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Activity-Dependent Potentiation and Depression of Visual Cortical Responses to Optic Nerve Stimulation in Kittens

HIROSHI TAMURA, TADAHARU TSUMOTO, AND YOSHIO HATA
Department of Neurophysiology, Biomedical Research Center, Osaka University Medical School, Yamadaoka, Suita 565 Japan

SUMMARY AND CONCLUSIONS

1. To see whether long-lasting changes in synaptic efficacy are induced in the developing visual cortex (VC), field potentials evoked by test stimulation given alternatively to each of the optic nerves (ONs) were recorded from VC of kittens ranging in age from 4 to 8 wk. In some experiments, field potentials were recorded simultaneously from the dorsal lateral geniculate nucleus (LGN) in addition to VC.

2. Tetanic stimulation was applied to one of the ONs for 1–60 min at 5 Hz. Homosynaptic potentiation of cortical responses, defined as an increase lasting >2.5 h in the cortical field potential evoked by test stimulation of the ON that was tetanized, was induced without any changes in LGN responses in 3 of the 12 kittens tested. Heterosynaptic depression, defined as a decrease lasting >0.5 h in the field potential evoked by stimulation of the ON that was not tetanized, was also induced in two of those three kittens.

3. To elucidate a role of inputs originating from spontaneous activity of retinal ganglion cells in induction of potentiation and depression in the cortex, tetrodotoxin (TTX) was injected into both eyes of 11 kittens. After we confirmed the suppression of retinal activity by TTX, tetanic stimulation was applied to ON. Homosynaptic potentiation of cortical responses was induced in 6 of the 11 kittens, and the ratio of the mean amplitude of posttetanic responses to that of pretetanic responses for the 11 kittens was on average larger than that for the 12 control kittens. Heterosynaptic depression was not observed in any of the 11 kittens.

4. To see a role of postsynaptic activity in induction of potentiation and depression, γ-aminobutyric acid (GABA) was applied continuously to the VC by an infusion pump in 10 kittens. Tetanic stimulation was given to ON while cortical activities were suppressed by GABA. After recovery of cortical activities, homosynaptic depression was found to be induced in 3 of the 10 kittens, but homosynaptic potentiation was not observed at all. The ratio of amplitude of posttetanic to pretetanic responses at the tetanized side for the 10 kittens was on average smaller than that for the 11 TTX-injected kittens.

5. These results can be accounted for by the modified covariance model in which the relation of postsynaptic activity and direction of changes in synaptic efficacy is formulated. It is suggested that temporal correlation between presynaptic background activity and postsynaptic activity plays a role in inducing use-dependent changes in synaptic efficacy and determining the direction and magnitude of the changes in the developing visual cortex.

INTRODUCTION

Since Hebb's original proposal that the co-occurrence of activity of pre- and postsynaptic neurons increases synaptic strength (Hebb 1949), a number of experimental and theoretical studies have been carried out to find the existence of such a modifiable synapse in the brain (see Brown et al. 1990 for review). Theoretical studies suggested that a decrease in synaptic strength also should take place after temporally uncorrelated activity of pre- and postsynaptic neurons (Bear et al. 1987; Bienenstock et al. 1982; Sejnowski 1977a,b; Stent 1973). These two opposing directions of changes, increase or decrease, in synaptic efficacy were observed experimentally in the hippocampal formation as long-term potentiation (LTP) and long-term depression (LTD) of synaptic transmission (Bliss and Lømo 1973; Levy and Steward 1979; Lynch et al. 1977; see Bliss and Lynch 1988 and Brown et al. 1990 for reviews). LTP and LTD can be classified further into the three types, i.e., heterosynaptic, homosynaptic, and associative modification, respectively (Abraham and Goddard 1983; Dunwiddie and Lynch 1978; Gustafsson and Wigstrom 1986; Levy and Steward 1979; Stanton and Sejnowski 1989; see Tsumoto 1992 for review), although the existence of associative LTD in the hippocampus is still a matter of some controversy (Goldman et al. 1990).

In the visual cortex of kittens, Tsumoto and Suda (1979) reported that cortical field potentials evoked by electrical stimulation applied to one of the optic nerves (ONs) were potentiated after repetitive stimulation (2 Hz for 15 min–1 h) of the same ON (homosynaptic potentiation), whereas those evoked by test stimulation of the nontetanized ON were depressed (heterosynaptic depression). In this early study, however, no attempt was made to look for a possible dependency of the effects on presynaptic background activity and postsynaptic responsiveness. In the present study, therefore, we attempted to elucidate such conditions for induction of potentiation and depression in the kitten visual cortex. To reveal conditions necessary for heterosynaptic modification, slice preparations of the cortex, which are currently very popular in studies on synaptic plasticity, seemed not to be appropriate, because it is difficult to separately activate afferent fibers transmitting visual inputs from each eye to a cortical neuron in such preparations. So, in the present study, we rather used in vivo preparations in which afferents from the two eyes can be activated separately. To see possible long-lasting changes in synaptic efficacy, we observed field potentials rather than intracellulary recorded synaptic potentials, because the latter potentials are difficult to observe for a long time in in vivo preparations.

In in vivo and physiological conditions, retinal ganglion cells and relay cells of the dorsal lateral geniculate nucleus (LGN) have high spontaneous activity (see Levick 1973; Meister et al. 1991), and this spontaneous activity is sug-

gested to play an important role in afferent segregation in the primary visual cortex (Stryker and Harris 1986; see Shatz 1990). From the viewpoint of the Hebbian rule, spontaneous inputs from the nontetanized ON to postsynaptic neurons may play some role in induction of potentiation or depression in visual cortex because these inputs activate postsynaptic neurons sporadically and such sporadic activation is not correlated with tetanic inputs from the other ON. In the first type of experiment, therefore, we tested this possibility by blocking retinal spontaneous activity with an intraocular injection of tetrodotoxin (TTX), a sodium channel blocker (Stryker and Harris 1986). From the Hebbian rule of synaptic modification, the responsiveness of postsynaptic neurons to tetanized inputs also is expected to play a role in induction of potentiation and depression. In the next set of experiments, therefore, we tested this possibility by inhibiting cortical activity with the application of γ-aminobutyric acid (GABA), an inhibitory transmitter in the visual cortex (Iversen et al. 1971; Sillito 1975; Tsumoto et al. 1979).

