Neuroscience Research 66 (2010) 7–13

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Direct synaptic connections between superior colliculus afferents and thalamo-insular projection neurons in the feline suprageniculate nucleus: A double-labeling study with WGA-HRP and kainic acid

Kaeko Hoshino [a,*], Masao Horie [a], Attila Nagy [b], Antal Berényi [b], György Benedek [b], Masao Norita [a]

[a] Department of Neurobiology and Anatomy, Niigata University School of Medicine, 1-757 Asahimachi, Chuo-ku, Niigata 951-8510, Japan
[b] Department of Physiology, Faculty of Medicine, University of Szeged, Dóm tér 10, H-6720 Szeged, Hungary

ARTICLE INFO

Article history:
Received 3 July 2009
Received in revised form 8 September 2009
Accepted 9 September 2009
Available online 13 September 2009

Keywords:
Extrageniculate visual system
Degeneration
Tracer
Insular visual area
Tectum
Electron microscopy

ABSTRACT

The suprageniculate nucleus (Sg) of the feline thalamus, which subserves largely unimodal sensory and orientation behavior, receives input from the deep layers of the superior colliculus (SC), and projects to the suprasylvian cortical areas, such as the anterior ectosylvian visual area and the insular visual area (IVA), which contain visually responsive neurons. Through a double tract-tracing procedure involving the injection of wheat germ agglutinin conjugated with horseradish peroxidase (WGA-HRP) into the IVA and the injection of kainic acid into the SC, this study sought to determine the nature of the synaptic relationship between the SC afferents and the thalamo-cortical projection neurons. WGA-HRP injections labeled numerous neurons in the Sg, while kainic acid injections destroyed many tectothalamic terminals in the Sg. The distributions of the WGA-HRP-labeled neurons and the degenerated axon terminals overlapped in the dorsal part of the Sg. Electron microscopic observations demonstrated that the degenerated axon terminals made synaptic contacts with the dendrites of the WGA-HRP-labeled neurons in this overlapping region of the Sg. These results provide the first anatomical evidence that the Sg may play a role in the key relay of visual information from the SC to the IVA, within an identified extrageniculo-cortical pathway.

1. Introduction

There are two major visual projection pathways from the retina to the cerebral cortex in the cat: one of these is the geniculo-striate system, which conveys information to the primary visual cortex by way of the lateral geniculate nucleus. The other is the extra-geniculo-extrastriate system, in which information flows first to the superior colliculus (SC), and then to the nucleus lateralis posterior-pulvinar (LP-Pul) complex of the thalamus, from where projections lead to what has traditionally been called the associational cortices, including such structures as the lateral suprasylvian cortex (LS) and the associational cortices along the anterior ectosylvian sulcus that include the anterior ectosylvian visual area (AEV), and the insular visual area (IVA) (Benedek et al., 1988). Physiological and anatomical studies have demonstrated that the suprageniculate nucleus (Sg) of the posterior thalamus is another relay nucleus of the tectal extrageniculo-extrastriate visual information toward the associative cortical areas.

The Sg receives input from subcortical areas, most intensively so from the SC (Graham, 1977; Huerta and Harting, 1984; Takada et al., 1985; Hicks et al., 1986; Katoh and Benedek, 1995; Sato and Ohtsuka, 1996), but also from brainstem regions such as the pedunculopontine tegmental nucleus (PPT) (Edley and Graybiel, 1983; Hoshino et al., 1997) and the substantia nigra (Takada et al., 1984; Hoshino et al., 2009). It is also known that neurons of the Sg project to extrastriate cortices such as the LS (Norita et al., 1996; MacNeil et al., 1997), the AEV (Olson and Graybiel, 1987; Benedek et al., 1988) and the IVA (Benedek et al., 1986; Hicks et al., 1988a,b; Norita et al., 1991). Electrophysiological studies have revealed that the Sg subregion has neurons that are sensitive primarily to visual stimuli (Hicks et al., 1984; Krupa et al., 1984). The deep layers of the SC, which sends projection fibers to the Sg, have constituent neurons whose activity is related largely to the orientational behavior of the eye and head in relation to concomitant visual, auditory and somatosensory stimuli (Schiller, 1984; Sherk, 1986). This is interesting in the context that the IVA, a cortical area closely connected with the Sg, contains visually responsive neurons that express considerable directional selectivity (Benedek et al., 1986; Hicks et al., 1988a). It has therefore been widely assumed, though never proven, that the afferent fibers of the SC neurons make synaptic contacts with thalamo-cortical Sg projection neurons that have axons leading to the IVA.

