Thalamic posterior ventral neurons with bifurcating axons to the first and second somatosensory areas in the cat, demonstrated by the fluorescent retrograde double labeling technique.

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Abstract

The thalamic posterior ventral neurons with bifurcating axons to both the first and second somatosensory cortical areas (SI and SII) in the cat were examined by the fluorescent retrograde double labeling technique. After injection of Evans blue (EB) into the SI, and of 4',6-diamidino-2-phenylindol.2HCl (DAPI) into the SII of the same hemisphere, EB- and DAPI-labeled cells were observed predominantly in both the posterolateral ventral and the posteromedial ventral nuclei of the thalamus. Although EB single-labeled and DAPI single-labeled cells tended to occupy separate regions within the posterior ventral nuclei, a small number of cells double-labeled with both EB and DAPI were detected in the border zone between two single-labeled cell groups. These observations indicate that some cells in the posteromedial and posterolateral ventral nuclei project both to the SI and SII by bifurcating axons.

KEYWORDS: thalamus, somatosensory area, posterior ventral nuclei, axon collateral

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Thalamic Posterior Ventral Neurons with Bifurcating Axons to the First and Second Somatosensory Areas in the Cat, Demonstrated by the Fluorescent Retrograde Double Labeling Technique

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The thalamic posterior ventral neurons with bifurcating axons to both the first and second somatosensory cortical areas (SI and SII) in the cat were examined by the fluorescent retrograde double labeling technique. After injection of Evans blue (EB) into the SI, and of 4′,6-diamidino-2-phenylindole·2HCl (DAPI) into the SII of the same hemisphere, EB- and DAPI-labeled cells were observed predominantly in both the posterolateral ventral and the postero medial ventral nuclei of the thalamus. Although EB single-labeled and DAPI single-labeled cells tended to occupy separate regions within the posterior ventral nuclei, a small number of cells double-labeled with both EB and DAPI were detected in the border zone between two single-labeled cell groups. These observations indicate that some cells in the posteromedial and posterolateral ventral nuclei project both to the SI and SII by bifurcating axons.

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It has been well established that the thalamic posterior ventral nuclei project to the first and second somatosensory cortical areas (SI and SII) (1-13). Electrophysiological data have also indicated that some thalamic neurons send fibers both to the SI and SII (14-17). On the other hand, an anatomical investigation using retrograde cell degeneration in combination with retrograde transport of HRP suggested that some cells of the thalamic ventrobasal nucleus send axon branches to both the SI and SII (18). Recently, a noteworthy advance in neuroanatomical methods using retrograde tracers has made it possible to use two or three different kinds of tracers simultaneously to search for neurons with diverging axons to different areas (19-21). Recent studies using the retrograde double-labeling technique have shown that several neurons in the posterolateral ventral nucleus (VPL) project to both the SI and SII by axonal branching (21-23). In the present study, posterior ventral neurons projecting to both the SI and SII were examined by the fluorescent retrograde double labeling technique. A preliminary report of this study was given earlier (24).

Materials and Methods

Material for this study was collected from 23 hemispheres obtained from 16 adult cats. Animals were sedated with ketamin (8 mg/kg, i.m.) and surgical levels of anesthesia were induced by
sodium pentobarbital (35 mg/kg, i.p.). After the exposure of the somatosensory cortical areas, a total of 0.2–0.5 μl of 10% Evans blue (EB) (W/V) solution containing 1% poly-L-ornithine was injected over two or three needle penetrations into the SI (Fig. 1). On the same side of the brain, 0.2–0.4 μl of a 2.5% 4,6-diamidino-2-phenylindol·2HCl (DAPI) (W/V) aqueous suspension was injected in one or two needle penetrations into the SII. Three days later the animals were deeply anesthetized again and perfused transcardially with saline followed by 10% formalin. After 4–5 h, the brains were removed from the skull and kept overnight in 30% cacodylate-buffered sucrose (pH 7.2). Frozen sections were cut transversely at 50 μm, mounted from cacodylate buffer solution (pH 7.2) onto gelatine coated slides, air dried, and coverslipped with glycerol.

