Neuroscience Letters, 26 (1981) 5–10
Elsevier/North-Holland Scientific Publishers Ltd.

THE CORTICAL PROJECTIONS OF THE THALAMIC INTRALAMINAR NUCLEI, AS STUDIED IN CAT AND RAT WITH THE MULTIPLE FLUORESCENT RETROGRADE TRACING TECHNIQUE

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(Received June 15th, 1981; Accepted July 7th, 1981)

Two retrograde fluorescent tracers were injected in two different areas of the cerebral cortex in rats and in cats. In all the experiments many single labeled cells and only some double labeled ones were seen in the thalamic intralaminar nuclei. The present results suggest that the diffusely distributed intralaminar-cortical projections mainly consist of axons of separate cells, and only to a minor extent of axon collaterals of the same cells.

The efferent projections of the intralaminar nuclei have represented one of the most debated problems of the anatomical organization of the thalamo-cortical system. Studies based on the degeneration techniques finally provided some evidence of direct cortical projections of the intralaminar nuclei, which were considered a major component of the so-called 'non-specific' thalamo-cortical system (see review in ref. 8). Studies based on horseradish peroxidase retrograde transport [5,7] confirmed the widespread distribution upon the cortex of the intralaminar afferents, which represents a characteristic feature of the non-specific thalamic projections. The recently introduced fluorescent retrograde double labeling technique [6] provided a tool for investigating anatomically whether these widely distributed thalamic projections are presented by axonal branches of the same cells, or whether they take origin from different cells. Fluorescent retrograde double labeling has been successfully applied to the detection of divergent axon collaterals in several systems in rat and cat [6].

Two tracers were injected in different areas of the cerebral cortex in 11 rats and 6 cats (Tables I and II). In rats the fluorescent retrograde tracers True Blue (TB) [2] and Fast Blue (FB) [3] were used in combination with the tracer Nuclear Yellow (NY) [3] (Table I). In cats, FB was used in combination with NY (Table II). TB and FB mainly label the neuronal cytoplasm while NY mainly labels the neuronal nucleus; moreover TB, FB and NY fluoresce in different colors at the same excitation wavelength [6]. Therefore TB and NY, as well as FB and NY, can be simultaneously visualized in one and the same double retrogradely labeled neuron [6]. Each tracer was injected with a microsyringe in one cortical region at a depth of

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TABLE I

EXPERIMENTAL DATA FOR RATS

Case	%	Tracer	µl	Injected cortex	%	Tracer	µl	Injected cortex	Survival time
Rat 1	2%	TB	0.3	Pregenual frontal	1%	NY	0.3	Agranular frontal	TB: 7 days NY: 18 hours
Rat 2	1%	NY	0.5	Pregenual frontal	2%	TB	0.4	Agranular frontal	TB: 4 days NY: 18 hours
Rat 2	2%	TB	0.6	Pregenual frontal	1%	NY	0.6	Parietal	TB: 6 days NY: 19 hours
Rat 4,5	1%	NY	0.4, 0.6	Pregenual frontal	3%	FB	0.4, 0.5	Parietal	FB: 4,5 days NY: 19,17 hours
Rat 6	1%	NY	0.4	Pregenual frontal	2%	TB	0.6	Parietal	TB: 5 days NY: 19 hours
Rat 7	1%	NY	0.4	Agranular frontal	2%	TB	0.4	Occipital	TB: 5 days NY: 19 hours
Rat 8	1%	NY	0.7	Agranular frontal	3%	FB	0.8	Occipital	TB: 5 days NY: 19 hours
Rat 9	2%	TB	0.3	Parietal	1%	NY	0.4	Occipital	TB: 8 days NY: 19 hours
Rat 10	1%	NY	0.5	Frontal & parietal	2%	TB	0.5	Occipital	TB: 6 days NY: 18 hours
Rat 11	1%	NY	0.6	Agranular frontal	2%	TB	0.7	Parietal and occipital	TB: 6 days NY: 18 hours

The injections were placed in the dorsolateral convexity.

