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The effects of glutamate iontophoresis on single cell responses and inter-neuronal interactions in the kitten visual cortex

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Abstract. We studied the effect of glutamate (Glu) micro-iontophoresis on visual responses (PSTHs) and cross-correlograms of neurones recorded simultaneously in the cortex of 4-6 weeks old kittens. PSTHs and cross-correlograms were tested before, during and after Glu injections and compared with adult cats data from previous experiment. Before the injection cross-correlogram shapes and numbers did not differ in young and adult cats, whereas PSTHs differed dramatically. Glu iontophoresis typically elevated background PSTHs levels rather than peak responses in young kittens. In adult animals the opposite effect predominated. After the termination of iontophoresis visual responses were enhanced in 21% of kitten neurones. In adult cats this effect was not observed. In kittens, a clear increase of cross-correlogram strength was recorded in one neuronal pair (3.6%) after the injection terminated. In adult cats similar effect was also found in 3% of pairs.

Key words: kitten, striate cortex, single neurone, cross-correlation, glutamate

INTRODUCTION

Visual information in the cortex is transmitted by excitatory glutamate synapses that activate both NMDA and non-NMDA postsynaptic receptors (Tsumoto 1990). The large body of experimental data, derived from the study on rat hippocampal slice preparation indicate that, besides the normal operation of the neuronal network, these synapses are involved in a number of plastic processes (Malenka and Nicoll 1993). High frequency, tetanic afferent stimulation results in a long lasting, stable increase in synaptic strength, known as long-term potentiation (LTP) (Collingridge et al. 1983). With lower frequency of stimulation it is possible to generate a short-term synaptic potentiation (STP) that decays to control levels within a few tens of minutes (Malenka 1991, Hanse and Gustafsson 1992). Prolonged, low frequency stimulation causes a stable long-term depression (LTD) (Dudek and Bear 1992, Mulkey and Malenka 1992). All three phenomena critically depend on NMDA receptors. Moreover, Glu receptors may also control the type of reaction. It has been suggested that the bidirectional control of synaptic strength that differentiates between potentiation and depression, can be mediated by the level of calcium ions. Calcium level depends on the activity of NMDA receptors (Lisman 1989). Concomitant activation of different Glu receptors (NMDA and metabotropic) seems to be necessary to turn short- into long-term potentiation (Bashir et al. 1993, Behnisch and Reymann 1993, Musgrave et al. 1993). The activity thresholds for inducing both potentiation and depression may not be fixed, but may be adjusted according to the recent history of the synapse i.e. also Glu receptors (Christie and Abraham 1992, Wexler and Stanton 1993).

While much was learned about LTP, STP and LTD in hippocampal slices, it seems important to establish the role of these phenomena in the intact cortex where the response to tetanic electrical stimulation is replaced by a much weaker response to natural stimulation. In addition, the large number of modulatory inputs remain intact. To study plastic processes in the cortex, the model of monocular, visual deprivation has been used extensively. Behavioural, electro-physiological and anatomical data indicate that there is a critical period - in kittens between 1 and 3 months after birth - during which the developing visual cortex is particularly sensitive to environmental manipulations (Wiesel 1982). Closing one eye during this period causes a long term decrease in the responsiveness of the visual cortex to the closed eye, accompanied by a number of changes in receptive field structure and in the organization of cortical eye-dominance columns. However, the comparison of data from rat hippocampal slices and visually deprived cats is very difficult (for rewiev see Fox and Dow 1993). Visual deprivation is a long-lasting and far-reaching process that alters the shapes of dendritic trees in the cortex and the morphology of afferent neurones in the lateral geniculate nucleus. A model of cortical plasticity that addresses the questions raised by experiments on hippocampal slices should have comparable time course. The experimental data indicate that, during the critical period in cats, successful modifications of neuronal visual responses can be obtained after a few hours of visual stimulation (Imbert and Buisseret 1975). This is still much longer than the time required to produce changes in hippocampal slices. Moreover, the procedure seems unreliable and usually some additional requirements have to be fulfilled. Greuel and co-authors (1987) found that response modification could be obtained only if visual stimulation was paired with high, unspecific activation of the cortex. It is difficult to induce changes in the cortex of anaesthetized and paralysed kittens (Buisseret et al. 1978, Freeman and Bonds 1979). However, changes can be obtained in anaesthetized and paralysed kittens when visual stimulus is reinforced with iontophoretic application of inhibitory or excitatory substances (Frégnac et al. 1984, Greuel et al. 1988) or when it is paired with electrical stimulation of the midbrain reticular formation (Singer and Rauschecker 1982).

In our experiments we concentrated on the possible contribution of Glu receptors to plastic processes in the visual cortex. We studied the alter-

ation of response strength to continuous visual stimulation induced by short, local injections of Glu. Using the aforementioned hippocampal data and the coincidence theory of synaptic modification (Bienenstock et al. 1982), we assumed that the increased level of Glu at the time of postsynaptic depolarization caused by bursts of visual responses could alter the strength of involved inputs and affect future responses. In the same experiment we injected Glu into the vicinity of two simultaneously responsive neurones. With the cross-correlation method we tested whether simultaneous activation could change the strength of the connection between these cells. Cross-correlation analysis (based on extracellular recordings that facilitate longer experiments on small neurones) provides information about interneuronal interactions similar to the analysis of intracellularly recorded postsynaptic potentials.