The materials were examined with an Olympus reflecting fluorescence microscope equipped with filter systems UV and G providing excitation lights of 360 nm and 550 nm wavelength, respectively. The DAPI-labeled cells showed a blue fluorescence in the cell nucleus at a wave length of 360 nm, whereas the EB-labeled cells displayed a red fluorescent cytoplasm at a wave length of 550 nm. Individual sections were photographed under the fluorescence microscope. Then, coverslips were carefully removed from the slides, and sections were counterstained with 0.1% cresyl violet. By this method it was possible to determine the exact location of labeled cells within the thalamus. The posterior ventral nuclei were identified using the criteria of Niimi and Kuwahara (25).

Results

In 9 cases, both EB-labeled and DAPI-labeled cells were detected in the posterior ventral nuclei. In cat 49L, EB was injected into the lateral part of the posterior sigmoid gyrus near the dorsal end of the coronal sulcus (SI), while DAPI was injected into the anterodorsal portion of the anterior ectosylvian gyrus (SII) (Fig. 2). In the posterior ventral nuclei, EB-labeled cells, which sent axons to the SI, were seen in the dorsal central portion of the VPL at anterior levels, and caudally increased in number spreading somewhat ventrally. With the appearance of the lateral geniculate nucleus (LGN) the labeled cells decreased in number and were not detected at the anterior level of the LGN. On the other hand, DAPI-labeled cells, which sent

Abbreviations used: aeg, anterior ectosylvian gyrus; aes, anterior ectosylvian sulcus; asg, anterior sigmoid gyrus; ass, anterior suprasylvian sulcus; cg, coronal gyrus; CL, central lateral nucleus; crs, crusiate sulcus; cs, coronal sulcus; LD, lateral dorsal nucleus; LG, lateral geniculate nucleus; LP, lateral posterior nucleus; ls, lateral sulcus; MD, mediadorsal nucleus; meg, medial ectosylvian gyrus; MG, medial geniculate nucleus; psg, posterior ectosylvian gyrus; pes, posterior ectosylvian sulcus; Pf, parafascicular nucleus; psg, posterior sigmoid gyrus; PL, lateral pulvinar nucleus; PM, medial pulvinar nucleus; Rt, thalamic reticular nucleus; Sg, suprageniculate nucleus; sg, suprasylvian gyrus; Sm, submedial nucleus; ss, sylvian sulcus; SI, first somatosensory area; SII, second somatosensory area; VL, ventral lateral nucleus; VM, ventral medial nucleus; VPL, posterolateral ventral nucleus; VPM, posteromedial ventral nucleus.
Fig. 2  Schematic representation of the distribution of single- and double-labeled cells in the posterior ventral nuclei of selected thalamic sections (10-15) following injections of EB (black area) into SI, and DAPI (faint-line shaded area) into SII in cat 49L. Dotted lines indicate the limitation of diffused dye. Each solid dot and open circle represents three or four cells single-labeled with EB and DAPI, respectively, and each half-solid circle represents one double-labeled cell.
Fig. 3  Schematic representation of the distribution of single- and double-labeled cells in the posterior ventral nuclei of selected thalamic sections (3–9) following injections of EB into SI, and DAPI into SII in cat 55L. Symbols as in Fig. 2.
axons to the SII, were observed in both the VPL and VPM. Within the VPL, the DAPI-labeled cells were distributed in its ventral and dorsomedial portions, but caudally they were widely scattered throughout the nucleus. DAPI-labeled cells were also found in the VPM, particularly in its marginal region. The cells double-labeled with both EB and DAPI were detected in the central portion of the VPL at about the most rostral level of the LGN.

