Descending Projections From the Cortical Accommodation Area in the Cat

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Purpose. Results in previous studies indicated that the lateral suprasylvian (LS) area, the cortical area surrounding the middle suprasylvian sulcus (Mss) of the cat, has important roles in the control of accommodation. The current study was conducted to investigate descending projections from the accommodation-related area in the LS area to the brainstem.

Methods. Wheat germ agglutinin–horseradish peroxidase (WGA–HRP) was injected into the accommodation-related area in the pretectum or in the rostral superior colliculus (SC) of the cat. These regions are thought to be involved in the control of accommodation based on results of previous studies. The authors investigated locations of retrogradely labeled cells in the LS area. In addition, the authors compared amplitudes of accommodative responses evoked by stimulation of the LS area before and after neuronal activities in the rostral SC were inhibited by the injection of muscimol (γ-aminobutyric acid agonist) into the accommodation-related area in the rostral SC.

Results. After injections of WGA–HRP into the accommodation-related area in the rostral SC, retrogradely labeled cells were observed in the lower part of the medial bank of the Mss, which corresponded to the accommodation-related area in the LS area. Conversely, after injections of WGA–HRP into the accommodation-related area in the pretectum, retrogradely labeled cells were seen in the upper part of the medial bank of the Mss, which did not correspond to the accommodation-related area in the LS area. Accommodation responses evoked by stimulation of the LS area were abolished by the injection of muscimol into the accommodation-related area in the rostral SC.

Conclusions. These findings suggest that accommodation-related signals from the LS area mainly project to the rostral SC, but not to the pretectum. Invest Ophthalmol Vis Sci. 1996;37:1429–1436.

Results of previous studies have suggested that the lateral suprasylvian (LS) area, the cortical area surrounding the middle suprasylvian sulcus (Mss) of the cat, is related to the control of lens accommodation.1–3 The LS area receives visual input. Some neurons in this area responded to changes in ocular disparity and target size and to motion in depth, which are important visual cues for accommodation.4–6 Some LS neurons also exhibited burst discharges preceding the onset of spontaneous accommodation.2 It is likely that these neurons have an important role in the control of accommodation. Microstimulation of the LS area evoked accommodative responses.1,3 We conducted systematic microstimulation of the LS area in the cat and found that low-threshold areas for evoking lens accommodation were located in the lower parts of the medial banks of the middle suprasylvian sulcus (Mss) from A1 to A4 and at A8 in the stereotaxic coordinates.3 The LS area projects to many areas, such as other cortical areas, the thalamus, the pulvinar, the striatum, the pretectum, the superior colliculus (SC), and the pontine nuclei.7–11 Bando et al2 reported that approximately 70% of accommodation-related neurons were antidromically activated by stimulation through electrodes placed in the pretectum, the SC, or both with average latencies of 2.4 to 2.5 msec.

It was previously reported that the pretectum and the rostral SC may be involved in the control of accommodation.12–14 Therefore, descending projections to these areas are thought to be important in the control
of accommodation. However, it is unknown whether projections to the pretectum and to the rostral SC are involved in the control of accommodation. In the current study, we injected wheat germ agglutinin horseradish peroxydase (WGA–HRP) into the accommodation-related area in the pretectum or the rostral SC of the cat and investigated locations of retrogradely labeled cells in the LS area, in which low-threshold sites for evoking accommodation previously were identified. In addition, muscimol, an agonist of inhibitory neurotransmitter γ-aminobutyric acid (GABA), was injected through a glass micropipette into the rostral SC, where accommodative responses were elicited by microstimulation with weak currents. Amplitudes of accommodative responses evoked by microstimulation of the cortical accommodation-related area were compared before and after the injection.

METHODS

The general paradigm of the current experiments was to determine locations of retrogradely labeled cells in the LS area by injecting WGA–HRP through a glass micropipette into a region in the accommodation-related area in the pretectum or the rostral SC immediately after the region was mapped electrophysiologically. This study was conducted in four cats, each weighing 2.5 to 3.5 kg. In addition, accommodative responses evoked by stimulation of the LS area were compared before and after injection of muscimol into the accommodation-related area in the SC to determine whether the projection from the LS area is related to the control of accommodation. Two cats weighing 2.5 to 3.0 kg were used for this experiment.

