Retrograde Tracer Studies of Tecto-Reticulospinal Pathway and Dorsal Lateral Geniculate Nucleus on GluR1- and GluR4-Immunoreactive Neurons in the Hamster Superior Colliculus

Jae-Sik Choi, Jea-Young Lee, Yu-Jin Jang, Eun-Shil Lee and Chang-Jin Jeon*

Department of Biology, College of Natural Sciences, Kyungpook National University, Daegu 702-701, South Korea

Received September 18, 2009 / Accepted October 29, 2009

We recently reported the distributions of AMPA (α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) receptor subtypes glutamate receptors (GluR) 1 and GluR4 in the superior colliculi (SC) of hamsters with antibody immunocytochemistry and the effect of enucleation on these distributions. We also compared these labelings to those of calcium-binding proteins calbindin D28K, calretinin, parvalbumin, and GABA. In the present study, we investigated whether the GluR1- and GluR4-immunoreactive (IR) neurons are interneurons or projection neurons by injection of the retrograde tracer horseradish peroxidase (HRP) into one of each major ascending and descending pathways of the SC. HRP injections were made into a tecto-reticulospinal pathway (TRS) and dorsal lateral geniculate nucleus (dLGN). Animals were then allowed to recover and to survive for 48 hr before perfusion. Sections containing retrograde-labeled neurons were then treated for GluR-immunoreactivity. HRP injections proved that only a small population of the GluR1-IR cells project into TRS (1.4%) and dLGN (2.6%). However, a large subpopulation of GluR4-IR cells project into TRS (32.7%). The differential compositions of inter/projection neurons, along with our previous studies on the separate distribution of the GluR subunits, its differential co-localization with calcium-binding proteins and GABA, and differential reactions to enucleations, strongly imply the functional variety of the receptor subunits in visual behavior responses.

Key words : AMPA glutamate receptors, retrograde tracer; localization, tecto-reticulospinal pathway, dorsal lateral geniculate nucleus

Introduction

The mammalian superior colliculus (SC) serves as a visuo-motor integration center and is a seven laminated structure that can be divided into superficial and deep layers [4,5,15,19,39]. The superficial layers consisted of three layers and these are stratum zonale (SZ), stratum griseum superficiale (SGS), and stratum opticum (SO). The superficial layers receive visual information primarily from the retina and the visual cortex. The deep layers consisted of four layers and these are stratum griseum intermediale (SGI), stratum album intermediale (SAI), stratum griseum profundum (SGP), and stratum album profundum (SAP). The deep layers are multimodal and receive projections from many functionally different areas of the brain.

The SC is characterized in the topographical distribution of its afferent fibers and efferent cells [11,15,17]. An example is the patch-like organization of afferent fibers from sub-

stantia nigra in the SGI of the cat SC [6]. Many other cortical and subcortical inputs to the SC also form distinctive puffs or patches [11]. The topographical distribution of SC afferent fibers and efferent cells correspond to transmitter-specific labeling patterns in the SC [11]. The most distinctive example is the patchy-like distribution of cholinergic fibers in the SGI [7,9,20]. Another example is the specific efferent cell clusters of tectofugal pathways. For example, neurons projecting cuneiform region formed clusters and were found to overlap precisely the acetylcholine patches in the SGI [20].

Glutamate and glutamate receptors (GluR) play a crucial role in various cellular mechanisms such as synaptogenesis, formation of neuronal circuitry, and in synaptic plasticity including long-term potentiation and long-term depression [37,38,41]. The three subtypes of ionotropic glutamate receptors are AMPA (α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate), NMDA (N-methyl-D-aspartate) and kainate receptors. There are at least four forms of AMPA receptor subunits (GluR1-4) [13,30]. The function of AMPA subunits is not clear yet, but the functional properties of GluR are determined by their subunit compositions. For example, the

*Corresponding author

Tel : +82-53-950-5343, Fax : +82-53-953-3066

E-mail : cjjeon@knu.ac.kr

GluR2 subunit plays a major role in the determination of the Ca^{2+} permeability of the receptors [21]. The AMPA receptors that contain GluR2 subunits are only weakly permeable to Ca^{2+} while AMPA receptors lacking GluR2 subunits were highly permeable to Ca^{2+} [16].

