Exp Brain Res (1985) 60: 179–183

Experimental Brain Research

Research Note

Efferent connections of area 20 in the cat: HRP-WGA and autoradiographic studies

S. Kuchiiwa[1], T. Kuchiiwa[1], H. Matsue[2], and K. Sukekawa[1]

[1] Department of Anatomy, Hirosaki University, School of Medicine, Hirosaki 036, Japan
[2] Department of Biochemistry, Hirosaki University, School of Medicine, Hirosaki 036, Japan

Summary. Following injections of horseradish peroxidase-wheat germ agglutinin conjugate (HRP-WGA) and tritiated leucine into area 20 of the cat, terminal labeling was observed in visual areas 19, 21, the splenial visual area, the lateral suprasylvian area as well as in premotor, association and limbic related cerebral cortical regions. Labeled terminals in the subcortex were distributed in the caudate nucleus, the claustrum, the putamen, the anterior ventral nucleus, the intralaminar nuclei, the caudal division of the intermediate lateral nucleus, the lateralis posterior-pulvinar complex, the parvocellular C laminae of the dorsal lateral geniculate nucleus and the ventral lateral geniculate nucleus. In HRP-WGA preparations, retrogradely labeled somata were observed in these regions with the exception of certain subcortical structures. The projections are discussed with respect to the possible role area 20 plays in the cortical control of pupillary constriction.

Key words: Area 20 – Corticofugal connections – Pupilloconstrictor area – Near reflex – Cat

Introduction

Area 20 of the cat was defined anatomically by Heath and Jones (1971). A retinotopic organization of this area was determined electrophysiologically by Tusa and Palmer (1980), and its possible role as a cortical center for accommodative pupilloconstriction was suggested by Shoumura et al. (1982) and Kuchiiwa et al. (1984) based on results of electrical stimulation and on anatomical grounds after discussing its afferent connections with the retrograde horseradish peroxidase (HRP) method. The afferent connections of this area were further investigated by Cavada and Reinoso-Suárez (1983) using the retrograde HRP method. In the present study, the efferent connections of this area were investigated using anterograde HRP-WGA and tritiated leucine labeling methods in an attempt to delineate the pupilloconstrictor path.

Offprint requests to: S. Kuchiiwa (address see above)

Material and methods

Experiments were performed on seven adult cats using HRP-WGA and tritiated leucine. The cats were anaesthetized and placed in a stereotaxic head holder. The occipital cortex of one hemisphere of the brain was exposed under sterile conditions, and the extents of the pupilloconstrictor areas (PCA's) were identified by the method described in a previous paper (Shoumura et al. 1982), and injections of 0.05–0.07 µl of 4–10% HRP-WGA (4 cats) or tritiated leucine in saline (total 30–50 µCi; 3 cats) were made into the centers of the PCA's using a 1 µl-Hamilton microsyringe. It is known that the PCA's are confined to area 20 and extend almost the whole extent of the lateral surface of area 20 (Shoumura et al. 1982). *HRP-WGA method:* 45–51 h after the injections of HRP-WGA into area 20, the animals were deeply re-anaesthetized and perfused with a 0.1 M phosphate-buffered saline (pH 7.4) followed by a buffered 1.25% glutaraldehyde and 2.5% formalin mixture. The brain was removed and immersed in a cold buffer solution containing 30% sucrose. 50 µm frozen sections were cut in the frontal plane. Every third section of the series was reacted with tetramethyl benzidine (TMB; Mesulam 1978), and neighboring sections were counterstained with neutral red and the others were treated with acetylcholinesterase (Hardy et al. 1976). The HRP-WGA used in the present study was prepared and purified in our laboratories according to the method of Nakane et al. (1974). In our experience, the HRP-WGA method is as sensitive as autoradiography for tracing anterograde connections. *Autoradiography:* After a survival period of 5–7 days the animals were re-anaesthetized deeply and perfused transcardially with saline solution followed by a 0.1 M phosphate buffer (pH 7.4) containing 10% formalin. The brain was removed from the skull, fixed in the same fixative for a week, saturated with a cold solution of 30% sucrosed buffer, and cut serially at 25 µm in the frontal plane. Every tenth section was mounted on a gelatinized slide, dipped in Kodak NTB-2 nuclear track emulsion, exposed at 4° C for 3–6 weeks, developed in Kodak D-19 and counterstained with thionine.

