

All-or-none Excitatory Postsynaptic Potentials in the Rat Visual Cortex

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Abstract

Intracellular recordings were obtained from supragranular neurons in slices of the rat visual cortex. In ~25% of the cells large (0.5–1.6 mV) excitatory postsynaptic potentials (EPSPs) of constant amplitude were observed after minimal, presumably single-fibre stimulation. The amplitude variance of these large EPSPs was surprisingly small and within the range of the variance of the noise. These EPSPs could be reduced in amplitude by paired-pulse and low-frequency stimulation or by raising extracellular Mg^{2+} concentration. Reduced EPSPs could either continue to behave as all-or-none responses, or they could fluctuate between several amplitude levels. Conversely, responses where the amplitude fluctuated from trial to trial under control conditions could be converted into large all-or-none responses by paired-pulse facilitation. This indicates that the large all-or-none EPSPs were composed of several subunits, probably reflecting the action of several different release sites. It is concluded that these release sites are either independent and operate with a probability close to 1 or, if operating with a lower probability, are coordinated by a mechanism which synchronizes release. Several observations suggest that release probabilities can switch from values close to 1 to 0 with repetitive stimulation or high Mg^{2+} concentration. Thus, a substantial fraction of single-fibre inputs to supragranular cells possess synapses which operate with high synaptic efficiency and extremely low variance under control conditions but can undergo drastic changes in efficacy when release probabilities are interfered with. Such modifications of release probability could serve as an effective mechanism to regulate the gain of synaptic transmission.

Introduction

Synaptic transmission in the neocortex has become a subject of considerable interest, especially since there is now good evidence for use-dependent long-term modifications of synaptic efficacy (for reviews and references see Tsumoto 1992; Artola and Singer, 1993). A powerful tool for the investigation of synaptic transmission and its modification is the quantitative analysis of responses evoked by a single fibre. In several recent studies using dual recording techniques (Kang *et al.*, 1988; Thomson *et al.*, 1988, 1993; Mason *et al.*, 1991; Thomson and West, 1993) or focal stimulation of neighbouring cells (Stern *et al.*, 1992), it has been shown that in the neocortex, excitatory postsynaptic potentials (EPSPs) or excitatory postsynaptic currents (EPSCs) evoked by single afferent fibres can vary considerably in amplitude, ranging from <0.05 to >2 mV whereby a substantial portion of these EPSPs (20–30%; Thomson *et al.*, 1988; Mason *et al.*, 1991) had amplitudes >0.5 mV. In the majority of cases these large EPSPs exhibited considerable amplitude fluctuations (Stern *et al.*, 1992; Thomson and West, 1993; Thomson *et al.*, 1993), but on rare occasions large EPSPs have also been observed with remarkably constant amplitudes (Thomson and West, 1993).

Most of the studies on use-dependent synaptic plasticity in the neocortex have examined modifications of excitatory input to supra-

granular neurons (see above). In order to obtain more detailed information about the synaptic input to these cells we applied the technique of minimal stimulation in slices of rat visual cortex to record single-fibre responses in supragranular neurons. In the course of this study we encountered large EPSPs which exhibited surprisingly little amplitude fluctuations and resembled those observed by Thomson and West (1993) in infragranular cells of the somatosensory cortex. To further characterize these EPSPs we examined their modifiability by applying paired-pulse and low-frequency stimulation or by raising the extracellular magnesium concentration ($[Mg^{2+}]$), and analysed the fluctuation of response amplitudes.

Some of these results have been published in abstract form (Volgushev *et al.*, 1994).

Materials and methods

Visual cortex slices of adult rats (4–6 weeks) were prepared by conventional methods (Artola and Singer, 1987). Standard perfusion medium contained (in mM) 124 NaCl, 5 KCl, 2 $CaCl_2$, 1.5 $MgSO_4$, 1.25 $NaHPO_4$, 26 $NaHCO_3$ and 10 D-glucose bubbled with 95% O_2 and 5% CO_2 . Intracellular recordings were obtained with glass

microelectrodes (60–100 M Ω) filled with 3 M potassium acetate. Only cells with stable resting membrane potential, more negative than -70 mV (mean -79.0 ± 4.8 mV, $n = 71$) and stable input resistance (mean 66 ± 6 M Ω) were recorded and their responses were analysed. Here and throughout the text means are given together with \pm SEM. Statistical significance was evaluated by the t -test; differences were considered to be significant at $P < 0.05$, if not stated otherwise.

