MODULATION OF SINGLE CELL RESPONSES AND NEURONAL INTERACTIONS PRODUCED BY IONTOPHORESIS OF GLUTAMATE IN ADULT CAT VISUAL CORTEX

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Abstract. Single neuron responses and interneuronal coordinations after microiontophoretic injections of L-glutamic acid (glutamate) were examined in cats striate cortex with an aid of multielectrode extracellular recording and crosscorrelation analysis. The effects of 15 min long stimulation with glutamate were compared with spontaneous mutability observed within at least 1.5 h. A mutability coefficient was suggested for quantitative data analysis. Spontaneous changes were analyzed in 144 neuronal pairs in two anesthetized and seven pretrigeminal cats. Differences in spontaneous mutability between these groups were not significant. Fluctuations of crosscorrelograms were smaller than fluctuations of poststimulus time histograms (PST). The type of correlogram and receptive field preferred orientation and direction never changed. The effect of glutamate injection was studied in 157 neuronal pairs in four anesthetized and eight pretrigeminal cats. Excitatory responses were observed in 69% of neurons, inhibitory in 18%. The excitatory response to glutamate was accompanied with enhanced visual response in 47% of cases and with reduced visual response in 18%. Crosscorrelogram strength was reduced in 43% of pairs with excitatory response to glutamate and increased only in 23%. After the termination of iontophoresis a persistent increase of the shared input coordination was observed in two pairs in anesthetized animals and in three pairs in pretrigeminal cats. The appearance of direct excitatory coordination was found in one case in pretrigeminal cats. Alterations of PST histograms did not
exceed the range of spontaneous mutability. We infer that in a small percent of neuronal pairs the short glutamate stimulation can affect interneuronal links even in adult cat.

INTRODUCTION

The techniques of multiple neuron extracellular recording, together with computer aided techniques permit the detection of different types of coincidences of neuronal firing. These coincidences can be interpreted in terms of underlying afferents and interconnections among the observed neurons. This technique also yields quantitative measurements of the effectiveness of synaptic connections. With extracellular recording functionally related neurons are available for study for a long time even in the case of small neurons in the mammalian cerebral cortex. This in turn makes it feasible to search for possible changes in the strength of synaptic connections, either “spontaneous” or in association with different types of stimulation, i.e. visual, electrical or chemical.

The modulation of synaptic efficacy is thought to be the basic mechanism underlying both the reorganization of the cerebral cortex during early postnatal life and at least some forms of adult learning (16). Recent experiments suggest that the excitatory synapses mediated with L-glutamic acid (glutamate) may well be involved in these plastic processes. A number of studies have indicated that glutamate is the main specific excitatory transmitter in the cerebral cortex (1, 3, 5, 24). There are three different types of glutamate postsynaptic receptors, named respectively N-methyl-D-aspartate (NMDA), quisqualate and kainate receptor (26, 45). The NMDA receptor is involved in modulating synaptic efficacy as it is linked to a ionophore that allows the passage of Ca\(^{2+}\) ions (10, 25). This Ca\(^{2+}\) permeable channel can be opened only if the postsynaptic membrane is already sufficiently depolarized to eliminate a voltage dependent block of the channel by Mg\(^{2+}\) ions (7, 25, 29). It has been shown that the NMDA-preferring receptors are involved in long-term potentiation in the hippocampus (4, 15). In the visual cortex of young kittens the blockade of these receptors disrupted the experience dependent ocular dominance plasticity (23). Also in young kittens pairing an unpreferred visual stimulus with the iontophoretic injection of glutamate (or even acetylcholine or noradrenaline) could produce a persistent shift in the receptive field preferred orientation (14).

In adult cats iontophoretic administration of glutamate to the visual cortex resulted in a small enlargement of neuronal receptive fields and an increase in the response amplitude during and shortly after iontophoresis (18). No information is available as to whether the glutamate
stimulation can evoke persistent changes in receptive field properties and other aspects of electrophysiologically tested neuronal responses or in the pattern and/or strength of interneuronal interconnections.

The purpose of the present study was to characterize the fluctuation in neuronal responses and in strength of interneuronal interactions tested with the crosscorrelation method. The fluctuations that occurred spontaneously in time or under different visual stimulation were compared with those related to iontophoretic injections of glutamate through the recording electrode.

