reported that projections from the intralaminar thalamic nuclei are distributed within the CPu in a topographical arrangement (Groenewegen and Witter, 2004; Vertes et al., 2014), we predicted that gustatory and oral mechanosensory information from the MPB and MPBE is conveyed to the CPu topographically via the ventrocaudal part of the intralaminar thalamic nuclei. However, only a few reports are available regarding the thalamo-striatal projections from the ventrocaudal part of the intralaminar thalamic nuclei. Anterograde tracer studies that were performed in the ventrocaudal part of the intralaminar thalamic nuclei have shown projections from the caudal part of the CM to the ventral and ventromedial parts of the CPu (Vertes et al., 2012), from the lateral part of the PF to the lateral part of the CPu, and from the medial part of the PF to the medial part of the CPu (Berendse and Groenewegen, 1990). Thus, projection fibers from the caudal part of CM and medial and lateral parts of the PF are likely to be distributed within the CPu topographically, as our expectation. However, the OPC and ventral part of the PF projections are unknown, and further, it is unclear whether neural pathways exist from the MPB and MPBE to the CPu via the ventrocaudal part of the intralaminar thalamic nuclei.

The aim of the present study is to reveal the topographical arrangement of the parabrachio-thalamo-striatal pathways within the CPu. To identify neural pathways from the MPB or MPBE to the CPu via the ventrocaudal part of the intralaminar thalamic nuclei in the rat brain, we (1) confirmed the presence of projections from the MPB or MPBE to the ventrocaudal part of the intralaminar thalamic nuclei; (2) examined projections from the ventrocaudal part of the intralaminar thalamic nuclei to the CPu using an anterograde tract-tracing method; and (3) examined overlaps between axonal varicosities from the MPB or MPBE and projection neurons from the ventrocaudal part of the intralaminar thalamic nuclei to the CPu using an anterograde and retrograde double-labeling method.

EXPERIMENTAL PROCEDURES

Animals

The present study was approved by the Animal Experiment Committee of Kagoshima University and performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used. The present study used 46 male Wistar rats weighing 250–350 g (Kyudo, Kumamoto, Japan). Tracer injections were performed under anesthesia induced by an intraperitoneal injection of chloral hydrate (350 mg/kg).

Anterograde labeling

In 40 rats, 2% biotinylated dextran amine (BDA; Invitrogen, Carlsbad, CA, USA) in 0.1 M phosphate buffer (pH 7.3) was injected unilaterally into the MPB (n = 5), MPBE (n = 5), OPC (n = 5), caudal part of the CM (n = 5), ventral part of the PF (n = 10), VPMpc (n = 5) or retroreuniens area (RRe) (n = 5) by iontophoresis (positive, 4 μA, 3 Hz, 6–15 min) through a glass micropipette (tip diameter: 30–50 μm). After a survival period of 3–7 days, rats were deeply reanesthetized, transcardially perfused with phosphate-buffered saline (PBS; pH 7.3), and then fixed with 4% formaldehyde in phosphate buffer. Subsequently, the brains were removed, postfixed with the same fixative solution at 4 °C for 12 h, and immersed in 30% sucrose in PBS at 4 °C for 1–2 days. Serial frontal sections (50 μm) of the brains were cut on a freezing microtome. Sections were divided into two sets, one for BDA visualization and one for counterstaining using 0.5% Cresyl Violet. BDA was visualized according to the procedure of Veenman et al. (1992). Sections were incubated with 0.2% Triton X-100 in PBS for 3 h and 1:100 avidin-biotin-peroxidase complex (Elite ABC Kit; Vector Laboratories, Burlington, CA, USA) in PBS for 1 h. Finally, sections were reacted with 0.02% diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St Louis, MO, USA), 0.07% $NiCl_2$ and 0.005% H_2O_2 in 0.05 M Tris-HCl buffer for 20 min. BDA-labeled axonal varicosities were identified as black staining. Sections were mounted on gelatin-coated glass slides, air-dried, and coverslipped.

Anterograde and retrograde double-labeling

In six rats, BDA was injected into the MPB (n = 3) or MPBE (n = 3) as described above, and 0.5% cholera toxin B subunit (CTb; List Biological Laboratories, Campbell, CA, USA) in phosphate buffer was injected unilaterally into the CPu by iontophoresis (positive, 4 μA, 2 Hz, 15 min) through a glass micropipette (tip diameter: 50 μm). After a survival period of 5–7 days, reanesthesia, perfusion fixation, and sectioning were performed as described above. BDA and CTb were visualized according to the procedure of Coolen et al. (1999). After the BDA reaction described above, for CTb visualization, sections were incubated with 3% normal rabbit serum, 1:10,000 goat anti-CTb (List Biological Laboratories, Campbell, CA, USA), and 0.2% Triton X-100 in PBS at 4 °C overnight; 1:200 biotinylated rabbit anti-goat IgG (Vector Labs, Burlington, CA, USA) in PBS for 3 h; and then 1:100 avidin-biotin-peroxidase complex in PBS for 1 h. Finally, sections were reacted with 0.02% diaminobenzidine tetrahydrochloride and 0.005% H_2O_2 in 0.05 M Tris-HCl buffer for 20 min. CTb-labeled neurons were identified as brown staining. Sections were mounted on gelatin-coated glass slides, air-dried, and coverslipped.

Photomicrographs of injection sites, labeled axonal varicosities, fibers and neurons were taken using a digital slide scanner (Toco; Claro, Aomori, Japan). Labeled axonal varicosities and neurons were drawn from photomicrographs using Illustrator CS6 (Adobe Systems, San Jose, CA, USA) and confirmed by light microscopy. Brain structure identification was based on Paxinos and Watson (2007). In addition, the parabrachial and thalamic nuclei were classified according to Fulwiler