In the present study, we found that presynaptic spontaneous activity and postsynaptic responsiveness of cortical neurons regulate the direction, induction probability, and magnitude of long-lasting changes in synaptic efficacy in the developing visual cortex. Furthermore, depression at the nontetanized synapse could be taken as associative rather than heterosynaptic modification. A preliminary account of some of these experiments has been presented in abstract form (Tamura et al. 1990).

METHODS

Animal preparation

Thirty-five kittens obtained from our house colony, ranging in age from 4 to 8 wk, were used in the present experiments. Each kitten was studied in a single nonsurvival experiment. Initial anesthesia was carried out with 3–4% halothane in a gas mixture of 70% N_2O-30% O_2. During the surgical operation, all the wounds were infiltrated with local anesthetic (xylocaine) and halothane was maintained at 1–2%. Subsequently the animal was positioned in a stereotaxic head holder and immobilized by a continuous intravenous infusion of gallamine triethiodide (7–11 mg · kg^{-1} · h^{-1}) and maintained under artificial respiration. During recordings, the animal was anesthetized with 0.1–0.5% halothane in a gas mixture of 70% N_2O-30% O_2. The end-tidal CO_2 concentration was kept at 3.5–4.0%, and the rectal temperature was at 37–38°C. The electrocardiogram (ECG) and heart rate were monitored continuously throughout the experiments. Except for the control experiments, 8 μg of TTX dissolved in 8 μl of citrate buffer (pH 7.4) was injected intravitreally into both eyes by a 10-μl Hamilton syringe to block spontaneous activity of retinal ganglion cells (Stryker and Harris 1986). The effectiveness of the TTX administration was indicated by a complete elimination of mass fiber responses of the ON to direct photic stimulation of the eyes.

Stimulation of the ONs and recording of field potentials

For electrical stimulation, both ONs were exposed at the outset of the surgical preparation of the animal by a retrobulbar approach (Tsumoto 1978). A J-shaped electrode was inserted under the ON to hook it. Another silver wire was brought into touch with the ON so as to make up a bipolar electrode. Thus the ON was softly caught between the electrodes to be stimulated in constant conditions during the experiments. The silver wires were insulated except for the part touching the ON. Resistance of this electrode was usually 0.1 MΩ and was confirmed to be stable for ≥2–3 days, during which the experiments were carried out. By aural monitoring of the mass fiber responses to a flash of light, the tips of the stimulating electrode were judged to be in contact with the ON. For recordings of the field potentials from the primary visual cortex (VC), a bipolar electrode, consisting of two insulated wires with bared tips separated vertically by ~0.5 mm, was inserted into the VC. In some of the experiments, responses from the LGN were also recorded through a stereotaxically inserted bipolar electrode. An arrangement of stimulating and recording electrodes is shown in Fig. 1.

The intensity of ON stimulation was set at 2 times the threshold of induction for maximal field potentials of VC, i.e., 0.9–1.0 mA for 0.1- to 0.5-ms pulses. Test stimulation was applied at 0.2 Hz alternatively to the ONs so that each ON was stimulated at every 10 s. Field potentials evoked by ON stimulation were recorded through conventional amplifiers and processed by a computer (7T17 NEC-Sanei, Japan). Usually 10 responses to stimulation of each ON were averaged, and the amplitude of each component was measured with the computer. Initially, pretetanic control responses to test stimulation of each ON were recorded over 1 h to check consistency of the responses. After the responses were confirmed to be stable, tetanic stimulation was applied to one of the ONs at 5 Hz for 1 min with the same intensity but double the duration. Usually, the tetanus was given to the ON contralateral to the hemisphere from which recordings were made. After tetany, responses to ON stimulation with the same parameters as the pretetanic stimulation were recorded again for ≥30 min. If the tetanus failed to induce any significant changes, another tetanus was applied to the same ON for 5 min. If it was also ineffective, tetanus was given again for 15 min. If the same failure took place, tetanus was applied finally for 60 min. Responses to test ON stimulation were recorded 1, 10, and 30 min after the final tetanus, and then every 30 min for ≥2.5 h. In some of the experiments, the responses were recorded for ≥5 h. In such cases, the responses were further recorded every 1–2 h until the end of experiments (longest duration 20 h).

In the second type of experiment, 100 mM GABA dissolved in sterile saline was infused into the VC to suppress cortical activity. For this purpose, a cannula (27 gauge) was glued to the side of the bipolar recording electrode and used for the GABA infusion to the recording site in the VC. GABA was infused at a flow rate of 2.5 or 5.0 μl/min by an infusion pump (Harvard Apparatus, USA). The

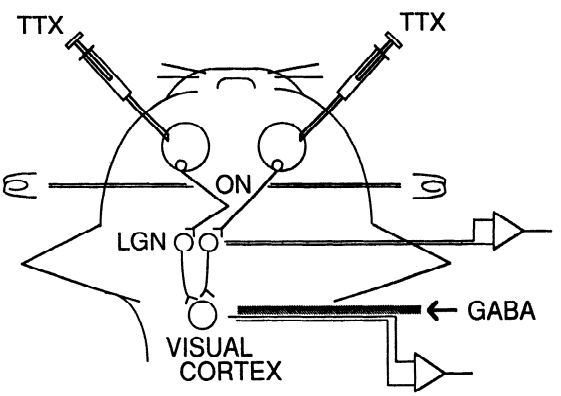

FIG. 1. Schematic depiction showing experimental procedures. Stimulating electrodes were placed on both optic nerves (ONs), and recording electrodes were inserted into the dorsal lateral geniculate nucleus (LGN) and primary visual cortex (VC). Tetrodotoxin (TTX) was injected into both eyes by Hamilton syringes. γ-aminobutyric acid (GABA) was applied to the VC through a cannula glued to the recording electrode by an infusion pump.

duration of the infusion was usually 15–45 min. In this type of experiment, tetanic stimulation was applied after the infusion of GABA was confirmed to suppress cortical activity (see RESULTS).

Histological procedures

After each experiment, the animal was anesthetized deeply by an overdose of pentobarbital sodium and was perfused transcardially with Ringer solution followed by 3.3% formaldehyde solution in 0.9% saline. The brain was removed, cut into blocks, and sectioned at 60 μm. The sections containing electrode tracks were mounted on glass slides and stained with cresyl violet. The tips of the cortical and geniculate electrodes were confirmed to be located within area 17 of the cortex and in the LGN, respectively. The advanced tips of the cortical bipolar electrode were mostly located in layers 4–5 of the cortex, and the trailing tips were in 2/3. The two tips of the geniculate electrodes were located mostly in laminae A and A1, respectively.

