主論文

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EFFECTS OF CHOLINERGIC DEPLETION ON NEURON ACTIVITIES IN THE CAT VISUAL CORTEX

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SUMMARY AND CONCLUSIONS

1. Unilateral lesions of the nucleus basalis magnocellularis (nBM), a source of cholinergic projection to the cerebral cortex, were produced by injection of kainic acid in the cat. The lesions caused a significant reduction in density of choline acetyltransferase-immunoreactive terminals in the visual cortex ipsilateral to the lesions.

2. In the primary visual cortex ipsilateral to the lesions (ACh-depleted cortex), about half of the cells had weak or undetectable visual responses, while in the contralateral visual cortex almost all the cells had normal responsivity. The response selectivity, such as orientation and direction selectivities, of cortical cells was not affected by the depletion of ACh.

3. The microionophoretic application of acetylcholine (ACh) to cells under observation facilitated visual responses in 83 % of the cells recorded from the ACh-depleted cortex while it suppressed the responses in only 9 %. The application of a muscarinic antagonist, atropine, to cells in the ACh-depleted cortex was ineffective, suggesting no residual ACh activity.

4. The mean current required to induce facilitation in the cortex ipsilateral to the lesion was significantly smaller than that required in the contralateral cortex and the visual cortex of the normal cat, suggesting a supersensitivity of receptors mediating the effect or a reduction in catabolism of exogeneous ACh in the ACh-depleted cortex.

5. More than half of the cells which had been unresponsive to visual stimuli became clearly responsive during the ACh application. The response magnitude of cortical cells, as a whole, increased to the same degree as that observed during the ACh application in the normal cat.

6. In addition to the decrease in the average response magnitude,

there was a remarkable variability in responses of cells to motion of the slit from sweep to sweep in the ACh-depleted cortex. The application of ACh to cortical cells decreased the variability of responses, and consequently made the responses much more consistent.

7. These results suggest that without ACh supplied from the nBM, most of the cortical neurons could not respond briskly and consistently to excitatory inputs and that exogeneously applied ACh could reverse such an impairment of cortical neurons through intact or even supersensitive postsynaptic receptors.

INTRODUCTION

The nucleus basalis magnocellularis (nBM) in the basal forebrain is known as a main source of the cholinergic projection to the cerebral cortex in several animal species (4,21,24-26,34,35,39,47). Recently, a selective loss of cholinergic neurons in the nBM, a decrease in acetylcholine (ACh) content, and a reduction in activity of the ACh-synthesizing enzyme were found in the neocortex and hippocampus of patients with senile dementia of Alzheimer type (5,8,10,38). From these findings it was suggested that the impairment of memory and cognition in those patients may be related to a functional deterioration of the cholinergic system in the cerebral cortex. Indeed, it was reported in the rat that a chemical or electrical lesion of the nBM led to a severe impairment in learning tasks (12,17,18,40,46).

In the preceding paper (41) we suggested that a functional role of the cholinergic innervation to the visual cortex may be to improve responsivity of cortical neurons to the main visual inputs from the dorsal lateral geniculate nucleus (LGN). Therefore, a depletion of ACh in the visual cortex may lead to an impairment of visual information processing of cortical neurons and consequently to a deterioration of visual cognition. The present experiments were designed to confirm this suggestion and further to see whether exogeneously applied ACh could reverse such an impairment. Thus, we studied effects of chemical lesions in the nBM on responses of cortical neurons in the cat visual cortex and further analyzed effects of ionophoretically applied ACh and its antagonist on their responses. The effects of ionophoretically applied ACh and its antagonists on cortical neurons in the normal visual cortex have been described in the preceding

paper (41).

METHODS

Twelve adult cats, weighing 2.2-6.0 kg, were used in the present experiments.

Chemical Lesions of the nBM

The animals were initially anesthetized with ketamine (i.m., 20-25 mg/kg), and then sodium pentobarbital (20-30 mg/kg) was given intraveneously. Animals were placed in a stereotaxic head holder. After 2 % lidocaine was subcutaneously injected for local anesthesia, the skin overlying the skull was cut and the skull above targets for lesion was removed. A bipolar, enamel coated stainless-steel electrode was attached to the needle of a Hamilton microsyringe filled with kainic acid (5 mM, pH 7.4). The electrode was used to identify the location of the optic chiasm (OX) by monitoring field responses of optic nerve fibers to photic stimulation of the both eyes. Once the location of the OX was identified, the needle of microsyringe was moved at regular intervals to inject 1.0-1.5 μl of kainic acid. It was injected at six sites in the unilateral basal forebrain. Stereotaxic co-ordinates of these sites were as follows; (A, 0.0; L, 3.5; D, 3.0), (1.5, 2.0, 3.0), (1.5, 3.5, 3.0), (1.5, 5.0, 3.0), (3.0, 2.0, 3.0) and (3.0, 3.5, 3.0), where A is the distance rostral from the OX, L is the distance from the midline, and D is the distance above the OX. In two cats, Ringer solution was injected into the corresponding sites of the contralateral

hemisphere to control for the possibility of mechanical destruction of the brain due to the syringe itself. After finishing the injection, phenobarbital (25 mg/kg) and cephamycin (30 mg/kg) were given intramuscularly to prevent possible convulsions and bacterial infections. Penicillin G was further given to prevent infections of the wounds for 5 successive days after the operation. The animals were allowed to survive 14 to 46 days and then used for recording experiments.

Recordings from Cortical Neurons and Application of Drugs

Details of the preparation and anesthesia of animals, and of visual and electrical stimulations have been described in the preceding paper (41). For ionophoretic application of drugs, two barrelled micropipettes with tip diameter of 2-4 μm were attached to a recording pipette filled with 0.5 M sodium acetate with 2 % pontamine sky blue. Tips of the recording pipette protruded 10-15 μm from those of the drug pipettes. Procedures for extracellular single-unit recordings and for drug applications have been described previously (41). Drugs applied were acetylcholine chloride (0.5 M, pH 4.5) and atropine sulphate (0.2 M, pH 5.0). Receptive fields of cells recorded from area 17 of the cortex ipsi- and contralateral to the nBM lesions were analyzed quantitatively using a computer (Signal Processor 7T17, NEC-Sanei). Also, with the computer we analyzed effects of ionophoretically applied ACh and atropine on cell's responses to visual and afferent electrical stimulations. Details for the assessment of effects of drug application have been described in the preceding paper (41).

Immunocytochemical Procedures for Staining Cholinergic Fibers

To evaluate the effect of the nBM lesions on the cholinergic innervation to the primary visual cortex, cholinergic fibers and terminals were stained immunohistochemically by using the monoclonal antibody to the ACh-synthesizing enzyme, choline acetyltransferase (ChAT) in a biotin-avidin detection system (23). At the end of recording experiments, extracellular dye marks were produced for histological identification of recorded cells using methods described earlier (45). Then, the animals were deeply anesthetized by intravenous injection of sodium pentobarbital and perfused through the heart with Ringer solution followed by a fixative solution of 4 % paraformaldehyde and 1 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The small block of tissue including the visual cortex was cut out and infiltrated with a solution of 30 % sucrose in 0.1 M phosphate buffer for 24 to 48 h at 4^{o} C. Thirty μm-thick frontal sections were cut on a cryostat. Sections were rinsed three times (10 min each) with phosphate buffered saline solution (PBS: 0.02 M phosphate buffer, pH 7.4, containing 0.85 % NaCl) and then, incubated for 72 h with monoclonal antibody to ChAT (Boehringer Mannheim; diluted 1:10 in PBS) at room temperature. After rinsing three times (10 min each) with PBS, sections were incubated for 24 h with biotinized anti-rat IgG (Amersham; diluted 1:100 in PBS) at room temperature. After rinsing three times (10 min each) with PBS, sections were incubated for 24 h with peroxidase-labelled avidin (HistoGen; not diluted) at room temperature. The sections were rinsed three times (10 min each) with PBS. Then, tissues were transferred to the reaction solution (a drop of 2.2 % 3-amino-9-ethylcarbazole as the substrate and a drop of 0.5 % hydrogen peroxide in 2.5 ml acetate buffer (0.05 M, pH 5.0)) where the reaction product was developed within 60 min after which the reaction was stopped

by quick rinses in PBS (19). The sections were separated and mounted in a PBS-glycerine mixture. The reaction products were dark red. To make a quantitative evaluation of the effect of the nBM lesion on the cortex, immunoreactive varicosities and terminals were counted in a 700-μm-wide square, which was located mainly in layer III of the primary visual cortex. Counts were done in at least 10 squares of samples for each section to calculate an average density of varicosities and terminals.

RESULTS

Lesions in the nBM and Loss of Cholinergic Fibers in the Ipsilateral Visual Cortex

The injection of kainic acid successfully destroyed the nBM in the basal forebrain. An example of kainate-induced lesions in the nBM is shown in Fig. 1. The lesion included the major portion of the nBM, the ventral part of the putamen and globus pallidus, and the ventral part of the internal capsule (Fig. 1, arrow) where the large cholinergic neurons are located (25,47). Also, the lesion included a part of the vertical and horizontal limbs of the diagonal band of Broca (not seen in this section). Although the extent of the lesions varied slightly from animal to animal, the center of the lesions was always within the nBM. In most cases, lesions extended rostrocaudally from A 14.0 to 19.0, mediolaterally from L 1.0 to 6.0 and horizontally from D 7.0 to 10.0 in the standard stereotaxic co-ordinates. The lesions did not include the structures directly related to the visual cortex, such as the optic chiasm and the dorsocaudal sector of the claustrum; This sector of the claustrum is known to have a dense

connection with the visual cortex, but is located much more caudally and laterally (A 9.0-12.5, L 11.0-13.5 in the stereotaxic co-ordinates) than the lesions (27,46). In the basal forebrain where Ringer solution was injected as a control such a lesion was not observed, although the fine and straight-formed gliosis by the insertion of the needle of microsyringe was seen (not shown).

A loss of cholinergic fibers in the primary visual cortex ipsilateral to the lesions was demonstrated with the immunohistochemical method. Examples of ChAT-immunoreactivity of the visual cortex are shown in Fig. 2. In the visual cortex contralateral to the nBM lesions, the ChAT-immunoreactive fibers and varicosities along them were clearly visible as black dots like strings of beads (Fig. 2A). In agreement with the previous reports (25,43) the ChAT-positive fibers were densely distributed in layers I, II and III, and sparsely in layers IV, V and VI. The density of the ChAT-positive fibers and varicosities in each layer was approximately the same as seen in the visual cortex of the normal cat. In the visual cortex ipsilateral to the lesions, on the other hand, the ChAT-positive terminals were very sparsely distributed (Fig. 2B). The average density of the ChAT-positive terminals and varicosities was 107.8 ± 41.0 (S.D.)/mm^2 which was significantly smaller than that (970.6 ± 99.0 /mm^2) for the cortex contralateral to the nBM lesions ($p<0.005$, t-test). Therefore, ChAT-positive terminals and varicosities were estimated to reduce by about 90 % by the lesions. The average value of the density in the contralateral cortex was about the same as that in the visual cortex of the normal cat (908.4 ± 99.8 /mm^2). We could not find intrinsic cholinergic neurons in any layers of the visual cortex of the normal as well as the lesioned cats.

Effects of Ionophoretically Applied ACh on Neurons in the Cortex from Which ACh Was Not Depleted

As the first step of analysis, we studied 51 cells recorded from area 17 of the cortex contralateral to the nBM lesions. The facilitatory and suppressive effects of ACh on their visual responses were judged as significant when the total number of spikes in the optimal response was more than 150 % and less than 50 %, respectively, of the average number of spikes in the control responses before and after the ACh application. In 69 % of the 51 cells, an application of ACh facilitated their visual responses while in 14 % it suppressed the responses (Table 1). These values are quite similar to those obtained from the normal cat (41). The mean currents of the ACh application to induce the maximum effects were 48.2 ± 25.1 (S.D.) nA for the facilitation and 58.6 ± 31.8 nA for the suppression. These values were not significantly different from those obtained in the normal cat (41). Also, the magnitude of visual responses and their receptive field properties were generally not different from those of the normal cat, suggesting that the unilateral lesions in the basal forebrain did not affect activities of neurons in the contralateral cortex. This is consistent with the immunohistochemical results described above.

From the cortex ipsilateral to the injection site of Ringer solution we recorded 19 cells, and found that the magnitude and stimulus-selectivity of their visual responses were not different from those of the cells in the normal cortex. This is compatible with immunohistochemical results mentioned in the preceding section.