Previously, we reported the distribution and morphology of GluR-immunoreactive (IR) neurons to determine if these receptor subtypes are localized in the specific lamina and specific cell types [2,35]. We also examined whether GluR-IR neurons are specifically localized in subpopulations of calcium-binding proteins calbindin D28K-, calretinin-, parvalbumin-, and GABA-IR neurons. We also investigated whether GluR-immunoreactivities are affected by enucleation. The understanding of the neural pathway is the key features in understanding of the neural coordination. However, whether there is a relationship between the GluR1- and GluR4-IR and specific efferent cell groups in the SC has not been established.

Thus, the present study is undertaken to investigate whether the GluR1- and GluR4-IR are inter/projection neurons. The mammalian SC contains complex tectofugal pathways. Projection neurons in the SC have been divided into two primary pathways. The ascending pathways from superficial SC reach several targets including pretectal, the lateral posterior, and lateral geniculate nuclei. The descending pathways from deeper SC reach numerous motor and sensory nuclei. The major descending pathways from deep SC are tectopontine bulbar and tecto-reticulospinal pathway (TRS) [5,15]. Our results show that the organizational features of inter/projection neurons of the GluRs found in hamster SC are strikingly different among GluRs.

Materials and Methods

Animals

Adult hamsters (8-10 weeks old, 20-30 g, $n=30$, mixed-sex) were used in these experiments. The animals were divided into three groups. First, intact hamsters ($n=6$) were used to determine the normal distribution of immunoreactivity to the AMPA receptor subunits GluR1 and GluR4 in the SC. Second, twenty four hamsters were used to label retrogradely SC neurons that make ascending and descending pathways. The National Institute of Health guideline for the use and care of animals were followed for all experimental procedures. All efforts were made to minimize animal suffering as well as the number of animals used.

Retrograde tracing experiments

Horseradish peroxidase (HRP, Sigma Type IV, St Louis, MO, USA) was used as the retrograde tracer in all experiments. Each hamster was anesthetized with a mixture of ketamine hydrochloride (30-40 mg/kg) and xylazine (3-6 mg/kg), supplemented as needed to maintain anesthesia. The animals were mounted in a Kopf stereotaxic apparatus. A 5 μl Hamilton microsyringe with a 30 gauge needle mounted on a specially designed injection device attached to the stereotaxic apparatus was used to inject the HRP solution. Injections consisted of 0.3-1.5 μl of a 30% HRP solution in distilled water. Animals were then allowed to recover and to survive for 48 hr before perfusion. HRP injections were made into a TRS and dorsal lateral geniculate nucleus (dLGN) identified by stereotaxic coordinates obtained from the Morin and Wood atlas [32]. Among the twenty four cases, substantial retrograde labeling of SC cells with HRP was obtained in four animals, two from the TRS injections and two from the dLGN.

Perfusion and tissue processing

All animals were anesthetized deeply with a mixture of ketamine hydrochloride (30-40 mg/kg) and xylazine (3-6 mg/kg) before perfusion. They were perfused transcardially with 4% paraformaldehyde and 0.3-0.5% glutaraldehyde in 0.1 M sodium phosphate buffer (PB, pH 7.4) with 0.002% calcium chloride added. Following a pre-rinse with approximately 30 ml of phosphate-buffered saline (PBS, pH 7.2) over a period of 1-4 min, each hamster was perfused with 30-50 ml of fixation for 5-10 min via a syringe needle inserted through the left ventricle and aorta. The head was then removed and placed in the fixation for 2-3 hr. The brain was removed from the skull, stored for 2-3 hr in the same fixation, and left overnight in 0.1 M PB (pH 7.4) containing 8% sucrose and 0.002% calcium chloride. The blocks of diencephalon and midbrain including part of the hindbrain were removed, mounted onto a chuck, and cut coronally into 50 μm thick sections with a Vibratome. For three or four sections, two or three sections were used for immunocytochemistry and one was used for thionin. The thionin stained sections were used to identify the nuclei and collicular layers.

HRP Immunocytochemistry

The polyclonal antibodies against GluR1 (AB 1504) and GluR4 (AB 1508) were obtained commercially from