Electrical stimuli (1–10 V, 0.04–0.1 ms) were applied with a frequency of 0.03–0.06 Hz through two bipolar tungsten stimulation electrodes positioned laterally (layers I–II) and below (layer IV) the recording site respectively. The test stimuli were applied as double pulses with an interval of 50 ms between the first and second stimulus of the pair. Usually stimulus strength was adjusted to induce EPSPs with occasional failures of fibre activation (Fig. 1a). Stimuli of these intensities corresponded to the beginning of the first plateau of the stimulus–response curves (Fig. 1d,e) (Voronin *et al.*, 1977; McNaughton *et al.*, 1981; Stern *et al.*, 1992) and with all likelihood activated a single presynaptic fibre. After conventional amplification (Axoclamp-2A, Axon Instruments, Foster City, MI; or EPC-7, List-electronic, Darmstadt, Germany), data were digitized at 10 kHz and fed into a computer (PC-386; Labmaster, TL-1 DMA interface and pCLAMP software; Axon Instruments). Some records were smoothed by a computer program which averaged 5–9 consecutive points. EPSP amplitudes were measured as the difference between average voltage in two windows of 1–3 ms duration, one being positioned immediately

before the response or in the pre-stimulus period and the other one over the peak or over the initial slope at two thirds of the peak latency of the averaged response. The two measures yielded similar results with respect to the shape of amplitude distributions and the effects of paired-pulse and repetitive stimulation. The measure of the initial slope allowed us to exclude a possible contribution of polysynaptic (including inhibitory) components discernible in some cases as notches on the EPSP slope around its peak. The noise was measured in windows of the same duration and temporal separation that were both placed in the pre-stimulus period.

EPSP amplitude distributions were plotted for the periods during which averaged response amplitudes were stable. These could comprise the whole recording period if responses were not depressed by continuous low-frequency testing. In cases where low-frequency depression occurred, we selected for analysis stable regions before the onset and after the stabilization of depression respectively (Kandel, 1976). These regions had to be free of statistically significant drifts; only periods in which averages of 10–20 consecutive responses did not differ from all other points within the selected period at the $P < 0.01$ level (t -test) were accepted for further analysis. Standard deviation of response amplitudes (excluding failures) was calculated and compared with the standard deviation of the noise.

In experiments in which $[Mg^{2+}]$ was raised in the perfusion medium, responses were first recorded under control conditions for ~ 1 h. Then $[Mg^{2+}]$ was raised from 1.5 to 4.5 or 6 mM to decrease the probability of transmitter release (Katz, 1969) and after 15 min

