

Short Communications

Ascending thalamic projections from the obex region in ranid frogs

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Amphibian forebrains display few obvious specializations and have frequently served as models of a primitive tetrapod condition from which more specialized sauropsid and therapsid forebrains have been derived. This approach to tetrapod forebrain evolution has yet to be fully exploited, as our knowledge of amphibian forebrain pathways is embryonic. Most experimental studies have focused on primary and secondary visual connections, and information concerning other systems is relatively sparse. Information on forebrain somatosensory pathways is particularly slight, and experimental data is available only for anurans. Anatomical studies using selective silver impregnation methods have failed to reveal a direct spinothalamic projection in frogs^{4,5,9}. Although a projection to the thalamus has been described following hemisection near the obex in *Rana*, it appears to be light and is restricted to a very small portion of the caudalmost thalamus⁵. The apparent paucity of somatosensory projections to the forebrain seen in anatomical studies contrasts strikingly with the electrophysiological results of Vesselkin et al.¹⁷, who described an extensive thalamic somatosensory representation on the basis of recordings following sciatic nerve stimulation in *Rana temporaria*.

Since there appears to be some conflict between anatomical and physiological studies regarding the extent of thalamic somatosensory representation in anurans, and since a precise determination of this representation is essential to an understanding of tetrapod forebrain evolution, we have initiated a series of experiments on the ascending somatosensory system in ranid frogs. This preliminary report will describe our major findings to date.

Nine bullfrogs, *Rana catesbeiana*, received single unilateral injections of 1–4 μCi of [³H]proline or a [³H]proline-leucine mixture at a concentration of 20 $\mu\text{Ci}/\mu\text{l}$. Injections were made at various levels between the caudal (lumbar) enlargement of the spinal cord and the obex. Survival times ranged from 2–9 days at temperatures of

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20–23 °C. Following fixation in AFA, the brains were processed for autoradiography using either Ilford K2 or Kodak NTB-3 emulsions. Autoradiographs were exposed for 4 weeks at 7 °C, developed in D-19, and counterstained with cresyl violet. Of these 9 cases, three (SCI-7, SCI-8 and SCI-9) will be discussed here.

In case SCI-7, 1 μ Ci of [3 H]proline was injected 1.5 mm caudal to the obex. Survival time was three days. Examination of the injection site and surrounding area revealed the presence of labelled cells from just caudal to the second spinal nerve (S2) through levels coincident with the caudal two-thirds of the hypoglossal nucleus. In this case, as in all others, there was some spread of label to the contralateral side. In no case, however, was there any indication of isotope leakage into the ventricular system. Ependymal cells rostral to the injection sites were unlabelled as were the populations of CSF-contacting cells in the hypothalamus and preoptic area.

In case SCI-8, 4 μ Ci of a [3 H]proline-leucine mixture were injected near the caudal end of the rostral (brachial) enlargement of the spinal cord. Survival time was 6 days. Labelled cells were present throughout most of the rostral enlargement and were found as far rostral as one-third the distance between S2 and the obex.

In case SCI-9, 4 μ Ci of the [3 H]proline-leucine mixture were injected just behind S2. Survival time was also 6 days. The entire rostral enlargement was labelled, with

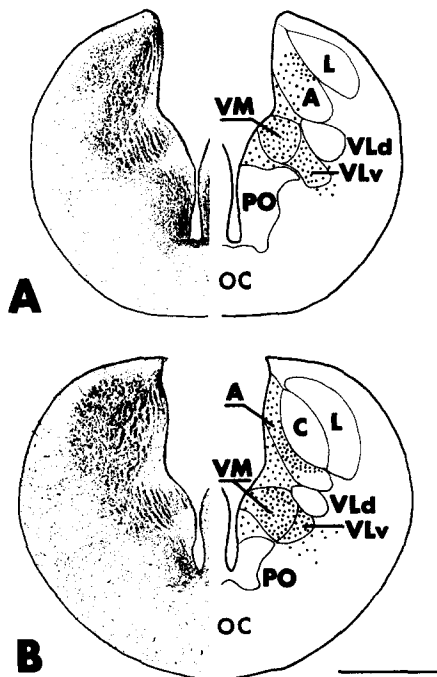


Fig. 1. Transverse sections at the levels of the rostral (A) and middle (B) optic chiasm showing contralateral projections seen in SCI-7. High contrast photographs taken from standard Bodian-impregnated, cresyl violet counterstained reference series. Abbreviations: A, anterior nucleus; C, central nucleus; L, lateral nucleus; OC, optic chiasm; PO, preoptic area; VLd, dorsal ventrolateral nucleus; VLv, ventral ventrolateral nucleus; VM, ventromedial nucleus. Nomenclature after Neary¹³. Calibration bar = 1 mm.

labelled cells present as far rostral as three-quarters of the distance between S2 and the obex.

In cases SCI-8 and SCI-9, where labelled cells were confined to the spinal cord proper, no indication of a projection to the thalamus was seen, a result confirming the lesion studies of Hayle⁹ and Ebbesson^{4,5}. The distribution of silver grains in the rhombencephalon and mesencephalon corresponded closely to the results of Ebbesson⁵, although the overall density of projections, particularly to the mesencephalon, was greater in our material.