RESULTS

In response to test stimulation of the ON, a biphasic field potential was recorded from the VC (Fig. 2, *A* and *C*). In some cases, a small negative deflection preceeded the biphasic potential. The initial small potential was probably presynaptic in origin, and the later biphasic potential was thought to be postsynaptic (Malis and Kruger 1956). In the present study, the peak-to-peak amplitude of the prominent biphasic potential (C_4–C_5 component, according to Malis and Kruger 1956) was measured as an indicator of cortical responsiveness. Usually, cortical field potentials evoked by stimulation of ON are, to a substantial degree, variable in amplitude. Initially, therefore, averaged cortical potentials consisting of 10 responses to stimulation of each ON were recorded at least three times at intervals of 0.5–2 h to confirm stability of the responses. Only if these responses were judged to be stable and did not show any significant drift in amplitude (33 of 35 cases) was tetanic stimulation applied to one of the ONs. Changes in amplitude of the field potentials were judged as significant when these changes were different ($P < 0.05$, Student's two-tailed t test) from those of the last control responses before tetanus. Only if the significant increase in amplitude lasted during the observation period (≥2.5 h), was it defined as potentiation.

There was an asymmetry of maintenance of potentiation and depression. For example, heterosynaptic depression seen in two of the three control kittens in which potentiation was induced did not show the same time courses of changes as the potentiation did; the depression was usually shorter-lived. In the present study, therefore, the change is called depression only if the significant decrease in amplitude of the field potentials lasted for ≥30 min.

Induction of potentiation and depression in control kittens

Homosynaptic potentiation of cortical field potentials was induced in 3 of the 12 control kittens. Figure 2 shows an example of potentiation in the tetanized pathway (homosynaptic potentiation) and depression in the nontetanized pathway (heterosynaptic depression) observed in a 5-wk-old kitten. Test stimulation was applied to each ON, and recordings were done from the left VC and LGN. Earlier tetanic stimulation applied to the right ON for 1, 5, and 15 min had been ineffective. After the 15-min tetanus, responses were stable for ≥30 min. Then tetanic stimulation was applied to the same ON for 60 min. In this case, VC responses to test stimulation of the tetanized ON increased to 161% of pretetanic responses 30 min after the tetanus was stopped (Fig. 2*A*, *left* and *right*). The difference is statistically significant ($P < 0.01$, two-tailed t test), and this potentiation lasted for 9 h. The VC responses to test stimulation of the nontetanized ON were depressed to 69% of pretetanic responses 30 min after the tetanus was stopped (Fig. 2*C*, *left* and *right*), and this difference is also significant ($P < 0.05$, two-tailed t test). Field potentials of the LGN were recorded simultaneously, and the amplitude of the first biphasic response (t_1–r_1 component, according to Bishop and MacLeod 1954) was measured. The later components were not taken as an index of activity of the LGN proper, because these components might be contaminated with the corticogeniculate activity. In contrast to cortical responses, both of the LGN responses to test stimulation of the tetanized and nontetanized ON did not significantly change from the control values (Fig. 2, *B* and *D*). So the observed changes in the VC responses were judged to take place within the cortex.

Figure 3 shows an example of homosynaptic potentiation and heterosynaptic depression seen in another 5-wk-old kitten. In this case, responses to test stimulation of the tetanized ON were significantly potentiated 1 min after stopping the tetanus and attained 252% of the pretetanic control value 5.5 h later (Fig. 3, *A1* and *A2*). Such a remarkable potentiation lasted throughout the observation period (14.5 h). The responses to nontetanized ON stimulation were depressed 30 min after stopping the tetanus. Although this heterosynaptic depression was not fully persistent, the responses were reduced to 43% of the pretetanic value ~6.5 h after tetanus (Fig. 3, *B1* and *B2*).

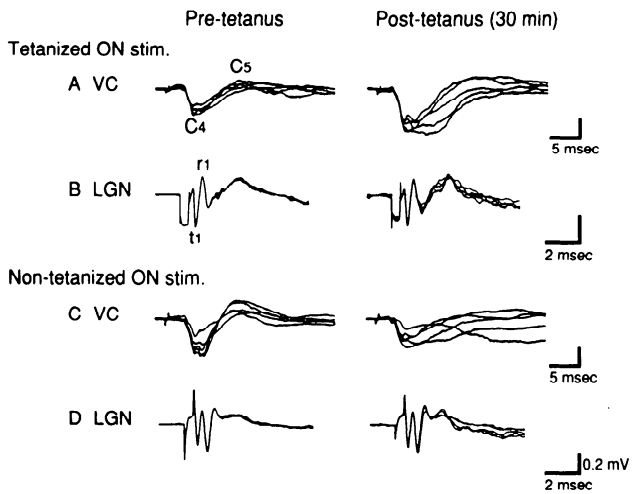

FIG. 2. An example of homosynaptic potentiation (*A*) and heterosynaptic depression (*C*) of cortical field potentials induced by tetanic stimulation (5 Hz for 60 min) applied to the optic nerve (ON) contralateral to the recording sites in a 5-wk-old kitten. Field potentials simultaneously recorded from the lateral geniculate nucleus (LGN) ipsilateral to the visual cortex (VC) are shown in *B* and *D*. *A* and *B*: responses were evoked by test stimulation of the tetanized ON. *C* and *D*: responses were evoked by test stimulation of the nontetanized ON. "Pretetanus" responses were recorded just before the application of tetanus. "Posttetanus" responses were recorded 30 min after stopping the tetanus. Five sweeps are superimposed. Vertical scales at the *bottom right* apply to all the records.