Effects of Ionophoretically Applied ACh on Neurons in the ACh-depleted Cortex

A total of 103 cells recorded from area 17 of the cortex ipsilateral to the nBM lesions were studied in detail. In 83 % of these cells, an application of ACh facilitated their responses to visual stimuli, while in only 9 % it suppressed the responses (Table 1). The proportion of facilitated cells was significantly larger in the cortex ipsilateral to the lesions than in the contralateral cortex ($p<0.05$, x^2-test). In the ipsilateral cortex, the mean current required to induce the maximal ACh effect were 34.8 $\pm$ 23.1 (S.D.) nA for facilitation and 51.1 $\pm$ 20.3 nA for suppression. The value for the facilitation is significantly smaller than that obtained in the contralateral cortex (48.2 $\pm$ 25.1 nA) and in the cortex of the normal cat (45.7 $\pm$ 22.1 nA, from Ref. 41) ($p < 0.05$, t-test). These results suggest a supersensivity of receptors mediating the facilitatory effect or a reduction in catabolism of exogeneous ACh in the ACh-depleted cortex. This point will be discussed later.

Facilitatory Effects of ACh

In the great majority of neurons in the ACh-depleted cortex, visual responses were facilitated by an application of ACh. An example of this is shown in Fig. 3. This cell had a receptive field of complex type and was located in layer III of the cortex. Before an application of ACh this cell had a preference to unidirectional motion of the slit oriented as depicted at the top of Fig. 3. The mean number of spikes evoked by a sweep of the slit in the preferred direction was 4.4. During ACh application the optimal response was clearly facilitated and the mean number of spikes to one sweep of the

slit increased to 24.1 (Fig. 3-2). In addition, weak responses to motion of the slit in the non-preferred direction appeared (Fig. 3-2, arrow) so that the direction preference was obviously weakened by ACh. The visual responses were not affected by an application of atropine using currents as high as 100 nA (Fig. 3-4). Although a small number of spikes was elicited by the stimulus in the non-preferred direction during atropine application, the directionality index (0.24, according to the formula described in the preceding paper) was not significantly different from that of control responses before and after the application (0.15 and 0.25, respectively). The ineffectiveness of atropine suggests that there was no endogenous ACh which might operate continuously to improve visual responsivity of cortical neurons as shown in the normal visual cortex (41). In the present experiments, we applied atropine to the 14 cells which were facilitated by ACh. In all cases, except one, their activities were not affected by the application of atropine.

In the ACh-depleted cortex, 20 % of the cells had no detectable visual responses or very weak responses (less than one spike per one sweep of the optimal stimulus). In the majority of these cells (11 of the 18 cells), visual responses became unambiguously detectable or more consistent during the application of ACh. An example of this is shown in Fig. 4. Before ACh application this cell was almost unresponsive to any visual stimuli (Fig. 4A-1 and B-1). During ACh application it became clearly responsive to leftward motion of the vertically oriented slit (Fig. 4A-2). However, it remained unresponsive to any motion of the horizontally oriented slit (Fig. 4B-2), indicating that the responses uncovered by ACh were orientation-selective.

Cells which were unresponsive or very weakly responsive to visual stimuli

were located predominantly in layers II+III of the striate cortex. They made up 56 % of the cells recorded from these layers. The unresponsive or very weakly responsive cells made up 23 and 36 % in the upper and lower parts of layer VI, while they were rare in layers IVab (0 %), IVc (8 %) and V (7 %). These results suggest that the activity of cells in layers II+III of the striate cortex is influenced by the cholinergic input more effectively than that of cells in layers IV and V. This suggestion seems consistent with the above-mentioned histological observation that the cholinergic terminals were most densely distributed in layers II+III of the normal visual cortex.

The appearance or facilitation of responses during ACh application was also seen in the case of responses evoked by electrical stimulation of the LGN. For example, the cell in Fig. 4 was originally unresponsive to the LGN stimulation. During the ACh application, however, it became consistently responsive to stimulation (not shown). This was the case for most of the cells which had been unresponsive to LGN stimulation before ACh application. In the cells already responsive to LGN stimulation, we observed an improvement in the response probability and in some cases the appearance of double-spike responses, as seen in the visual cortex of the normal cat (41). These results suggest that responsivity of cortical neurons to excitatory inputs from the LGN was considerably weakened in the ACh-depleted cortex.

Effects on Orientation and Direction Selectivities of Cortical Neurons

In the visual cortex of the normal cat, it is suggested that the facilitatory effect of ACh is not necessarily related to an improvement in response selectivity, and in fact the cholinergic facilitation was

sometimes accompanied by a significant reduction in orientation and/or direction selectivity (41). Nevertheless, there is still a possibility that endogenous ACh might contribute to the response selectivity of visual cortical neurons through the intracortical inhibitory system (31,33, 42,44) If so, the lesions in the nBM should cause a significant reduction of the response selectivity. To test this possibility, we analyzed the degree of direction selectivity of cells recorded from the ACh-depleted cortex by calculating the directionality index (DI) of visual responses from the formula of Daw and Ariel (11). The details of this formula have been described in the preceding paper (41). In short, the DI is 0 when no spikes are elicited by motion of a slit in the non-preferred direction. Conversely, the DI is 1.0 when equal numbers of spikes are evoked by motion of a slit in the both directions. This index was calculated for 72 of the 86 cells facilitated by ACh and compared between controls and tests with ACh. The other 14 cells were not included in this analysis because their response areas could not clearly be demarcated in peristimulus time histograms (PSTHs) of spike discharges.

The distribution of the DIs for the 72 cells is shown in Fig. 5. In the control, 32 % of the cells had a strong preference to unidirection of stimulus movement ($DI \leq 0.25$). This proportion is the same as that in the visual cortex of the normal cat (41). Also, the overall distribution of DIs seems similar to that in the normal cat, although the proportion of the cells with the DIs larger than 0.51 is slightly larger than that in the normal cortex. During the ACh application, the proportions of the strongly selective ($DI \leq 0.25$) and very weakly selective ($0.51 \leq DI \leq 0.75$) cells were decreased and those of the weakly selective ($0.26 \leq DI \leq 0.50$) and non-selective ($0.76 \leq DI \leq 1.00$)

cells were increased (Fig. 5, filled and hatched columns). This distribution pattern of DIs was very similar to that seen also during the ACh application in the normal cat (see Fig. 4 in the preceding paper). Although we did not systematically analyze changes in orientation selectivity of cortical cells in the present experiments, most of the cells responsive to visual stimuli seemed to have normal orientation tuning of responses. Even in cells which had been almost unresponsive to visual stimuli, the responses uncovered by ACh had orientation selectivity as exemplified in Fig. 4. These results suggest that cortical mechanisms responsible for making cells orientation- and direction-selective may remain intact in the ACh-depleted cortex.

Variability of Visual Responses

Another finding in the ACh-depleted visual cortex was that there was a substantial variability in responses to motion of a slit from sweep to sweep even in briskly responsive cells. An example of this is shown in Fig. 6-1. In the control PSTH, it is seen that this cell had weak responses with no apparent direction preference. A remarkable variability in the number of spikes to each sweep of the slit is obvious in a raster display of the responses (Fig. 6-1). During the application of ACh this cell responded consistently to each motion of the slit in the downward direction, except for the initial two sweeps, so that prominent responses with a strong direction preference appeared in the PSTH (Fig. 6-2). In most of the cells, responses to motion of slits in the non-preferred direction also became consistent so that the direction selectivity remained the same or even became worse during ACh application, as described above. To show such a change in response variability by ACh more quantitatively, we calculated the coefficient

of variation termed the variability index (VI) as follows: The standard deviation (S.D.) of the number of spikes per sweep was divided by the mean (m) of the spike numbers. Thus, the formula is VI = S.D./m. For example, the VI of the cell shown in Fig. 6 was 0.92 without ACh, and it decreased to 0.69 during ACh application.

The VIs were thus calculated for the 84 cells facilitated by ACh, and the distribution of these values is shown as percentage histograms in Fig. 7. Two cells facilitated by ACh were not included in this analysis because they failed to respond to visual stimuli without ACh. In the control, the distribution of cells with each category of VIs was widespread and had no distinct peaks (Fig. 7, empty columns). Twenty-six percent of the cells had consistent responses with VIs smaller than 0.40 while other 24 % had extremely variable responses with VIs larger than 1.21. During the ACh application, however, the distribution of VIs shifted to the left, and the consistently responsive cells with VIs smaller than 0.40 made up 50 % of the total (Fig. 7, filled columns). Therefore, the facilitatory effect of ACh was accompanied with an improvement in the consistency of responses in most of the cells in the ACh-depleted cortex.

Suppressive Effect of ACh

As described previously, the small proportion of the cells in the ACh-depleted cortex was suppressed by ACh (Table 1). An example of the suppressive effect of ACh is shown in Fig. 8. This cell was of the complex type and located in layer III of the cortex. In the control PSTH, it had clear responses to motion of a slit with relatively high background activity (Fig. 8-1). This background activity was almost completely abolished during

ACh application while the visual responses were only weakly suppressed (Fig. 8-2). Consequently, the signal to noise ratio of the responses was obviously improved by the ACh application. The same type of ACh effect was observed in spontaneously active cells in the normal cat (41). A single application of atropine did not significantly change the activity of this cell (Fig. 8-4), suggesting that there were no sustained influences of endogeneous ACh on this cell.

Changes in the Magnitude of Visual Responses

As described in a previous section, a substantial number of cells was unresponsive or only weakly responsive to visual stimuli, but these became clearly responsive during the ACh application. To demonstrate these findings more quantitatively, we rated the magnitude of visual responses into four classes (from class 0 to 3). The criteria for this classification have been described in the preceding paper (41). In short, no detectable visual responses examined as PSTHs were rated as class 0. Weak responses just detectable in PSTHs were rated as class 1. Clear responses to each of the repeated stimuli were rated as class 2. Vigorous responses with more than 40 spikes to one stimulus presentation were rated as class 3. The proportion of cells with each class of response magnitude is shown as percentage histograms of the total (Fig. 9). Cells suppressed by ACh were not included in these histograms because we attempted to draw a quantitative statement as to how strongly ACh enhanced visual responses in the facilitated cells. Without ACh, 52 % of the total number of cells analysed had responses rated as classes 0 and 1 (Fig. 9A). The proportion of cells rated as class 0 was 20 % and this value was significantly larger than that obtained in the normal

cat (41) ($p< 0.05$, x^2-test). During ACh application, the percentage of cells rated as class 2 increased from 35 to 45 %, and the class 3 cells from 14 to 34 % (Fig. 9B). The distribution pattern of each class of cells during ACh application became quite similar to that seen during ACh application in the normal cat (compare the histogram B with that of Fig. 10B in the preceding paper (41)).

DISCUSSION

The nBM Lesions by Kainic Acid and ChAT-immunoreactivity of the Cortex

The injection of kainic acid successfully produced restricted lesions in the basal forebrain of the cats which probably spared fibers of passage (7). It seemed practically impossible, however, to destroy all the cholinergic neurons in the basal forebrain. They are located in widely distributed structures, some of which are very thin (24,25,36). Furthermore, a more extensive application of kainic acid to produce more widespread lesions would have killed the animals because of long-lasting convulsions. Therefore, it was unlikely that we could have destroyed all the sources of cholinergic projections to the visual cortex in the present experiments. Nevertheless, the present results with immunohistochemical techniques have demonstrated that ChAT-immunoreactive fibers and terminals were dramatically reduced in the primary visual cortex ipsilateral to the lesions.

In the rat there are some reports indicating an existence of intrinsic cholinergic cells in the cerebral cortex, particularly in layers II and III (13,21,22,28,37). In the visual cortex of normal as well as lesioned cats, however, we could not find cell bodies with ChAT-immunoreactivity.

This is consistent with previous reports on cat visual cortex (25,43). Therefore, it is unlikely that ACh might be supplied from intrinsic neurons in the visual cortex in spite of the ipsilateral nBM lesions. Also, a compensatory reinnervation from the nBM in the intact side was unlikely at least before the time we carried out the recording experiments (2-7 weeks after making the lesions). Indeed, Fine et al. (16) reported that cholinergic reinnervation from the contralateral nBM to the rat cerebral cortex did not take place between one week and 6 months after lesioning. Thus, it is reasonable to conclude that ACh was almost completely depleted from the visual cortex ipsilateral to the nBM lesions in the present experiments.