METHOD

The study consisted of two parts. First, the change of interneuronal connectivity in time and with variable light stimuli was analyzed. Second, the effects of glutamate injections on neuronal responses and interconnections were measured and compared with spontaneous fluctuations. The first part of the study was performed on seven cats with brainstem transection at the pretrigeminal level (46) and two cats anesthetized with pentothal sodium (Nembutal). The second part was performed on eight pretrigeminal cats and four animals under Nembutal anesthesia. The pretrigeminal transection was done under ether anesthesia. Animals without the transection were initially anesthetized with 35 mg/kg of Ketalar (Parke Davis) followed after 30 min by the intraperitoneal injection of 15 mg/kg of Nembutal. Additional doses of 12-15 mg/kg of nembutal were given every 8 h during the experiment. In all animals a tracheal cannula allowed control of respiration. CO₂ level was monitored and maintained at about 4%. The animals were paralyzed with an initial dose of 100 mg gallamine triethiodide (Flaxedil) and then maintained with an intravenous infusion of a mixture: 0.9% NaCl, 5% glucose and 4 mg/ml Flaxedil, given at a rate of 5 ml/h. Arterial blood pressure was monitored and maintained between 100-160 mm Hg. Rising blood pressure was used as indicator for the requirement of additional anesthesia. The body temperature was maintained between 37 and 39°C. Once the animal was prepared an opening was made in the skull over the visual cortex of the left hemisphere. The area between the stereotaxic coordinates A2 and P2, and between L0 and L4 was exposed. After removal of the dura mater the electrodes were lowered until the tips touched the cortical surface. The opening was covered with 3% agar. A layer of wax was added if necessary to reduce pulsations. The pupils were dilated using Neosynephrine and the cornea of the eye contralateral to the opening was protected with a neutral glass lens. The ipsilateral eye was covered with wet cotton. Neuronal responses were
recorded from the border between areas 17 and 18 within the region receiving a projection from the area centralis. The recordings began 4 h after the pretrigeminal transection or 6 h after the initial application of Ketalar.

Glass pipettes with 1.5-3 \( \mu m \) tip diameters and 3-10 M\( \Omega \) tip impedance were used as recording electrodes. Two channel theta glass pipettes of 2-4 \( \mu m \) tip diameters were used for recording and for the injection of glutamate. Recording electrodes were filled with 2 M NaCl solutions. Injection channels were filled with 0.2 M solution of monosodium L-glutamic acid with the pH adjusted to 7.5 using NaOH. In the first part of experiment two single channel electrodes were used. For recording and the simultaneous iontophoresis of glutamate one single channel and one theta glass electrode were used. One electrode was inserted perpendicularly to the cortical surface, the other one at an angle of 15°, with the initial distance between the tips being 400 \( \mu m \). The distance was decreased with depth.

Small bars of light projected onto a tangent screen set 57 cm from the cat's eye were used as visual stimuli. Unless otherwise stated the stimulus intensity was 10 cd/m\(^2\) and background level was 0.5 cd/m\(^2\). For the initial testing of receptive fields a hand held projector was used. During the recording this was replaced by an automatic mirror galvanometer system. In the first part of the experiment the spontaneous activity of each neuronal group was recorded followed by recordings for at least four different orientations of the moving light stimuli: the near optimal orientation of at least one of the neurons, the null orientation and two orientations between them (with a 45° step). In the majority of cases due to the relative location of both electrode tips the preferred orientations of simultaneously recorded neurons were similar. As soon as these measurements were accomplished the recording of the spontaneous activity and then the data from all the orientations of the light slit were undertaken again. The time delay between the first and second recording for the same stimulus orientation was longer than 1.5 h.

In the second part of study receptive field sizes and locations, the optimal stimulus orientations and velocities were determined using the hand held projector. The automatic projector was then set to the near optimal orientation of the light stimulus and the neuronal activity was recorded for 15 min before the iontophoretic injection of glutamate, 15 min during and 15 min after the injection. The current of iontophoresis was adjusted to the smallest value that induced a clear response of at least one recorded neuron. This value varied between 40 nA and 100 nA. In some cases a retention current of 5-10 nA was used to prevent spontaneous diffusion of glutamate ions.
Signals from each electrode channel were amplified, displayed on a multichannel oscilloscope and recorded, together with stimulus markers using a Racal 4DS FM tape recorder. Only if all the recordings were stable and artifact free in all channels were the data considered for analysis. Full analysis, including the triggering procedure was done off-line from the magnetic tape with a Cromemco Z-2 microcomputer. For better comparison sections of recordings of equal lengths of 8 min were selected from the longer recordings.

The following set of histograms was used to analyze each group of simultaneously recorded units:

1. Poststimulus time (PST) histograms for each neuron for each stimulus orientation.
2. Low and high resolution crosscorrelograms (0.5 and 5 ms bin width) for each neuronal pair, for each stimulus orientation, spontaneous activity and iontophoretic stimulation.
3. Shift predictors corresponding to all crosscorrelograms computed from stimulated unit activity.