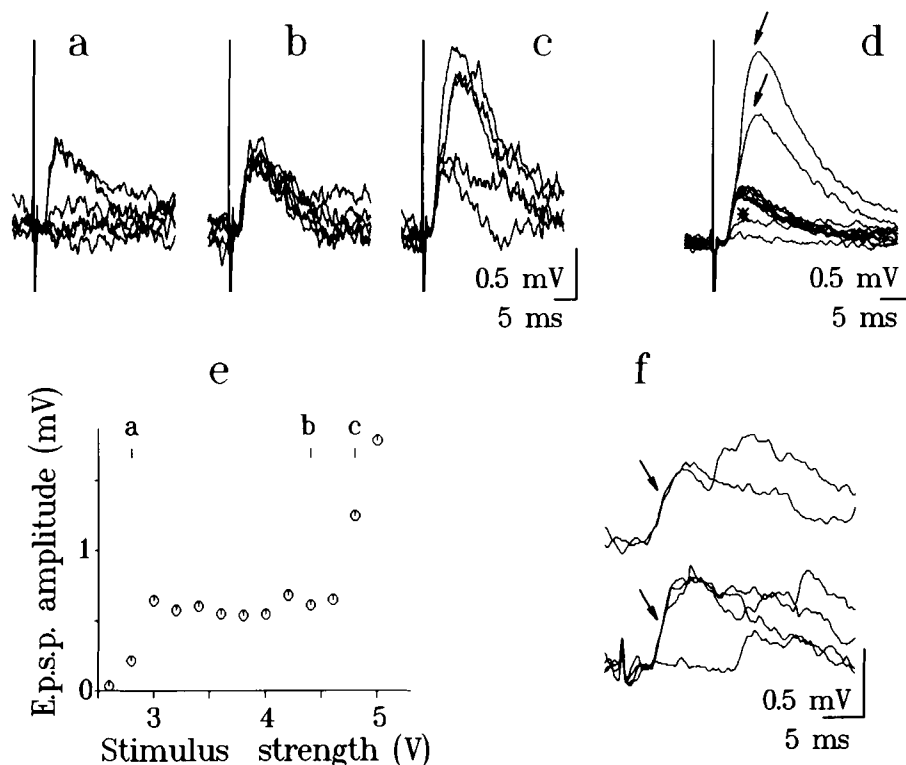


FIG. 1. Excitatory postsynaptic potentials recorded from a layer II–III cell of the rat visual cortex. (a–c) Superimposed responses to six consecutive stimuli of increasing intensity. Increasing stimulus strength from 2.8 V (a) to 4.4 V (b) abolished failures but did not change response amplitude. At 4.8 V (c), responses of larger amplitude appeared occasionally. (d) Averaged responses ($n = 6$) of the same cell evoked by stimuli of increasing strength. Note the slightly longer latency of the additional component in the two larger responses which appeared at high stimulus intensities (arrows). The small response indicated by the asterisk has been obtained at minimal stimulation intensity where failures were present (see a and e). (e) Stimulus–response curve for the same cell with points corresponding to the responses shown in (a–c) marked by the respective letters. The small response below the plateau level (a) is due to occasional failures occurring at the low stimulus intensity. (f) Examples of spontaneous depolarizing potentials (arrow, upper traces) resembling the large EPSPs evoked by minimal stimulation (arrow, lower traces).

the responses were recorded again. In all cases, stimulation parameters were kept constant during the control period and after elevation of extracellular $[Mg^{2+}]$. Only cells with no significant drift of EPSP amplitude during the control period were subject to high $[Mg^{2+}]$.

Results

Intracellular recordings were obtained from 71 neurons in layers II–III of rat visual cortex slices. EPSPs were evoked by minimal intracortical stimulation, the intensity of which was adjusted to produce occasional failures and corresponded to the beginning of the first plateau of the stimulus–response curve. In the example documented in Figure 1, increasing stimulus strength from 2.8 V (Fig. 1a) to 4.6 V (Fig. 1b) led to the disappearance of failures without changes in response amplitude. At 4.8 V (Fig. 1c), responses of larger amplitude appeared occasionally, presumably resulting from the recruitment of another presynaptic fibre. The stimulus–response curve (Fig. 1e) has a clear plateau in the interval from 3.0 V to 4.6 V, where response amplitude was independent of stimulus strength. The lower amplitude of the averaged response at 2.8 V (point 'a' in Fig. 1e and the averaged response marked by an asterisk in Fig. 1d) is due to the occurrence of several failures but not to an amplitude decrease of the EPSPs (Fig. 1a). The presence of a plateau in the stimulus–response curve indicates that in all likelihood the respective stimuli had activated a single presynaptic fibre (Voronin *et al.*, 1977; McNaughton *et al.*, 1981; Stern *et al.*, 1992). In some cells, spontaneous EPSPs were observed which closely resembled those evoked with minimal stimulation (Fig. 1f).

The EPSPs evoked with minimal stimulation which were analysed

in the present study are likely to be monosynaptic (Mason *et al.*, 1991; Stern *et al.*, 1992; Thomson and West, 1993) because their latencies were short (1.8–4.5 ms for different cells) and remained stable (within 1 ms) during paired-pulse and repeated stimulation.

Two distinct response types were observed following stimulation in layers II–III and layer IV and there appeared to be no systematic relation between response type and stimulation site. The first type of response is distinguished by its large amplitude (0.5–1.6 mV; mean 0.91 ± 0.06 mV) and minimal amplitude fluctuations. It will be addressed as 'large all-or-none EPSP' throughout the text. Such responses were evoked in 18 of 71 cells from 23 stimulation sites (Figs 1a,b,f and 2a). The second type of response consisted of smaller EPSPs exhibiting considerable amplitude fluctuations ($n = 97$). These responses were excluded from further analysis.

The membrane potential and input resistance of the 18 neurons with large all-or-none EPSPs did not differ from the rest of the sample (-78 ± 5 versus -79 ± 5 mV and 66 ± 5 versus 66 ± 8 M Ω). For 17 out of the 23 inputs evoking large all-or-none EPSPs, the response amplitudes remained stable over the whole recording period of 0.5–4 h (Fig. 2) but for 6 inputs (27%) mean response amplitude diminished due to repetitive stimulation in spite of the low test frequency used (0.03–0.06 Hz). Since responses recovered upon cessation of testing (data not shown), we interpret this stimulation-dependent reduction (Fig. 3) as low-frequency depression (Kandel, 1976).