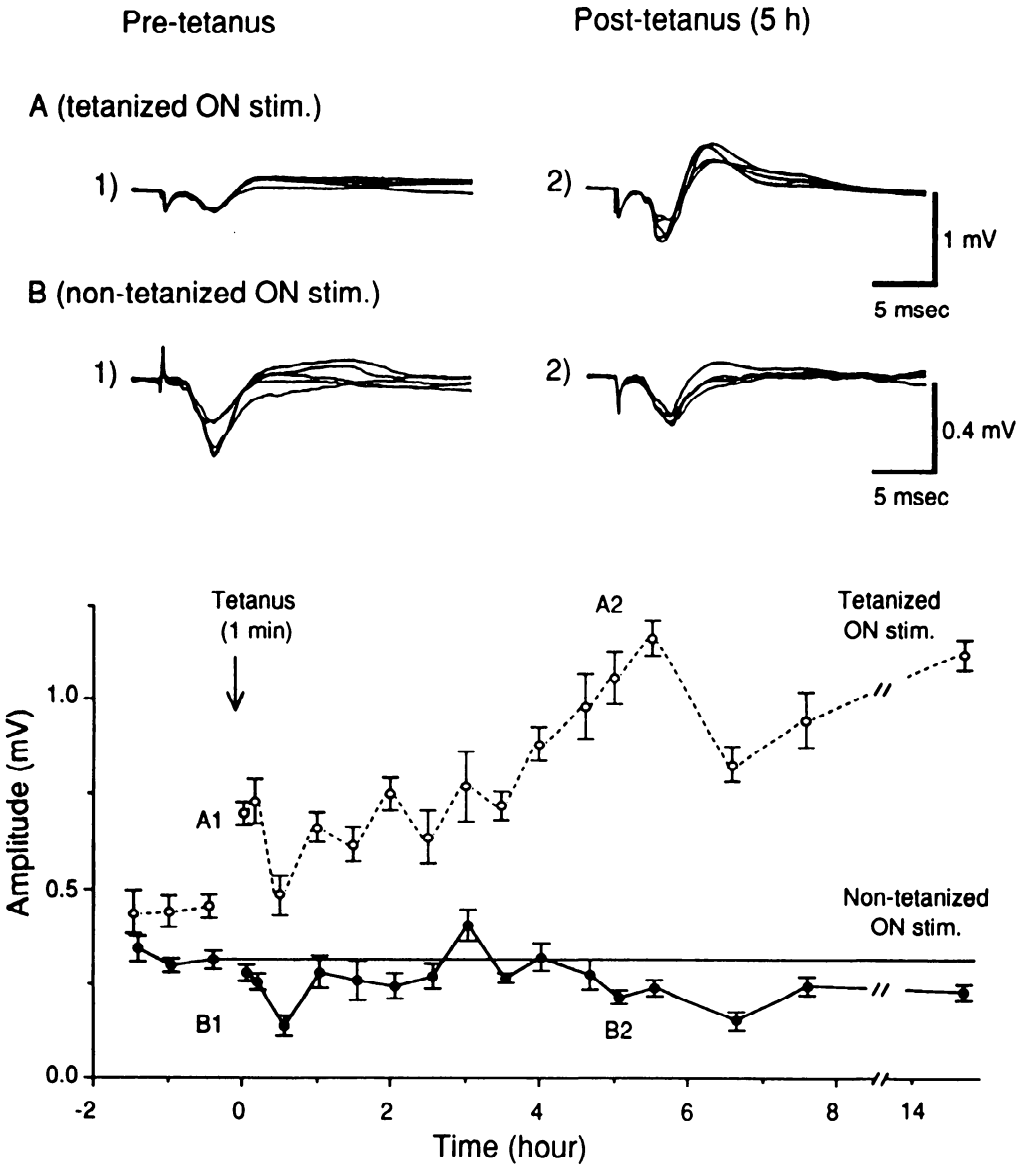

FIG. 3. Homosynaptic potentiation and heterosynaptic depression induced by tetanic stimulation for 5 min in a 5-wk-old kitten and their time courses. Records at *top* show cortical field potentials before (*A1* and *B1*) and 5 h after (*A2* and *B2*) tetanus. Superimposition of 5 sweeps. In the graph, each point and vertical bar represent the mean and standard error of mean (SEM) of 10 responses. Open circles represent the responses to tetanized optic nerve (ON) stimulation, and filled circles represent those to nontetanized ON stimulation. Horizontal line in the graph indicates the mean amplitude of the latter responses just before tetanic stimulation. Tetanic stimulation was applied at the time 0, indicated by arrow.

Effect of TTX injection on the induction of potentiation and depression

To observe the effects of retinal spontaneous activity on the induction of potentiation and depression in the visual cortex, TTX was injected into both eyes of 11 kittens. Usually, 1 h after the TTX injection, responses of the ONs to visual stimulation were totally abolished, and this suppression of retinal activity lasted throughout the experiment (2–3 days). In 6 of the 11 TTX-injected kittens, homosynaptic potentiation was induced, but heterosynaptic depression was not observed in any of the 11 kittens. Figure 4 shows an example of homosynaptic potentiation observed in a 5-wk-old kitten that had received the TTX injection. In this case, tetanus was applied to the right ON for 5 min. VC responses to test stimulation of the tetanized ON were significantly potentiated 1.5 h after the tetanus was stopped, and this potentiation lasted through the whole observation period (6.2 h, see the graph in Fig. 4). The amplitude of the responses attained 203% of the control response 5 h after tetanus (Fig. 4, *A1* and *A2*). On the other hand, VC response to stimulation of the nontetanized ON was not significantly changed (Fig. 4, *B1* and *B2*).

Effect of cortical suppression on the induction of potentiation and depression

In the last series of experiments, we attempted to suppress postsynaptic activity by an intracortical infusion of GABA in the 10 kittens that had also received a TTX injection into their eyes. In the present study, we did not test the effects of GABA infusion in kittens with intact eyes, be-