The shift predictor is a necessary control required to isolate the correlation effects due purely to the stimulus when it affects both neurons simultaneously. It is calculated by shifting one of the two spike trains by one interstimulus period and by then recomputing the crosscorrelograms. The shift predictor shows the isolated effects of the simultaneous stimulation (30). Only if the original crosscorrelogram had peaks or valleys higher than 50% of the background levels which were not seen in the shift predictor or spontaneous activity correlogram, was the presence of interneuronal connection inferred and its possible changes with time, visual and iontophoretic stimulation analyzed.

RESULTS

Interneuronal interactions in the visual cortex

Pretrigeminal cats. Interneuronal interactions were analyzed in 15 cats. In eight of these animals the effects of glutamate injections were subsequently studied. The data base consisted of 46 neuronal assemblies. In 34 cases four neurons were recorded simultaneously; in seven cases three neurons and in five cases two neurons.

Cats under Nembutal anesthesia. Interneuronal interactions were tested in six cats. The effects related to glutamate injections were studied in four of these animals. Thirty three neuronal assemblies were recorded. In four cases four units were recorded simultaneously, 10 assemblies consisted of three neurons and in 19 cases neuronal pairs were studied.
Table I shows the percentages of responsive neurons and the distribution of different types of interneuronal interactions encountered in anesthetized and pretrigeminal cats. In both groups of animals all the neuronal receptive fields were located within 10° of area centralis. The preferred orientations of neurons in all correlated pairs were located within the same 45° angle sector. In eight pairs with perpendicular preferred orientations neuronal activity was never correlated. Neuronal receptive field types (simple, complex) were tested only during the initial analysis with hand hold stimulus projector. No consistent relationship between receptive field properties and the type and strength of correlation was found. At least the shared input type of coordination could clearly encompass any configurations of receptive field types. Thirty three neuronal pairs with strong receptive field overlaps were selected from pretrigeminal cats data. Fourteen such pairs were found in anesthetized animals. Neuronal activity in all these pairs was strongly correlated but the distribution of correlogram types and strengths in this sample did not differ significantly from the rest of the correlated pairs. Inhibitory troughs were observed in less than 10% of neuronal pairs recorded with one electrode. They were usually accompanied by excitatory phenomena and they never attained the level of chosen criterion. Despite of this the criterion was not changed since only strong, well expressed phenomena were adequate for further analysis of spontaneous or glutamate induced mutability.
Spontaneous mutability of crosscorrelation and PST histograms

In this part of study seven pretrigeminal cats and two animals anesthetized with Nembutal were used. Since the differences in spontaneous mutability of crosscorrelograms and poststimulus time histograms between anesthetized and pretrigeminal cats were not statistically significant, the results will be described jointly.

Recordings were done from 106 neurons in 29 neuronal assemblies recorded simultaneously. In 19 cases two neurons were recorded with the same electrode; in nine groups two neurons were recorded with one electrode and one neuron with the other. One hundred forty four simultaneously recorded pairs were formed. In 48 pairs both neurons were recorded with the same electrode. In 96 pairs the cells were recorded using separate electrodes. All neuronal assemblies were analyzed for more than 1.5 h.

No case was found in which the alteration of preferred orientation of the neuronal receptive field after 1.5 or even 2 h could be convincingly proven. No case was found in which the correlogram strength was dependent on the stimulus orientation. In spite the large differences in responses and numbers of spikes of orientation sensitive neurons, the crosscorrelogram peak to noise ratios were similar.

Peak to background ratios were calculated for 127 crosscorrelograms with 5 ms bin width, in which the background levels were high and smooth enough to make the measurements reliable. Crosscorrelograms built with the 0.5 ms bin width were usually much less smooth because of the smaller number of intervals and so the measurements of peak to noise ratios were much less accurate. The mutability coefficients (M) were calculated according to the formula:

\[ M = \frac{(S_1 - S_2)}{(\text{mean } S)} = 2\frac{(S_1 - S_2)}{(S_1 + S_2)}, \]

where \( S_1 \) and \( S_2 \) are the crosscorrelogram strengths (peak to background ratios) obtained for the same stimulus parameters after 1.5 h. Figure 1 shows the distribution of crosscorrelogram mutability coefficients obtained in present experiment. Table II summarizes the differences observed in the crosscorrelogram and poststimulus time histogram peak to noise ratios measured with the same stimulus parameters and with the time delay longer than 1.5 h. Statistical analysis of these data failed to reveal any significant trends. Low number of analyzed poststimulus time histograms is due to the frequently encountered low background levels making the calculation inaccurate. In crosscorrelograms the numbers of intervals in the backgrounds are usually much higher than the numbers of collected spikes. This is due to the crosscorrelation algorithm (30).
Fig. 1. Distribution of mutability coefficients in the sample of 127 crosscorrelograms. Centrally located, dashed bar represents the percentage of neuronal pairs in which the differences between crosscorrelograms peak to noise ratios were smaller than the accuracy of measurement. Two marginal bars represent all the cases with mutability coefficients higher than 100%.