Chemicon (Temecula, CA, USA). The tissues were processed free floating in small vials. For immunocytochemistry, the sections were incubated in 1% sodium borohydride (NaBH_4) for 30 min. Sections were rinsed 3 X 10 min in 0.1 M PB (pH 7.4) and incubated in 0.1 M PB with 4% normal goat serum for 2 hr with 0.5% Triton X-100. They were then incubated in the primary antiserum that had been diluted from 1:100 to 1:1000 (GluR1 and GluR4), in 0.1 M PB with 4% normal goat serum for 48 hr in the presence of 0.5% Triton X-100. Following a further 3 X 10 min rinse in 0.1 M PB, the sections were incubated in a 1:200 dilution of biotinylated secondary IgG in 0.1 M PB with 4% normal serum for 2 hr in the presence of 0.5% Triton X-100, then rinsed 3 X 10 min in 0.1 M PB and incubated in a 1:50 dilution of avidin-biotinylated HRP (Vector Lab., Burlingame, CA, USA) in 0.25 M Tris for 2 hr. The sections were rinsed as before, and staining was visualized by reaction with 3, 3'-diaminobenzidine tetrahydrochloride (DAB) and hydrogen peroxide in 0.25 M Tris for 3-10 min using a DAB reagent set (Kirkegaard & Perry, Gaithersburg, MD, USA). All sections were rinsed in 0.1 M PB before mounting. As a negative control, some sections were incubated in the same solution without the addition of the primary antibody. The negative control sections showed no GluR1 and GluR4 immunoreactivities. The stained tissues were finally mounted on Superfrost Plus slides (Fisher, Pittsburgh, PA, USA) and dried overnight in an oven set at 37°C. The mounted sections were dehydrated through alcohol, cleared with xylene, and coverslipped with the mounting medium, Permount (Fisher). The tissues were examined and photographed on a Zeiss Axioplan microscope using conventional or differential interference contrast (DIC) optics.

Tracer histochemistry

Retrogradely transported HRP was identified by a previously described, cobalt-nickel intensification procedure [34]. Briefly, the tissue sections were incubated in a solution of 0.05% DAB with 0.005% nickel chloride and 0.005% cobalt acetate in 0.1 M PB (pH 7.4) for 10 min, and further incubated in 0.05% DAB with 0.01% hydrogen peroxidase (H_2O_2) for 15-30 min. The sections were then rinsed several times in PBS (pH 7.2), before being reacted for immunocytochemistry.

Quantitative analysis

Distribution of neurons labeled by anti-GluR antibodies, by both anti-GluR antibodies and retrogradely transported

HRP, or by HRP only was produced with the aid of a Zeiss drawing tube attached to a Zeiss standard microscope with a 40X objective.

To determine the double-labeled neurons of GluR with HRP, the sequential fields, each 303 μm X 303 μm in area, across the medial-lateral extent of the SC were positioned in superficial (seven fields) and deep layers (seven fields) of SC. GluR-IR cells, HRP cells, and GluR and HRP double-labeled cells were counted from six different sections (two rostral, two middle, and two caudal sections) from each HRP injected animal. Images were obtained using Zeiss Axioplan microscope using 40X Zeiss Plan-Apochromat objective. The number of double-labeled cells was expressed as a percentage of the total populations of GluR1- and GluR4-IR cells.

Results

Distribution of GluR1- and GluR4-IR neurons in the SC

As we previously reported [2], GluR1-IR cells were found throughout the SC of the hamster (Fig. 1B, D-F). These cells

Fig. 1. Low power photomicrographs of the laminar distribution of AMPA subunits GluR1- and GluR4-IR neurons in the hamster SC. (A) Thionin-stained section showing the collicular lamination. (B) Anti-GluR1-immunoreactivity. GluR1-IR cells were distributed throughout the SC. (C) Anti-GluR4-immunoreactivity. GluR4-IR cells were selectively distributed in the lower lateral SGI and lateral SAI and formed clusters. Squares in (B-C) indicate regions that are shown in higher magnification in (D-I). SAI, stratum album intermediale; SGI, stratum griseum intermediale; SGS, stratum griseum superficiale; SP, stratum profundum; SO, stratum opticum; SZ, stratum zonale. Bar=200 μm (A-C) and 50 μm (D-I).

did not show any specific laminar or cluster distribution. We observed an almost equal density of labeled cells throughout the SC. By contrast, GluR4-IR cells were very selectively distributed in the hamster SC (Fig. 1C, G-I). Figs show that anti-GluR4-immunoreactivity in the normal hamster SC was distinctively located within the lower lateral SGI and lateral SAI and formed clusters. This cluster of highly GluR4-IR cells could be seen throughout the rostral-caudal extent of the SC. The thickness of this cluster was approximately 200 μ m wide from the central portion, and approximately 600-800 μ m long from the mid-colliculus. Fig. 1A shows a thionin-stained section for collicular lamination.