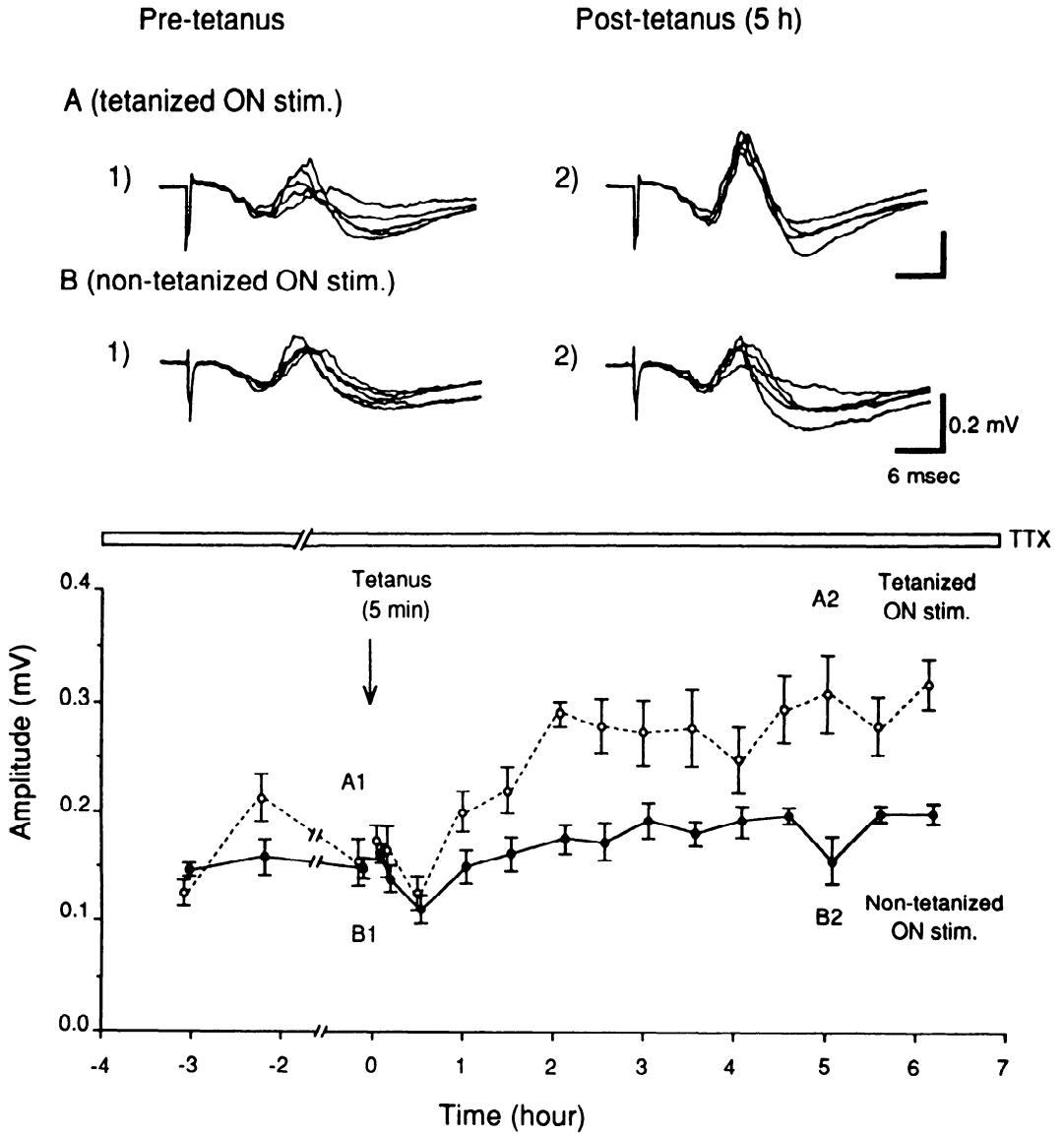

FIG. 4. Homosynaptic potentiation induced by tetanic stimulation (5 min) in a 5-wk-old kitten that received tetrodotoxin (TTX) injection and the time course of potentiation. Records at the *top* show cortical field responses to optic nerve (ON) stimulation before (*A1* and *B1*) and 5 h after (*A2* and *B2*) tetanus. Superimposition of 5 sweeps. In the graph, each point and vertical bar represent the mean and standard error of mean (SEM) of 10 responses. Open circles represent the responses to tetanized ON stimulation, and filled circles represent those to nontetanized ON stimulation. Horizontal long bar at *top* indicates the period when activities of retinal ganglion cells were suppressed by TTX.

cause the probability of induction of potentiation was rather low without the TTX injection and such preparations with the low induction probability seemed to be inappropriate for detecting possible effects of the GABA infusion on the induction of potentiation and depression.

In the initial preliminary experiments, we used a selective agonist for $GABA_A$ receptors, muscimol, but its action was very long-lasting, so that after stopping its application we could not see a recovery of cortical activity during the experiments. So, we decided to use GABA itself to suppress postsynaptic activity in a reversible manner. In the subsequent preliminary experiments, we attempted to determine the effective dose and rate of GABA infusion. The effectiveness of the GABA infusion was judged from a decrease in amplitude of cortical field responses to ON stimulation. The application of GABA usually decreased cortical responses <50% of the control responses. In two kittens, 4 and 5 wk old, respectively, however, the amplitude of the responses did not decrease <50% (data not shown). This may be due to the relatively late development of GABAergic inhibitory systems in kitten visual cortex (Komatsu 1983; Shaw et al. 1987; Tsumoto and Suda 1982). Thus, for these kittens, the applied GABA was assumed to be effective even if the amplitude was reduced by 38 and 42%. The effect of GABA lasted for 1.7 ± 0.89 (SD) h on average after stopping the infusion.

An example of the time course of changes in VC responses observed in an 8-wk-old kitten that had received TTX injection into the eyes is shown in Fig. 5. During the GABA infusion, VC responses to test stimulation of the tetanized ON were suppressed to 23% of the control responses and those to stimulation of the nontetanized ON

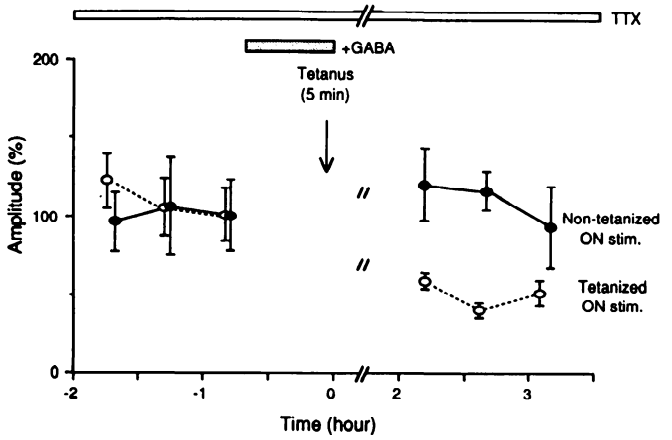

FIG. 5. Time course of homosynaptic depression induced in the visual cortex (VC), into which γ-aminobutyric acid (GABA) was infused, in an 8-wk-old kitten. Each point and vertical bar represent the mean and standard error of mean (SEM) of 10 responses. Open circles represent the responses to tetanized optic nerve (ON) stimulation, and filled circles represent those to nontetanized ON stimulation. Open bar at *top* indicates the period when retinal activities were suppressed by tetrodotoxin (TTX). Filled short bar indicates the period when GABA was infused into the cortex.

also were suppressed to 36% of the control (not plotted in Fig. 5). Two hours after stopping the GABA infusion, the responses to nontetanized ON stimulation recovered to the preinfusion level, but the responses to tetanized ON stimulation were still depressed to 60% of the pretetanic level and stayed depressed during the observation period (Fig. 5, open circles and dotted line). This type of homosynaptic depression was observed in 3 of the 10 GABA-infused kittens. In these kittens, however, potentiation and heterosynaptic depression were not observed.