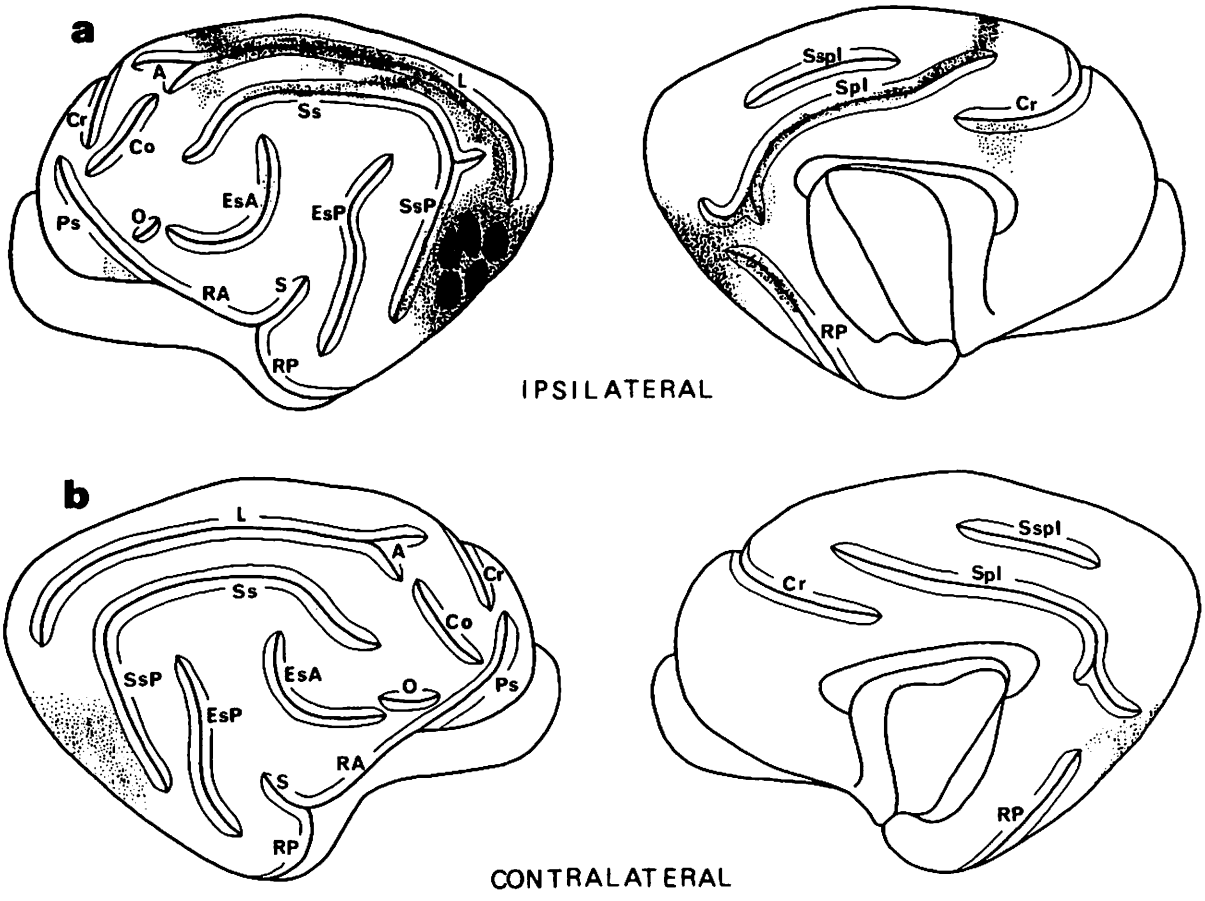

Fig. 1a and b. Drawings showing the distribution of cortical terminal labeling in lateral and medial views of the ipsilateral (a) and contralateral (b) hemispheres after injections of HRP-WGA into area 20 of the cat. Large black dots represent injection site and small black dots represent terminals. Abbreviations: A, Sulcus (S) ansatus; Co, S. coronalis; Cr, S. cruciatus; EsA et EsP, S. ectosylvius anterior et posterior; L, S. lateralis; O, S. orbitalis; Ps, S. presylvius; RA et RP, S. rhinalis anterior et posterior; S, S. sylvius; Spl, S. splenialis; Sspl, S. suprasplenialis; Ss, S. suprasylvius; SsP, S. suprasylvius posterior

Results and discussion

In all experiments, except in one case, the tracer was found to be restricted to area 20 and the patterns of distribution of terminals labeled anterogradely with each tracer were similar. Terminal labeling was observed bilaterally with a dominance of the ipsilateral distribution in the cerebral cortex, the basal ganglia and the brain stem, and ipsilaterally in the thalamus.

In the neocortex, ipsilateral to the injections, a large amount of terminal labeling was observed in the banks and fundus of the rostral and intermediate portions of the lateral sulcus. This labeling extended laterally at more caudal levels, and medially at more rostral levels, where it encroached upon the rostral lateral gyrus and continued caudally over the labeling in the splenial visual area of Kalia and Whitteridge (SVA, 1973, Fig. 1a). This distribution of labeling closely corresponds to the hodologically and cytoarchitectonically defined area 19 (Heath and Jones 1971). Labeled terminals were also found in the intermediate portion of the suprasylvian gyrus and the banks and fundus of the intermediate and posterior suprasylvian sulcus (Fig. 1a). This distribution of labeling seemed to involve areas 7, 21, and the lateral suprasylvian area (LS). Labeled terminals were also observed in the ventral proreal gyrus (Fig. 1a), a region from the ventral bank of the cruciate sulcus (area 6, Hassler and Muhs-Clement 1964) to the anterior limbic area of Rose and Woolsey (1948, Fig. 1a), the posterior portion of the ventral bank of the anterior ectosylvian sulcus (anterior ectosylvian visual area of Mucke et al., AEV, 1982), and the caudal portion of the posterior rhinal sulcus (Fig. 1a). In the contralateral cerebral cortex, densely labeled terminals were observed in an area homotopical to the injections (Fig. 1b). Labeled cortical terminals were mainly found in layer I except in the posterior rhinal sulcus, although abundant labeling was also