Visual Responsiveness of Neurons in the ACh-depleted Cortex

In the visual cortex ipsilateral to the nBM lesions, more than half of the cells had no detectable or weak visual responses. Although visually unresponsive cells have been reported to exist near the bottom of layer VI of the visual cortex, they make up only a small fraction of cortical cells throughout all layers in the normal visual cortex (45). Thus, it was a rather unexpected finding that 20 % of the visual cortical cells, which were recorded from all layers except layer I, were virtually unresponsive to visual stimuli. This failure to drive them was not due to an inadequacy of the visual stimuli employed because we could successively plot receptive fields for other cells encountered in the same penetration of electrodes. Indeed, most of the unresponsive cells became clearly responsive to the visual stimuli during ACh application. Thus, it is reasonable to assume that the ACh depletion resulted in a

significant reduction in visual responsiveness so as to make the originally weakly responsive cells virtually unresponsive to visual stimuli. This suggestion is confirmed by the quantitative analysis of the magnitude of visual responses (Fig. 9) which showed that the response magnitudes of cortical cells as a whole increased dramatically during ACh application and became virtually the same as those facilitated by ACh in the normal visual cortex.

Neurons in the ACh-depleted visual cortex seemed to maintain the stimulus selective responsiveness, although their responses were often weak and inconsistent. In other words, the depletion of ACh may not reduce response selectivity (e.g., orientation and direction selectivity), of visual cortical neurons. Therefore, it is possible to suggest that the cholinergic system may not directly be involved in mechanisms responsible for the response selectivities of visual cortical neurons. This suggestion may be consistent with previous findings that intracortical inhibition by gamma-aminobutyric acid (GABA) may be directly responsible for the selectivities of visual cortical neurons (42,44).

In a study to explore the modulatory role of ACh and noradrenaline in plasticity of the kitten visual cortex, Bear and Singer reported that visual response properties of the cells recorded from the ACh- and noradrenaline-depleted cortex did not differ from controls, although they did not state this in detail (2). In that study, however, they focused their attention on the ocular dominance of cortical neurons and thus did not analyze response magnitude quantitatively. Therefore, it seems possible that they observed the response selectivity mainly in the group of cells which were

still clearly responsive.

Facilitatory Effect of Exogenous ACh in ACh-depleted Cortex

The ionophoretic application of ACh on neurons in the ACh-depleted cortex induced a facilitation of visual responses in the great majority of cells tested. This indicates that receptors for ACh, probably muscarinic receptors (41), remain sensitive to ACh during its depletion for as long as 7 weeks. Furthermore, the proportion of the facilitated cells in the ACh-depleted cortex was significantly larger than that in the normal visual cortex. To account for this finding the following two interpretations are possible, although they are not mutually exclusive. First, the sensitivity of receptors of neurons in the ACh-depleted cortex became higher than the normal, i.e., the denervation supersensitivity seen in cholinergic receptors in muscles and autonomic ganglia (6). This possibility seems consistent with the present findings that the mean currents of ACh ionophoresis required to obtain maximum effects in ACh-depleted cortex was significantly lower than that in normal or intact cortex. Second, a breakdown of exogeneously applied ACh may be retarded and consequently actions of the ACh be potentiated in the ACh-depleted cortex, because a reduction in activity of the ACh-hydrolyzing enzyme, acetylcholinesterase is known to occur after lesions of the nBM (1,2,16,26).

Recently, McCormick and Prince reported evidence indicating that the cholinergic suppression seen in cingulate cortical neurons is mediated by a rapid excitation of adjacent inhibitory neurons (31,33). They further reported that cholinergic receptors at these inhibitory neurons are relatively insensitive to a muscarinic antagonist, pirenzepine. With this

drug muscarinic receptors can be classified into two subtypes, pirenzepine-sensitive (M_1) and -insensitive (M_2) receptors (3,14,15,20,32). In the ACh-depleted cortex of the rat and in the cortex of patients with senile dementia of Alzheimer type (30), it was reported that M_2 receptors were reduced in the number while M_1 receptors were not changed. Although it is not clear whether these M_2 receptors correspond to the pirenzepine-insensitive receptors of McCormick and Prince or not, the above-mentioned reports suggest another possibility to account for the increase in the proportion of the facilitated cells in the present study, i.e., the loss of M_2 receptors in the ACh-depleted cortex might result in insensitivity of inhibitory interneurons to the ACh applied ionophoretically. From the present results, any case, it is reasonable to conclude that M_1 receptors in the visual cortex may remain functional with normal or even supernormal sensitivity after cholinergic denervation.

Variability of Responses of Neurons in the ACh-depleted Cortex

In the present experiments we found that there was a remarkable variability of responses to motion of a slit from sweep to sweep even in the briskly responsive cells. This may be due to a lack of the action of ACh which enables excitatory inputs to cortical neurons to generate repetitive spikes through blockade of the voltage-dependent potassium channel, as discussed in the preceding paper (41). In other words, without ACh the possibility for visual inputs to induce responses with high frequency discharge of spikes may be influenced simply by the spontaneous fluctuation of membrane potentials of visual cortical neurons (9) so that the responses are more highly variable than normal. Such a change in visual

responsivity of corical neurons may result in the worsening of the signal to noise ratio of activity of cortical neurons, as discussed in the preceding paper (41).

Cells Suppressed by ACh

Only 9 % of the cells in ACh-depleted cortex were suppressed by application of ACh. As described above, it is possible that this is due to the loss of pirenzepine-insensitive muscarinic receptors of inhibitory interneurons. Another possibility is a sampling bias. A difference of the proportion of suppressed cells between ACh-depleted and normal cortices may be due to a difference in sampling ratio of cells in the superficial and deep layers of the cortex, because the suppressed cells were mostly located in the former layers. If the suppressed cells are continuously inhibited by endogeneous ACh in the normal cortex, then they should have a relatively high activity in the ACh-depleted cortex. In fact, the cell shown in Fig. 8 had a high spontaneous firing rate. However, the total number of the suppressed cells is too small to compare the activity quantitatively between the ACh-depleted and normal cortices. So, in the present study we can only state that there were a small fraction of cells in the superficial layers of the ACh-depleted cortex which were released from the suppressive effect of ACh so as to have a relatively high spontaneous activity.

Deterioration of Cortical Function due to Depletion of ACh

In the ACh-depleted cortex, more than half the of cells were unresponsive or weakly responsive to visual stimulation. This suggests that a significant reduction in responsivenss of cortical neurons to afferent inputs and a

considerable worsening of the signal to noise ratio of neuron activity must occur in other cortical areas as well as visual areas, because the cholinergic projections from the basal forebrain are spread throughout the entire cortex (24,25,34,39). Consequently, a significant malfunction in the various cortical areas must take place after the depletion of ACh. This may be one of reasons why cognitive or learning functions are impaired after mechanical or chemical lesions of the nBM in the rat (17,18,29,40). Also, such an impairment of cortical neuron activities may explain why patients with senile dementia of Alzheimer type suffer from severe impairment of cognition as well as memory. In the present study we have demonstrated that ionophoretic application of ACh to most of the neurons in the ACh-depleted cortex improved their responsiveness to visual inputs. These results may have possible therapeutic implications. If ACh itself or other agonists for M_1 receptors could be applied directly to cortical neurons, it might improve the symptoms from which patients with senile dementia of Alzheimer type are suffering.

ACKNOWLEDGMENTS

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Legends for Figures

Fig. 1 Photomicrograph showing a lesioned site in the basal forebrain. The lesion is indicated by arrow. Remarkable expansion of the lateral ventricle (VL) ipsilateral to the lesion is visible. nBM, nucleus basalis magnocellularis. GP, globus pallidus. PU, putamen. CI, internal capsule. NC, caudate nucleus. Frontal section at a level of A +16. Cresyl violet stain. Scale, 2 mm.

Fig. 2. Choline acetyltransferase-immunoreactive terminals and varicosities (dots) in layer III of the primary visual cortex contralateral (A) and ipsilateral (B) to the nBM lesion. A remarkable decrease in density of cholinergic varicosities and fibers was seen in B. Scale, 50 μm.

Fig. 3. Facilitatory effect of ACh on visual responses of a neuron in the cortex ipsilateral to the nBM lesion and ineffectiveness of atropine (ATROP.). Each histogram shows peristimulus time histogram (PSTH) of spike discharges to a slit moving back and forth over the receptive field. Direction of motion and orientation of the slit is indicated at the top. Eight sweeps for each PSTH, bin width 14 ms. Stimulus speed was 5.7 deg/s. 1), 3) and 5), control PSTHs without application of any drug. 2), test PSTH during application of ACh with 60 nA. Arrow indicates that responses to motion of the slit in non-preferred direction was also enhanced. 4), test PSTH during application of atropine with 100 nA.

Fig. 4 Facilitatory effect of ACh on visual responses of a neuron which had almost no responsivity in the cortex ipsilateral to the nBM lesion. PSTHs to motion of the optimally oriented slit are shown in column A and those to the slit orthogonal to the optimal are shown in column B. Twelve sweeps for each PSTH, bin width 8 ms. Stimulus speed was 5.7 deg/s. A-1), B-1) and A-3), control PSTHs. PSTH corresponding to B-3 was omitted because there were no spikes. Almost no responses were seen in A-1) and A-3). A-2) and B-2), test PSTHs during application of ACh with 40 nA.

Fig. 5. Distribution of directionality index (DI) of 72 cells in the ACh-depleted visual cortex at control (open columns) and during the ACh application (filled and hatched columns). DIs were calculated from the formula described in text. Hatched columns represent cells with reversal of preferred direction during the ACh application. The ordinate represents the number of cells in each range of DI as percentages of the total number of cells analyzed.

Fig. 6. Improvement in response variability of a cortical cell by ACh. Visual responses were shown as PSTH (at the top) and raster display of spike discharges (below the middle). Ten sweeps for each PSTH. Bin width, 14 ms. Filled triangles at the left of the raster display indicate the beginning of each stimulus sweep. Stimulus speed was 4.6 deg/s. 1) control runs without ACh. 2) test runs during ACh application with 40 nA.

Fig. 7. Distribution of variability index (VI) of 84 cells in the ACh-depleted cortex in control (open columns) and during the ACh

application (filled columns). VIs were calculated from the formula described in text. The ordinate denotes the number of cells in each range of VI as percentages of the total number of cells analyzed.

Fig. 8. Suppressive effect of ACh on activities of a cortical neuron with relatively high spontaneous activity and ineffectiveness of atropine. The spontaneous activity was suppressed much more clearly than visual responses. Eight sweeps for each PSTH, bin width 14 ms. Stimulus speed was 7.3 deg/s. Other conventions are same as in Fig. 3.

Fig. 9. Proportion of cells with each category of response magnitude in control (A) and during ACh application (B) in the cortex ipsilateral to the nBM lesion. Criteria for the four categories of response magnitudes, see text. The ordinate represents the number of cells in each category as percentages of the total number of cells analyzed.

Table 1

Effects of ionophoretically applied ACh on visual cortical neurons

A) Cortex contralateral to the nBM lesion

	Facilitation	No effect	Suppression	Total
Number of cells	35	9	7	51
(percentage)	(69)	(18)	(14)	(100)

B) Cortex ipsilateral to the nBM lesion

	Facilitation	No effect	Suppression	Total
Number of cells	86	8	9	103
(percentage)	(83)	(8)	(9)	(100)