Animal Preparations

Each cat was deeply anesthetized with 2% to 4% halothane. After the trachea and the femoral vein were cannulated, the halothane anesthesia was replaced by the administration of ketamine hydrochloride (initial dose, 25 mg/kg intramuscularly) and α-chloralose (25 mg/kg intravenously). For accurate measurement of the dioptric change of the lens, each animal was immobilized with pancuronium bromide (initial dose, 0.1 mg/kg intravenously) and artificially ventilated. Pancuronium bromide (0.05 mg/kg intravenously) was administered every 40 minutes. The animal was attached to a stereotaxic head-holder frame, and a small craniotomy was made in the parietal skull for lateral insertions of microelectrodes and glass micropipettes into the LS area, the rostral SC, and the pretectal area. All incisions and pressure points were infiltrated with 2% lidocaine hydrochloride. Rectal temperature was maintained at 38°C using a feedback-controlled heating pad. During the experiment, supplemental doses of ketamine hydrochloride (15 mg/kg intramuscularly) and α-chloralose (10 mg/kg intravenously) were administered every 30 minutes. The right pupil was dilated for accurate measurement of accommodation with 5% L-phenylephrine hydrochloride, a drug that produces no measurable effect on the accommodative response. All experimental protocols were approved by the Sapporo Medical University Animal Care and Use Committee and complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Mapping and Recording Procedures

Accommodative responses of the right eye were recorded continuously with an infrared optometer (model AR-1100; Nidek, Tokyo, Japan). This system basically was analogous to the system developed by Cornsweet and Crane. 16 For accurate measurement, the ocular alignment of the right eye was monitored continuously with an infrared television monitor mounted in the optometer. This system has a resolution of 0.01 D. Accommodation and trigger pulses for stimulation were recorded on magnetic tapes for subsequent computer analysis, using a data recorder. The data recorded on magnetic tapes were digitized by a computer at a sampling rate of 200 Hz.

Systematic mappings of the LS area were performed in all the cats used in the current study according to previously reported techniques. 3,11 Tungsten microelectrodes insulated with Isoneel 31 (Nishihoku, Osaka, Japan) were used for electrical microstimulation. The electrodes were introduced into the pretectum and the rostral SC along the vertical axis, whereas the electrodes were introduced into the cortex at an angle of 25° from the vertical axis in a coronal plane for mapping of the LS area. To identify the accommodation-related area in the LS area, systematic mapping of the LS area was conducted in all the cats for the study of WGA–HRP injection and the study of muscimol injection. Results of our previous studies on the LS area 3,11 indicated that the accommodation-related area is located in the posterior portion of the medial bank of the Mss. Thus, systematic mapping of the thresholds for evoking accommodative responses in the LS area was carried out at 1-mm intervals rostrocaudally along the medial bank of the Mss. A train of negative pulses (0.2 msec) of 30 to 200 μA was used (intratrain frequency, 100 Hz; train duration, 500 msec). The accommodation-related area in the LS area was defined as the area in which accommodative responses were evoked by stimulation with currents of <100 μA according to our previous study. 3,11

To identify the accommodation-related area in the pretectum or the rostral SC, systematic mapping of the pretectum or the rostral SC was conducted in two cats according to previously reported techniques. 13,14 Approximately 5 to 10 electrode penetrations (at intervals of 0.5 to 1.0 mm) were made to
determine the low-threshold area for evoking accommodation in the pretectum or the rostral SC in each cat. Routinely, the maximum current (60 μA) was first applied to determine whether accommodative responses could be evoked. If no accommodative responses were evoked, the electrode was advanced 500 μm. If accommodative responses were evoked, the stimulus intensity was lowered to the threshold, defined as the intensity that elicited seven or eight accommodative responses of 0.03 D or larger out of 10 stimulations.

WGA–HRP Injection

After mapping the thresholds for evoking accommodative responses in the pretectum or the rostral SC was completed, a track, in which accommodative responses were elicited with the lowest threshold, was selected in each cat. The microelectrode was replaced with a glass micropipette pulled from 1-mm glass tubing and beveled to a tip diameter of 70 to 90 μm, which was filled with a 5% solution of WGA–HRP in a 0.1 M phosphate buffer containing 0.2 M NaCl. Before the injection, we confirmed that accommodation was evoked by microstimulation of the injection site with low current (<20 μA) using the glass micropipette (Fig. 1). In two cats for each of the studies on the pretectum and the rostral SC, 0.02 μl of the solution was injected into the low-threshold site under pressure using the microdriver of a 10-μl microsyringe connected to the micropipette over a 10- to 15-minute period.