It is generally assumed that the synaptic potentiation observed in hippocampal slices is biologically significant, because it is correlated with either electrical or chemical stimulation. In our study we tried to evaluate the functional significance of effects produced by Glu injections into the intact cortex by comparing them with spontaneous fluctuations of visual responses and interneuronal interactions. In our previous study of adult cats (Kraszewski and Michalski 1989) we compared the effects of 15 min of Glu stimulation with spontaneous variability measured within 1.5 h. In the present experiment we repeated the same tests on kittens of the age close to the peak of maximum sensitivity to plastic modifications.

METHODS

The data was obtained form 6 kittens 4-6 weeks of age, ranging in weight between 400 and 700 g. Anaesthesia was induced with an initial dose of 35 mg/kg Ketalar (Parke-Davis) followed by 15 mg/kg Nembutal (Abbot) at 30 min. The kittens were then paralysed, using an initial dose of 20 mg Flaxedil and maintained with an intravenous infusion consisting of: 0.9% NaCl, 1 mg/ml Sodium Pentobarbital, 5% glucose and 4 mg/ml Flaxedil and given at a rate of 2 ml/h. Additional doses of Nembutal were used when indicated by rising blood pressure. The time between the application of Ketalar and the beginning of recording was always more than 6 h. Arterial blood pressure, end-tidal CO_2 level and body temperature were continuously monitored. The bone covering the visual cortex was removed at stereotaxic coordinates A2 to P2 with a laterality of 0 to L3.

Two theta glass micropipettes with tip diameters 2-4 μm were used. Each electrode was inserted into a separate drive, the first was set perpendicular to the cortical surface and the second at an angle of 15^{o} to the first. The distance between the two electrode tips on the cortical surface was 400 μm. Recording channels were filled with 2 molar NaCl, whereas the injection channels were filled with a solution of mono-sodium L- Glutamic acid (Glu, 0.2M, pH = 7.5) The recording channel impedance varied between 4 and 10 MΩ. Glutamate ejecting current was in a range between -20 and -50 nA and retaining current 0 to +20 nA. The current of iontophoresis was adjusted to the smallest value that induced a clear response from the recorded neurone, usually equivalent to a 30-50% change in spontaneous activity or in visually evoked responses. The stimulating effect of the ejecting current alone was tested occasionally (because of the difficulties of long time recording from young kittens cortex). In all cases this effect was negligible.

The visual stimuli consisted of a moving bar of light 0.25^{o} x 4^{o} projected onto a translucent tangent screen at 57 cm from the cats eye. The stimulus intensity was 10 cd/m^2, with a background of 0.5 cd/m^2. The speed of the stimulus movement was adjusted for maximum neuronal response and varied between 2 and 10^{o}/s. The amplitude of the slit movement was typically 10^{o}. Thus, depending on the velocity, the stimuli were presented at a rate between 6 and 30 cycles/min. Initial receptive field plotting was done with a hand held projector. The automatic projector was then set to an optimal orientation and the neuronal activity was recorded for at least 15 min with continuous stimulation.

Subsequently, the injection current was adjusted to obtain the response of at least one cell to Glu and the neuronal activity was again recorded for 15 min. A third block of recording for at least 15 min was made after termination of iontophoresis. The visual stimulus conditions were kept identical during all three recording periods. The majority of neuronal pairs were recorded from the single orientation column and both neurones had very similar preferred stimulus orientations. When differences occurred an intermediate orientation was used.

Neuronal spike trains and stimulus markers were recorded using an FM recorder (Racal 4DS FM). All the computer analysis was done off-line. Only artefact-free data was used. All recordings with sudden changes in spike amplitude or spontaneous activity (not correlated with Glu injections) were discarded. At least three sets of artefact-free data were selected for each neurone (before, during and after iontophoresis). For better comparison all data sets had the same length (8 min of continuous recording) and included both the response peaks and the intervals when the stimulus was outside of the receptive field. The analysis of each simultaneously recorded neuronal group included: (1) The PSTH for each neurone stimulated with a moving light bar, computed before, during and after Glu injection, (2) Computation of total number of spikes and mean frequencies in all these situations, (3) Low and high resolution cross-correlograms (0.5 and 5 ms bin width) for each neuronal pair established before, during and after Glu injection, (4) Shift predictors corresponding to all cross-correlograms. The shift predictor was a necessary control required to isolate those stimulus-induced correlation effects due entirely to the stimulus affecting both neurones simultaneously (Perkel et al. 1967). Only if the original cross-correlogram had peaks or troughs absent in the shift predictor could the presence of an interneuronal connection be inferred. Cross-correlogram and PSTH peak-to-background ratios were used as a measure of interaction and response intensity. Peak value was defined as the number of events (intervals for cross-correlograms or spikes for PSTH) in the highest bin of the histogram. Background value represented the averaged number of events in the bins located outside of the operator defined boundaries of the peak. Since the larger number of intervals provided more reliable results, cross-correlograms built with 5 ms bin width were chosen for peak-to-background calculations. Cross-correlograms variability was tested only in the correlated pairs i.e. when the peak was at least twice as high as the background. The similar criterium was used to select the neurones responsive to visual stimulation.