In case SCI-7, where labelled cells were present rostral to the obex, an extensive, above background, distribution of grains was seen in the thalamus, primarily contralateral to the injection site. This distribution appears to represent the projections of labelled fibers which ascended via the spinal lemniscus of Ebbesson⁵. The heaviest concentration of grains was found throughout the rostrocaudal extent of the ventral thalamus (Figs. 1 and 2A). Rostrally, where three divisions of the ventral thalamus can be recognized (Fig. 1), grains were distributed in moderate density over the medial (VM) and ventrolateral (VLv) divisions. The dorsolateral division (VLd) was, however, unlabelled. Slightly lighter, but significant, grain densities were seen over the medial half of the posterior nucleus (Fig. 2A) and over a portion of the anterior nucleus (Fig. 1). The rostradorsal pole of the anterior nucleus was unlabelled,

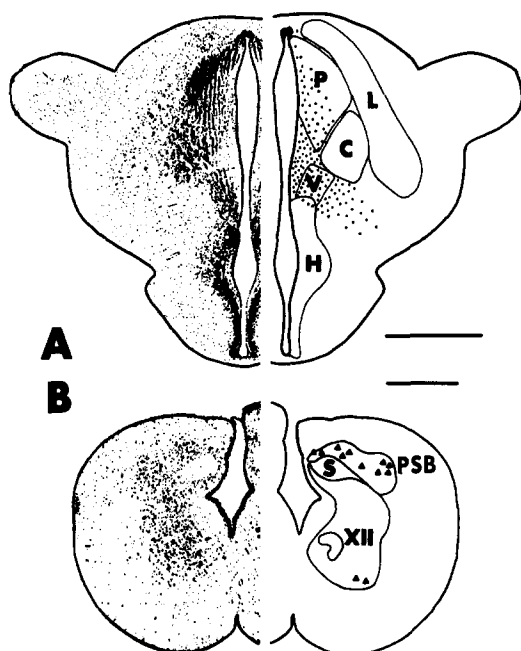


Fig. 2. A: distribution of contralateral projections in the caudal thalamus in case SCI-7. Abbreviations: H, hypothalamus; P, posterior nucleus; V, ventral thalamus. Calibration bar = 1 mm. B: transverse section just rostral to the obex showing distribution of HRP-positive cells (triangles) contralateral to HRP injections in cases DX-6,8. Abbreviations: PSB, perisolitary band; S, solitary tract; XII, hypoglossal nucleus. Calibration bar = 0.5 mm.

as was its ventrolateral aspect. Within the labelled intermediate portion a notably higher grain density was seen just ventromedial to the lateral and central nuclei. Finally, some indication of higher grain densities was observed over the caudal portions of the lateral nucleus, but because of light and irregular grain distribution in this area we are unwilling to claim a somatosensory projection to this nucleus at this time.

The injection in case SCI-7 indiscriminately labelled cells in several populations in the spinal cord and caudal medulla; therefore, we have also begun a series of HRP experiments to determine the cells responsible for this presumed somatosensory projection to the thalamus. At present, 8 injections of 40% HRP (Sigma type VI) have been made into the rostral one-third of the thalamus in both *Rana catesbeiana* and *Rana pipiens*. Injection volumes varied from 25 to 200 nl, and survival times ranged from 5 to 9 days at temperatures of 20–23 °C. Following fixation in buffered 2% glutaraldehyde, frozen sections were processed by modifications of previously published methods^{2,7}.

In 5 cases where HRP injections were confined primarily to the habenular complex and rostral anterior nucleus, HRP-positive cells were not detectable in the spinal cord or caudal medulla. In the remaining three cases, where the rostral ventral thalamus was injected as well, HRP-positive cells were found in the caudal medulla. These cells were localized in two areas: the reticular formation ventrolateral to the hypoglossal nucleus, and a band of cells lying dorsal and lateral to the solitary tract (Fig. 2B). Of 112 cells counted in two cases (DX-6,8), 26 were found in the reticular area, with 15 of these ipsilateral to the injection sites. Eighty-six cells were found in the perisolitary band, with 73 of these contralateral to the injection sites. The majority of these cells were found in the lateral portion of the band. All 13 of the ipsilateral cells were also located in the lateral portion. No labelled cells were evident in the spinal cord proper.

The cytoarchitecture of the perisolitary band is very complex and a variety of cell types is present. Although the band appears closely associated with the solitary tract, it also lies in close proximity to three major superficial tracts. The dorsal funiculus lies adjacent to the medial portion of the band^{11,19}, while the descending tract of V is superficial to the lateral portion^{3,6,10,18}. Many of the HRP-positive cells in the medial and lateral areas of the band possessed filled dendritic processes radiating toward their associated tracts. The intermediate portion of the band may lie deep to the descending tract of VIII^{8,12,14}. Many of the cells labelled in this area were of bipolar variety, with dendritic ramifications extending medially and laterally.

Several authors have equated the lateral portion of the perisolitary band with the spinal nucleus of V^{3,6,10,18}, and our results are in accord with this interpretation. Some investigators have also described a dorsal column nucleus^{4,5,16,19}. There is, however, considerable disagreement over its position, extent, and existence as a distinct cytoarchitectonic unit^{1,11,14,15}. On the basis of our results, we suspect that the medial portion of the perisolitary band may be related to the amniotic dorsal column nuclei. Nevertheless, it must be remembered that in mammals (and, undoubtedly, in other amniotes) the obex region contains many nuclear groups relaying somatosensory

information to the thalamus, and this portion of the perisolitary band may be related to any number of them.

Finally, our results indicate that anurans may, like mammals, possess multiple thalamic somatosensory areas. Unlike most mammals, however, none of the somatosensory input to the thalamus appears to come directly from the spinal cord. Thus it seems that a direct spinothalamic system may not represent a primitive tetrapod condition.

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