Muscimol Injection

In three cats, muscimol was injected into the accommodation-related area in the rostral SC. Accommodative responses evoked by stimulation of the same site in the LS area were compared before and after injection of muscimol into the ipsilateral rostral SC. The SC contains substantial numbers of GABAergic terminals. Therefore, neuronal activity of the SC is expected to be inhibited after the injection of muscimol. If the accommodation-related area in the LS area projects to the accommodation-related area in the rostral SC, accommodation evoked by microstimulation of the LS area will be reduced after muscimol administration. Tungsten microelectrodes for microstimulation were introduced into the accommodation-related area in the LS area at an angle of 25° from the vertical axis in a sagittal plane. Glass micropipettes, which were filled with 1 μg/μl saline solution of muscimol, were introduced stereotaxically into the accommodation-related area in the rostral SC along the vertical axis on the same side of the tungsten microelectrode. The solution was stained with fast green for later identification of the injection sites. Single injection of muscimol was made, and accommodative responses were evoked with low-current stimuli <20 μA. The total amount of muscimol injected ranged from 0.2 to 0.3 μl. In the control, 0.2 to 0.3 μl of saline was injected into the pretectum.

In each cat, 20 responses of accommodation evoked by stimulation of the LS area were recorded before the injection, and the mean amplitude of the 20 responses was calculated. After the injection, 10 accommodative responses were recorded every 10 minutes until the mean amplitude of the 10 responses recovered to the level before the injection.

Histologic Processing and Data Analysis

For the WGA–HRP Experiments. After 48 hours of survival, the animals were deeply anesthetized with pen-
tobarbital sodium and were perfused transcardially. Two liters of physiological saline with heparin sodium were introduced, followed by 2 l of fixative solution containing 1% paraformaldehyde and 1% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4). After perfusion, the brains were exposed, blocked in the stereotaxic plane, placed in a 0.1 M phosphate buffer with 20% sucrose, and kept in a refrigerator overnight. The next day they were sectioned into 70-μm coronal sections on a freezing microtome and collected in compartmentalized trays. The sections of the brains were treated with tetramethylbenzidine according to the method of Meslam. After the reaction, sections were mounted on gelatin-coated slides and counterstained with neutral red.

For the Muscimol Experiments. After the experiments, the animals were perfused transcardially in the same manner as in the WGA-HRP experiments. Brains were sectioned to 80-μm serial coronal sections on a freezing microtome and collected in compartmentalized trays. The sections were then mounted on gelatin-coated slides and stained with neutral red.

Each section was examined with both low- and high-magnification lenses using bright- or dark-field illumination. Distributions of retrogradely labeled cells or injection sites of muscimol were plotted on sheets of paper with the aid of a drawing tube attached to a microscope. Subdivisions of the pretectum were identified according to data of Berman and of Avendano and Juretschke.

RESULTS

WGA–HRP Injections Into the Superior Colliculus

The low-threshold sites for evoking accommodation were located in the superficial and intermediate layers of the rostral SC in the two cats used in this experiment, which corresponded to the results obtained in our previous study. Figure 1 shows distributions of the injection sites in coronal sections through the rostral SC in the two cats and accommodative responses evoked by microstimulation of the injection site using the glass micropipette. WGA–HRP was injected into the site at which accommodative responses were evoked by stimulation with weak currents of <20 μA. Figures 1C and 1E show surface reconstructions of the SC, indicating that the injection sites were localized mainly in the superficial and intermediate layers in the rostral SC (Fig. 2). Figure 3 shows distributions of retrogradely labeled cells in the LS area in the two cats after WGA–HRP injection into the accommodation-related area in the rostral SC. The shaded areas in this figure represent low-threshold areas for evoking accommodation with weak currents of <50 μA. The low-threshold area was located in the lower part of the medial bank of the Mss in both cats, which corresponded to the results obtained in our previous studies. Almost all the retrogradely labeled cells were observed in the low-threshold area in both cats, although relatively few labeled cells were located in the lateral bank of the Mss and in the region rostral to the low-threshold area in the medial bank of the Mss. Figure 4 shows a dark-field photomicrograph of labeled cells in the LS area after WGA–HRP injection into the accommodation-related area in the rostral SC. Retrogradely labeled cells were found in layer V.
FIGURE 4. A dark-field photomicrograph of a coronal section of the lateral suprasylvian area showing retrogradely labeled cells. Bar = 200 μm.