Fig. 2. Distribution of neurons labeled by anti-GluR antibodies (asterisks), by both anti-GluR antibodies and retrogradely transported HRP (filled diamond), or by HRP only (filled circle). Drawings of three sections after injection into the dLGN. The rostral-caudal sequence is from bottom to top. Approximate laminar boundaries and the distance from bregma are indicated. Location of HRP injection is illustrated in the bottom. As the GluR1-IR neurons are too numerous, the drawings do not contain GluR1-IR cells. Au, primary auditory cortex; dLGN, dorsal lateral geniculate nucleus; LP, lateral posterior thalamic nucleus; PF, parafascicular thalamic nucleus; PH, posterior hypothalamic area; SAI, stratum album intermediale; SGI, stratum griseum intermediale; SGS, stratum griseum superficial; SP, stratum profundum; SO, stratum opticum; SZ, stratum zonale; V1, primary visual cortex; VPM, ventral posteromedial thalamic nucleus.

Retrograde filling of SC neurons

To determine whether the GluR1- or GluR4-IR cells in the hamster SC were projection neurons, HRP injections were placed into one major ascending projection site, dLGN, and into one major descending pathway, TRS of the SC of twenty four hamsters. Sections containing retrogradely labeled neurons were then treated for GluR- immunoreactivity.

Among the twelve cases of dLGN injections, Fig. 2 shows the one injection that was successfully centered into the dLGN. The low successful case is due to the exceedingly small size of the targeted nucleus of the brain. In this successful dLGN injection, the substantial spread of HRP involved a lateral posterior nucleus (LP) that also has terminal projections of the superficial layers of SC [15]. Many retrogradely labeled neurons were found on the side ipsilateral to the injection (Fig. 2). The vast majority of HRP-labeled neurons were located within the superficial layers. The majority of HRP backfilled neurons were relatively small neurons with round or oval shape (Fig. 3A). However, stellate, horizontal, and vertical fusiform neurons were also back-filled with HRP. Figs. 3B and 3C show HRP backfilled vertical fusiform and horizontal neurons, respectively. Some GluR1-IR neurons were labeled by the retrograde tracer of HRP in the present study. The majority of double-labeled neurons of GluR1 and HRP were relatively small and had round or oval morphology (Fig. 3D). Fig. 3E shows a horizontal cell double-labeled with anti-GluR1 antibody and HRP. Quantitatively, we counted total 7451 GluR1-IR cells from seven sequential fields, each 303 μ m X 303 μ m in area,

Fig. 3. Morphology of neurons retrogradely labeled by HRP (A-C) and neurons labeled by both anti-GluR antibodies and HRP (D-E) after injection into the dLGN. (A) Round or oval neurons. (B) A vertical fusiform neuron. (C) A horizontal neuron. (D) A round or oval neuron. (E) A horizontal neuron. (F) A large round GluR4-IR neuron. None of the GluR4-IR neurons was backfilled with HRP. Bar=20 μ m.

across superficial layer of SC from six sections. We counted total 990 HRP-labeled cells in these fields. Among 990 HRP-labeled cells, 195 cells (19.70% or 195 of 990 cells) were double-labeled with GluR1. Thus, 2.62% (195 of 7451) of GluR1-IR cells in the superficial layer are dLGN projection neurons in the present study. None of the GluR4-IR neurons (Fig. 3F) was double-labeled by the retrograde tracer of HRP in the present study after dLGN injection.

Among the twelve cases of TRS injections, Fig. 4 shows HRP injection that was successfully placed in TRS. The HRP-labeled neurons were located within the deep layers (Fig. 4). More HRP-labeled cells were located in the lateral half of the SC than the medial half in the present injection.

Fig. 4. Distribution of neurons labeled by anti-GluR antibodies (asterisks), by both anti-GluR antibodies and retrogradely transported HRP (filled diamond), or by HRP only (filled circle). Drawings of three sections after injection into the TRS. The rostral-caudal sequence is from bottom to top. Approximate laminar boundaries and the distance from bregma are indicated. Location of HRP injection is illustrated in the bottom. As the GluR1-IR neurons are too numerous, the drawings do not contain GluR1-IR cells. CnF, cuneiform nucleus; RRF, retrorubral field; rs, rubrospinal tract; SAI, stratum album intermediale; SGI, stratum griseum intermediale; SCS, stratum griseum superficial; SP, stratum profundum; SO, stratum opticum; SZ, stratum zonale; TS, tectoreticulospinal tract; TRS, tectoreticulospinal pathway; V2M, secondary visual cortex, medial; V2L, secondary visual cortex, lateral.