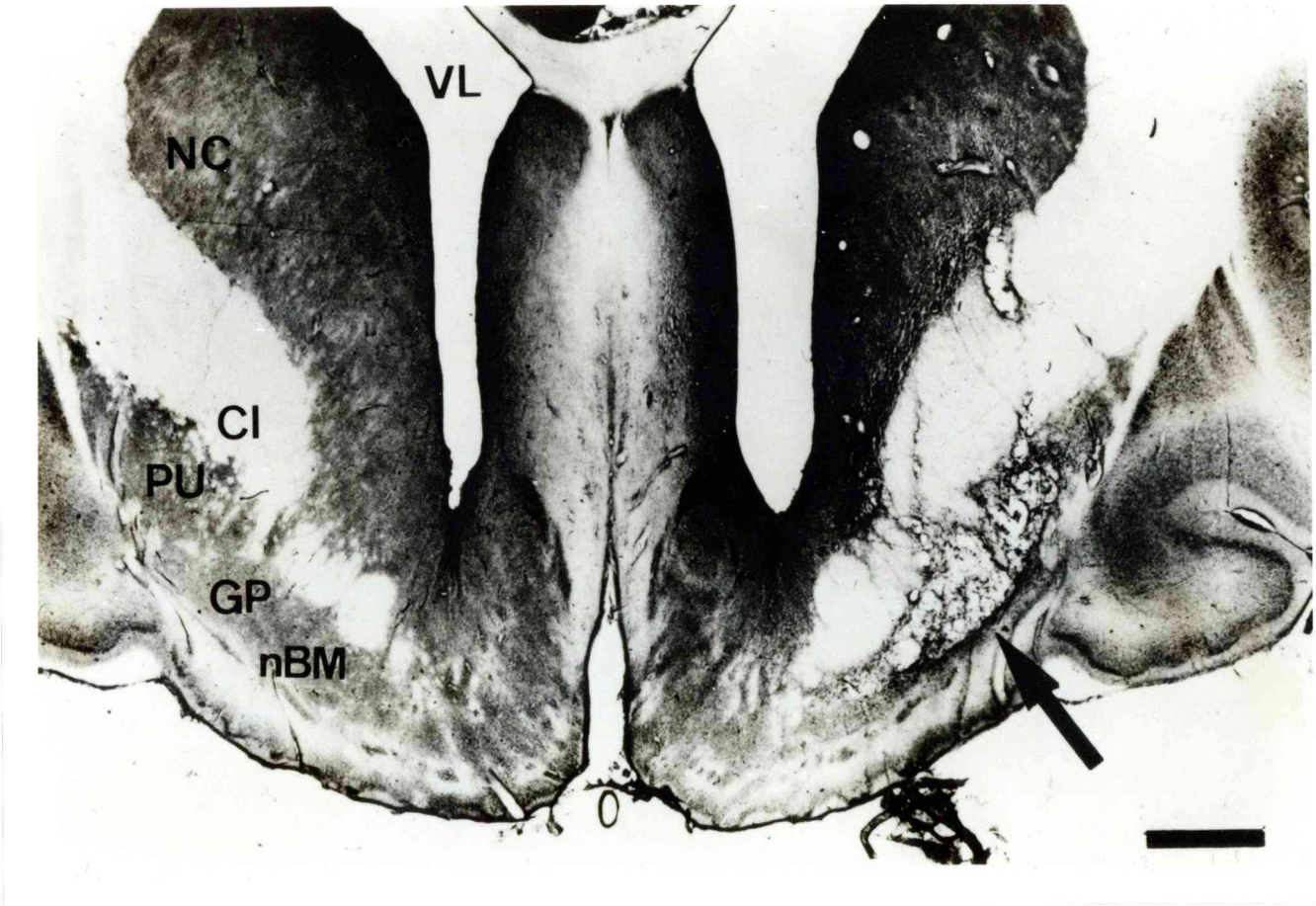

Figure 1

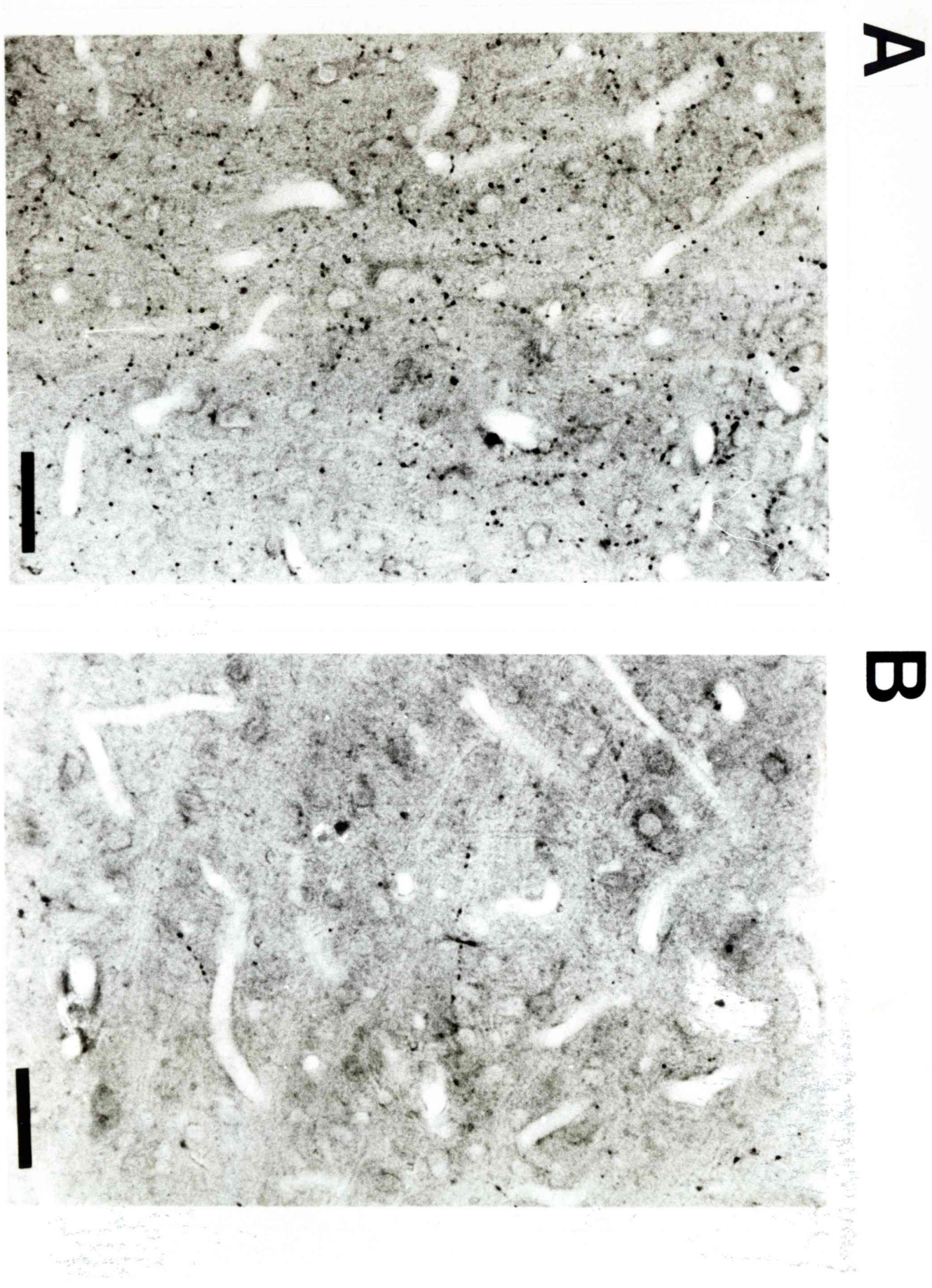

Figure 2

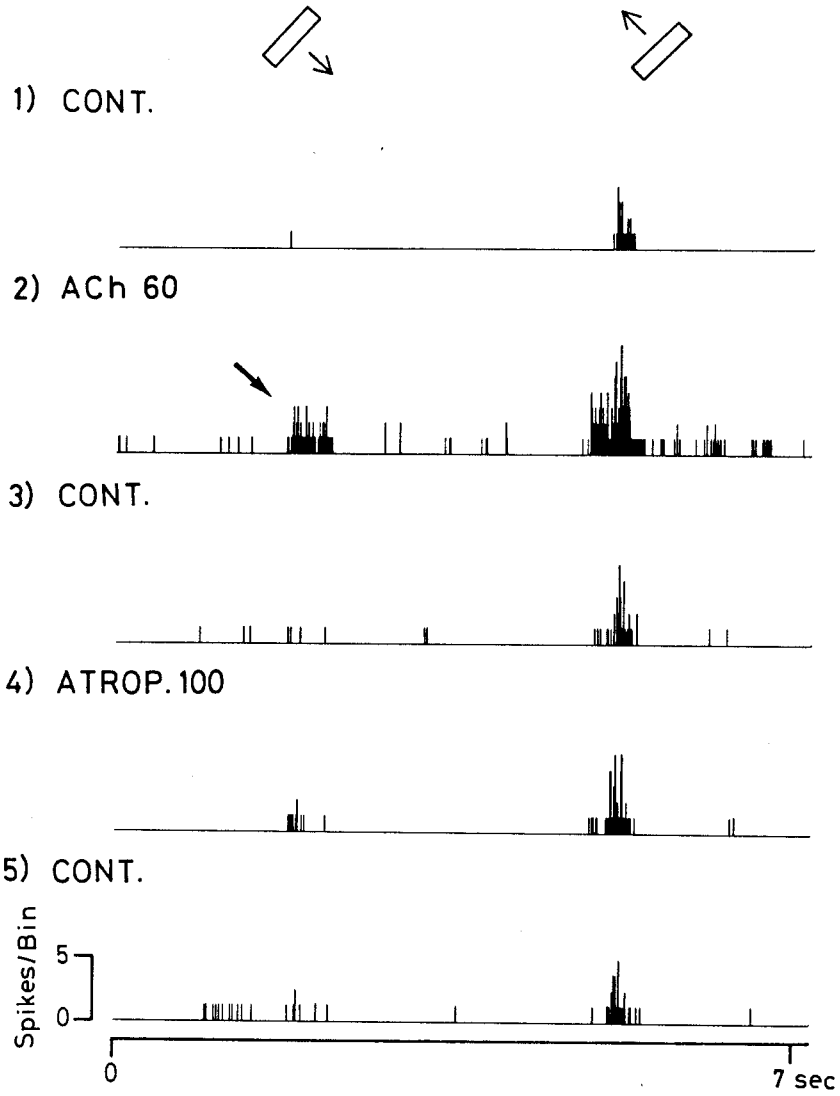

Figure 3.

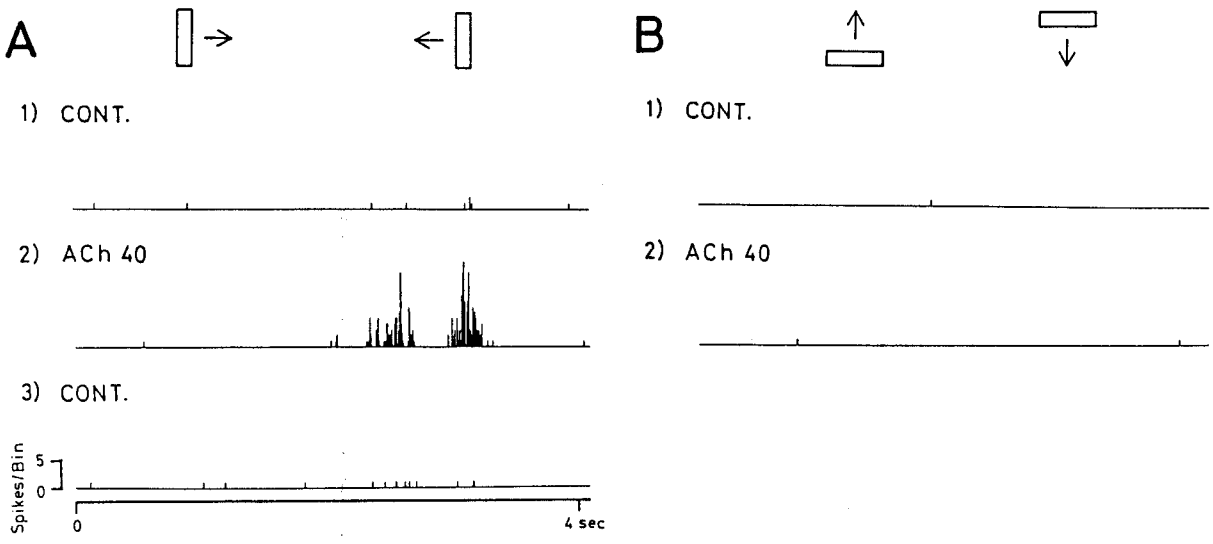

Figure 4.