**Table II**

The comparison of crosscorrelogram and poststimulus time histogram peak to noise ratios measured before and after the delay of 1.5 h. Differences calculated according to the formula of mutability coefficients (see text)

<table>
<thead>
<tr>
<th>Peak to noise ratio</th>
<th>Number of cases</th>
<th>Mean difference</th>
<th>Maximal difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crosscorrelograms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased</td>
<td>60</td>
<td>56%</td>
<td>160%</td>
</tr>
<tr>
<td>Decreased</td>
<td>32</td>
<td>60%</td>
<td>160%</td>
</tr>
<tr>
<td>Unchanged</td>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Poststimulus time histograms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased</td>
<td>13</td>
<td>66%</td>
<td>120%</td>
</tr>
<tr>
<td>Decreased</td>
<td>13</td>
<td>75%</td>
<td>130%</td>
</tr>
<tr>
<td>Unchanged</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The increase of crosscorrelogram strength exceeded 100%/o in six cases. In four cases the decrease was higher than 100%/o. In all these cases however the background level was substantially lowered at the end of the recording session, making the measurements less accurate.

**Neuronal activity during glutamate injections**

Eight pretrigeminal cats and four animals under Nembutal anesthesia were used. Since no differences between these animal groups were found, the results will be presented together. Seventy four neurons were subjected to microiontophoretic injections of glutamate with currents varying from 40 nA to 100 nA. In 51 cases (69%/o) the mean frequency of neuronal activity increased during injection, in 13 cases (18%/o) it
decreased and in nine cases (13%) it remained unaffected. In the group of 51 neurons in which the effect of glutamate was excitatory 40 were responsive to the visual stimuli. Table III shows the effects of increased or decreased mean spike frequency on the crosscorrelogram and post-stimulus time histogram peak to noise ratios. Pairs were classified as having excitatory or inhibitory response to glutamate if at least one cell showed this response (the response of the other cell could be the same or none). Data presented in Table III indicate that the excitatory effect of glutamate reduced the crosscorrelogram strength whereas the glutamate induced inhibition enhanced it ($\chi^2$ test $P < 0.001$). The effect of glutamate injections observed with poststimulus time histograms was opposite ($\chi^2$ test $P < 0.001$). Figure 2 shows this typical excitatory effect of glutamate on the activity of the adult cat neuron as measured by poststimulus time histograms. The peaks of neuronal responses to the visual stimulation are strongly increased with only a slight elevation of the background level. An increase in the background level was mainly observed with the higher iontophoretic currents. In all cases this increase followed the increase of response peaks noticeable earlier with small currents. Even the cells that responded to glutamate with strong excitation hardly showed any increase in the sizes of their visual receptive field. If inhibitory areas could be detected before iontophoresis they appeared to be unchanged during glutamate stimulation.

### Table III

<table>
<thead>
<tr>
<th>Mean spike frequency</th>
<th>Mean spike frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>increased by glutamate</td>
<td>decreased by glutamate</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Crosccorrelograms</th>
<th>POSTstimulus time histograms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pairs</td>
<td>65</td>
</tr>
<tr>
<td>Percent of pairs with increased peak to noise ratio</td>
<td>23</td>
</tr>
<tr>
<td>Percent of pairs with decreased peak to noise ratio</td>
<td>43</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of neurons</th>
<th>POSTstimulus time histograms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of neurons with increased peak to noise ratio</td>
<td>47 measurements unreliable</td>
</tr>
<tr>
<td>Percent of neurons with decreased peak to noise ratio</td>
<td>18 background level</td>
</tr>
</tbody>
</table>
Fig. 2. Typical excitatory effect of glutamate on the activity of adult cat neuron. The animal was anesthetized with Nembutal. The cell was stimulated with a light slit (0.25°×4°, 10 cd/m²) moving horizontally as indicated by the dark bars above the upper histogram. PST histograms show the neuronal responses before (a), during (b) and after (c) iontophoretic injection of glutamate. Neuron was recorded 700 μm below the cortical surface. Eight minute recordings were used for all histograms. Total number of recorded spikes before the injection — 542, during — 1206 and after — 641. Glutamate ejection current — 40 nA.