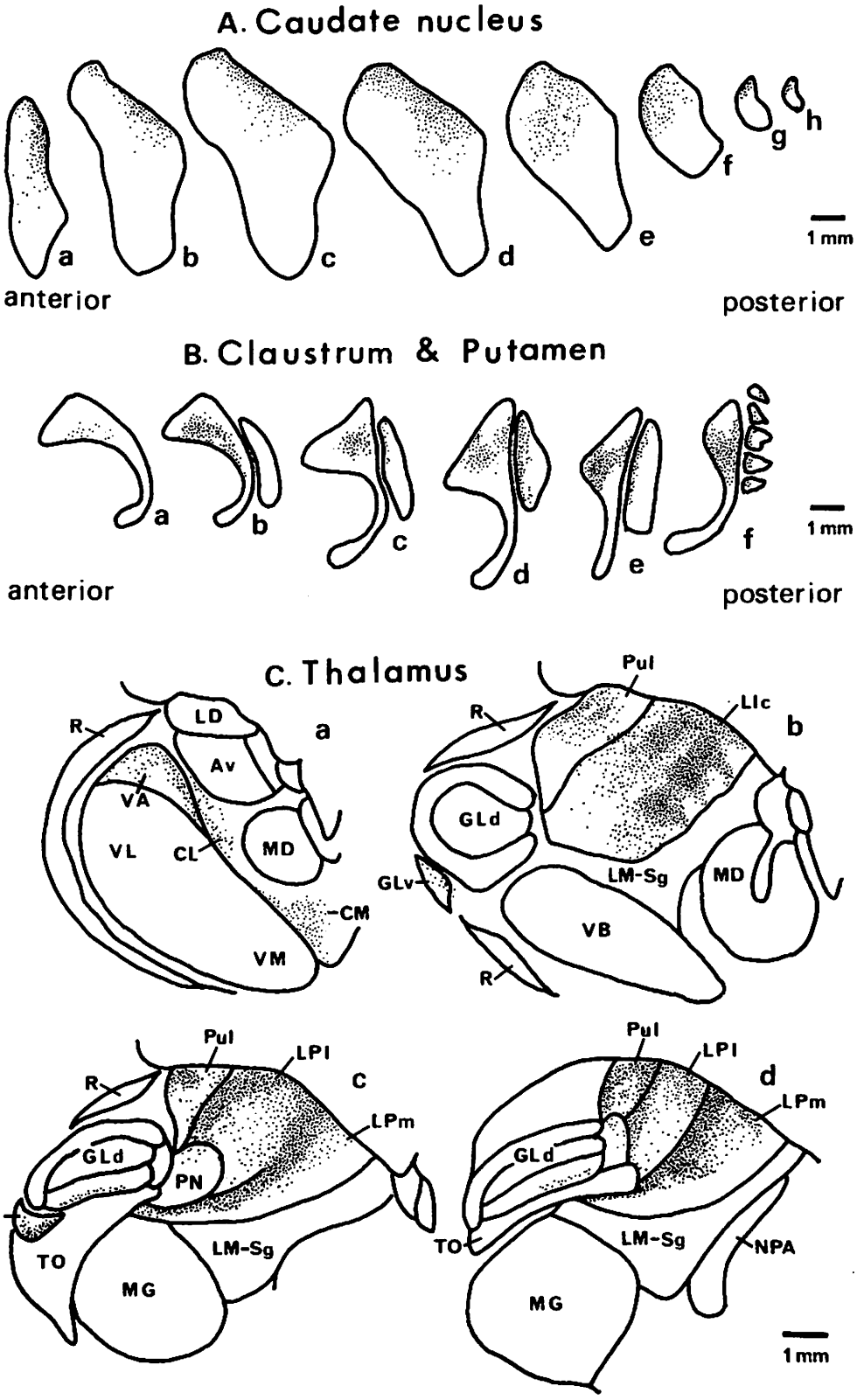

Fig. 2A–C. Distribution of labeled terminals in the caudate nucleus (A), the claustrum (B), the putamen (B) and the thalamus (C) in the frontal sections following injections into area 20. Abbreviations: Av, Nucleus (N) anteroventralis; CL et CM, N. centralis lateralis et medialis; Gld et Glv, N. geniculatus lateralis dorsalis et ventralis; LD et LM, N. lateralis dorsalis et medialis; LIc, N. lateralis intermedius, pars caudalis; LPl et LPm, N. lateralis posterior, pars lateralis et medialis; MD, N. medialis dorsalis; MG, N. geniculatus medialis; NPA, N. pretectalis anterior; PN, N. posterior; Pul, Pulvinar; R, N. reticularis thalami; Sg, N. supragenicularis; TO, Tractus opticus; VA, VL et VM, N. ventralis anterior, lateralis et medialis; VB, ventrobasal complex

observed in deeper layers in certain cortical regions such as the SVA and the region from area 6 to the anterior limbic area. In HRP-WGA preparations, retrogradely labeled neurons were observed in all of the above-mentioned cortical regions, and fewer retrogradely labeled cells were found in other cortical regions, viz. in the ventral part of the posterior ectosylvian gyrus, the ventral part of the posterior sylvian gyrus, the cingulate gyrus and the retrosplenial area.