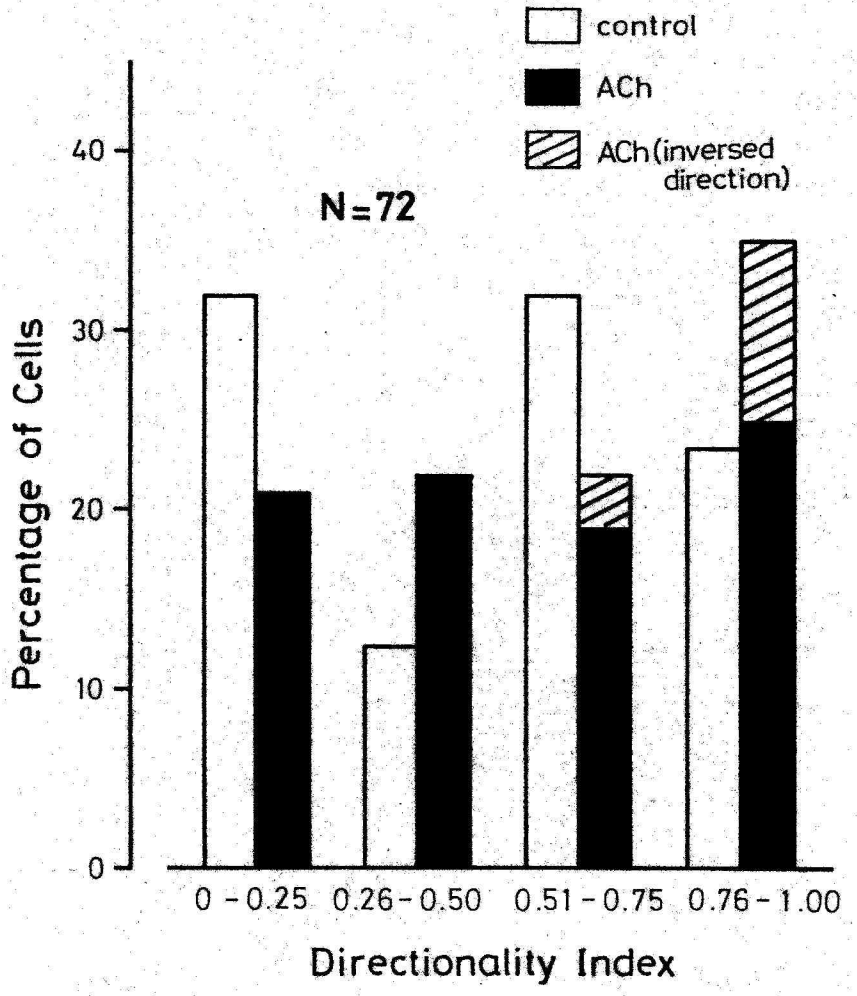

Figure 5

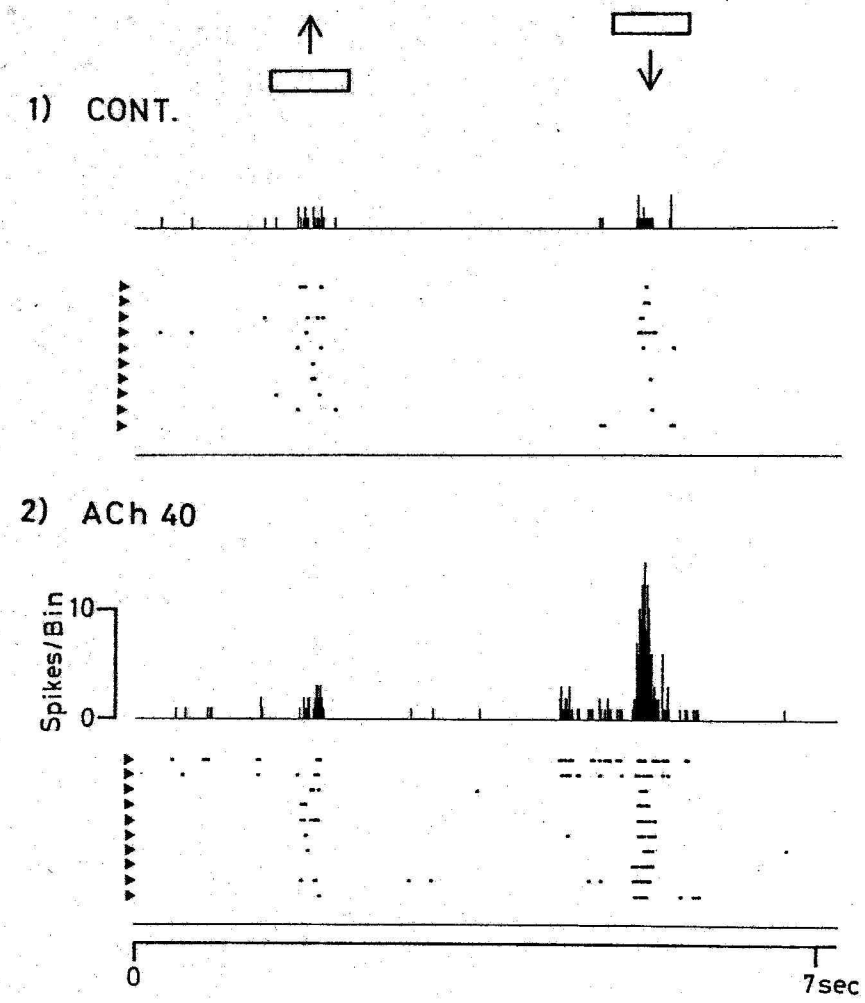

Figure 6

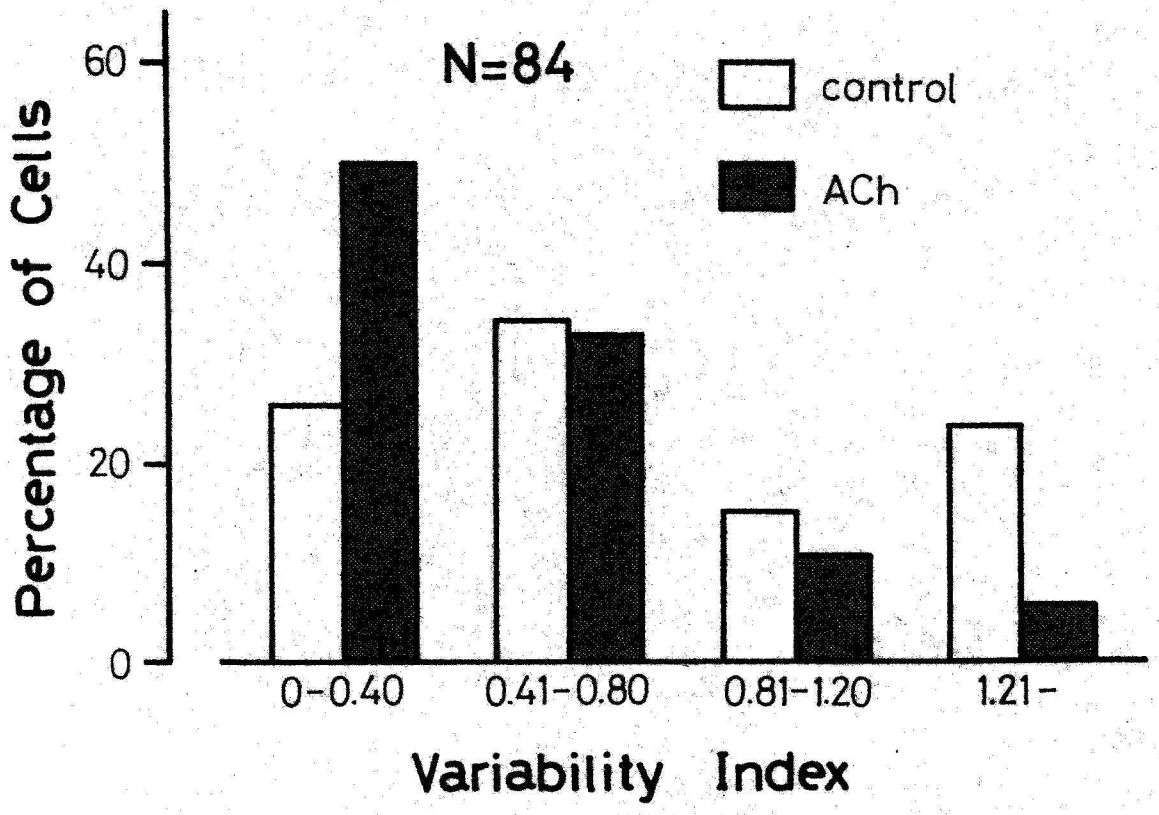

Figure 7

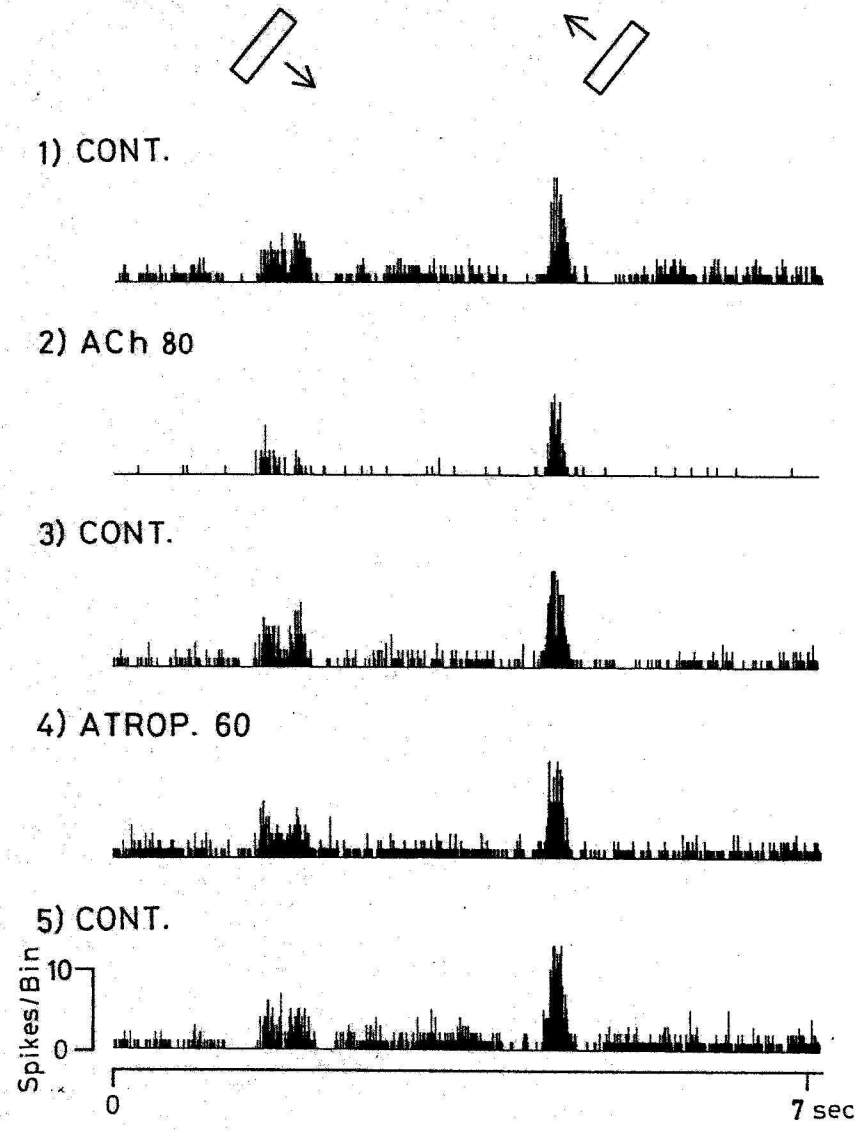

Figure 8

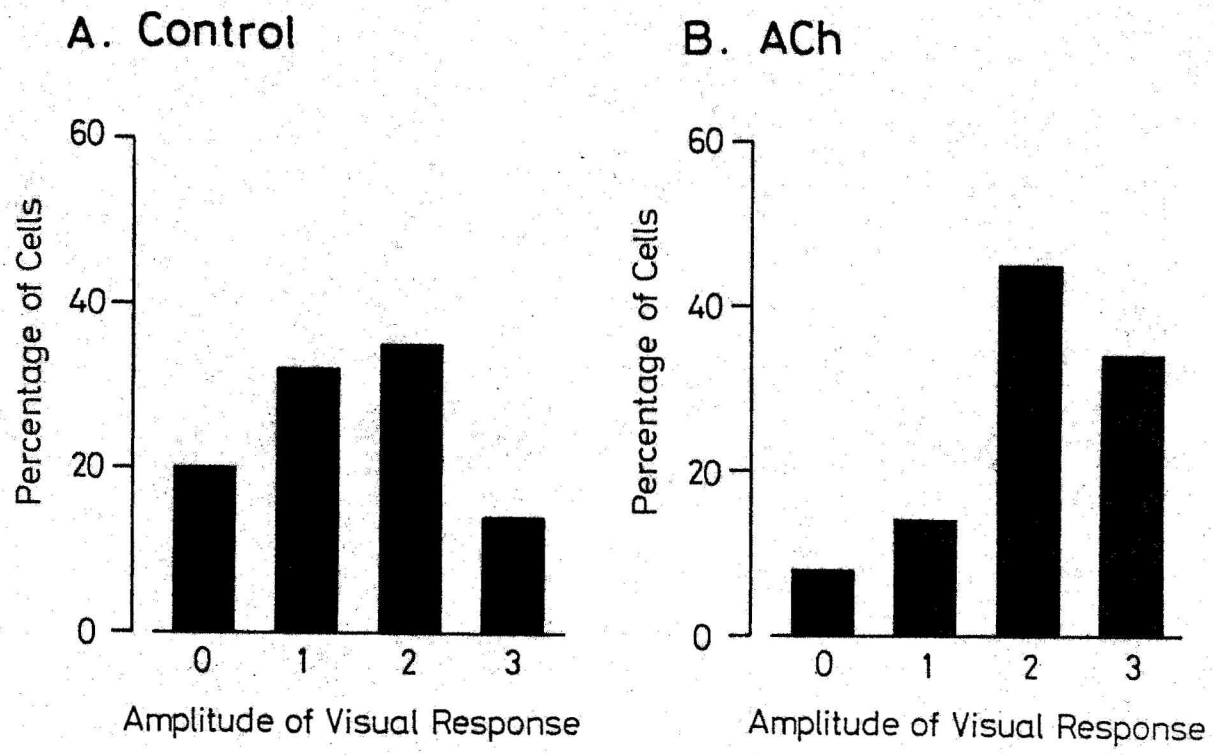
A. Control
B. ACh
Percentage of Cells
60
40
20
0
0
1
2
3
Amplitude of Visual Response

Figure 9

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参考論文

Short Communication

GABAergic inhibition already operates on a group of neurons in the kitten visual cortex at the time of eye opening

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Key words: visual cortex — GABAergic inhibition — bicuculline — development — orientation selectivity — kitten

Effects of iontophoretic application of γ-aminobutyric acid (GABA) and its antagonist, N-methyl-bicuculline (BIC), on visual responses of striate cortical neurons were studied in kittens 6–13 days old. Visually responsive cells were classified into 3 groups, i.e., orientation-selective, orientational bias and non-oriented cells. In almost all of the orientation-selective cells their responses were completely suppressed by GABA while the majority of the others were not significantly or only weakly suppressed. BIC abolished or reduced the selectivity of all the orientation-selective cells while it did not affect any of the non-oriented cells tested. These results suggest that GABAergic inhibition already operates on a group of cortical neurons to make them orientation-selective at the time of eye opening.