Fig. 5. Morphology of neurons retrogradely labeled by HRP (A-C) and neurons labeled by both anti-GluR and HRP (D-F) after injection into the TRS. (A) Round or oval neurons. (B) A large stellate neuron. (C) A horizontal neuron. (D) A round or oval neuron. (E) A large round or oval neuron. (F) A large stellate neuron. Bar=20 μ m.

The HRP backfilled neurons were varied in size with round or oval (Fig. 5A), or stellate morphologies (Fig. 5B). However, vertical fusiform and horizontal neurons were also HRP backfilled. Fig. 5C shows a horizontal neuron backfilled with HRP. Some GluR1- and GluR4-IR neurons were also labeled by the retrograde tracer of HRP in the present study. The majority of double-labeled neurons of GluR1 and HRP were relatively medium to large in size and had round or oval morphology (Fig. 5D). The majority of double-labeled neurons of GluR4 and HRP were relatively medium to large in size and had round or oval (Fig. 5E) and stellate (Fig. 5F) morphologies. Quantitatively, we counted total 7490 GluR1-IR cells and total 355 GluR4-IR cells from seven sequential fields, each 303 μ m X 303 μ m in area, across deep layer of SC from six sections. We counted total 408 and 418 HRP-labeled cells from GluR1- and GluR4-IR cells in these fields, respectively. Among 408 HRP-labeled cells, 106 cells (25.99% or 106 of 408 cells) were double-labeled with GluR1. Thus, 1.42% (106 of 7490) of GluR1-IR cells in the deep layer is TRS projection neurons in the present study. Among 418 HRP-labeled cells, 116 cells (27.75% or 116 of 418 cells) were double-labeled with GluR4. Thus, 32.67% (116 of 355) of GluR4-IR cells in the superficial layer are TRS projection neurons in the present study.

Discussion

The present study has revealed that only a few of GluR1-IR neurons are projection neurons while a large number of GluR4-IR neurons are projection neurons. In our pre-

vious and present studies we have clearly shown differences in the pattern of distribution among different AMPA subunits in hamster SC [2,35]. GluR1-IR cells were found throughout the SC and did not form any specific laminar or cluster distribution. By contrast, GluR4-IR cells were distinctively located within the lower lateral SGI and lateral SAI, and formed clusters. These results indicate that there are considerable subunit differences in the distributions of GluRs in the hamster SC. The functional significance of the remarkable different laminar distributional pattern of GluR-IR neurons is not obvious yet.

Although there are some animal variations [14], neurons projecting to the dLGN and ventral are located primarily in the upper half of the SGS in various mammalian SC, while neurons that project to lateral posterior pulvinar are located in ventral SGS [3,10,11,22]. TRS projection neurons contained many larger round or oval, or stellate neurons and showed some tendency of grouping in cluster in deep SC [33,36,40]. We believe that the majority of GluR1-IR cells in the hamster SC are not projection neurons for the following reasons: 1) The dLGN and TRS injections in the present study showed that only a few GluR1-IR cells are projection neurons; 2) Many GluR1-IR neurons co-occur with GABA [2]; and 3) Previous studies have shown the morphology of GluR1-IR neurons in a variety of areas of mammalian brain [8,24,25, 28,29]. The strongly labeled neurons were non-pyramidal interneurons. In general, interneurons specifically express the calcium-binding proteins calbindin, calretinin, or parvalbumin in the central nervous system [1]. GluR1-IR was also found mainly in non-pyramidal neurons that contain calcium-binding proteins [23,25,26,28,29]. In accordance with the previous study, the majority of GluR1-IR cells of the hamster SC were small round or oval cells and some GluR1-IR cells co-express calbindin, calretinin, or parvalbumin [2]. Taken together, a large subpopulation of GluR1-IR cells in the hamster SC appears to be primarily interneurons.

By contrast, we believe that the majority of GluR4-IR cells in the hamster SC are projection neurons for the following reasons: 1) The TRS injection in the present study showed that a large number of GluR4-IR cells is projection neurons; 2) None of the GluR4-IR neurons co-occurs with GABA [2]; and 3) More than half of the GluR4-IR cells of the hamster SC are large round or oval, or stellate cells in our previous study and none of GluR4-IR cells co-express calbindin, calretinin, or parvalbumin [2]. Taken together, a large number of GluR4-IR cells in the hamster SC appears to be primarily

projection neurons. However, differentiating interneurons and projection neurons on the basis of co-expression of calcium-binding proteins is not confirmative as there are interspecies differences. Many calbindin D28K-containing rat SC neurons are projection neurons [27], while most calbindin D28K-containing cat SC neurons are interneurons [31].