Persistent effects of glutamate observed after the termination of iontophoresis

Cats under Nembutal anesthesia. Twenty five neuronal assemblies were recorded in four cats. In 20 assemblies two neurons were recorded, one with each electrode. In four cases three neurons were recorded simultaneously, two of them with the injection electrode and one with the recording electrode. In one case three neurons were recorded but only one of them with injection electrode. Thirty five neuronal pairs were analyzed in which at least one neuron was subjected to glutamate
stimulation. Figure 3 shows the typical effects of iontophoresis. Glutamate was injected in the vicinity of neuron 2. The poststimulus time histogram of this cell shows strong enhancement of the responses to visual stimulation. The shared input peak, visible on the crosscorrelation histogram becomes broader but not higher, whereas the background level of the crosscorrelogram is elevated. After the termination of iontophoresis both the spontaneous activity and the responses of cell 2 fall beneath their initial level. This effect was often observed, being usually stronger with higher iontophoretic currents. The corresponding crosscorrelogram still shows the peak of shared input. This is slightly lower than the peak before the injection but the associated background level is also lower. This kind of effects seemed to be stronger than the spontaneous mutability observed within short time delays comparable with the duration of the test (15 min), but they were not stronger than the spontaneous mutability observed within 1.5 h.

![Diagram](Image)

**Fig. 3.** Typical effect of glutamate stimulation on the responses and interconnection in the neuronal pair. The animal was anesthetized with Nembutal. Left column, shows the PST histograms of cell 1; middle column, PST histograms of cell 2 and right column, crosscorrelation histograms. Upper row shows the activity of neuronal pair before the glutamate stimulation; middle row, during the injection and lower row, after its termination. Directions and orientations of moving light slit are shown by the thick bars above the upper PST histograms. Neurons were recorded with separate electrodes 700 and 600 µm below the cortical surface. Overlapping receptive fields were located 4° from area centralis. Eight minute recordings were used for all histograms. All the shift predictors were flat. Glutamate was injected in the vicinity of neuron 2. Ejection current, 70 nA. Bin width for PST histograms, 22.5 ms; for crosscorrelograms, 0.5 ms. Numbers of recorded spikes: a, 673; b, 407; d, 449; e, 1179; g, 445; h, 310.
In 29 cases among the 35 analyzed pairs the crosscorrelogram peaks were strong enough to measure the peak to noise ratio and calculate the mutability coefficients (M). Crosscorrelogram alterations evoked by the 15 min long glutamate stimulation were compared with spontaneous mutability observed during at least 1.5 h. In two cases strong changes of crosscorrelograms were observed. The mutability coefficients in these cases were only 100% and 130%. Such mutability was comparable with the spontaneous changes observed in six neuronal pairs. In all of these cases however the effect could be explained by a strong decrease in spontaneous activity. Since after glutamate injection a strong alteration of crosscorrelogram peak to noise ratio occurred rapidly it could not be related to the slow changes in neuronal excitability. Figure 4 shows one of these two cases. The injection of glutamate in the vicinity of neuron 2 enhanced this neuron's responses to light stimuli, as well as the shared input peak and the background level in the crosscorrelation histogram. After the termination of iontophoresis the spontaneous activity of cell 2 and its responses to visual stimuli were lowered but the peak of shared input remained much higher then before the injection.

Fig. 4. The injection of glutamate can rapidly reinforce the shared input interaction. The animal was anesthetized with Nembutal. Figure convention and stimulus parameters are the same as in Fig. 3. Neurons were recorded with separate electrodes at the depths of 570 and 520 μm. Slightly overlapping receptive fields were located 3° from area centralis. Eight minute recordings were used. All the shift predictors were flat. Glutamate was injected in the vicinity of neuron 2. Glutamate ejection current, 40 nA. Number of recorded spikes: a, 242; b, 419; d, 228; e, 1033; g, 257; h, 408. Bin width for PST, 22.5 ms, for crosscorrelograms, 0.5 ms.
In another neuronal pair a strong increase of the shared input peak was also observed after the injection, whereas the spontaneous activity and response to light stimuli of the neuron subjected to the glutamate iontophoresis came back to the initial level after the termination of the injection.

**Pretrigeminal cats.** Twenty six neuronal assemblies were recorded in eight cats. In 18 assemblies four neurons were recorded simultaneously, two with each electrode. In two cases two neurons were recorded with the injection electrode and one with the recording electrode. In one case one neuron was recorded with injection electrode and two with the recording electrode. In five cases two neurons were recorded, one with each electrode. The total data base consisted of 122 neuronal pairs. In 83 pairs the neurons were recorded with separate electrodes, in 20 cases both neurons were recorded with injection electrode and in 19 pairs cells were recorded with the recording electrode.