RESULTS

Activity in the absence of Glu

The activity of 28 neuronal pairs were recorded in 6 kittens. Neuronal pairs were always recorded with separate electrodes with the possibility of simultaneous iontophoresis of Glu. The PSTH of 38 neurones (68%) showed responses to visual stimulation. The responses, however, were usually much weaker and less regular than those observed in adult cats (Kraszewski and Michalski 1989). For 18 of these neurones (32%) receptive field sizes and preferred stimulus orientations could not be clearly evaluated. All the receptive fields that could be plotted were located within the central 10^{o} of the visual field.

The cross-correlograms of 16 neuronal pairs (57%) showed the broad (between 60 and 200 ms), centrally located peaks, indicating shared input type of interaction (Michalski et al. 1983). In two pairs (7%) peaks were narrower than 3 ms and shifted from the correlogram centre by 0.5 and 1.5 ms. Such peaks indicate direct excitatory connection between recorded neurones. Unequivocal inhibitory correlations were not found.

Neuronal activity during Glu iontophoresis

Recording from a young kitten's cortex is difficult and iontophoretic current can damage the cells a lot easier in young than in adult cats. For this reason Glu injections always started with negligible

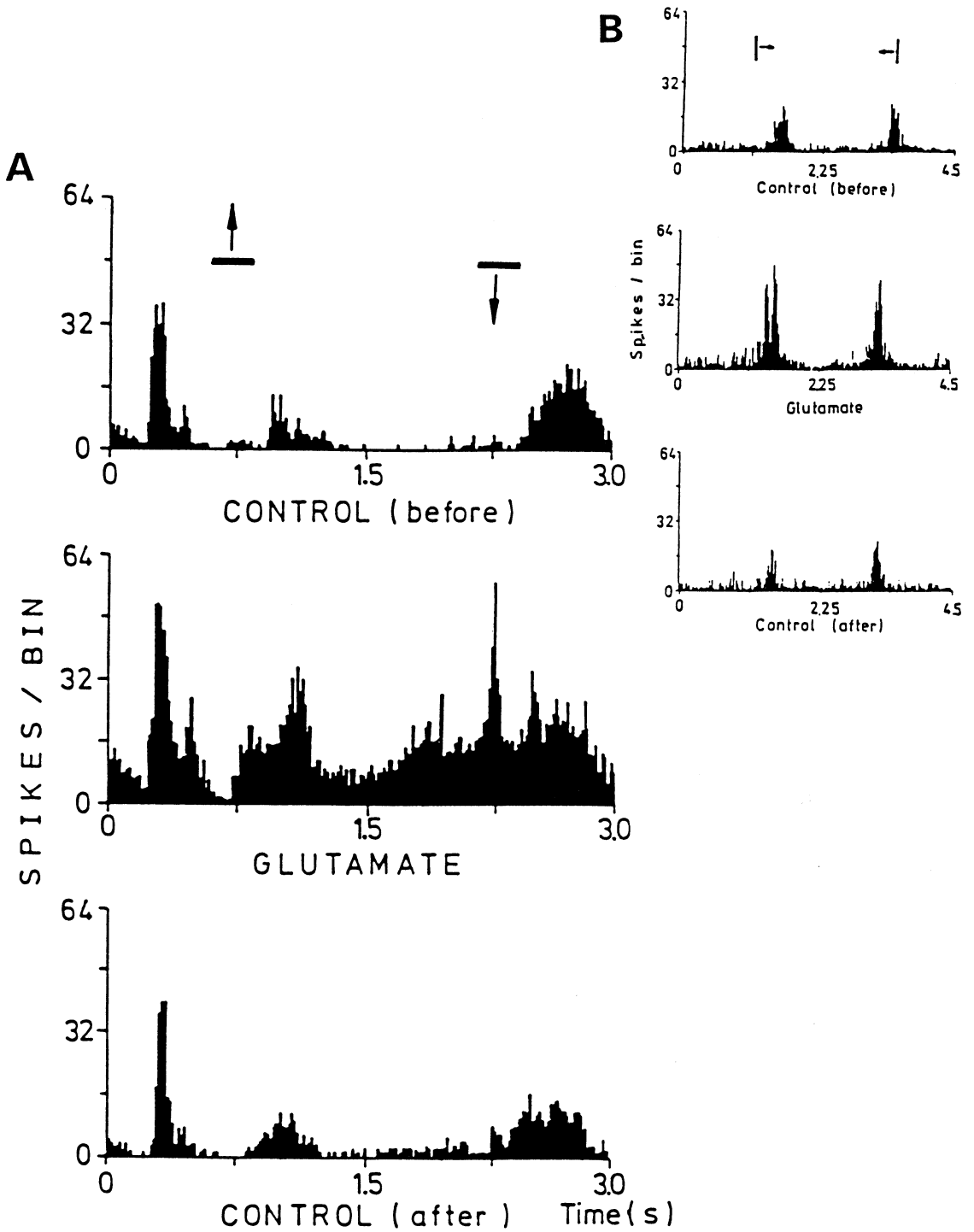

Fig. 1. A, typical effect of Glu injection on visual responses and spontaneous activity of young kitten neurone. The cell was stimulated with the light slit moving continuously (without periods of stationary stimulus) in the directions indicated by dark bars above the upper histogram. All PSTHs were built with 8 min long pieces of continuous recordings. Total numbers of recorded spikes were: before the injection - 902, during the injection-2859, after the injection - 790. Glu ejection current - 20 nA. Bin width - 30 ms. Inset B: The typical behaviour of adult cat's neurone in an identical experiment. Figure conventions the same as in 1A.

current that was then slowly increased until the response to Glu could be detected. Subsequent quantitative analysis showed that detectable responses were equivalent to 30-50% change in neuronal activity. In all responsive cells this change was obtained with ejection current between 20 nA and 50 nA. The cells that did not respond were tested with higher current (up to 120 nA) but this was never successful.