Amplitude histograms of the large all-or-none responses exhibited only a single peak which had nearly the same variance as the noise (Figs 2c, 3c and 4c). This shape of the amplitude histogram was independent of sample size and was similar for small and large samples (Fig. 2c, 1 and 2) in experiments ($n = 3$) in which stable

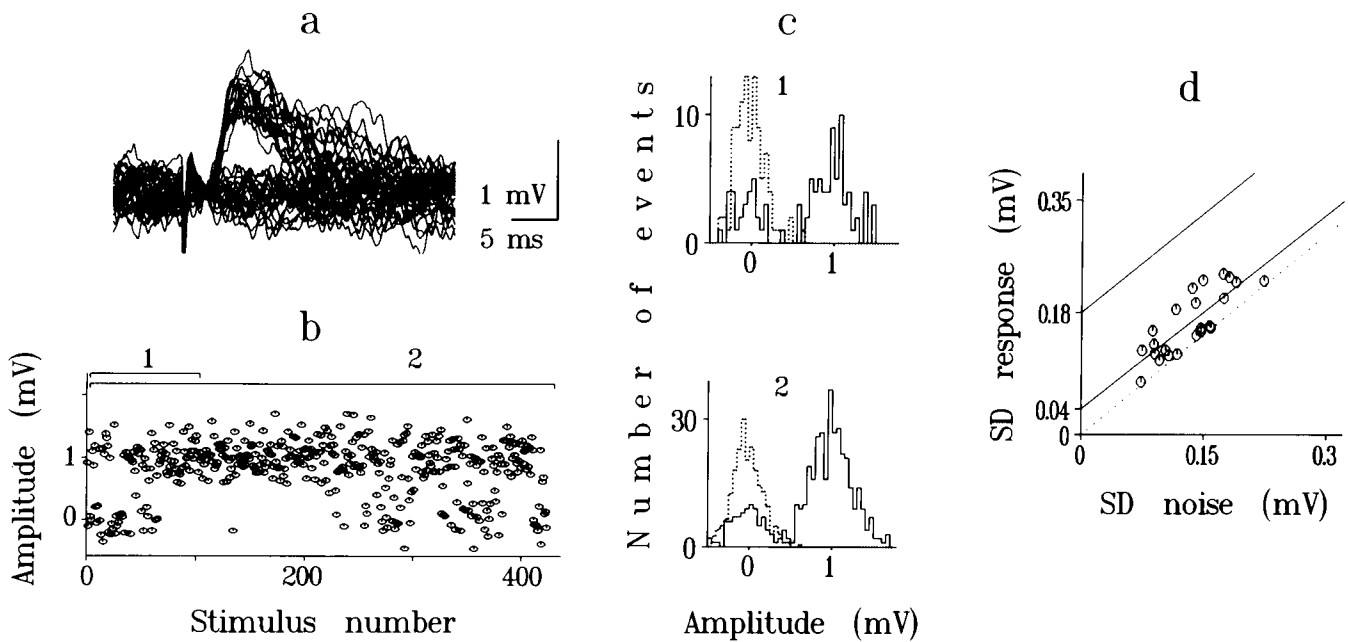


FIG. 2. Example of a large all-or-none EPSP. (a) Superposition of 40 consecutive responses. (b) Amplitudes of EPSPs (ordinate) evoked by repeated stimulation at 0.05 Hz. Each point represents the amplitude of a single response. Response regions from which data were taken for statistical analysis (see c and d) are marked by horizontal bars 1 and 2. (c) Amplitude distributions of the initial 100 responses (1, upper) and of the whole sample (2, bottom, $n = 428$). In this and the following figures abscissa scaling gives the mean amplitude of responses; noise distributions are shown with a dotted line. (d) Scatter diagram with the standard deviation of response amplitudes (ordinate) plotted against the standard deviation of the noise (abscissa). Each point represents a single cell. The dotted line indicates the main diagonal. The continuous line intersecting the ordinate at 0.04 represents the regression line computed from the data points. The continuous line intersecting the ordinate at 0.18 represents the regression line calculated from a sample of 20 EPSPs with fluctuating response amplitude.