In four pairs strong changes in the crosscorrelogram peak to noise ratio were found that persisted after the termination of glutamate iontophoresis. Mutability coefficients calculated for these pairs exceeded

![Fig. 5. Narrow unsymmetrical peak of direct excitatory connection appears after glutamate stimulation. Pretrigeminal cat. Neurons were recorded with separate electrodes at the depths of 720 and 760 μm. Both receptive fields were located 5° from area centralis. Eight minute recordings were compared. Crosscorrelograms were computed before the injection (a) and after its termination (b). Vertically moving light slit (0.25°×4°, 10 cd/m²) was used as visual stimulus. Glutamate ejection current, 60 nA. Crosscorrelogram bin width, 0.5 ms.](image-url)
100%. In all these pairs one of the cells showed an excitatory response to glutamate. In all cases the responses to light stimuli were enhanced during injection, but after its termination both the response peaks and the mean spike frequency dropped to the initial level. In three of these four pairs an increase in the shared input strength was observed. This increase persisted after the termination of the injection. In one pair the narrow peak of direct excitatory coordination appeared in a previously uncorrelated pair. Figure 5 shows the crosscorrelogram histograms of this pair before and after the injection of glutamate.

Table IV

Table IV shows the distribution of neuronal pairs with enhanced and reduced coordination in the groups of neurons with different responses during glutamate stimulation.

<table>
<thead>
<tr>
<th>Neuronal pairs with excitatory effect of glutamate</th>
<th>Cats under Nembutal anesthesia</th>
<th>Pretrigeminal cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pairs</td>
<td>24</td>
<td>67</td>
</tr>
<tr>
<td>Number of pairs with enhanced coordination</td>
<td>8</td>
<td>33</td>
</tr>
<tr>
<td>Number of pairs with reduced coordination</td>
<td>9</td>
<td>21</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neuronal pairs with inhibitory effect of glutamate</th>
<th>Cats under Nembutal anesthesia</th>
<th>Pretrigeminal cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pairs</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Number of pairs with enhanced coordination</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Number of pairs with reduced coordination</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neuronal pairs with no effect of glutamate</th>
<th>Cats under Nembutal anesthesia</th>
<th>Pretrigeminal cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pairs</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Number of pairs with enhanced coordination</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Number of pairs with reduced coordination</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Reaction to glutamate was measured as the alteration of mean frequency of spikes produced by neurons during iontophoresis. Statistical analysis did not reveal any significant relations in this table. With the exception of cases mentioned above all the mutability coefficients were within the range of spontaneous instabilities.

The peak to noise ratios were measured in poststimulus time histograms formed before and after the termination of glutamate iontophoresis. Since no differences between pretrigeminal and anesthetized animals were found, Table V presents the data from both groups tog-
The effects of glutamate related changes in neuronal activity on the visual responses measured after the termination of iontophoresis

<table>
<thead>
<tr>
<th>Responses to glutamate injection</th>
<th>Excitatory</th>
<th>Inhibitory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of neurons</td>
<td>35</td>
<td>7</td>
</tr>
<tr>
<td>Number of neurons with enhanced visual responses</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Number of neurons with reduced visual responses</td>
<td>9</td>
<td>3</td>
</tr>
</tbody>
</table>

ether. All the alterations were within the range of spontaneous mutability observed without glutamate iontophoresis. The statistical analysis of all the poststimulus time histogram peak to noise ratios measured before and after the glutamate injections failed to show the statistically significant differences.

**DISCUSSION**

The distribution of shared input and direct excitatory interconnections encountered among the neuronal pairs recorded with the same and separate electrodes correspond well to earlier data (27, 40, 41). Inhibitory troughs found by other authors in about 10% of pairs were also observed despite the fact that they were too weak for their mutability to be analyzed. For comparable distances between neurons in pairs the distribution of different types of interconnections was similar.

*The effects of Nembutal anesthesia and pretrigeminal transection*

The percent of neuronal pairs with shared input type of coordination was slightly lower in pretrigeminal cats than in animals under Nembutal anesthesia. Interestingly, this difference was found only in pairs recorded with separate electrodes, i.e. with larger distances between cells. We can assume that unspecific inputs are more scattered over the cortical surface than the specific visual inputs (9, 28, 36). In pairs with small distances between the neurons the specific inputs should predominate, but for longer distances the polysynaptic long-range, unspecific inputs can play a more important role. These inputs should be inhibited by Nembutal but our data suggest that the effects of the transection of the entire brain stem were stronger. On the other hand the percent of visually responsive neurons in pretrigeminal cats was significantly lower than in animals under Nembutal anesthesia. Neurons in pretrigeminal cats also showed weaker responses and higher spontaneous activity.
Similar effects of barbiturate anesthesia were observed earlier by other authors (31, 32). It is generally assumed that the inhibitory effect of Nembutal was proportional to the number of synapses along each input to the analyzed neuron. Thus Nembutal causes unspecific, polysynaptic inputs to be more attenuated than the relatively direct visual input, thereby lowering the spontaneous activity and inducing a more pronounced visual response. One of the possible explanations of the fact that the single unit activities are affected more strongly by Nembutal anesthesia while the pretrigeminal transection to a larger extent eliminates shared input connections could be as follows. Light Nembutal anesthesia attenuates to some degree the majority of the large number of unspecific inputs to the tested neuron, whereas pretrigeminal transection eliminates completely particular fraction of the inputs whilst leaving the rest intact. The global effect of Nembutal can predominate but crosscorrelogram analyses only the inputs from one specific source shared by the two tested cells. Weaker coordinations in anesthetized animals can still be detectable but transection must eliminate the coordination completely in a number of cases.