Neurons in the visual cortex are selectively responsive to particular visual stimuli, such as to a light slit moving in one direction at a specific orientation[5]. There is ample evidence suggesting that these direction and orientation selectivities of cortical neurons may, at least, in part be due to γ-aminobutyric acid (GABA)-mediated intracortical inhibition[11,12,14]. In very young kittens with eyes just opened, a certain group of cortical neurons already have such selectivities[2,3,6,15]. These neurons can be called 'innately-specified cells'[2] because the kitten had virtually no visual experiences at the time of experiments. A question of whether these innately-specified properties are due to the intracortical inhibition or to excitatory convergence of afferents as originally suggested by Hubel and Wiesel[5] in the adult remains open. With the microiontophoretic method we have applied GABA and its antagonist, N-methyl-bicuculline (BIC) to neurons in the primary visual cortex of kittens around the time of eye opening. The results suggest that GABAergic inhibition already exerts its action onto the innately-specified neurons to make them orientation-selective.

Eleven kittens were used at ages from 6 to 13 days. Three of them, aged 6, 6 and 7 days, had not yet opened their eyes at the initiation of the experiments, but their eyelids were easily parted by experimenter's hands. The others (two at 10 days, one at 11 days, 3 at 12 days and two at 13 days) were 1–3 days after the time of natural eye opening. Except for anesthesia, experimental procedures for preparing and maintaining the animals, electrical and visual stimulations, and extracellular single-unit recordings were essentially the same as described previously[15]. Under ketamine anesthesia (i.m., 35–40 mg/kg) the trachea and femoral vein were cannulated for artificial ventilation and infusion, respectively. The animals were then paralyzed with Flaxedil and anesthetized with a gas mixture of 70% N_2O–30% O_2 and 1–2% Halothane. All wound margins were carefully covered with 2% Xylocaine jelly. During recordings the animals were maintained by a 70% N_2O–30% O_2 mixture with the addition of 0.5–1% Halothane when neccessary to maintain an adequate depth of anes-

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TABLE I

Effects of GABA and BIC applications on visual responses of cortical neurons

Weak and complete GABA effects; reduction of the optimal response to 6–49 and 0–5% of the control, respectively. Tests with BIC were done only on the cells with weak or complete GABA effects. Responsiveness of a few cells did not recover to the original control level after stopping BIC application so that these cells were discarded.

Type of cells	*No. of cells*	*GABA effect*			*BIC effect*	
		no	*weak*	*complete*	*no*	*present*
Orientation-selective	10	0	1	9	0	6
Orientational bias	22	4	10	8	8	9
Non-oriented	16	6	5	5	9	0
Vaguely or non-responsive	29	—	—	—	—	—
Total	77					

thesia. Judging from the recording sites in the cortex, the receptive fields of units studied were within 15° of the area centralis. Five-barrel micropipettes, consisting of one double-chambered pipette (θ-pipette) and 3 single-chambered pipettes, were fabricated such that the tips of the θ-pipette were protruded 10–20 μm from those of the drug electrodes. One channel of the θ-pipette was filled with 2 M-NaCl solution for recording, and the other with a solution of dyes (Fast Green or Pontamine blue) for histological identification of the recording site. The drug electrodes contained GABA (0.5 M, pH 4.0), BIC (5 mM, pH 4.0), and normal saline or glutamate (1.0 M, pH 8.4). Retaining currents of 10 nA were passed through the drug electrodes. Since the spontaneous activity of most of cortical neurons was extremely low around the time of eye opening[2,3,6,15], electrical stimulation through electrodes placed in the dorsal lateral geniculate nucleus was used to activate neurons in addition to various kinds of visual stimuli[15]. Once a single unit was isolated, its visual response properties were studied and peristimulus time histograms (PSTHs) to a light slit moving back and forth at the optimal and other orientations were constructed. In order to assess drug effects, PSTHs to the same set of stimuli were compiled before, during and after administration of the drugs.

A total of 77 cells were studied in detail, of which 29 were visually unresponsive or only vaguely responsive (Table I). The other 48 cells, which had relatively consistent visual responses, were classified into 3 groups according to the degree of orientation selectivity as follows[2,3,15]. Orientation-selective cells had no responses to motion of a slit oriented 90° to the optimal; orientational bias cells had responses but the response magnitude (total number of spikes in the preferred direction at the orientation 90° to the optimal) was less than 49% of that of the optimal; and non-oriented cells had responses of the magnitude more than 50% of the optimal. Other selectivities, such as the direction selectivity, will not be analyzed in detail in this report. Tests with GABA were completed on the visually responsive cells, and the results were classified into 3 categories, i.e., no effects, a weak but significant suppression of visual responses and a complete elimination of responses (Table I). The GABA tests were usually done with ejecting currents of 10–30 nA, since almost all the cells in the adult cortex were completely suppressed with the currents of this range.

In almost all of the orientation-selective cells their visual responses were completely suppressed by GABA application (see Fig. 1B), while nearly half of the orientational bias cells were only weakly inhibited. Six of the 16 non-oriented cells were not significantly suppressed by GABA application, but the others were weakly or completely inhibited (Table I). Cells with the weak or complete GABA effects were then tested with BIC. Effects of BIC were judged as present only when orientation-selective cells changed to orientational bias and orientational bias cells to non-oriented or responses of non-oriented cells significantly increased during BIC application. Effectiveness of BIC was closely related to the degree of orientation selectivity. All of the orientation-selective cells, on which the BIC test was completed, changed their responsiveness whereas all of the non-oriented cells tested did not (Table I). An example of

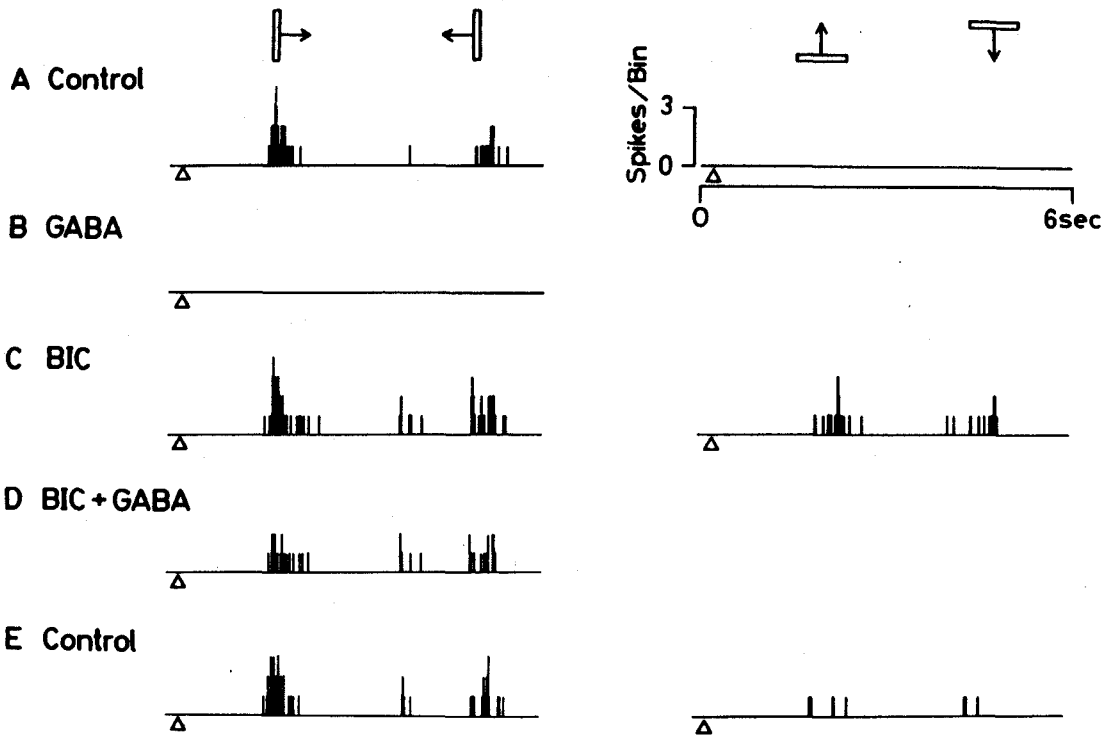

Fig. 1. Effects of GABA and BIC application on visual responses of an orientation-selective cell at 10 days of age. Each PSTH shows responses to a slit moving back and forth over the receptive field. Direction of motion and orientation of the slit are indicated at the top of each column of PSTHs. Fifteen sweeps for each PSTH, bin width 6 ms. Stimulus speed was 4.8 deg/s. Markers (triangles) beneath each PSTH indicate the beginning of the stimulus sweep. In case of GABA and BIC+GABA applications, PSTHs to motion of the slit orthogonal to the optimal were omitted because PSTHs to the optimally oriented slit were enough for assessment of drug effects. Ejecting currents for GABA and BIC were +10 and +60 nA, respectively.

the action of BIC together with that of GABA on an orientation-selective cell is illustrated in Fig. 1. This cell, recorded from a 10-day-old kitten, responded clearly to motion of a slit at the vertical orientation (Fig. 1A, left). Although this cell responded weakly to motion of the slit oriented ± 45° to the vertical (not shown), it had no responses at all to motion of the horizontally oriented slit (Fig. 1A, right). During application of GABA, responses to the optimal stimulus were completely abolished (Fig. 1B). The responses recovered to the original control level a few minutes after stopping the GABA application (not shown). During application of BIC, responses to motion of the vertical slit slightly increased in either direction (Fig. 1C, left). A more dramatic change was that responses to motion of the slit orthogonal to the optimal appeared in either direction during BIC application (Fig. 1C, right). The number of spikes elicited by the preferred motion of the orthogonal slit became 52% of that of the optimal response. It is to be noted that the spontaneous activity did not significantly increase during the BIC application and the enhancement of the optimal response was not as marked as in the adult[12,14]. This is not due to a pharmacological inefficiency of the BIC applied because it effectively blocked the GABA action (Fig. 1D). Therefore, it is possible to suggest that the intracortical tonic inhibition[12,14] has not fully developed yet around the time of eye opening. Responsiveness of this cell had almost completely recovered to the original control level several minutes after ceasing the application of both drugs (E). This type of BIC effects was seen in all of the orientation-selective cells tested (Table I). Sixty percent of the orientation-selective cells, recording sites of which were histologically identified, were located in layer V of the cortex.