TABLE II

EXPERIMENTAL DATA FOR CATS

Case	%	Tracer	µl	Injected cortex	%	Tracer	µl	Injected cortex	Survival time
Cats 1,2	3%	FB	3.5	Pericruciate	1%	NY	2.3	Ant. suprasylvian	FB: 6,3 days NY: 25,23 hours
Cat 3	3%	FB	2.5	Pericruciate	1%	NY	3	Pregenual and ant. cingulate	FB: 6 days NY: 23 hours
Cat 4	3%	FB	4	Pericruciate	1%	NY	3.5	Middle and post. cingulate	FB: 6 days NY: 23 hours
Cat 5	3%	FB	1.5	Proreus gyrus	1%	NY	1.2	Pericruciate	FB: 3 days NY: 24 hours
Cat 6	3%	FB	0.8	Ant. suprasylvian	1%	NY	0.8	Middle suprasylvian	PB: 5 days NY: 23 hours

1–1.5 mm from the cortical surface, in the concentrations and volumes indicated in Tables I and II. The NY survival time was limited in order to prevent migration of NY out of the retrogradely labeled neurons to adjacent cells, including glial cells [1]. The animals were perfused transcardially with saline followed by 10% buffered formalin (pH 7.2). The brains were removed and after a few hours' soaking in 30% buffered sucrose (pH 7.2) they were cut on a freezing microtome into 30 μm thick transverse sections. Every third section was floated in distilled water, immediately mounted on slides and air dried. The material was studied without coverslipping with a Leitz Ploemopack fluorescence microscope, using filter-mirror system A which provides an excitation wavelength of 360 nm. The distribution of labeled cells in the thalamus was studied with the aid of an X–Y plotter attached to the microscope stage by means of transducers. The sections were counterstained with cresylviolet after plotting.

The distribution of labeled cells in the intralaminar nuclei in rat 1 (Table I), which received TB injections in the dorsolateral pregenual frontal cortex and NY injections in the agranular frontal cortex, is shown in Fig. 1. TB retrogradely labeled cells as well as NY retrogradely labeled cells were scattered in the intralaminar

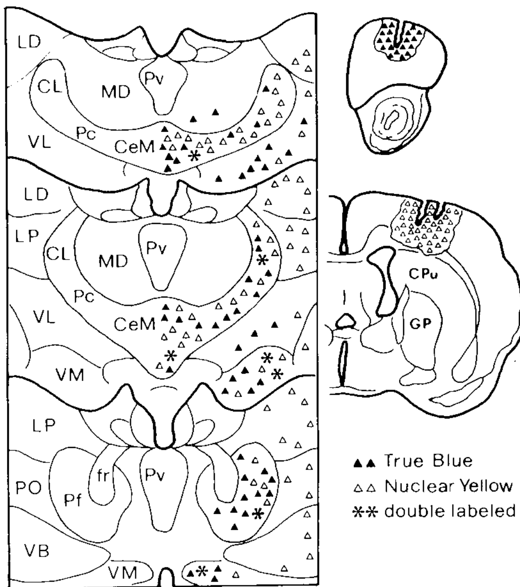

Fig. 1. Diagrammatic representation of the distribution of retrogradely labeled cells in the intralaminar nuclei in rat 1 (Table I). Each symbol represents approximately two cells. Abbreviations: CM, central medial nucleus; CL, central lateral nucleus; CPu, caudoputamen; fr, fasciculus retroflexus; GP, globus pallidus; LD, laterodorsal nucleus; LP, lateroposterior nucleus; MD, mediodorsal nucleus; Pc, paracentral nucleus; Pf, parafascicular nucleus; PO, posterior complex; Pv, paraventricular nucleus; VB, ventrobasal complex; VL, ventrolateral nucleus; VM, ventromedial nucleus.