The different laminar organization of AMPA subunits and inter/projection neurons may be associated with functionally different neuronal mechanisms or pathways. Neurons in the superficial layers concerned with vision receive input from the eye as well as other sensory systems, while the deeper SC concerned with motor play a role in integrating sensory information into motor signals that help orient the head toward various stimuli [5,11,12,15,18]. We have demonstrated that GluR4-IR cells form a distinctive cluster within specific domains of labeled neurons in the lower lateral SGI, and lateral SAI, and many GluR4-IR neurons are projection neurons. Many efferent neurons of SC are located within specific domains of the SC forming compartmental architectures [11,15]. We previously demonstrated that neurons projecting cuneiform nucleus are located in clusters within the SGI, and these cuneiform nucleus projecting neurons in the SC lie within the acetylcholine-rich patches [20]. The prefrontal cortex and the nigral inputs overlap the acetylcholine-rich patches [11]. Thus, it is possible that the clusters of GluR4-IR neurons in the present study receive convergent motor inputs which are related with to saccadic eye movements [11]. Therefore it is reasonable to suppose that the GluR4-IR cell clusters which are characteristically positioned in the lower SGI and SAI may reflect neurochemically or modulatory specific functional domains. However, the distributional pattern of GluR1-IR cells in the present study suggests that GluR1-IR cells receive both visual inputs and multisensory inputs from many central nervous systems. However, further studies are needed to explore the functional significance of these features.

In conclusion, the present study suggests that most of the GluR1-IR cells are primarily interneurons, while a large number of GluR4-IR cells are primarily projection neurons. Our previous study demonstrated that the distinct distributional pattern of the GluRs-IR neurons differential co-localization with calcium-binding proteins or GABA, and differential responses to enucleation. The previous and the present results of the differential compositions as interneurons or projection neurons strongly suggest the diverse functional physiology of the AMPA subunits in visuo-motor in-

tegration in the SC. The questions on the diverse functional significance on the complex differences in distribution and in composition of inter/projection neurons in SC expressing should be carry out in the future.

Acknowledgement

This work was supported by a Grant (KRF-2008-313-E00367) from the Korea Research Foundation.