About half of the orientational bias cells and all of the non-oriented cells tested with BIC did not significantly change their visual responses (Table I). As an example, visual responses of a non-oriented cell are shown in Fig. 2. This cell responded almost equally to motion of the slit at any orientation. During appli-

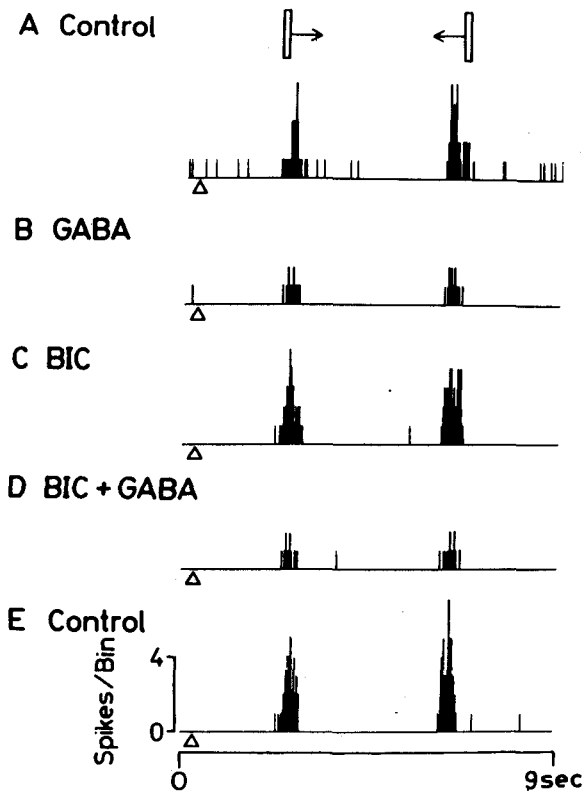

Fig. 2. A weak effect of GABA and inefficiency of BIC application on responses of a non-oriented cell at 13 days of age. Each PSTH shows responses to motion of a slit oriented as indicated at the top. This unit had the receptive field similar tc those of afferent fibers, but its action potentials were judged to be generated post-synaptically because of inconsistent responses to electrical stimulation of the lateral geniculate nucleus in addition to the standard criteria for soma potentials[2,15]. Number of sweeps, 10 and bin width, 9 ms. Stimulus speed was 4.4 deg/s. Ejecting currents for GABA and BIC were +10 and +40 nA, respectively.

cation of GABA, responses were significantly suppressed but not completely abolished (Fig. 2B). BIC was applied after the responsiveness had recovered to the original control level, but responses were not significantly changed (Fig. 2C). GABA was then ejected while continuing application of BIC. Again, responses reduced to the same magnitude as during the single application of GABA (compare Fig. 2D with B), indicating that BIC did not block the weak action of GABA. One might argue that the BIC electrode did not effectively work or BIC ejected was not pharmacologically effective. We believe that this is unlikely, because other cells sampled before and after this cell along the same electrode penetration had clear BIC effects with the same currents as used for this cell.

In the rat visual cortex, activity of the GABA-synthetizing enzyme, glutamic acid decarboxylase (GAD), develops relatively late and remains very low at the time of eye opening[9,10]. This might suggest that the intracortical GABAergic inhibition has not fully developed yet in the cat visual cortex when eyes open. Therefore, selective properties of innately-specified cells might not be due to such an inhibitory system. The present results have demonstrated, however, that the GABAergic inhibition already operates in the visual cortex at the time of eye opening as suggested in the sensorimotor cortex[8], but its action is localized on the restricted number of neurons, mostly innately-specified cells, to make them orientation-selective. This does not necessarily mean, of course, that the GABAergic inhibition is the sole factor for the orientation selectivity of such cells, because in a strict sense the elimination of selectivity by BIC was not complete in most of the orientation-selective cells, and about one-third of LGN cells were reported to have somewhat orientation-biased responses in very young kittens[1]. The late development of GAD activity observed with neurochemistry may reflect the fact that the proportion of the orientation-selective cells in the visual cortex as a whole increases markedly around 4 weeks of age[2,3,15] and that the tonic inhibition also develops late.

The result that most of the orientation-selective cells were located in layer V seems consistent with the recent findings that layer V cells are one of the earliest-maturing groups of cells[15], and inhibitory post-synaptic potentials of cortical neurons evoked by afferent electrical stimulation develop in layer V initially and then in other layers[7]. Blakemore and his colleagues reported, however, that innately-specified cells were located around layer IV[2,13]. Although their terms 'around layer IV' might include layer V, further works to sample much more cells seem necessary to reconcile these differences. Responses of a substantial number of non-oriented cells were completely or weakly inhibited by GABA application. In most of these cells the GABA action was not effectively blocked by BIC. This suggests that such cortical cells or afferent terminals innervating them have transient $GABA_B$ receptors which are insensitive to BIC[4]. This type of receptors might not play a role for forming orientation-selective receptive fields and disappear or become insensitive during postnatal de-

velopment of the cortex.

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Effects of Noradrenaline Applied Iontophoretically on Rat Superior Collicular Neurons

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SATO, H. AND Y. KAYAMA. *Effects of noradrenaline applied iontophoretically on rat superior collicular neurons.* BRAIN RES BULL **10**(4) 453–457, 1983.—In rats anesthetized with urethane effects of iontophoretically applied noradrenaline (ejection currents, less than 20 nA) were examined on spontaneous and/or photically evoked discharges of neurons in the superior colliculus (SC). In a given SC neuron NA acted either to facilitate or to inhibit both spontaneous and evoked discharges. Though the inhibition was more frequently observed than the facilitation in all layers of the SC, the ratio of the facilitated neurons to the inhibited ones increased as one advanced through the SC from superifical to deep. These findings are in line with those obtained when SC neurons were subject to repetitive electrical stimulation of the locus coeruleus.

Superior colliculus Spontaneous discharges Visual responses Noradrenaline Locus coeruleus

THE action of noradrenaline (NA) upon neuronal discharges is inhibitory in some cases [5, 14, 15] or excitatory in the others [12,13]. This is true whether the phenomenon is tested by electrical stimulation of the locus coeruleus (LC) or by iontophoretic application of the drug. Such dual effects of NA are observed even in the same nucleus; in the rat lateral geniculate nucleus relay neurons are excited while interneurons are inhibited [7].

A previous experiment [8] tested the effects of repetitive electrical stimulation of LC on neuronal discharges of the rat superior colliculus (SC); NA was found to be either excitatory or inhibitory in this neuronal site too, depending possibly upon the nature of the postsynaptic receptor of the neurons under examination. The purpose of the present experiment is to confirm the previous results by applying NA directly to the rat SC neurons by the method of iontophoresis.

METHOD

Fifty-three Sprague-Dawley male rats of 8–12 weeks old, weighing 210–330 g, were used. Under urethane anesthesia (1.2 g/kg, IP) the trachea was cannulated and the head was fixed in a stereotaxic instrument by the method of König and Klippel [9]. A constant level of light anesthesia was maintained by injecting about one fifth of the initial dose of urethane every 3 to 4 hours before the animal began to show such signs of waking as blinking and acceleration of breathing. Some of the urethane-anesthetized animals were further immobilized with a single dose of 20 mg/kg of gallamine triethiodide and maintained by artificial respiration. One percent lidocaine was injected to the pressure points and around the surgical wounds. The rectal temperature of the animals were kept between 36 and 37.5°C.

A bipolar electrode, made of insulated stainless steel wires, was introduced to the optic chiasm (OX). It was placed at a depth where responses to flash stimulation of the eyes, which were audible as swishes from a speaker connected to the amplifier, were judged to be maximal. For stimulation of OX, square pulses of 0.05 msec duration were applied every 1–2 sec with intensities lower than 60 V. The OX stimulation was utilized to identify from which layer of SC unit activities were recorded. As noted by previous workers, the potential configuration of the field response of SC to OX stimulation changed in a characterstic manner as the recording electrode advanced deep [3]. This helped us to classify units according to the depths from which they were recorded. Each unit was judged to belong one of the following three classes: (1) Upper visual layer neurons which are in stratum zonale and the upper half of stratum griseum superficiale (zone of horizontal cells [10]), (2) lower visual layer neurons which are in the lower half of stratum griseum superficiale and stratum opticum (zone of vertical cells [10]), and (3) non-visual layer neurons which are in stratum griseum intermediale, stratum album intermediale and stratum griseum profundum.

A small portion of the skull in the occipital area was trephined and the dura was opened under microscopic control. After a microelectrode for unit recordings was inserted through this trephined hole toward SC, the cortical surface was covered with agar.

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Double-barreled electrodes were used to examine the effects of iontophoretic application of NA upon SC neurons. These electrodes consisted of one pipette for drug application and the other for unit recordings. The tip of the recording pipette was protruded by 5–10 μm from the drug electrode, which was filled with 0.1 M noradrenaline HCl (Sigma) with pH between 3.5 and 4.0. The drug electrodes filled with such NA solutions had the resistance between 20 and 200 MΩ. A retaining current of 10 nA was passed through the NA electrode. Ejection of NA was made with currents of 10–20 nA supplied from iontophoresis programmers (Model 160, WP-Instruments).

In 4 rats a bipolar stimulating electrode was placed in LC ipsilateral to the recording side of SC. It was appropriately positioned by observing the field response to electrical stimulation of the dorsal bundle of ascending axons of LC neurons [11]. LC was stimulated with trains of 0.05 msec square pulses of 8–15 V repeated at 20 Hz for several to 40 sec. In these experiments effects of iontophoretically applied NA were compared with those of LC stimulation on the same SC neurons.

Visual stimuli were projected onto a tangent screen (80 cm high and 110 cm wide) placed 30 cm in front of the eye contralateral to the recording site. Receptive fields of SC neurons were plotted on the screen and stimulated by a flashing spot with the same size as the receptive field. The pupil was dilated with a mixture of 0.5% tropicamide and 0.5% phenylephrine (Mydrin-P, Santen Pharmaceutical) and the cornea was protected from drying with a plastic contact lens with no refractive power.

Unit activities were monitored with a speaker, displayed on the screen of an oscilloscope, photographed on running film and stored in magnetic tape. Frequently, rates of spike discharge were recorded on paper with a conventional rate meter.

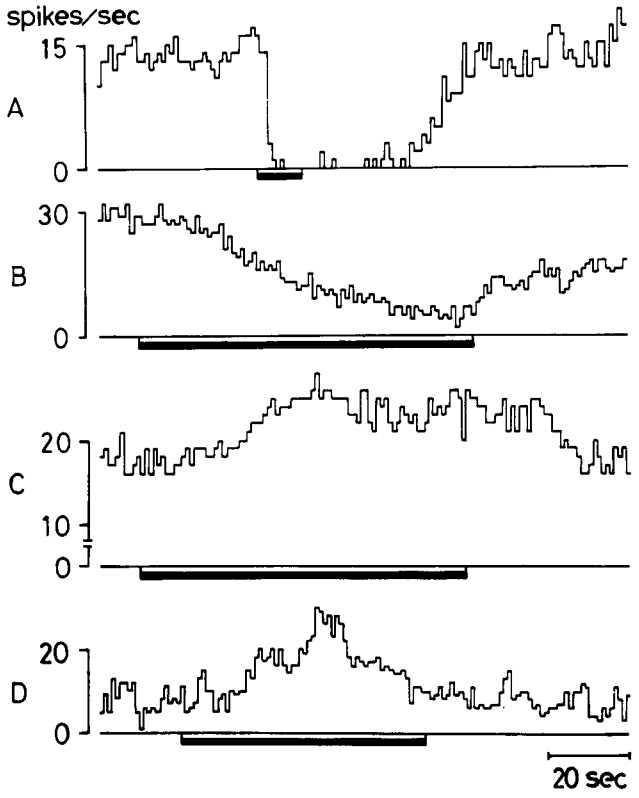

FIG. 1. Specimen records for NA-induced inhibition (A and B) and facilitation (C and D) of spontaneous discharges in 4 different neurons of the lower visual layer. In these and all subsequent records periods during which currents were flowed for application of NA are indicated by thick horizontal lines below each record. In all cases noradrenaline was applied with 20 nA.

RESULTS

The effects of NA application were investigated in 160 visual layer neurons and in 38 non-visual layer neurons. In 16 SC neurons the effects of iontophoretic NA were studied in comparison with those of electrical stimulatin of LC.

Visual Layer Neurons

Spontaneous discharges. The effects of NA application on spontaneous discharges were examined in 103 visual layer neurons by flowing ejection currents of 20 nA for several seconds to several tens of seconds. Except for a few units, NA acted to enhance or suppress the spontaneous discharge. Sample records are shown in Fig. 1; A and B for the suppressing effect and C and D for the facilitatory effect. In A, the discharge rate was very promptly reduced and reached zero, whereas in B, there was a gradual decrease of the discharge rate and no complete suppression could be attained even though the ejection current was continued for as long as 1.5 min. As exemplified by these records, the onset time for the minimal effect of NA application varied from unit to unit; it generally ranged from 3 to 30 sec. In both A and B, the inhibitory effect of NA lasted long after the ejection current was ceased. Usually the recovery of the discharge rate started 3 to 30 sec after the cessation of the ejection, but complete recovery to the control level needed 15 to 90 sec.