WGA–HRP Injections Into the Pretectum

In both cats, the low-threshold sites for evoking accommodation were located in the laterocaudal portion of the pretectum, which included the nucleus of the optic tract and the posterior pretectal nucleus. Figure 5 shows distributions of the injection sites in coronal sections through the pretectum in the two cats and accommodative responses evoked by microstimulation of the injection site using the glass micropipette. WGA–HRP was injected into the site at which accommodation was evoked by stimulation with weak currents of <20 μA. In cat 201, WGA–HRP was injected mainly into the nucleus of the optic tract and the posterior pretectal nucleus (Fig. 5B). In cat 202, WGA–HRP also was injected into the nucleus of the optic tract and the posterior pretectal nucleus, although deposits of WGA–HRP were seen in the thalamus (Fig. 5C).

FIGURE 5. Drawings of coronal sections of the pretectum showing accommodative responses evoked by microstimulation of the injection site in cat 201 (A) and distributions of the injection sites of wheat germ agglutinin–horseradish peroxidase in two cats (cats 201 and 202) (B,C). APN = anterior pretectal nucleus; HB = habenular nucleus; LGN = lateral geniculate nucleus; LPi = interjacent division of the lateral posterior nucleus; LPm = medial division of the lateral posterior nucleus; LPi = lateral division of the lateral posterior nucleus; MPN = medial pretectal nucleus; NOT = nucleus of the optic tract; NPC = nucleus of the posterior commissure; OPN = olivary pretectal nucleus; PAG = periaqueductal gray; PL = pulvinar; PPN = posterior pretectal nucleus; SC = superior colliculus. The horizontal bar under the accommodation traces indicates the period of stimulation. Bar = 2 mm.

FIGURE 6. A dark-field photomicrograph of a coronal section of the pretectum showing the injection site in cat 201. Bar = 2 mm.

FIGURE 7. Camera-lucida drawings of coronal sections through the lateral suprasylvian area in the two cats, of which the injection sites were indicated in Figure 4, showing distributions of retrogradely labeled cells after an injection of wheat germ agglutinin–horseradish peroxidase into the physiologically identified accommodation-related area in the pretectum. Each dot indicates the location of a retrogradely labeled cell. Shaded area represents the accommodation-related area. Coronal sections represent the region delineated by a solid line on a coronal section in the inset. Mss = middle suprasylvian sulcus. Bar = 2 mm.
gradely labeled cells in the LS area in the two cats after WGA-HRP injection into the accommodation-related area in the pretectum. In the two cats, no retrogradely labeled cells were seen in the low-threshold area for evoking accommodation in the LS area (shaded area in Fig. 7). In cat 201, the retrogradely labeled cells were observed mainly in the upper part of the medial bank of the Mss. In cat 202, the retrogradely labeled cells were observed in the suprasylvian gyrus and the upper part of the lateral sulcus. Figure 8 shows a dark-field photomicrograph of labeled cells in the LS area after WGA-HRP injection into the accommodation-related area in the pretectum. Retrogradely labeled cells were found in layer V.

Muscimol Injections Into the Rostral Superior Colliculus

In three cats, amplitudes of accommodative responses evoked by stimulation of the LS area were compared before and after muscimol injection into the accommodation-related area in the rostral SC. Muscimol was injected into the area in the rostral SC in which accommodative responses could be evoked by stimulation with weak currents of <20 μA. Figure 9 shows the methods used in this experiment and accommodative responses evoked by stimulation of the LS area before and after the injection. The amplitude of the accommodative responses evoked by microstimulation of the LS area gradually decreased within 10 minutes of the injection. Accommodative responses were abolished within 15 to 30 minutes of the injection in all three cats. The degree of deficits in accommodative responses did not increase beyond 30 minutes after the injection. We observed substantial recovery 40 minutes after the injection and complete recovery after 60 minutes. The injections in each cat had qualitatively the same effect as those in the other cats. Histologic examinations revealed that the injection sites—the areas stained by fast green—were confined to the rostral SC. Muscimol had diffused into neither the pretectum nor the mesencephalic reticular formation (MRF) in all three cats. Saline injections into the rostral SC had no effect on accommodative responses evoked by stimulation of the LS area.