nuclei. The vast majority of cells were single labeled. However, a few double labeled cells were seen in the anterior intralaminar nuclei, i.e. the central medial, paracentral and central lateral nuclei, as well as in the parafascicular nucleus. Double labeled cells displayed TB-labeled blue fluorescent cytoplasm and dendrites and a NY-labeled yellow fluorescent nucleus [6]. In this case some double labeled cells were also seen in the ventromedial nucleus (Fig. 1). In the other cases listed in Table I the pattern of labeling in the anterior intralaminar nuclei was similar to that described above, such that they contained cells labeled from the TB or FB injections, cells labeled from the NY injections and a minor number of double

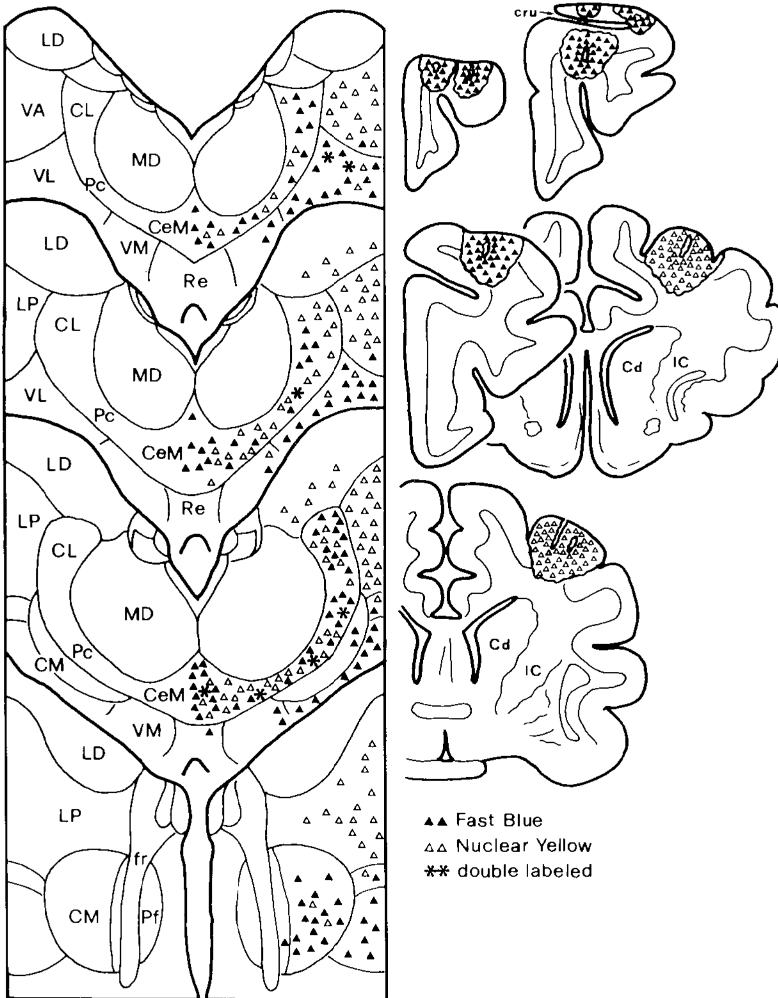

Fig. 2. Diagrammatic representation of the distribution of retrogradely labeled cells in the intralaminar nuclei in cat 1 (Table II). Each symbol represents approximately two cells. Abbreviations: Cd, caudate nucleus; CM, centre median nucleus; cru, cruciate sulcus; IC, internal capsule; Re, reuniens nucleus: VA, ventral anterior nucleus. Other abbreviations as in Fig. 1.

labeled cells. Labeled cells were observed in the centre median-parafascicular complex almost exclusively from the injections placed in the frontal cortex. Similar results were also obtained in the experiments performed in cats, as shown in Fig. 2 which portraits the distribution of labeled cells in the intralaminar nuclei (Fig. 3) in cat 1. In cats 1 and 2, which received FB injections in the pericruciate cortex and NY injections in the anterior suprasylvian gyrus, a few double labeled cells were also seen in the anterior and dorsal part of the ventrolateral nucleus (Fig. 2).