An increase in the mean frequency of spikes during Glu iontophoresis was observed in 40 neurones (71%). In 9 cases (16%) neurones decreased their activity and in 7 cases (13%) no effect could be seen. Figure 1A shows the responses of young kitten cells to visual stimulation before, during and after Glu iontophoresis. The main effect of injection was to increase the activity between the peaks visible in the middle histogram. The peaks themselves were only slightly elevated. For comparison, the inset 1B shows the typical behaviour of the adult cat's neurone (from our previous experiment). In adult animals the dominating effect of Glu iontophoresis was the enhancement of visual response. The narrow peak that appears in Fig. 1A, during Glu iontophoresis and the downward movement of the light slit, can be interpreted as an additional component of the neuronal response to visual stimulus evoked by Glu iontophoresis. This effect was not, however observed in other neurones.

Of the group of 40 neurones that increased their mean spike frequency during iontophoresis, 30 were responsive to the visual stimulation. The PSTH peak-to-background ratios were compared before and during Glu injections. In 12 cases (40%) the PSTH peak-to-background ratio decreased during the injection, in 5 cells (17%) it increased and in 13 cases (43%) it remained unchanged. Figure 2 compares the effects of Glu iontophoresis on spontaneous activity and visual responses of neurones in young and adult cats. The data from adult cats were taken from our earlier experiments (Kraszewski and Michalski 1989) in which the same anaesthesia and electrode configuration were used. It is apparent that, in young kittens, the excitatory effect of Glu iontophoresis affects mainly the background activity whereas, in adult cats, the peak of response to the light stimuli were more often enhanced. This difference was statistically significant (X^2 test, $P<0.05$). Amongst those cells that were inhibited during Glu iontophoresis both the peaks of responses to visual stimulation and the background activity were lowered. In these cases the measurements of the peak-to-background ratios were unreliable due to the low spike activity.

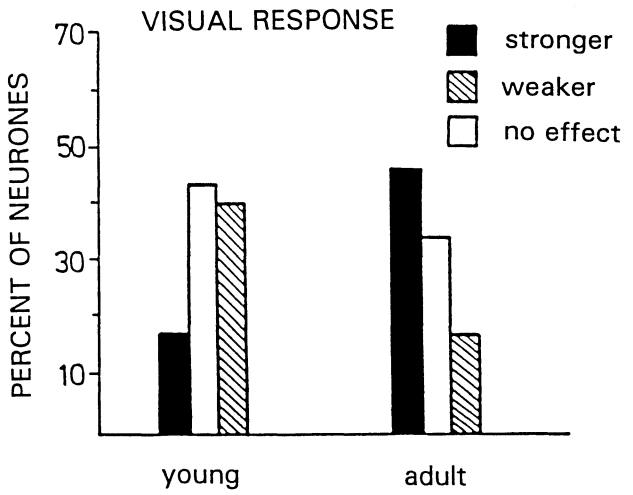

Fig. 2. The distribution of different effects of Glu stimulation among young and adult cat neurones. Visual responses were defined as peak-to-background ratios of visual PSTHs and were compared before and during the Glu injection. Thirty neurones were analysed in young and 40 in adult animals. All neurones were visually responsive before the injection and all showed the excitatory response to Glu. Adult cat data were obtained with identical electrodes and anaesthesia (Kraszewski and Michalski 1989). The difference between young and adult cat's data is statistically significant (X^2 test, $P<0.05$).

Effects of glutamate injection on consecutive neuronal activity

In 12 neurones (21% of all cells) the alterations of PSTHs persisted after cessation of Glu iontophoresis. In all neurones this effect was observed for 15 min and in some cases up to 20 min after termination of the injection. Five of these neurones were originally unresponsive to visual stimulation but showed the weak responses after the injections. These responses were weak, but reproducible dur-