Extent of potentiation and depression in the different conditions

As mentioned above, the induction of homosynaptic potentiation and depression and of heterosynaptic depression seemed to depend on the types of experiments. To show this finding more quantitatively, the mean amplitude of responses after tetanus of 1 or 5 min duration was divided by that before the tetanus, and the ratio thus obtained was plotted for each kitten (Fig. 6). Such plotting was not always available with tetanus of 15- or 60-min duration for all the kittens, because the long tetani were not applied in case significant changes had already been observed with the shorter tetani.

At the tetanized side of the control kittens, most of the points were ~1.0, although a marked deviation toward potentiation was seen in a few kittens (Fig. 6*A*). In one of them, the change was statistically ($P < 0.01$, Student's two-tailed t test) significant. Two other kittens showed significant potentiation when tetanus of 15- or 60-min duration was given again. At the nontetanized side, on the other hand, a substantial deviation toward depression was seen in most of the kittens (Fig. 6*B*), and thus the mean ratio of amplitude of posttetanic to pretetanic responses was 0.80 ± 0.26 (mean ± SD). This value was significantly ($P < 0.05$) smaller than those of the TTX-injected (1.05 ± 0.19) and the GABA-infused kittens (0.99 ± 0.09). At the tetanized side of the TTX-injected kittens, almost all the points showed a clear shift toward potentiation, and their mean ratio (1.37 ± 0.31) was significantly ($P < 0.05$) larger than that of the control kittens (1.10 ± 0.18), indicating that homosynaptic potentiation with larger magnitude is more frequently induced in the condition of TTX injection. At the tetanized side of the GABA-infused kittens, most of the points were ~1.0, but a clear shift toward depression was seen in two kittens. When the data obtained with tetani of 15- or 60-min duration were included, three kittens showed homosynaptic depression. The mean ratio at the tetanized side for the 10 GABA-infused kittens (0.92 ± 0.22) was significantly ($P < 0.01$) smaller than that for the 11 TTX-injected kittens. Regarding responses at the nontetanized side, significant changes were not seen in the TTX-injected and the GABA-infused kittens (Fig. 6 *B*).

The magnitude of changes might be related to the amplitude of pretetanic responses. For example, a smaller pretetanic value would have resulted in a larger ratio of amplitude. To test this possibility, the mean amplitude of responses before tetanus was compared among the three groups of kittens (Table 1). As seen in this table, there was no significant difference in the amplitude of pretetanic responses between each group. In addition, we explored the

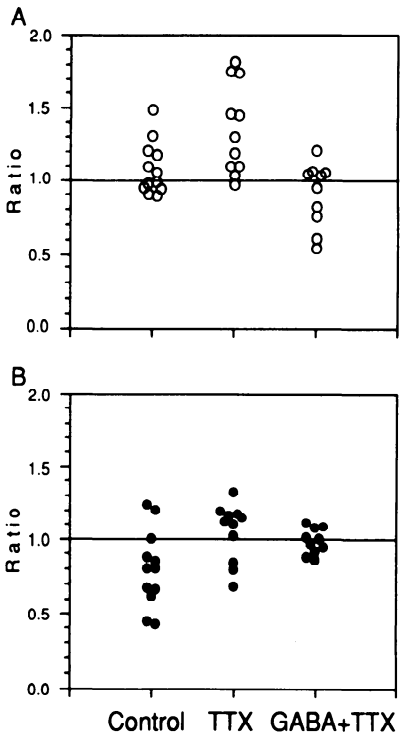

FIG. 6. Ratio of the mean amplitude of posttetanic responses to that of pretetanic responses evoked by test stimulation of the tetanized (*A*) and nontetanized (*B*) optic nerve. Control, tetrodotoxin (TTX), and γ-aminobutyric acid (GABA) + TTX along the abscissae indicate the data obtained from the control kittens, the kittens that had received TTX injection and those that had received GABA infusion in addition, respectively. The pretetanic amplitude at the tetanized and nontetanized sides was an average of respective responses recorded 10–30 min before tetanus. The posttetanic amplitude at the tetanized side was an average of responses during the period of 120–150 min after tetanus, and that at the nontetanized side was an average of responses during the period of 10–30 min after tetanus, because the modified amplitudes of the responses were most stable during these periods in almost all the cases.

TABLE 1. *Amplitude of pretetanic responses in the three types of experiments*

	Number of Kittens	Tetanized Side, mV	Nontetanized Side, mV
Control	12	0.20 ± 0.04	0.22 ± 0.04
TTX	11	0.21 ± 0.03	0.34 ± 0.07
GABA + TTX	10	0.24 ± 0.05	0.27 ± 0.09

Values are means ± SE. TTX, tetrodotoxin; GABA, γ-aminobutyric acid. Amplitude of responses for each kitten was obtained from the initial ten responses.

possibility that there might be any correlation between the pretetanic amplitude and the posttetanic-to-pretetanic ratio of amplitude in all the 33 kittens. The correlation coefficient of linear regression analysis was 0.07 ($P > 0.1$) at the tetanized side and 0.01 ($P > 0.1$) at the nontetanized side, and thus there was no significant correlation between these two measures.

DISCUSSION

First of all, it is to be pointed out that the potentiation and depression of the cortical field potentials we observed may represent a collection of many different polarities and magnitudes of changes at a number of different synapses in the visual cortex. Thus the phenomena we found in the present study might not correspond to those studied in slice preparations of the hippocampus (Bliss and Lomo 1973; see Bliss and Lynch 1988 and Brown et al. 1990 for reviews) and the developing visual cortex (Komatsu et al. 1981; see Tsumoto 1992 for review). In these previous studies, the terms LTP and LTD have meanings as a modification of the efficacy of a particular synaptic pathway. In the present study, however, there is no basis for identifying the changes in the cortical responses with changes in the efficacy of any particular population of synapses. Nevertheless, the changes in the cortical responses we observed may reflect a long-lasting modification of the efficacy of some population of synapses in the visual cortex, because, for example, LTP of field potentials observed in slice preparations of visual cortex of kittens and young rats was demonstrated to be due to an enhancement of synaptic currents at geniculocortical and corticocortical synapses with current source density analysis (Komatsu et al. 1981; Perkins and Teyler, 1988).