References

1. Celio, M. R. 1990. Calbindin d-28k and parvalbumin in the rat nervous system. *Neuroscience* **35**, 375-475.
2. Choi, J. S., J. Y. Lee, and C. J. Jeon. 2009. Glutamate receptor GluR1 and GluR4 in the hamster superior colliculus: distribution and co-localization with calcium-binding protein and GABA. *Acta Histochem. Cytochem.* **42**, 29-38.
3. Graham, J. and V. A. Casagrande. 1980. A light microscopic and electron microscopic study of the superficial layers of the superior colliculus of the tree shrew (*Tupaia glis*). *J. Comp. Neurol.* **191**, 133-151.
4. Grantyn, A. A. and A. K. Moschovakis. 2004. Structure-function relationships in the superior colliculus of higher mammals. pp. 107-145, In Hall, W. C. and A. Moschovakis (eds.), *The Superior Colliculus*, CRC Press Inc., Boca Raton.
5. Grantyn, R. 1988. Gaze control through superior colliculus: structure and function. *Rev. Oculomot. Res.* **2**, 273-333.
6. Graybiel, A. M. 1978. Organization of the nigrotectal connection: An experimental tracer study in the cat. *Brain Res.* **143**, 339-348.
7. Graybiel, A. M. 1978. A stereometric pattern of distribution of acetylcholine-esterase in the deep layers of the superior colliculus. *Nature* **272**, 539-541.
8. Gutiérrez-Igarza, K., D. J. Fogarty, F. Pérez-Cerdá, F. Doñate-Oliver, K. Albus, and C. Matute. 1996. Localization of AMPA-selective glutamate receptor subunits in the adult cat visual cortex. *Vis. Neurosci.* **13**, 61-72.
9. Hall, W. C., D. Fitzpatrick, L. L. Klatt, and D. Raczowski. 1989. Cholinergic innervation of the superior colliculus in the cat. *J. Comp. Neurol.* **287**, 495-514.
10. Harrell, J. V., R. B. Caldwell, and R. R. Mize. 1982. The superior colliculus neurons which project to the dorsal and ventral lateral geniculate nuclei in the cat. *Exp. Brain Res.* **46**, 234-242.
11. Harting, J. K. 2004. Puffs and patches: a brief chronological review. pp. 83-105, In Hall, W. C. and A. Moschovakis (eds.), *The Superior Colliculus*, CRC Press Inc., Boca Raton.
12. Harting, J. K., B. V. Updyke, and D. P. Van Lieshout. 1992. Corticotectal projections in the cat: anterograde transport studies of twenty-five cortical areas. *J. Comp. Neurol.* **324**, 379-414.
13. Hollmann, M. and S. Heinemann. 1994. Cloned glutamate receptors. *Annu. Rev. Neurosci.* **17**, 31-108.
14. Huerta, M. F., A. Frankfurter, and J. K. Harting. 1983. Studies of the principal sensory and spinal trigeminal nuclei of the rat: projections to the superior colliculus, inferior olive, and cerebellum. *J. Comp. Neurol.* **220**, 147-167.
15. Huerta, M. F. and J. K. Harting. 1984. The mammalian superior colliculus: studies of its morphology and connections. pp. 687-772, In Vangega, H. (ed.), *The Comparative Neurology of the Optic Tectum*, Plenum Press Inc., New York.
16. Hume, R. I., R. Dingledine, and S. F. Heinemann. 1991. Identification of a site in glutamate receptor subunits that controls calcium permeability. *Science* **253**, 1028-1031.
17. Illing, R. B. 1996. The mosaic architecture of the superior colliculus. *Prog. Brain Res.* **112**, 17-34.
18. Isa, T. 2002. Intrinsic processing in the mammalian superior colliculus. *Curr. Opin. Neurobiol.* **12**, 668-677.
19. Isa, T. and S. Sasaki. 2002. Brainstem control of head movements during orienting; organization of the premotor circuit. *Prog. Neurobiol.* **66**, 205-241.
20. Jeon, C. J. and R. R. Mize. 1993. Choline acetyltransferase-immunoreactive patches overlap specific efferent cell groups in the cat superior colliculus. *J. Comp. Neurol.* **1**, 127-150.
21. Jonas, P., C. Racca, B. Sakmann, P. H. Seeburg, and H. Monyer. 1994. Differences in Ca^{2+} permeability of AMPA-type glutamate receptor channels in neocortical neurons caused by differential GluR-B subunit expression. *Neuron* **12**, 1281-1289.
22. Kawamura, S., N. Fukushima, S. Hattori, and M. Kudo. 1980. Laminar segregation of cells of origin of ascending projections from the superficial layers of the superior colliculus in the cat. *Brain Res.* **184**, 486-490.
23. Kerr, R. C., D. J. Maxwell, and A. J. Todd. 1998. GluR1 and GluR2/3 subunits of the AMPA-type glutamate receptor are associated with particular types of neuron in laminae I-III of the spinal dorsal horn of the rat. *Eur. J. Neurosci.* **10**, 324-333.
24. Kharazia, V. N., R. J. Wenthold, and R. J. Weinberg. 1996. GluR1-immunopositive interneurons in rat neocortex. *J. Comp. Neurol.* **368**, 399-412.
25. Kondo, M., R. Sumino, and H. Okado. 1997. Combinations of AMPA receptor subunit expression in individual cortical neurons correlate with expression of specific calcium-binding proteins. *J. Neurosci.* **17**, 1570-1581.
26. Kwok, K. H., Y. C. Tse, R. N. Wong, and K. K. Yung. 1997. Cellular localization of GluR1, GluR2/3 and GluR4 glutamate receptor subunits in neurons of the rat neostriatum. *Brain Res.* **778**, 43-55.
27. Lane, R. D., D. M. Allan, C. A. Bennett-Clarke, D. L. Howell, and R. W. Rhoades. 1997. Projection status of calbindin- and parvalbumin-immunoreactive neurons in the superficial layers of the rat's superior colliculus. *Vis. Neurosci.* **14**, 277-286.
28. Leranth, C., Z. Szeideemann, M. Hsu, and G. Buzsaki. 1996. AMPA receptors in the rat and primate hippocampus: a possible absence of GluR2/3 subunits in most interneurons.