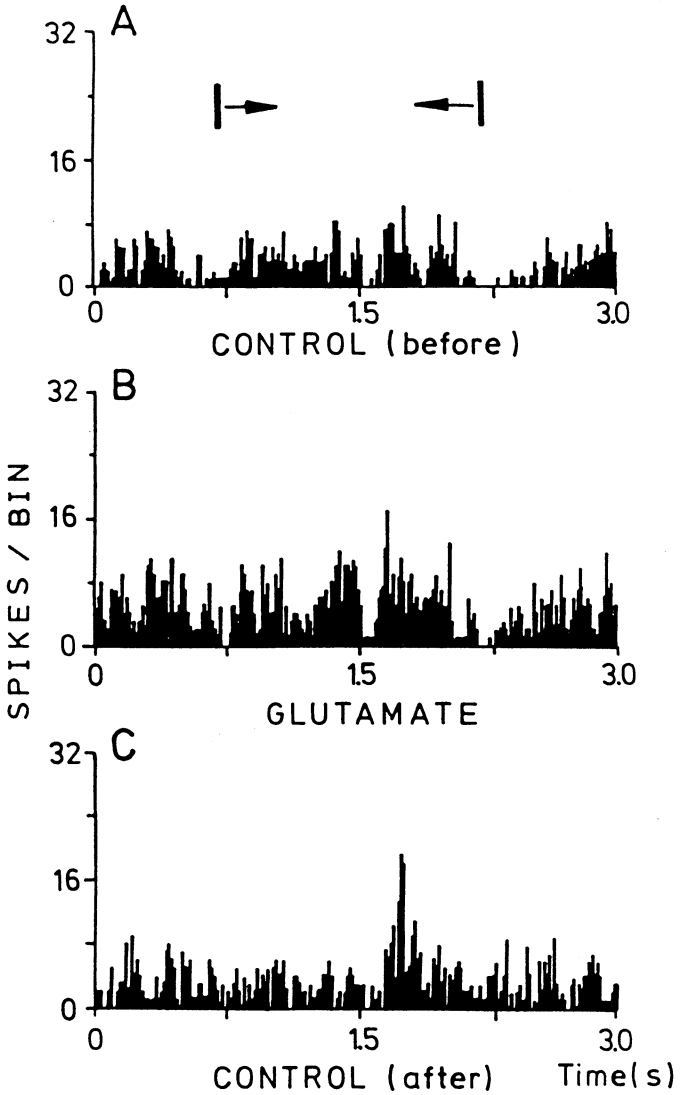

Fig. 3. Weak response to visual stimulation appears after Glu injection. The response is very immature but, after Glu iontophoresis, similar stimulus related peaks appeared in PSTHs of 5 previously unresponsive neurones. Conventions the same as in Fig. 1. All histograms were built with 8 min long pieces of recordings. Total numbers of recorded spikes were: a-545, b-927, c-654. Glu ejection current-20 nA. Bin width - 30 ms.

ing consecutive presentation of visual stimuli and were better than responses recorded during iontophoresis. This was possibly due to the fact that all these neurones increased their activities during Glu injection and their visual responses were hidden in the elevated background. Figure 3 shows the PSTH of one of these cells taken (A) before, (B) during and (C) after the Glu iontophoresis. Before the injection this neurone could be either classified as unresponsive or the weak inhibitory trough could be recognized as the response. During the injection the mean spike frequency increased from 1.13 spikes/s to 1.93 spikes/s. After termination of Glu iontophoresis the mean frequency dropped down to 1.37 spikes/s and the peak of the excitatory response appeared in the PSTH. The off-line re-examination of the recording excluded the possibility of unreliable triggering. The stimulus projector was also working continuously during this analysis to avoid any accidental change. In 6 other neurones the weak visual responses were enhanced after Glu injections. The PSTH peak-to-background ratios of these neurones increased between 50% and 150% with the mean of 75%. Five of these cells increased their activity during iontophoresis, whereas one was inhibited.

It was demonstrated that in adult cats spontaneous changes in PSTHs peak-to-background ratios could reach 130% of the original value within 1.5 h (Kraszewski and Michalski 1989). Because of the technical difficulty associated with long recordings this test was not repeated in young kittens. Instead, the level of 130% was used as a threshold above which the effect of 15 min long injection of Glu was classified as significant. In 4 young kitten neurones (7% of all recorded cells) the alterations of PSTHs, after Glu injection, exceeded this level.

Smaller PSTHs alterations, frequently observed in young kittens, could also be significant if they showed a dominating trend. For this analysis peak-to-background ratios were measured in PSTHs of all neurones. A value of "1" was used for visually unresponsive cells. The mean peak-to-background ratio before Glu injection was 3.5, after the injection it increased to 3.7. To evaluate the probability that changes occurred randomly, the populations of peak-to-background ratios obtained before and after Glu iontophoresis were compared using a Student's *t*-test for paired data. The difference was statistically significant ($P<0.009$).

Among 28 simultaneously recorded pairs, in 16 cases (57%) neuronal activity was correlated (cross-correlogram peaks were at least twice as high as the background) and the shift predictors were flat