In the thalamus, ipsilateral to the injections the bulk of terminal labeling was found in the dorsolateral portion of the anterior ventral nucleus, the intralaminar nuclei, the caudal division of the intermediate lateral nucleus, the parvocellular C laminae of the dorsal lateral geniculate nucleus (GLd), the ventral lateral geniculate nucleus (GLv) and all three subdivisions of the lateralis posterior-pulvinar complex (LP-Pul, Fig. 2C). In HRP-WGA preparations, retrogradely labeled somata were distributed in all of the above-mentioned thalamic structures except in the GLv. Other terminal labeling regions in the prosencephalic structures were the almost entire rostrocaudal length of the caudate nucleus, the dorsal claustrum and the lateral border region of the putamen, as shown in Fig. 2A–B. In HRP-WGA preparations, retrogradely labeled neurons were observed in the claustrum but not in the putamen and the caudate nucleus. In the lower brainstem, labeled terminals were identified in the superficial layers of the superior colliculus (SC) and the rostroventral division of the pontine nuclei, but in the HRP-WGA preparations, retrogradely labeled neurons were not found in these structures.

In summary, area 20 of the cat mainly receives multiple inputs from various cortical and subcortical structures related to visual functions including areas 7, 19, 21, LS, SVA, AEV, claustrum, LP-Pul and the parvocellular C laminae of GLd, and sends efferent fibers to most of these structures and other vision-related regions such as GLv and SC. Furthermore, area 20 receives less inputs from premotor (area 6), association (prefrontal and perirhinal cortices), and limbic (anterior limbic, cingular and retrosplenial areas) cortices, and also heavily projects to regions other than vision-related structures, especially the motor-related telencephalic regions such as area 6 and the striatum. Of these direct connections, reciprocal connections with the vision-related structures especially areas 19, 21, LS, SVA, the claustrum and the LP-Pul seem to be the most substantial.

These findings on the afferent connections are in good agreement with previous retrograde HRP studies (Cavada and Reinoso-Suárez 1983; Raczkowsky and Rosenquist 1983; Kuchiiwa et al. 1984). Concerning the efferent connections, area 20 is known to project to areas 17, 18, 19, 21, the medial wall of LS, the perirhinal cortex, the basolateral group of the amygdala complex, the LP-Pul, the parvocellular C laminae of GLd and the GLv (Heath and Jones 1971; Updyke 1977; Hughes and Chi 1981; Raczkowski and Rosenquist 1983; Bullier et al. 1984). In our experiments, the presumed labeling in areas 17 and 18 was observed in a few cases, but it was not constant and too weak to confirm the projections to areas 17 and 18. Our other experiments of HRP injections into area 17 or 18 resulted in retrograde labeling in the infragranular layers of area 20 (unpublished), so it is highly probable that area 20 projects to these visual areas. Labeling in the amygdala complex was not observed except in one case in which injection sites extended into the region more rostroventral to area 20, so that this projection to the amygdala complex originated from the cortex rostroventral to area 20.