Low probability of induction of potentiation in the control kittens

The present experiments have demonstrated that homosynaptic potentiation could be induced in the kitten visual cortex after tetanic stimulation of the ON. In the control kittens, however, the probability of its induction was rather low. Such a low probability may be due to the usage of in vivo anesthetized preparations, in which visual cortical neurons are under powerful influences of the GABAergic inhibitory systems (Sillito 1975; Tsumoto et al. 1979). The inhibition mediated through $GABA_A$ receptors is known to control the activity of a type of glutamate receptors called *N*-methyl-D-aspartate receptors (Jones and Baughman 1988; Shirokawa et al. 1989; Thomson 1986), which are supposed to play a role in induction of use-dependent synaptic plasticity (Artola and Singer 1987; Bear et al. 1990; Collingridge et al. 1983; Kleinschmidt et al. 1987; Tsumoto et al. 1987). In the slice preparations in which the $GABA_A$ receptor–mediated inhibition is reduced to substantial degree by the application of its antagonist, homosynaptic potentiation is induced with much higher probabilities (Artola and Singer 1987; Kimura et al. 1989; see Tsumoto 1992). In the motor cortex of the cat also, Baranyi et al. (1991) reported that an associative potentiation after paired activation of afferents and postsynaptic neurons is induced with higher probability in awake cats than in anesthetized cats, and suggested that this increase in the induction probability may be due to reduction of tonic inhibitory influences.

Effects of spontaneous activity on the induction of potentiation and depression

The intraocular injection of TTX, which eliminated spontaneous activity of the retina, increased the induction probability and magnitude of homosynaptic potentiation. An interpretation of these results is that the elimination of spontaneous background activities may improve the signal-to-noise ratio of stimulus-induced inputs so that effects of tetanic stimulation are relatively enhanced. An alternative interpretation, albeit not mutually exclusive, is based on a theoretical model modified from previously proposed ones (Bear et al. 1987; Bienenstock et al. 1982; Frégnac and Shulz, 1991); past activities of postsynaptic neurons or synapses determine the threshold for synaptic modification (Fig. 7). In this and the previous models, synaptic efficacy is supposed to vary as the covariance between pre- and postsynaptic activities. On the basis of various in vivo and in vitro tests of this scheme of synaptic plasticity, Frégnac and coworkers (Frégnac and Shulz, 1991; Frégnac et al., 1990) suggested the existence of two independent modification thresholds. In this latter model, synaptic changes induced by presynaptic activity can be predicted as a function of postsynaptic activity (c) in the following way: if c is larger than the more positive modification threshold, $\theta+$, the synaptic efficacy will be potentiated, and if it is below the other lower threshold, $\theta-$, it will be depressed. When the postsynaptic activity level remains within a value window defined by the two thresholds, the synapse stays in a nonadaptive transmitting mode. The simplest assumption to be made in the present study to give this two-threshold model the floating characteristics as in the original Bienenstock et al. (1982) model is to assume that both thresholds will be positively correlated with the past mean activity ($\bar{c}$). When $\bar{c}$ is increased by the previous postsynaptic activity, $\theta+$ and $\theta-$ will increase, and vice versa. In the control experiments, c of tetanized and nontetanized pathway is supposed to be partly beyond the $\theta+$ and $\theta-$, respectively (Fig. 7*A*, ⇔), so that potentiation and depression could be induced at each pathway with relatively low probability and small magnitude.

Regarding depression, it is to be pointed out that spontaneous inputs coming from the nontetanized pathway to cortical neurons are mostly uncorrelated with high-fre-