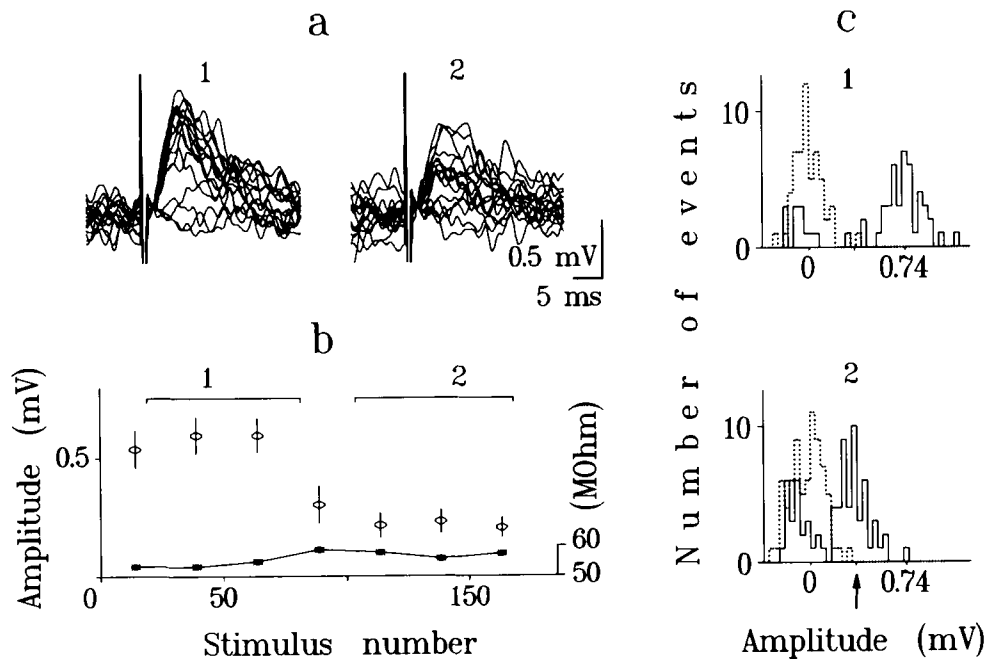


FIG. 3. Low-frequency depression of large all-or-none EPSPs. (a) Superposition of 15 consecutive responses from regions 1 and 2 in (b). (b) Time course of amplitude changes (left ordinate) during low-frequency depression. Abscissa: number of stimuli presented at 15 s intervals. Open circles with bars represent mean amplitudes ($n = 23$) with SEM. Regions of stable responses from which data were taken for statistical analysis (see c and d) are marked by horizontal bars 1 and 2. Filled symbols connected with a line represent input resistance of the cell (right ordinate). Note that the input resistance did not decrease during low-frequency depression. (c) Amplitude distributions of EPSPs (continuous lines) and the noise (dotted lines) from regions 1 and 2. Arrow below abscissa in (c,2) shows the mean amplitude of reduced responses (0.36 mV).

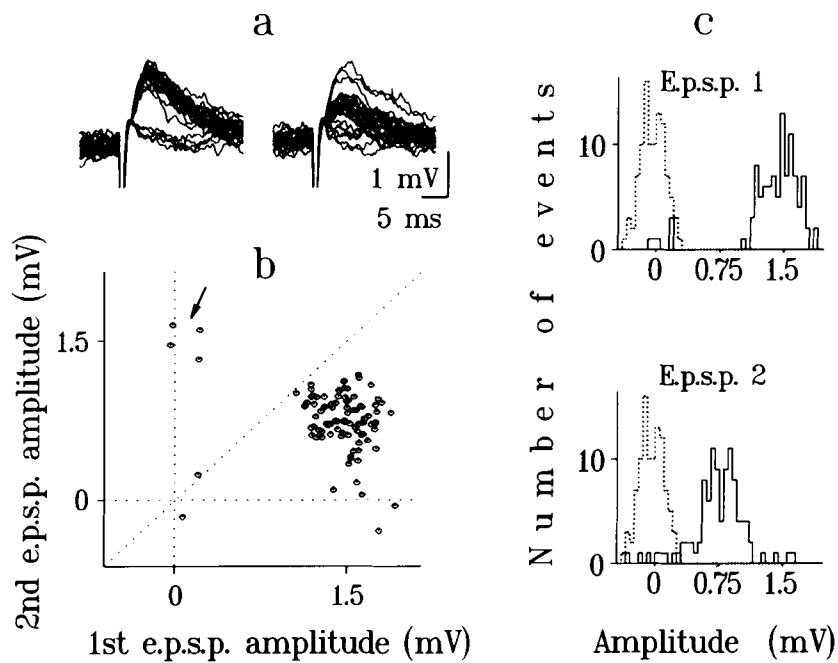


FIG. 4. Paired-pulse depression of large all-or-none EPSPs. (a) Superposition of 30 responses to the first (left) and the second (right) stimulus in a pair. (b) Scatter diagram with the amplitudes of the second EPSP in each pair (ordinate) plotted against those of the first EPSP (abscissa). Paired-pulse depression is reflected by the cloud of dots below the diagonal. The four large second responses (arrow) were evoked after response failures to the first stimuli. Their amplitudes were equal to those of the first EPSPs, suggesting that in these cases, response failures to the first pulse were due to activation failures of the presynaptic fibre. (c) Amplitude distributions of the EPSPs evoked by the first (upper graph) and the second (lower graph) pulse in a pair. Dotted histograms show the corresponding noise distributions.