- Neuroscience* **70**, 631-652.
29. McDonald, A. J. 1996. Localization of AMPA glutamate receptor subunits in subpopulations of non-pyramidal neurons in the rat basolateral amygdala. *Neurosci. Lett.* **208**, 175-178.
 30. Michaelis, E. K. 1998. Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging. *Prog. Neurobiol.* **54**, 369-415.
 31. Mize, R. R., C. J. Jeon, G. D. Butler, Q. Luo, and P. C. Emson. 1991. The calcium-binding protein calbindin-D28K reveals subpopulations of projection and interneurons in the cat superior colliculus. *J. Comp. Neurol.* **307**, 417-436.
 32. Morin, L. P. and R. I. Wood. 2001. *Stereotaxic Atlas of the Golden Hamster Brain*. Academic Press Inc., San Diego.
 33. Murray, E. A. and J. D. Coulter. 1982. Organization of tectospinal neurons in the cat and rat superior colliculus. *Brain Res.* **243**, 201-214.
 34. Nabors, L. B. and R. R. Mize. 1991. A unique neuronal organization in the cat pretectum revealed by antibodies to the calcium-binding protein calbindin-D 28k. *J. Neurosci.* **11**, 2460-2476.
 35. Park, W. M., M. J. Kim, and C. J. Jeon. 2004. Ionotropic glutamate receptor GluR2/3-immunoreactive neurons in the cat, rabbit, and hamster superficial superior colliculus. *Neurosci. Res.* **49**, 139-155.
 36. Sahibzada, N., D. Yamasaki, and R. W. Rhoades. 1987. The spinal and commissural projections from the superior colliculus in rat and hamster arise from distinct neuronal populations. *Brain Res.* **415**, 242-256.
 37. Sheng, M. and M. J. Kim. 2002. Postsynaptic signaling and plasticity mechanisms. *Science* **298**, 776-780.
 38. Song, I. and R. L. Huganir. 2002. Regulation of AMPA receptors during synaptic plasticity. *Trends Neurosci.* **25**, 578-588.
 39. Sparks, D. L. 1999. Conceptual issues related to the role of the superior colliculus in the control of gaze. *Curr. Opin. Neurobiol.* **9**, 698-707.
 40. Weber, J. T., G. F. Martin, M. Behan, M. F. Huerta, and J. K. Harting. 1979. The precise origin of the tectospinal pathway in three common laboratory animals: a study using the horseradish peroxidase method. *Neurosci. Lett.* **11**, 121-127.
 41. Whitlock, J. R., A. J. Heynen, M. G. Shuler, and M. F. Bear. 2006. Learning induces long-term potentiation in the hippocampus. *Science* **313**, 1093-1097.

초록 : Tecto-reticulospinal pathway (TRS)와 dorsal lateral geniculate nucleus (dLGN)에서 역행성이동추적물질 이용 햄스터 상구에서 GluR1-, GluR4- 면역반응 신경세포 연구

최재식 · 이제영 · 장유진 · 이은실 · 전창진*

(경북대학교 자연과학대학 생물학과)

본 연구자들의 앞선 연구에서는 햄스터 상구에서 AMPA 수용체의 아형인 GluR1과 GluR4의 분포와 눈 적출 이후 이들 수용체의 분포를 면역세포화학적 방법으로 연구하였다. 또한, 표지한 GluR1과 GluR4를 칼슘결합단백질인 calbindin D28K, calretinin, parvalbumin과 GABA로 표지하여 비교하였다. 본 연구에서 역행성이동추적물질(retrograde tracer)인 호스래디시퍼옥시다아제(horseradish peroxidase, HRP)를 상구의 각 주요 상행로와 하행로에 주입함으로써 GluR1- 면역반응 신경세포들과 GluR4- 면역반응 신경세포들이 투사신경세포(projection neurons)임을 밝혀내었다. Tecto-reticulospinal pathway (TRS)와 dorsal lateral geniculate nucleus (dLGN)으로 HRP를 주입한 후, 햄스터들은 회복을 위해 48시간 동안 살려둔 뒤 관류(perfusion)하였다. GluR- 면역반응 처리된 절편들은 역행성 표지된 신경세포를 지남을 확인하였다. HRP를 주입하였더니 단지 적은 수의 GluR1- 면역반응 신경세포들이 TRS (1.4%)와 dLGN (2.6%)으로 투사되었고, 반면에 많은 수의 GluR4- 면역반응 신경세포들이 TRS (32.7%)로 투사되었다. 사이/투사 신경세포(inter/projection neurons)들은 GluR 아단위들의 분류된 분포와 차별화된 양상을 보였고 이들의 이러한 분포는 칼슘결합단백질들과 GABA와는 겹쳐지지 않았으며, 일전에 발표했던 시각적 행동 반응에서 안구 적출 후 수용체 아단위들의 기능적 다양화와는 차별화된 양상을 보였다.