With regard to the cortical control of ocular parasympathetic functions by area 20, we suggested the possibility that area 20 plays a role in the cortical center of the near reflex from the observation of bilateral pupillary constriction following electrical stimulation of this area, and from the evidence of its afferents from various vision-related structures (Shoumura et al. 1982; Kuchiiwa et al. 1984). Furthermore, in our electrical stimulation experiments on the pretectal complex, we observed bilateral pupillary constriction by the stimulation of the medial and olivary (NPO) pretectal nuclei (unpublished data). It is, therefore, probable that one of these or both pretectal nuclei mediate the information for pupillary constriction from the cerebral cortex. However, because the evidence of direct projections from area 20 to these pretectal nuclei was not found in the present study, it seems that the information for pupillary constriction was mediated by another way-station and reached the midbrain. It is known that the pretectal complex receives afferent fibers from the GLv and the superficial layers of the SC, in particular, the NPO receives heavy afferent inputs from the GLv (Edwards et al. 1974; Berman 1977), and projections from area 20 to the GLv and SC were apparent in the present study. Because electrical stimulation of the SC could not induce pupillary constriction (unpublished data), it is considered that the GLv relays the pupilloconstrictor pathway rather than the SC. It seems probable that area 20 sends fibers subserving the near reflex indirectly to the NPO and controls the pre-ganglionic pupilloconstrictor neurons in the midbrain.

Acknowledgement. We are very grateful to Drs. K. Shoumura and M. B. Price for their help in improving the manuscript.

References

Berman N (1977) Connections of the pretectum in the cat. J Comp Neurol 174: 227–254

Bullier J, Kennedy H, Salinger W (1984) Branching and laminar origin of projections between visual cortical areas in the cat. J Comp Neurol 228: 329–341

Cavada C, Reinoso-Suárez F (1983) Afferent connections of area 20 in the cat studied by means of the retrograde axonal transport of horseradish peroxidase. Brain Res 270: 319–324

Edwards SB, Rosenquist AC, Palmer LA (1974) An autoradiographic study of ventral lateral geniculate projections in the cat. Brain Res 72: 282–287

Hardy H, Heimer L, Switzer R, Watkins D (1976) Simultaneous demonstration of horseradish peroxidase and acetylcholinesterase. Neurosci Lett 3: 1–5

Hassler R, Muhs-Clement K (1964) Architektonischer Aufbau des sensomotorischen und parietalen Cortex der Katze. J Hirnforsch 6: 377–420

Heath CJ, Jones EG (1971) The anatomical organization of the suprasylvian gyrus of the cat. Ergeb Anat Entwickl 45: 1–64

Hughes CP, Chi DYK (1981) Afferent projections to the ventral lateral geniculate nucleus in the cat. Brain Res 207: 445–448

Kalia M, Whitteridge D (1973) The visual areas in the splenial sulcus of the cat. J Physiol (Lond) 232: 275–283

Kuchiiwa S, Shoumura K, Kuchiiwa T, Imai H (1984) Afferents to the cortical pupillo-constrictor areas of the cat, traced with HRP. Exp Brain Res 54: 377–381

Mesulam M-M (1978) Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents. J Histochem Cytochem 26: 106–117

Mucke L, Norita M, Benedek G, Creutzfeldt O (1982) Physiologic and anatomic investigation of a visual cortical area situated in the ventral bank of the anterior ectosylvian sulcus of the cat. Exp Brain Res 46: 1–11

Nakane PK, Kawaoi A (1974) Peroxidase-labeled antibody a new method of conjugation. J Histochem Cytochem 22: 1084–1091

Raczkowski D, Rosenquist AC (1983) Connections of the multiple visual cortical areas with the lateral posterior-pulvinar complex and adjacent thalamic nuclei in the cat. J Neurosci 3: 1912–1942

Rose JE, Woolsey CN (1948) Structure and relations of limbic cortex and anterior thalamic nuclei in rabbit and cat. J Comp Neurol 89: 279–347

Shoumura K, Kuchiiwa S, Sukekawa K (1982) Two pupillo-constrictor areas in the occipital cortex of the cat. Brain Res 247: 134–137

Tusa RJ, Palmer LA (1980) Retinotopic organization of areas 20 and 21 in the cat. J Comp Neurol 193: 147–164

Updyke BV (1977) Topographic organization of the projections from cortical areas 17, 18, and 19 onto the thalamus, pretectum and superior colliculus in the cat. J Comp Neurol 173: 81–122

Received November 2, 1984 / Accepted May 15, 1985