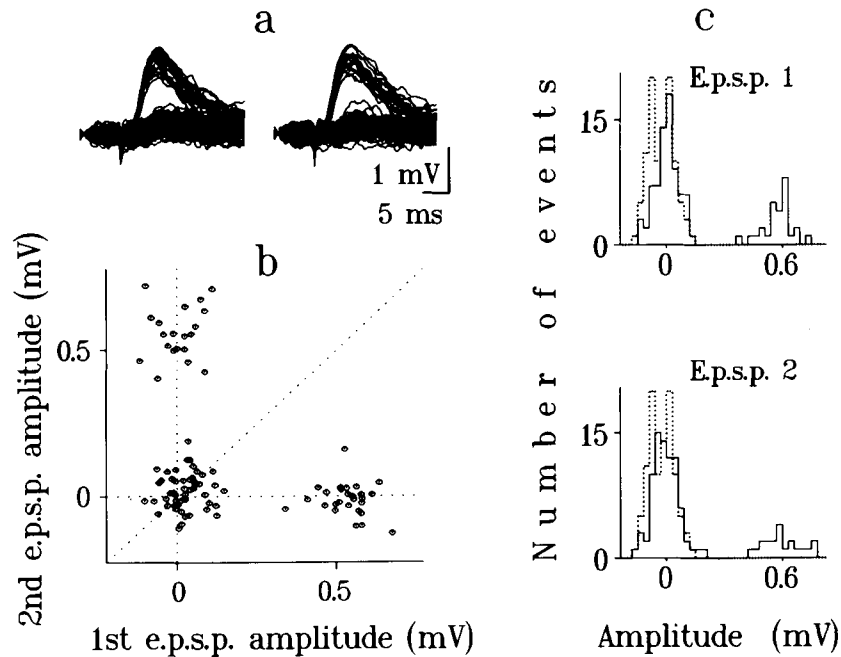


FIG. 5. Complete paired-pulse depression of large all-or-none EPSPs. (a) Superposition of 100 single responses to the first (left) and the second (right) stimulus in a pair. (b) Scatter diagram with the amplitudes of the second EPSP in each pair (ordinate) plotted against those of the first EPSP (abscissa). Note complete failure of responses to the second pulse after the occurrence of responses to the first pulse. (c) Amplitude distributions of the EPSPs evoked by the first and the second pulses in a pair. Dotted outlines show the noise distributions.

responses were recorded over several hours. Fluctuations in failure rate (Fig. 2b) were most likely due to the low stimulation intensity that was close to the threshold of the presynaptic axon but they could also reflect transitory changes in release probability (see below).

A discriminating feature of all-or-none EPSPs is their low amplitude variance. In Figure 2d the standard deviations of response amplitudes (failures excluded) are plotted against the standard deviation of the noise in the respective recordings for each of the 23 all-or-none EPSPs. The regression line calculated for this sample is close to the main diagonal indicating that fluctuations in response amplitude were mostly due to the noise. On average the standard deviation of the all-or-none responses was 128% of that of the noise, and in 9 cases it was less than 110%. For comparison we selected 20 EPSPs with large but fluctuating amplitude (mean amplitude 0.64 mV). The regression line for this sample lies well above the main diagonal, indicating the presence of fluctuations which are not attributable to the noise. For these fluctuating responses the mean standard deviation was 238% of that of the noise.

In order to examine the possibility that the large, unitary EPSPs were actually composed of several subunits we examined the cases where repeated low-frequency testing or paired-pulse stimulation had caused a reduction of response amplitude. Figure 3 shows one of the six cases where repetitive stimulation had led to a reduction of EPSP amplitude. Neither in this nor in the other cases was the decrease of the response amplitude accompanied by a significant change in input resistance (Fig. 3b). Two periods of stable responses, one before and one after the expression of low-frequency depression (Fig. 3b) were selected for quantification. The amplitude distribution of responses obtained after low-frequency depression still exhibits a single peak. It is shifted to the left but is as narrow as before depression. The standard deviation of the response was 0.144 mV before and 0.12 mV

after the depression, indicating that there is no increase of amplitude fluctuation (Fig. 3c, 1 and 2).