Spontaneous fluctuations of neuronal activity

Spontaneous fluctuations in neuronal maintained activity and in the amplitude of responses to identical visual stimuli were frequently observed (13, 17, 33, 34, 39). Such fluctuations were found in awake cats (35), in preparations with “encephale isole” transection (31) and in anesthetized animals at different levels of anesthesia (21). It is assumed that the fluctuations are strongest in awake cats and are suppressed by larger doses of anesthetics. In the present experiment statistically significant differences between animals with pretrigeminal brainstem transections and under light Nembutal anesthesia were not found.

The basic properties of neuronal receptive fields such as preferred orientation or direction of moving light slit remained unchanged during 1.5 or even 2 h of recording. Spontaneous changes in preferred orientations and directions of moving light stimuli have been described in area 17 neurons of adult, anesthetized and curarized cats by Donaldson and Nash (8), but these results could not be reproduced in other laboratories even in young kittens during the critical period (2, 11, 37, 38). It could be concluded that in the anesthetized and curarized adult or even young animal such fluctuations are very rare. The experiments of Tsutomo and Freeman (43) indicate that the effects of curare or Flaxedil, by causing a lack of feedback from the proprioceptors of eye muscles, can be even more important that the effects of anesthesia. This is in agreement with the results of the present experiments as no fluctuations
of preferred orientation and direction of visual stimuli were observed in either the anesthetized or pretrigeminal cats.

**Spontaneous fluctuations of interneuronal interactions**

Interneuronal interactions, tested with the crosscorrelation method revealed surprising stability, at least in pairs with the shared input type of coordination. Fluctuations in crosscorrelograms strength were smaller than the fluctuations in PST histograms. Variable parameters of visual stimulation usually caused a dramatic change in PST histograms and overall neuronal activity, whereas shared input type of crosscorrelograms were much less affected. This data supports the earlier findings of Dickson and Gerstein (6) on the cat's auditory cortex, that the functional connectivity between neurons shows a rather fixed, stimulus independent arrangement which consists mostly of shared input to cortical neurons. The higher stability of crosscorrelations than PST histograms, during the same kind of stimulation can be explained by different population of inputs that influence each of these statistics. It can be assumed that in the adult cats primary visual cortex, the majority of synaptic inputs to most neurons participate in producing the visual response. This response can be visualized in PST histograms. Crosscorrelogram on the other hand analyses only those inputs that are shared by both neurons under study or that form the direct link between them. This can be only a small fraction of the total number of inputs and the possible fluctuations of only this set of synapses can be also much smaller.

**Neuronal activity during glutamate iontophoresis**

Sixty nine percent of our analyzed neurons were excited by glutamate. With lower ejection currents, the amplitude of the response to the visual stimulation was increased more then the background. Higher currents also increased the background activity. Similar effects of glutamate stimulation have been observed earlier by Hess and Murata (18). In a number of studies (18-20) the inhibitory responses of some neurons to glutamate were also observed. These were interpreted as being glutamate excitatory effects on the neurons which in turn inhibit synaptically the analyzed cell.

Crosscorrelograms of neuronal pairs with excitatory response to glutamate usually showed both higher peaks and elevated background level. These are the typical effects of increased neuronal activity. In contrast, in the majority of cases (28 vs. 15) the peak to noise ratios were lowered during glutamate stimulation. In other words the increased number of spikes affected the correlogram background more than the peak. In the group of neuronal pairs exhibiting an inhibitory response
to glutamate the effect was opposite: peaks and backgrounds were lowered but the peak to noise ratios were increased in eight pairs and decreased only in five cases. These findings can be explained the same way as the relatively small spontaneous fluctuations of crosscorrelograms i.e. that only a relatively small number of synaptic inputs participate in the generation of the crosscorrelogram peaks and that these only partially overlap with the population of synapses stimulated with glutamate.

Persistent changes after the termination of iontophoresis

Strong changes in the crosscorrelogram strengths were observed only in seven cases among 157 tested pairs. The sample of seven pairs is too small to analyze the difference between the pretrigeminal and anesthetized cats, but we suggest that as such pairs were found in both groups of animals, there can not be dramatic differences between them.