The facilitation of spontaneous discharges following NA iontophoresis generally proceeded with a pattern that was the reverse of the suppression, as typically demonstrated in Figs. 1C and 4B. However, two points are noted as characterizing the NA-induced facilitation; (1) no unit showed such a prompt change of discharge rate as seen in the unit of Fig. 1A for the suppression; the time to the maximally increased discharge rate was usually longer than 30 sec. (2) In some units the facilitation was only transient, as seen in Fig. 1D; after the discharge rate was increased to a certain level, it gradually returned to the control level despite a continuous flow of the ejection current.

Photically evoked responses. As is the case with the spontaneous discharge, the iontophoretic NA acted either to facilitate or to suppress the photically evoked response. Specimen records are shown in Fig. 2. In neuron A, the on-response was weaker than the off-response in the control stage, and both responses were inhibited by NA with no appreciable changes in their relative magnitudes. In neuron B, before application of NA there was a high spontaneous activity, making the photically evoked responses unclear. However, the responses became distinct after application of NA, because the responses were inhibited by NA more weakly and gradually than the spontaneous discharges. The phenomenon that the spontaneous discharges were suppressed more strongly than the responses was observed in many SC neurons. Neuron C is presented as an example for the facilitation. In the control stage this unit

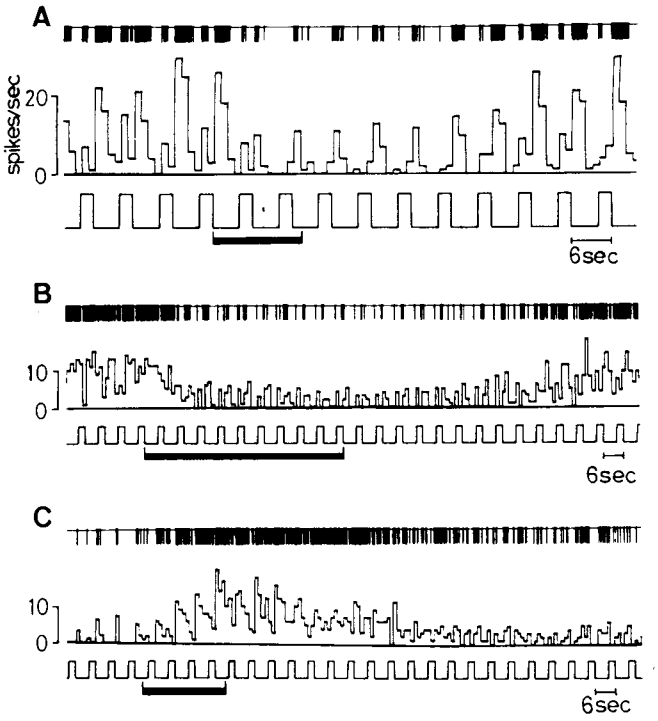

FIG. 2. Effects of iontophoretic NA on visual responses of 3 different neurons. Neurons A and B were in the upper visual layer, and neuron C in the lower visual layer. In each frame are mounted from above downward a train of constant amplitude pulses indicating spike discharges, discharge rates and marks for photic stimulation (upward deflections mean light on). In all cases NA was applied with 20 nA.

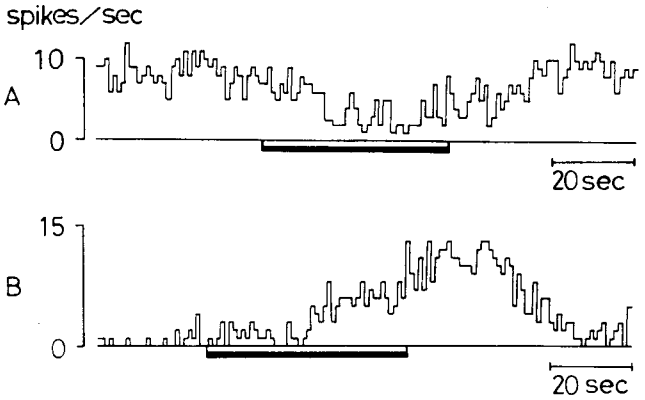

FIG. 3. Responses to iontophoretic NA in 2 different neurons of the deep, non-visual layer (A and B). NA was applied with 20 nA.

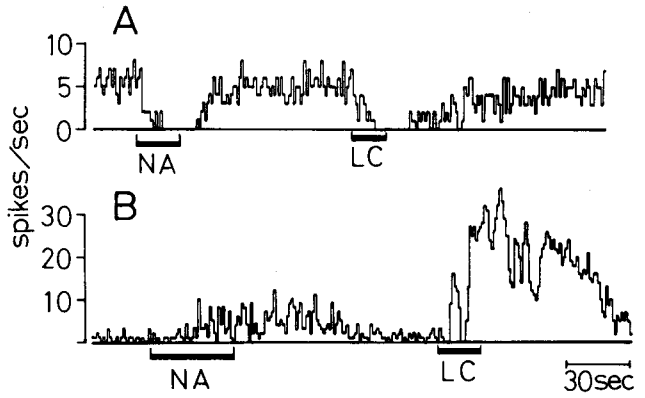

FIG. 4. Effects of iontophoretic NA and of locus coeruleus stimulation (LC) in 2 different neurons. A, neruon in the deep, non-visual layer. B, neuron in the lower visual layer. Periods during which currents for NA iontophoresis were flowed and reptitive stimuli were applied to the locus coeruleus are shown by thick horizontal lines under each record with labels NA and LC, respectively. In both cases NA was applied with ejection currents of 20 nA, and the locus coeruleus was stimulated with trains of 0.05 msec square pulses of 12 V repeated at 20 Hz.

showed distinct off-responses with no discharges in the background. In response to NA application, there was an increase in the response, being associated with enhanced background discharges.

Non-Visual Layer Neurons

A total of 38 non-visual layer neurons were recorded for testing effects of NA upon their spontaneous discharges. In these neurons too, NA acted either to inhibit or to facilitate spontaneous discharges. As exemplified by records A and B of Fig. 3, when the NA was ejected by a current of 20 nA, an increase or decrease of the spontaneous discharge occurred in essentially the same way as seen in the visual layer neurons.

Effects of Electrical Stimulation of LC

Effects of NA iontophoresis upon spontaneous discharges were compared with those of electrical stimulation of LC in 16 neurons (11 in the visual layer, 5 in the non-visual layer), and the two procedures were found to yield the same effect in all neurons tested, whether it was excitatory or inhibitory. It was often observed, however, that in some units the time to the maximal effect was significantly different between the two procedures. In neuron A of Fig. 4, NA iontophoresis and LC stimulation caused suppression of spontaneous discharges in a closely similar time course, whereas in neuron B the facilitation following NA iontophoresis occurred more slowly than that following LC stimulation.

Frequencies of Occurrence for NA-Induced Facilitation and Inhibition

Table 1 shows the number of units exhibiting facilitation and inhibition in response to NA iontophoresis. They are classified according to the layers the units were recorded from and as to whether the testing activity was the spontaneous discharge or the evoked one. Two points emerge from this Table; (1) as one passes from superficial to deep through SC, the facilitation of spontaneous discharges is encountered more frequently with a concomitant decrease of the inhibition, and (2) the same is true with the facilitation and inhibition of evoked discharges. A similar finding has been obtained in our previous experiment where effects of LC stimulation were tested upon SC neurons [8].

TABLE 1

NUMBERS OF NEURONS INHIBITED, FACILITATED AND NOT AFFECTED BY IONTOPHORETICALLY APPLIED NORADRENALINE

Layer	Inhibited	Facilitated	No effect	Total
Spontaneous discharges				
Upper visual	13 (87%)	1 (7%)	1 (7%)	15
Lower visual	56 (64%)	24 (27%)	8 (9%)	88
Non-visual	20 (53%)	13 (33%)	5 (13%)	38
Visual responses				
Upper visual	12 (86%)	0	2 (14%)	14
Lower visual	33 (60%)	9 (16%)	13 (24%)	55

DISCUSSION

The essential points of the present results are summarized as follows. (1) NA, applied directly to neurons by the iontophoretic technique, inhibited spontaneous and photically evoked discharges of many neurons of SC, while it facilitated those of some neurons. (2) As one passes through SC from superficial to deep, the frequency of occurrence of the facilitatory response to NA increases with a concomitant decrease in the frequency of the inhibitory response. (3) In each SC neuron the effect of repetitive electrical stimulation of LC was consistent with that of NA applied iontophoretically.

Straschill and Perwein [17] have also studied the effects of iontophoretically applied NA upon neuronal discharges in the SC. They found that NA produced inhibition of spontaneous, glutamate-induced and stimulus-induced discharges in 37 out of 65 units (53%), with no or questionable effects in the remaining units. Evidently their data are at variance with ours; we have observed not only the inhibitory effect but also the excitatory effect in a certain fraction of the samples, particularly frequently in the samples from deep layers of SC.

The finding that iontophoretically applied NA exerts an excitatory action upon some central nervous system neurons is not entirely new. Rogawski and Aghajanian [12] have examined the excitatory effect of NA on neurons of the rat dorsal lateral geniculate nucleus. They pointed out that if the ejection current of NA solution was too intense, the predominant effect was inhibition of neuronal discharges. Since this was confirmed in our preliminary experiment, the ejection current of NA solution was limited so as not to exceed 20 nA. Adherence to this limit probably helped us to establish the excitatory effect of NA in some of SC neurons.

Although Frederickson *et al.* [2] reported that iontophoretic application of NA in a highly acidic solution (pH lower than 3.5) favored the elicitation of the excitatory effect, we could not confirm their observation. In preliminary experiments discharges of SC neurons were subjected to effects of NA with solutions of different pHs. As long as the pH was between 3.0 and 4.5, no systematic variation in the relative frequency of the excitatory effect could be observed with varying pH, nor was it observed that with a given SC neuron the excitatory effect of NA tended to be associated with a high acidity of the solution. Therefore, acidity of NA solution used in the present experiment (pH 3.5–4.0) would not be a cause that neurons excited by NA were recorded.

We have not been able to demonstrate how the excitatory and inhibitory effects of NA were distributed among different kinds of SC neurons, which were categorized according to the position of evoked spike on the field potential induced by OX stimulation and to the depth in SC [4]. This result was in contrast with the response of SC neurons to iontophoretically applied GABA; SC neurons of some kinds had invariably high sensitivity to the depressing effects of GABA, while others consisted of GABA-resistant neurons [6]. Yet we did find that frequencies of incidence for the excitatory and inhibitory effects of NA were graded along the dorsoventral axis of SC; the relative preponderance of the excitatory effect increased with increasing depth, whereas the opposite was true for the inhibitory effect. The relevance of this laminar organization to visual information processing of SC remains unclear. However, it may be related to an Sprague's hypothesis that visual impulses are relayed from the superficial to the deep layer of SC [16].

In a total of 16 units electrical stimulation of LC was found to affect spontaneous or photically evoked discharges in the same way as did iontophoretic application of NA. In our previous experiment the excitatory and the inhibitory effects of LC stimulation were found to be graded in the frequency of occurrence along the dorso-ventral axis of SC [8], and these previous data are in good agreement with the data presented in Table 1. The effects of iontophoretic NA upon SC neuronal activities are said to be precisely mimicked by electrical stimulation of LC.

In many spontaneously active SC neurons which were suppressed by NA, the spontaneous discharges were affected more strongly and rapidly than the photic responses. Exact ratios of the evoked discharges to the spontaneous ones were not calculated in the present study, because long application of NA sufficient for analysis with averaged histograms was avoided to observe appearance of effects of NA and recovery from it successively. However, this is clearly the same phenomenon as "improvement of signal to noise ratio" proposed by Woodward and colleagues [18,19]. These workers thought this phenomenon was characteristic of the action of NA. However, Foote *et al.* [1] reported not only NA but also iontophoretically applied GABA depressed the discharges in pre-stimulus control periods more strongly than the vocalization-evoked discharges in the auditory cortical neurons. Similarly, we observed stronger depression of the spontaneous discharges than the somatosensory responses of ventrobasal thalamic neurons, when GABAergic inhibitory projection to the ventrobasal neurons were activated by infusion of glutamate into the somatosensory part of the thalamic reticular nucleus (Mushiake, Shosaku and

Kayama, in preparation). Furthermore, when excitatory inputs from the visual cortex was eliminated by cortical cooling, the photic responses of many lateral geniculate relay neurons became more conspicuous because of stronger depression of the spontaneous discharges than that of the responses (Kayama, Shosaku and Doty, in preparation). These results suggest that the improvement of signal to noise ratio is possibly not a specific phenomenon to NA action, but a rather general one when neurons are inhibited or disfacilitated.

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This paper is dedicated to our teacher, Dr. Kitsuya Iwama, professor emeritus, who has just retired from Osaka University medical School. We are very grateful for his kind advice and comments.

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