In 15 out of the 23 large all-or-none EPSPs, application of double-pulse stimuli led to paired-pulse depression. The average amplitude of the response to the second of the two stimuli diminished, individual responses began to fluctuate between several levels and often the number of failures increased. In the case shown in Fig. 4, the amplitudes of most of the responses to the second pulse were, on average, 50% (0.75 ± 0.013 mV, $n = 89$) of those of the responses to the first pulse (1.5 ± 0.013 mV, $n = 94$) (Fig. 4a,c). Plotting the amplitudes of EPSPs evoked by the first of the two pulses against the amplitudes of responses to the second reveals that large, non-depressed responses are only evoked by the second pulse if the first fails to elicit a response (Fig. 4b).

In three out of 23 cases of all-or-none EPSPs tested with paired pulses we observed a complete depression of the response to the second stimulus. One example is illustrated in Figure 5. Responses to the second pulse occurred only if the first failed to elicit a response and then they were of a similar amplitude to those of the first pulse (Fig. 5a,b). Accordingly, the amplitude distributions of the responses to the two stimuli were alike and fluctuated within the same narrow range as the noise (Fig. 5c).

Paired-pulse facilitation was observed only for one out of 23 all-or-none responses and then consisted of a reduction of failure rate rather than of an increase in response amplitude (data not shown). In contrast, paired-pulse facilitation was frequent for EPSPs which under control conditions exhibited scattered amplitude distributions (52 out of 97 examined cases). In three out of these 52 cases, fluctuating responses to the first pulse became converted into large all-or-none responses to the second stimulus. In the case shown in Figure 6, the responses to the first pulse were variable (Fig. 6a), their amplitude

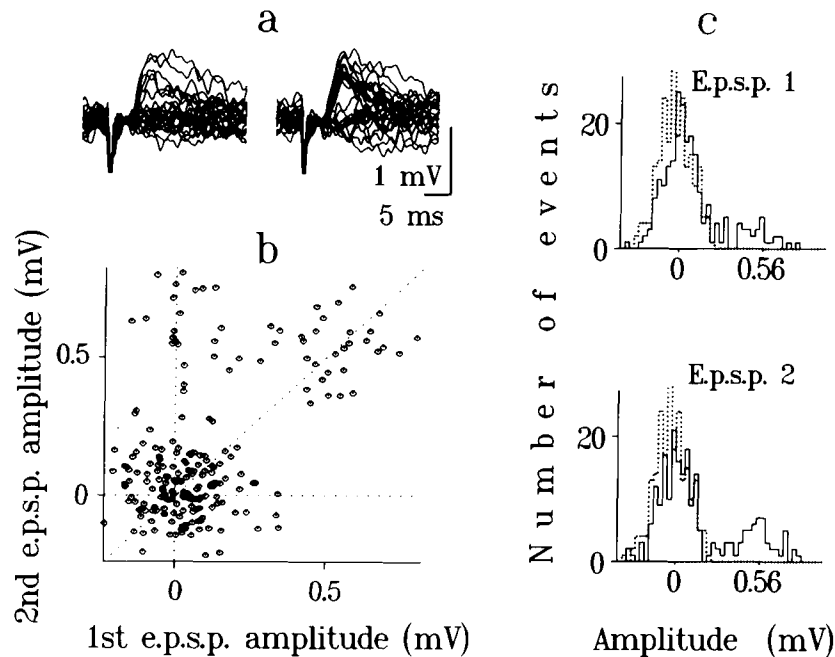


FIG. 6. Paired-pulse facilitation converting fluctuating into an all-or-none response. (a) Superposition of 25 single responses to the first (left) and the second (right) stimulus in a pair. (b) Scatter diagram with the amplitudes of the second EPSP in each pair (ordinate) plotted against those of the first EPSP (abscissa). (c) Amplitude distributions of the EPSPs evoked by the first and the second pulse in a pair. Dotted outlines represent the corresponding noise distributions.