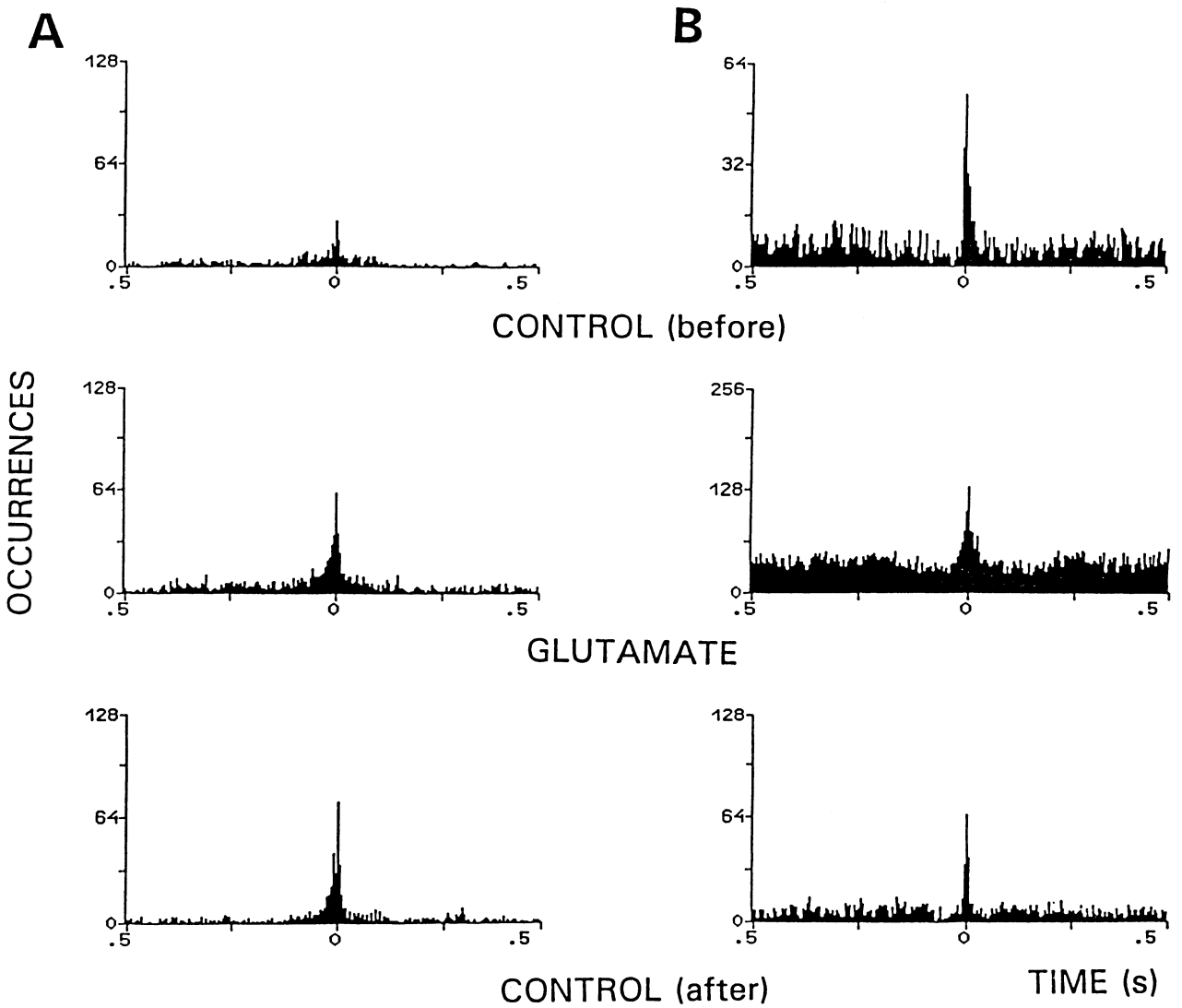

Fig. 4. Cross-correlograms of two neuronal pairs: with the strong (A) and weak (B) effect of glutamate injections. Cross-correlograms were recorded before (upper row) during (middle row) and after the cessation (lower row) of Glu iontophoresis. Neurones in both pairs showed the strong common input type of firing coordination (Michalski et al. 1983). Note the mature appearance of cross-correlogram peaks. Glu injection increased the mean rate of firing to 275% and 104% in pair A and to 374% and 170% in pair B. After the cessation of iontophoresis neuronal activity was 107% and 115% of the original values in pair A and 104% and 89% in pair B. Bin width - 5 ms.

indicating, that the interactions were genuine, due to the inter-neuronal connections and not to the simultaneous stimulation of neurones. For these pairs the variability coefficient (V) was calculated according to the formula:

$$V=(S2-S1)/\text{mean}S=2(S2-S1)/(S2+S1)$$

where S1 and S2 are the cross-correlogram peak-to-background ratios measured according to the formula given in the method section. A 5 ms bin width was used to compute the correlograms. S1 was measured before Glu iontophoresis and S2 after its termination. The parameters of visual stimulation were always kept identical during this test. In adult cats it was found that such variability coefficient varied by less than 100% during 1.5 h long observation (Kraszewski and Michalski 1989). This value was used as a threshold, above which the effects of 15 min long Glu injections were classified

as significant. If the same criterium is used in young kittens, only 1 pair (3.6%) will reach it (in adult cats, in the much larger sample, 3% of pairs showed the alterations higher than 100%).

Figure 4 shows the cross-correlograms of 2 neuronal pairs (A and B) recorded before, during and after Glu iontophoresis. Neurones in both pairs showed correlated activity with strong, centrally located peaks suggestive of a shared input to both cells. Unlike PSTHs the cross-correlogram peaks were high and regular, their appearance did not differ from adult cats cross-correlograms. Pair A showed the strongest change in the firing correlation after Glu injection. During iontophoresis one neurone in this pair increased its mean rate of firing to 275% of the original value, the other only to 104%. Both the peak and the background of the cross-correlogram were elevated, due to the increased activity, but the peak-to-background ratio increased only marginally. After the cessation of Glu stimulation the activity of the first neurone dropped to 107% and the other to 115%. The cross--correlogram background came back to its original value, but the peak remained high and the coefficient of variability reached 100%. Pair B exemplifies smaller changes. During Glu stimulation both neurones of this pair strongly increased their activity (to 374% and 170%). The corresponding cross-correlogram shows an increase of both peak and the background level (the change of scale was necessary here) and the peak-to-background ratio is clearly reduced. After the cessation of iontophoresis neuronal activity returned to the levels of 104% and 89% of the original values. The cross-correlogram peak, however, was clearly higher than before the injection and the background was slightly lover. The coefficient of variability in this pair was 30%.