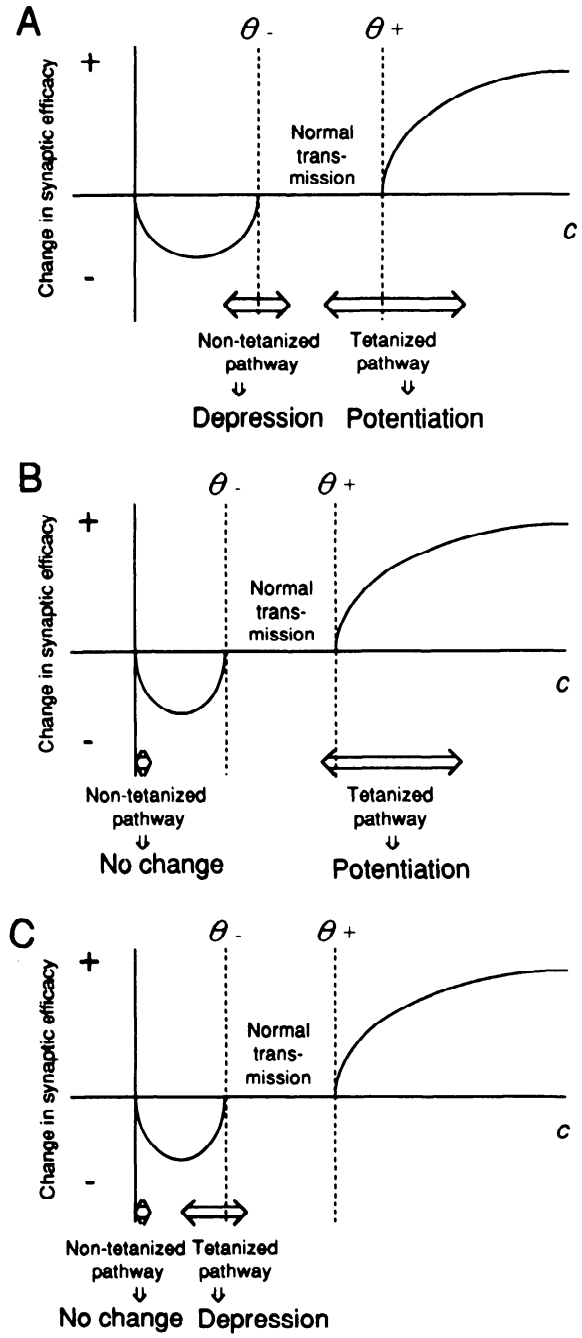

FIG. 7. A model accounting for the induction of potentiation and depression in each type of the experiments (modified from Bienenstock et al. 1982 and Frégnac and Shulz, 1991). Changes in synaptic efficacy (ordinates) depend on the postsynaptic activity, c (abscissae). The 2 modification thresholds ($\theta+$ and $\theta-$) are nonlinear functions of time averaged postsynaptic activity ($\bar{c}$). *A*: in the control kittens, c reached during tetanus is partly above $\theta+$, so that the efficacy of tetanized synapse changes to positive direction in some cases. c when the nontetanized pathway is active is in part between 0 and $\theta-$, so that the efficacy of nontetanized synapse changes to negative direction in some cases. *B*: in tetrodotoxin (TTX)-injected kittens, $\theta+$ and $\theta-$ decreases because of the elimination of spontaneous inputs. Consequently, c reached during tetanus is almost completely above $\theta+$, so that the efficacy of tetanized synapse changes to positive direction in most cases. On the other hand, TTX suppresses presynaptic activity almost totally, so that the efficacy of nontetanized synapse does not change. *C*: in γ-aminobutyric acid (GABA)-infused kittens, c during tetanus is mostly between 0 and $\theta-$, so that the efficacy of tetanized synapse is reduced in many cases. However, pre- and postsynaptic activities are almost completely suppressed, so that the efficacy of nontetanized synapses does not change.

quency inputs from the tetanized pathway. Such a negative association of inputs through the two pathways to postsynaptic neurons may facilitate the induction of depression at the nontetanized pathway, as in CA1 area of the hippocampus (Stanton and Sejnowski 1989). Therefore, in the present experiments, associativity of inputs from the two pathways may play a role in induction of heterosynaptic depression. In other words, heterosynaptic depression observed in the control kittens may be taken as a sort of associative depression induced in a condition where spontaneous inputs from the nontetanized ON are not temporally correlated with high-frequency inputs from the tetanized ON.

In the experiments with TTX, elimination of retinal spontaneous activity leads to almost complete loss of maintained inputs to the cortex. Consequently, $\bar{c}$ would decrease and $\theta+$ and $\theta-$ would shift to the left (Fig. 7 *B*), but the instantaneous postsynaptic activity induced by tetanus would exceed the $\theta+$, so that potentiation would be induced with higher probability and larger magnitude. In the TTX experiments, heterosynaptic depression could not be induced, despite the facilitation of induction of potentiation. This may be attributable to the absence of spontaneous inputs to the nontetanized synapse. Because no activity was present presynaptically, no change was expected to occur (Fig. 7*B*).

Postsynaptic activity regulates the direction of changes in synaptic efficacy

The suppression of postsynaptic activity led to the induction of homosynaptic depression rather than potentiation with substantial probability. This result may be explained in the above-mentioned model (Fig. 7*C*). During the application of GABA, the very weak postsynaptic activity induced by tetanus would be mostly below $\theta-$. Consequently, the synaptic efficacy would be decreased in many cases. On the other hand, no activity was present in the nontetanized pathway, and thus any change in synaptic efficacy would hardly be induced. The assumption that weak activation of postsynaptic neurons decreases the synaptic efficacy was predicted in the theoretical study (Bienenstock et al. 1982) and reported in an experimental study using goldfish (Yang and Faber 1991).

Homosynaptic depression observed in the present experiments is in line with the previous finding that visual responsiveness of cortical neurons to the opened eye was relatively weakened in monocularly deprived kittens in which visual cortex was infused with muscimol, a $GABA_A$ receptor agonist (Reiter and Stryker 1988). Also, the present results are consistent with the in vivo observation of Frégnac et al. (1988) that if postsynaptic activity of a cortical cell is iontophoretically reduced each time a given visual stimulus is presented, its subsequent level of response for that particular stimulus is selectively decreased. They further observed that the pairing of intracellularly imposed hyperpolarization with white matter or intracortical stimulation led to the depression of excitatory postsynaptic potentials in slice preparations of kitten and guinea pig visual cortex (Frégnac et al. 1990). On the other hand, Artola et al. (1990) reported that the moderate depolarization of the conditioned cell below a certain threshold rather than hyperpolarization was necessary to induce depression in the rat visual cortex.

Although the exact levels of postsynaptic membrane potential for the modification thresholds were difficult to predict from the present experiments, we suggest that the difference between these two studies may be accounted for from the model proposed above. In contrast to the study of Frégnac et al. (1990), which was performed in the normal perfusion medium, Artola et al. applied bicuculline methiodide (BMI) in the perfusion medium to block $GABA_A$ receptor–mediated inhibition for ≥1 h before tetanus. The blockade of inhibitory system for a long time might increase the $\bar{c}$, then would increase the modification threshold. Apart from the exact levels of modification threshold, these results and also the present results suggest that the balance of inhibitory and excitatory inputs determines the direction of use-dependent changes in synaptic efficacy in the developing visual cortex.

Sites of the action of GABA

In the present experiments, we have used GABA to block the postsynaptic activity. GABA is a well-known inhibitory transmitter in the visual cortex (Iversen et al. 1971; Sillito 1975; Tsumoto et al. 1979), and its receptors can be classified into the two types, $GABA_A$ and $GABA_B$ receptors (Bowery et al. 1980,1984). Thus the action of GABA applied through the cannula to visual cortical neurons was expected to be mediated through the both types of receptors located postsynaptically. However, $GABA_B$ receptors were reported to locate also at presynaptic sites and supposed to decrease the transmitter release in the cerebellum, hippocampus, striatum, and frontal cortex of the rat (Bowery et al. 1980; Lanthorn and Cotman 1981; see Bowery 1982 for review). So the effects observed in the GABA infusion experiments might be induced also by the GABA action on presynaptic sites. Although we cannot completely exclude this possibility, anatomic studies using autoradiographic labeling techniques showed that $GABA_B$ receptors were relatively sparse in the visual cortex (Bowery et al. 1984). In addition, the binding of GABA to the $GABA_B$ receptors was primarily restricted to layer 1–3 of the cortex (Chu et al. 1990). Therefore GABA may have minor effects on geniculocortical fiber terminals, which are located mostly in layer 4. In the cat VC, in fact, the suppression of visually evoked responses by iontophoretic application of GABA was not affected by phaclophen, a $GABA_B$ receptor antagonist, although it was antagonized by BMI (Baumfalk and Albus 1988). Taking these findings altogether, the effects of GABA observed in the present study may be mediated mainly through $GABA_A$ receptors located postsynaptically.

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Address reprint requests to T. Tsumoto.

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