* Corresponding author. Tel.: +81 25 227 2055; fax: +81 25 227 0753.
E-mail address: hoshinok@med.niigata-u.ac.jp (K. Hoshino).

0168-0102/$ – see front matter © 2009 Elsevier Ireland Ltd and the Japan Neuroscience Society. All rights reserved.
doi:10.1016/j.neures.2009.09.002

The present study was undertaken to evaluate the hypothesis that there are anatomically direct connections between the afferents of the SC and these thalamo-insular projection neurons. A combination of degeneration and retrograde tracing techniques at the light and electron microscopic levels was used to determine the distribution of degenerated fibers from the SC simultaneously with the distribution of retrogradely labeled neurons from the IVA, and to describe the synaptic contacts between the afferent fibers from the SC and the projection neurons of the Sg.

2. Materials and methods

2.1. Surgery and perfusion

The brains of 4 adult cats weighing 1.6–2.3 kg were used in this study. All experimental procedures undertaken were as approved by the Animal Care Committee of Niigata University. The animals were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and received unilateral injections of kainic acid (Sigma–Aldrich, St. Louis, MO, USA) dissolved in sterile saline and brought to a concentration of 1 μg/μl. Small injections (0.8 μl) of this solution were made via a Hamilton syringe into near the middle of the rostrocaudal extent of the SC (AP level 3.0–2.5 mm). Three days later, the animals were again anesthetized and, via a glass micropipette injection system, employing a needle tip diameter of about 70 μm, received 0.03 μl injection volumes of 3% wheat germ agglutinin conjugated with horseradish peroxidase (WGA-HRP, Sigma–Aldrich) into the ipsilateral portion of the insular cortex previously indicated electrophysiologically to be involved in the visual motor function (Hicks et al., 1988a; Norita et al., 1991).

2.2. Histochemical procedures

Two days following the WGA-HRP injection, the animals were again anesthetized deeply with sodium pentobarbital and perfused through the ascending aorta with isotonic saline, followed by 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.6. The brains were immediately removed from the skull and sectioned into 50 μm coronal serial sections with a Vibratome (Oxford Instrument). These sections were divided into four groups. The sections in the first and second groups were processed for WGA-HRP histochemistry with the tetramethylbenzidine (TMB) method. Following the TMB reaction, the sections in the first group were mounted on gelatin-coated slides and counterstained with neutral red for light microscopic observation. The sections in the second group were processed with the HRP-electron microscopic method of Carson and Mesulam (1982). They were post-fixed in 1% osmium tetroxide in 0.1 M sodium phosphate buffer (pH 6.0) at 45 °C for 1 h, dehydrated in a graded series of alcohols, and embedded in Epon. The ultra-thin sections, cut with an LKB ultramicrotome, were collected onto copper grids, stained with uranyl acetate and lead citrate, and examined with an H-7100 electron microscope.

The sections in the third group were processed by the silver impregnation method to reveal degeneration (Yamadori, 1975) and counterstained with Nissl, while those in the fourth group were used for acetylcholine esterase (AChE) histochemistry (Hardy et al., 1976) in order to identify the different subregions of the LP-Pul complex.