Both in rats and cats very few NY-labeled glial nuclei were observed in the intralaminar nuclei. However, more NY-labeled glial nuclei were seen throughout those specific thalamic nuclei which contained NY-labeled neurons (e.g. the lateroposterior nucleus in cat 1 (Fig. 2). Such labeling of glial nuclei could represent 'anterograde' glial labeling [1], in the terminal field of cortical fibers in the specific thalamic nuclei.

The present results indicate that the intralaminar projections to the different cortical areas here examined mainly consist of axons of separate cells and that some are represented by axon collaterals of the same cells. It has been reported in Golgi

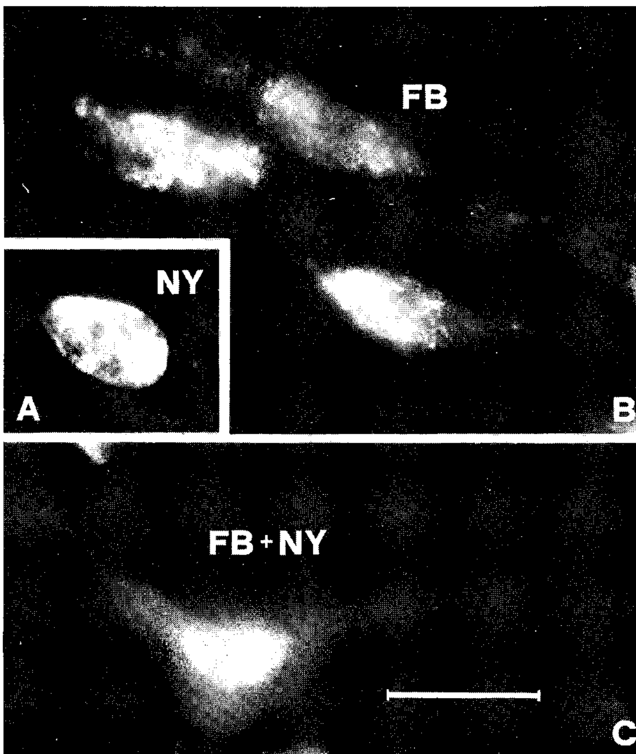

Fig. 3. Photomicrographs of fluorescent labeled neurons in the cat's intralaminar nuclei. A: NY single labeled neuron in the paracentral nucleus. B: FB single labeled neurons in the paracentral nucleus. C: FB–NY double labeled neuron in the central lateral nucleus. Note the NY labeling of the nucleus and the FB labeling of the cytoplasm. Light excitation wavelength: 360 nm. Scale bar: 20 μm.

studies [9], that single intralaminar fibers distribute collateral branches for several micra in the white matter underlying the orbitofrontal cortex. The present results suggest that the majority of such thalamic collaterals are not distributed over very wide cortical territories. These data are particularly interesting in view of the revision of the anatomical organization of the thalamo-cortical non-specific system which has been based on findings obtained by means of the axonal transport tracing techniques (see, for example, ref. 4). The results of the present study suggest that widely divergent axon collaterals do not represent the main anatomical substrate of the diffuse non-specific thalamo-cortical projections. Therefore, an extensive collateralization at cortical level cannot be considered as a characteristic distinguishing the cortical projections from the non-specific and the specific thalamic nuclei.

The authors are deeply grateful to Prof. T. Manzoni and all the staff of the Institute of Physiology of the University of Ancona who helped to make this work possible. The authors also thank Dr. M. Molinari for his help, Mr. A. Sbriccoli for excellent technical assistance, and Mrs. M. Bonfante for typing the manuscript. Samples of Nuclear Yellow were kindly provided by Dr. H. Loewe (Hoechst Company, Frankfurt); True Blue and Fast Blue were purchased from Dr. K. Illing (Makromolekulare Chemie-Gross Umstadt). This study was supported in part by CNR Grant 79.01936.04.

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