In cat 49R, EB was injected into the posterior sigmoid gyrus as in cat 49L, whereas DAPI was injected into the anterior ectosylvian gyrus, anterodorsal to the injection site of cat 49L. Double-labeled cells were detected in the central part of the VPL arranged dorsoventrally.

In cat 55L, EB injections were made into the posterior sigmoïd gyrus and the coronal gyrus with two needle penetrations (Fig. 3). The injection sites were located near both lips of the coronal sulcus. The DAPI injection site was similar to that in cat 49L. EB-labeled cells were seen in the medial half of the VPL at the frontal level just rostral to the LGN. Caudally they were localized in the central portion of the VPL, and were not detected at the middle level of the LGN. EB-labeled cells were also detected in the VPM, particularly in the centroventral portion at about the same thalamic level. A large number of DAPI-labeled cells were seen in the ventral part of the VPL and in the dorsal part of the VPM at levels just anterior to the LGN, and spread caudally as far as the caudal parts of the VPL and VPM. Double-labeled cells were found in the central portions of the VPL and VPM at anterior and posterior levels. However, at their middle levels, EB single-labeled and DAPI single-labeled cells were separated, and no double-labeled cells were detected.

In the other six cases, EB- and DAPI-labeled cells overlapped partially within the posterior ventral nuclei, but no double-labeled cells were observed. For example, in cat 64R, EB was injected into the posterior sigmoïd and coronal gyri, and DAPI was injected into the dorsal region of the anterior ectosylvian gyrus (Fig. 4). EB- and DAPI-labeled cells were found in both the VPL and VPM. Although these single-labeled cells overlapped in the central and lateral portions of the VPM and in the VPL, no double-labeled cells were encountered.

**Discussion**

Sprafico et al. (9) divided the VPL into the “core” zone which is composed mainly of neurons projecting to the SI, and the surrounding “shell” zone which contains mainly neurons projecting to the SII. They reported that neurons projecting to both the SI and SII by axonal branching were located mainly in the border zone between the core and the shell zones. Fisher et al. (23)
demonstrated these neurons with branching axons in the dorsolateral portion of the medial division of the VPL by the injection of HRP and retrograde fluorescent dye into the SI and SII regions, respectively. The present data show that these neurons with branching axons are scattered unevenly in the VPL and VPM, particularly in their ventral and posterior regions. Many such neurons were found at the posterior levels of the VPL, where the posterior tip of the core zone was assumed to be in contact with the shell zone.

Electrophysiological studies has indicated that neurons projecting to both the SI and SII by axonal branching exist in the VPM (14). Our anatomical investigation also revealed neurons with bifurcating axons to the SI and SII in the VPM, in addition to the VPL. In the anatomical approach, special care was required for two kinds of retrograde tracers which are injected into the SI and SII separately to keep them from diffusing and merging. In cat 64R, EB injection sites in the SI were located near DAPI injection sites in SII, but the dyes would not diffuse and mix with each other. No double-labeled cells were detected in the posterior ventral nuclei despite the interdigitation of EB single-labeled and DAPI single-labeled cells. In cat 55L, the injection sites of EB and DAPI in the SI and SII were far more separated from each other than in cat 64R, but many double-labeled cells were found in cat 55L, in contrast to cat 64R. Consequently, the double labeling in the VPM in cat 55 is not considered to be caused by the merging of the two dyes at the injection sites, but may be caused by the bifurcation of axons to both the SI and SII.

Both EB-labeled and DAPI-labeled cells were seen in nine cases, only three cases of which contained double-labeled cells. In all cases, the SI-projecting and SII-projecting cells tended to lie separated from each other in the posterior ventral nuclei as revealed by Spreatieco et al. (9, 22) in the VPL. This indicates that they project in parallel from the posterior ventral nuclei to the SI and SII. The channeling function using the filtering mechanism is generally considered as the function of the neurons with bifurcating axons (26–31). Aside from this, these branching neurons are assumed to play a role in the communication between the neuronal populations in the parallel neuronal pathways (32).

References


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