3. Results

As described above, the kainic acid injections were in all cases situated near the middle of the rostrocaudal extent of the SC, and the WGA-HRP injections were administered into the portion of the IVA previously indicated by electrophysiological experiments to be involved in the visual motor function (Fig. 1). In all cases, a number of degenerated axonal fibers and terminal-like puncta and numerous retrogradely labeled neuronal somata were found in the Sg. Although the distributions of the degeneration and the retrograde labeling differed slightly from case to case, only Case 140 will be described in detail, so as to avoid unnecessary repetition.

3.1. Light microscopic observations

The lesions produced by injections of kainic acid into the SC were centered mainly in the deep four layers, with slight involvement of the superficial three layers. Their extents were clearly delineated by Nissl staining as areas of reactive gliosis that contained no intact neurons and which included a needle track. The border of a lesion was characterized as an area that exhibited a rapid shift from normal tissue to an area completely devoid of normal elements. Fig. 2 presents a photomicrograph of a typical injection in Case 140. The injection of kainic acid into the SC produced many degenerated fibers and terminal-like puncta within the Sg (Fig. 3B and D). Similarly as in a previous study (Katoh et al., 1995), these puncta were distributed bilaterally, with an ipsilateral predominance in the Sg. The distribution of degenerated terminal-like puncta was not centered on the Sg, but rather produced a patchy profile close to the boundary between the LP, pars medialis (LPm) and the Sg (Fig. 4).

In Case 140, the WGA-HRP injected into the IVA was located mainly in the gray matter of the cortex (Fig. 2B and D) and labeled numerous neurons within the ipsilateral Sg (Fig. 3C and E), in accordance with the reports of previous studies (Norita and Katoh, 1988; Norita et al., 1991; Clascá et al., 1997). The distribution of these retrogradely WGA-HRP-labeled neurons throughout the Sg was not homogeneous. They formed a rather patchy pattern

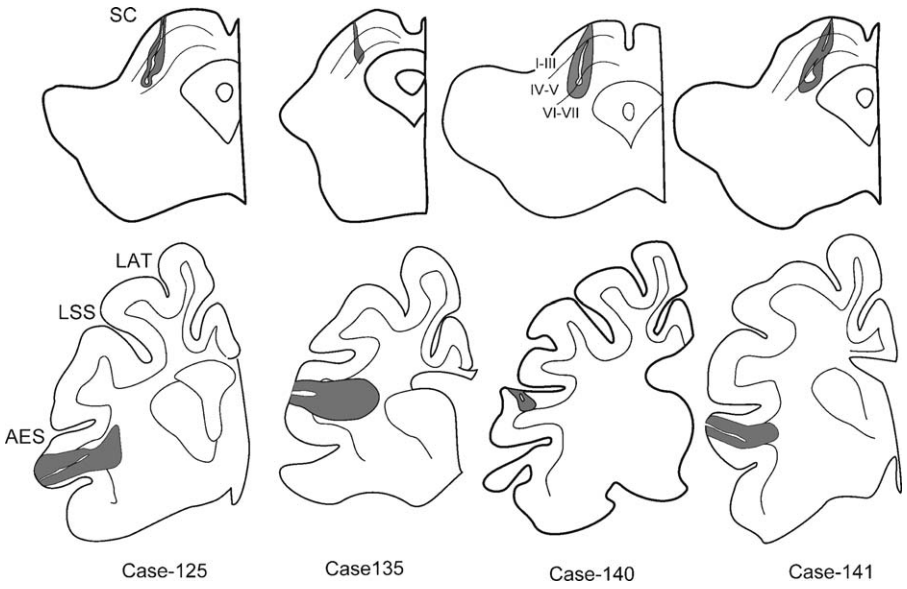

Fig. 1. Diagrams of coronal sections through the SC and IVA, depicting the center of kainic acid (upper) and WGA-HRP (lower) injections, respectively. The solid lines in SC denote the boundary of superficial (I–III), intermediate (IV–V) and deep layers (VI–VII). AES, anterior ectosylvian sulcus; LAT, lateral sulcus; LSS, lateral suprasylvian sulcus.