Ketalar which was used prior to induction of anesthesia contains ketamine that has been reported as being able to block the NMDA receptors (42). This effect could be responsible for the low number of plastic changes observed in the experiment. It has been shown however that the effects of ketamine given intramuscularly disappears within a few hours after injection (12). Nembutal anesthesia with the preliminary dose of ketamine was used in the pharmacological studies of the effects of microiontophoretic application of glutamate, aspartate and their antagonists (44). In those experiments recordings were started 6-8 h after the injection of ketamine. In the present study recordings were also started at least 6 h after the injection of Ketalar. Therefore it is unlikely that the present results were contaminated by the effects of ketamine.

In seven neuronal pairs the changes in crosscorrelogram strengths observed after 15 min of glutamate injection were stronger than spontaneous fluctuations observed within 1.5 h with the exception of a small percent of pairs where a strong change in overall neuronal activity was observed. In all these cases the neuronal spontaneous activity and responses to visual stimulation went back to normal or dropped slightly beneath the initial level after the termination of iontophoresis whereas the crosscorrelogram peak to noise ratios increased strongly. This criterion may have been set too high therefore although glutamate stimulation caused more effects these were not strong enough to exceed the limits of spontaneous fluctuations or they could only be inferred by the increased neuronal activity after the termination of the injection. It seems however that the low number of altered crosscorrelograms after glutamate stimulation can be related to the limitations of the experiment-
al technique employed. As was mentioned already, the crosscorrelogram analyses only the activity of those synapses that form either a shared input to both tested neurons or a direct link between them. The comparison of spontaneous fluctuations and the responses during glutamate stimulation visible on crosscorrelograms and PST histograms indicate that these synapses form only a small fraction of the total number of inputs to the analyzed neurons. The locations of these synapses on the dendritic tree are unknown. According to Hess et al. (19) glutamate ejected iontophoretically may spread at the most about 100 μm from the electrode tip. This is much less than the size of the dendritic tree of most cortical neurons. Thus in our experiments only a part of dendritic tree was stimulated and the synapses “responsible” for a particular crosscorrelogram could fall into the region of stimulation only by chance. An attempt to stimulate the entire dendritic tree would require very high currents of iontophoresis, large electrode tips and large amount of injected glutamate. These experimental conditions would lead to the stimulation of a large portion of the cortical tissue and so hundreds of neurons in the neighborhood. The activity of the large neuronal assembly would be totally altered and those neurons, close to the injection point would usually be injured. The activity of synapses that are not involved in the shared input to both tested neurons or direct connection between them, form the background of the crosscorrelogram. If the efficacy of some of these synapses is increased after the glutamate injection, the background level of crosscorrelogram should be elevated and the peak to background level should be decreased or remain constant if the shared input or direct link between neurons is also reinforced. Thus, the analysis of the mutability of interneuronal connections with the crosscorrelation method and the microiontophoretic drug injection can not provide information about the percentage of the neuronal pairs that show plastic phenomena. On the other hand this method can show if it is possible to evoke such changes with tested drug at least in a small percent of pairs when all the conditions mentioned above are fulfilled. It seems that in some cases even 15 min stimulation with glutamate can evoke a strong alteration of the interneuronal interaction which persists longer than 15 min.

The effects of microiontophoretic application of glutamate on the neuronal activity in dorsal LGN and visual cortex have been shown to disappear within seconds after the termination of iontophoresis, even if the ejection currents exceeded 100 nA (18, 22). Thus it can be assumed that the effects observed in the present study after several minutes were not directly caused by the glutamate remaining in the tissue. In all seven cases where persistent changes in crosscorrelograms were ob-
served, the direction of these changes were consistent with Hebb’s theory (16). All these neurons were excited during glutamate stimulation, and glutamate enhanced their responses to visual stimulation. After the termination of iontophoresis all crosscorrelograms showed an increased peak to noise ratio in spite of the fact that neuronal activity was even a little lower than before iontophoresis.

The analysis of PST histograms did not reveal strong persistent changes in spite of the fact that during iontophoresis these histograms were altered much more than the crosscorrelograms. This again can be explained with the assumption that the neuronal response to visual stimulation is produced with the aid of a large percent of synaptic inputs, but only a small fraction of these are subjected during the experiment to the glutamate stimulation. Even if some of them changed their efficacy, the global effect visible on the PST histograms would therefore be very weak. In the case of crosscorrelogram, if the relatively small population of synapses responsible for shared input to two specific neurons or for a direct link between them, overlap with the relatively small population of synapses stimulated by the glutamate, this effect would be much stronger.

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