The analysis of trends in the entire sample of correlated pairs showed that cross-correlogram peak-to-background ratios increased after Glu injections in 7 pairs (44% of pairs - the average increase was 44% in this group), remained unchanged in 5 (31%) and decreased in 4 pairs (25% - mean decrease was 43%). Apparently more pairs showed a strengthened correlation. But, when the populations of peak-to-noise ratios calculated before and after Glu injections were compared in the entire sample of pairs, the mean difference was only 14% and was statistically insignificant (Student t-test for paired data, $P<0.085$).

In order to find whether increased or decreased neuronal activity during Glu injection can be linked with the subsequent changes in cross-correlograms, a group of 7 neuronal pairs was selected in which either both neurones increased their activity during Glu injection or one cell was excited and the other unresponsive. Amongst these pairs the cross-correlogram peak-to-background ratio increased after the termination of injection in 3 cases, decreased in 2 and remained unchanged also in 2 pairs.

The assumption that increased activity during Glu iontophoresis may be directly linked with cross-correlogram alterations was also contradicted by the fact that in the neuronal pairs that showed the strongest increase and the strongest decrease of cross-correlogram peak after iontophoresis, all neurones increased their spike activity during Glu injection.

DISCUSSION

Table I shows the percentages of responsive neurones and different coordination types encountered in young and adult cats, under the same Nembutal anaesthesia. Only neuronal pairs recorded with separate electrodes and with an electrode configuration identical to that presently used were selected from the adult cats data (Kraszewski and Michalski 1989). In spite of the difference in the

TABLE I

Percentage of responsive neurones and different correlation types encountered in young and adult animals

Cats	Responsive neurones (%)	Pairs with shared input (%)	Pairs with direct excitatory connections (%)
Adult	86	55	6
Young	66	57	7

percentages of responsive units between young and adult cats (X^2 test, $P<0.05$), which was also reported in many earlier experiments (for review see Sherman and Spear 1982), the distributions of shared input and direct excitatory cross-correlograms in young and adult animals were strikingly similar. Not only the numbers but also the heights of cross-correlogram peaks and their smooth, regular shapes were similar to the adult cats data and very different from immature PSTHs (compare Fig. 1 with Fig. 4). Clearly cross-correlograms and PSTHs show the different aspects of cortical connectivity that behave differently in the process of development. More data is needed to explain this phenomenon, but it should by mentioned that some developmental processes were observed on cross-correlograms as well. Tsumoto and co-authors (1987) found that, in the young kitten cortex, shared input correlations were present even between neurones with very different preferred orientations, whereas in older animals correlated pairs usually had similar preferred orientations. The presence of developmental processes is also indicated by the fact that in binocularly deprived cats the number of shared input correlations was significantly reduced (Michalski et al. 1984).

We believe that the large number of centrally located, broad correlogram peaks - interpreted as shared input to recorded cells - reflects some unknown, but basic feature of the organization of neuronal network. These peaks are very common in the cortex of adult cats (Michalski et al. 1983) and present results show that they occur with the same frequency in 4 weeks old kittens. Broad, centrally located correlogram peaks are also very similar in shape and frequency of occurrence in different cortical areas (Dickson and Gerstein 1974, Michalski at al. 1983, Michalski and Czarkowska, unpublished observations). In subcortical structures, on the other hand, cross-correlograms have different shapes (Stevens and Gernstein 1976, Michalski and Wróbel 1994). Ts'o et al. (1986) suggested that in the visual cortex broad, centrally located cross-correlogram peaks reflect the activity of horizontal fibres linking the cortical columns with similar preferred orientation of the visual stimulus. Horizontal fibres with periodic pattern of dense arborizations, exactly as needed for such connections, were described in the visual cortex by Gilbert and Wiesel (1989).

On the grounds of coincidence theory of synaptic modification (Bienenstock et al. 1982) we expected that neurones that were interconnected, simultaneously responsive to visual stimulation and simultaneously stimulated with Glu injections would modify their interactions. Cross-correlograms however, showed relatively few modifications. Only small number of neuronal pairs revealed the cross-correlation changes after Glu injection and cross-correlogram analysis failed to show the significant difference between young and adult animals. Even if the criteria of a significant change (higher than spontaneous variability measured within 1.5 h) was set too high, the analysis of small changes also indicates that there were no significant trends altering the cross-correlogram strength. It should be remembered however, that synapses that form the link between the two specific neurones may constitute a very small percent of the total number of synapses of analysed neurone. The location of these synapses is unknown. The fact that the neurone increases its activity during Glu iontophoresis does not mean that these specific synapses are affected. In fact the probability of stimulating with iontophoretically injected Glu these specific synapses may be very low (see the discussion in Kraszewski and Michalski 1989). If this probability is low enough, it can also mask the difference between young and adult animals. Thus, the cross-correlation analysis may be comparable to intracellular studies but there was an important difference between the local microiontophoresis used in our experiment and the method of testing the drugs in slice experiments (adding to the bath) that affected much larger number of synapses in much more uniform way.