DISCUSSION

The current study indicated that the accommodation-related area in the rostral SC received input from the accommodation-related area in the LS area, whereas the pretectum did not receive input directly from the accommodation-related area in the LS. Results of previous anatomic studies also indicated that neurons in the medial bank of the caudal Mss project to superficial and intermediate layers of the rostral SC but not to the pretectum. Accommodative responses could not be evoked by stimulation of the LS area when the activity of rostral SC cells was inhibited by muscimol injection, suggesting that the descending projections from the LS area to the rostral SC convey accommodation-related signals.

On the other hand, the pretectum did not receive input from the accommodation-related area in the LS area. After WGA-HRP injection into the pretectum, retrogradely labeled cells were seen in the suprasylvian gyrus and the upper part of the medial bank of the Mss. Results of previous anatomic studies also indicated that neurons in the suprasylvian gyrus project to the pretectal nuclei. The upper part of the medial bank of the Mss is thought to include the pupillary-related area. Therefore, it is possible that the descending projections from the LS area to the pretectum convey partially pupillary-related signals. Neurons in the posterior pretectal nucleus and the olivary pretectal nucleus are sensitive to changes in light level.

FIGURE 8. A dark-field photomicrograph of a coronal section of the lateral suprasylvian area showing retrogradely labeled cells. Bar = 200 μm.

FIGURE 9. Schematic diagram showing the methods used for muscimol injections into the rostral superior colliculus, microstimulation of the lateral suprasylvian area, and monitoring of accommodative responses (A). Examples of accommodative responses evoked by microstimulation of the lateral suprasylvian area before and after the injection of muscimol into the rostral SC (B). The horizontal bar above the time scale in B indicates the period of stimulation.
and are thought to be involved in the control of pupillary movement.27 The LS area receives visual input, such as ocular disparity information, which is an important visual cue for accommodation and vergence eye movements.4–6 It is well known that the LS area also is involved in the control of vergence eye movements.28 The accommodation-related area almost corresponds to the vergence-related area in the LS area.11,28 The LS area in the cat is thought to provide command signals for controlling accommodation and vergence.2,24 The rostral SC may receive these command signals from the cortex. Results of our previous study20 indicated that the accommodation-related area in the rostral SC projects mainly to three parts of the brainstem: the pretectum, the dorsomedial portion of the MRF, and the raphe interpositus (RIP). The pretectum and the MRF are thought to be related to the control of accommodation, vergence, or pupillary movement.2,12,14,20,27,30–32 Therefore, the accommodation and vergence command signals from the cortex project to functionally related areas in the brainstem through the rostral SC.

Figure 10 shows a simplified diagram of the accommodation system. The accommodation and the vergence systems functionally interact with each other in the cortex.28 Signals from the accommodation and the vergence areas in the cortex converge in the rostral SC. The rostral SC projects to the pretectum and the MRF.23 The pretectum is involved in the control of both accommodation and pupillary movement. On the other hand, the MRF is involved in the control of both accommodation and vergence. The MRF could be a neural substrate for the interaction between accommodation and vergence.31,32 The rostral SC also projects to the RIP. The RIP has been indicated to be the location of omnipause neurons, which are tonically active in all periods of fixation and pause during saccades.33–37 Omnipause neurons inhibit activities of burst neurons in the paramedian pontine reticular formation during fixation. Recently, the rostral SC has been shown to be involved in the control of active visual fixation in both the monkey and the cat.36–41 Neurons in this portion also exhibit tonic discharges during fixation. Fixation neurons are thought to control the activity of omnipause neurons in the RIP.41 Saccade suppression may interact functionally with the ocular near response to provide a clear foveal image. The rostral SC has important roles in the interaction of the ocular near triad and the control of active fixation.

**Key Words**

accommodation, lateral suprasylvian area, pretectum, pupillarconstriction, superior colliculus

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