PSTHs, on the other hand, showed several effects of Glu injection that differed in young and adult animals. In young kittens Glu iontophoresis caused excitation mainly by enhancing the sponta-

neous activity of a neurone, while responses to visual stimulation were less significantly enhanced. The similar effect was found by Wolf and co-authors (1986). Ramoa and co-authors (1987), on the other hand, found that in young kittens, during Glu iontophoresis, visual responses appeared in previously unresponsive neurones. We observed this effect after the cessation of iontophoresis, but we can not exclude the probability that the high level of background activity during iontophoresis masked the weak responses. In adult cats, Glu iontophoresis typically enhanced the visual responses more than spontaneous activity (Hess and Murata 1974, Kraszewski and Michalski 1989). The simplest explanation of the difference between young and adult animals may be that, in young cats, visual responses were already saturated so that Glu increased only spontaneous activity. In most of our neurones, however, the responses were not saturated and it was possible to enhance them by increasing the stimulus contrast. The other possible explanation is based on the assumption that the adult cat neurones, showing strong visual responses, have a large proportion of their synapses 'wired properly' so thay can participate in producing the visual responses. In young kitten neurones, with weak, irregular responses, this proportion may be much smaller. Therefore, the probability of affecting 'response-producing' synapses with Glu injection must be higher in adult cats. In young animals the synapses unrelated to the visual response are more likely stimulated. This effect may be partly responsible for the observed differences, but other experiments data indicate that the organization of glutaminergic connections differ in young and adult animals. Fox and co-authors (1989,1990) found that the contribution of NMDA and non-NMDA postsynaptic receptors of glutamate synapses to visual responses is different in young and adult cats. The proportion of these two types of receptors also changes with age (Bode-Greuel and Singer 1989, Gordon et al. 1991, Reynolds and Bear 1991)

Interestingly, the proportion of neurones that increased and decreased their spike rates during Glu stimulation was almost identical in young and adult cats. The inhibitory response to Glu is usually interpreted as demonstrating the excitatory stimulation of neighbouring neurones which in turn inhibit the analysed cell (Hess and Murata 1974, Hicks 1983). If this interpretation is right, our results indicate that this mechanism is already well developed in 4 week old kittens.

In 21% of neurones we found an enhancement of the visual response that persisted after Glu injection. All these neurones showed very weak or no response before Glu iontophoresis but, after the injection, an increase in response occurred. In 7% of neurones these alterations exceeded the level of spontaneous variability measured in adult cats within 1.5 h. We failed to show similar effect in adult cats (Kraszewski and Michalski 1989). We assume that the alterations were not caused directly by exogenous Glu remaining in the nervous tissue. A fast mechanism for Glu inactivation exists and the effect of iontophoretic application of Glu disappears within seconds after termination of efflux (Hess and Murata 1974, Kemp and Sillito 1982). Thus, the efficiency of the visual input had to be altered. Persistent changes in neuronal responses to visual stimulation were observed in previous experiments on anaesthetized and paralysed cats (Frégnac et al. 1984, Greuel et al. 1988). In those experiments, projection of preferred and non preferred stimuli was combined with iontophoresis of excitatory or inhibitory substances. Changes in cell stimulus preferences were observed after several minutes of such conditioning. Visual stimulation alone was not effective enough to provoke plastic changes in the cortex of anaesthetized and paralysed kittens (Buisseret et al. 1978, Freeman and Bonds 1979). It should be noted here that visual responses in young kittens were usually much weaker than those observed in adult animals. In our experiments they were also paired with smaller Glu injections (due to the higher sensitivity of young kittens neurones!). The fact that, in spite of this, the alterations of visual responses after Glu injections were higher in young than in adult animals indicates that the threshold for synaptic modification is lower in young cats (Bienenstock et al. 1982) and supports the

aforementioned observations that glutaminergic input in young kittens and adult cats differ significantly (Bode-Greuel and Singer 1989, Fox et al. 1989, 1990, Gordon et al. 1991, Reynolds and Bear 1991).

To increase the probability of positive results we tested our cells within the first 15 min after the termination of iontophoresis. Thus, the time course was comparable with STP as observed in rat hippocampal slices, with possible component of developing LTP. To distinguish between these two phenomena further experiments are needed, based either on the very long recordings (which are technically difficult in young kittens) or the selective activation of different Glu receptors (Bashir et al. 1993, Behnisch and Reymann 1993, Musgrave et al 1993).

Our results indicate that, in spite of the immature responses to visual stimulation, inter-neuronal interactions in 4-6 weeks old kittens are relatively well developed. The percentage of cells with correlated firing is comparable with the adult cats value. Cross-correlogram peaks look mature, they are high and smooth. A local increase of excitation in the visual cortex, caused by iontophoretic ejection of Glu, can provoke changes in synaptic efficiency that are different in young and adult animals. These changes were reflected in PSTHs and cross-correlograms differently. In PSTHs Glu iontophoresis increased the peaks of visual responses in adult cats and the background in young kittens. PSTHs alterations persisted the cessation of injection in young but not in adult animals. Small percent of cross-correlograms in both groups of animals showed the differences that persisted the termination of iontophoresis.

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