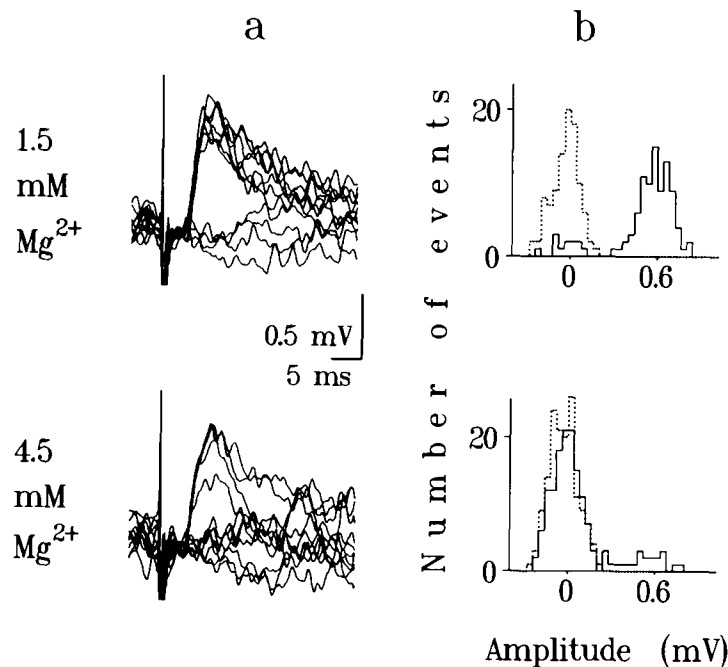


FIG. 7. The effect of increasing extracellular $[Mg^{2+}]$ on large all-or-none EPSPs. (a) Superposition of 10 consecutive single responses obtained under control conditions (1.5 mM $[Mg^{2+}]$) and after increasing $[Mg^{2+}]$ to 4.5 mM. Note the large, all-or-none EPSPs in the control condition and the increase of failures and appearance of responses with smaller amplitudes in high $[Mg^{2+}]$. (b) Amplitude distributions of the EPSPs in control medium (upper graph) and after increasing $[Mg^{2+}]$ (lower graph). Dotted outlines show the respective noise distributions.

distribution lacked a clear peak and was not well segregated from that of the noise (Fig. 6c). The standard deviation of the responses to the first pulse was 0.187 mV, which is 176% of the standard deviation of the noise. The response to the second pulse, by contrast,

had all-or-none characteristics, its amplitude distribution had a clear peak around 0.5 mV and a standard deviation of 0.137 mV (129% of the noise) (Fig. 6c). As indicated by the scatter diagram in Figure 6b, large responses to the first stimulus were always followed by

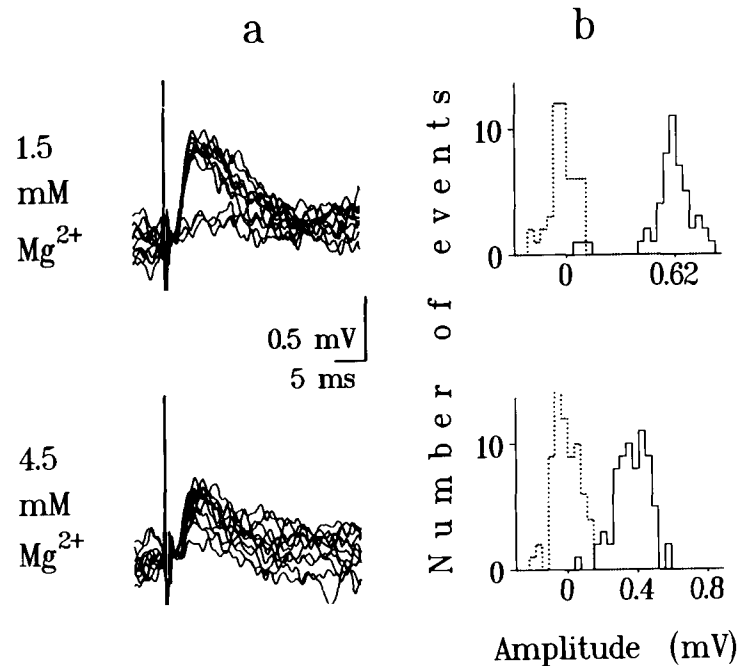


FIG. 8. Effect of increasing extracellular $[Mg^{2+}]$ on a large all-or-none EPSP. (a) Superposition of 10 consecutive single responses obtained under control conditions (1.5 mM $[Mg^{2+}]$) and after increasing $[Mg^{2+}]$ to 4.5 mM. (b) Amplitude distributions of the EPSPs in control medium (1.5 mM $[Mg^{2+}]$) and after increasing $[Mg^{2+}]$ to 4.5 mM. Dotted outlines indicate the noise distributions.

large second responses and never by failures or small responses. However, when the first responses were small, second responses failed or fluctuated between small and large amplitudes. Thus a consistent conversion of second responses to large all-or-none responses seems to occur only when the response to the first stimulus was also large.

The changes of response amplitude observed with low-frequency depression and paired-pulse interaction suggest that the large all-or-none EPSPs are composite in nature. To further test this possibility, we examined the effects of high extracellular $[Mg^{2+}]$ which is known to reduce transmitter release (Katz, 1969). In all examined cases ($n = 8$), synaptic transmission was reduced under high $[Mg^{2+}]$ due to an increase in the number of failures and an appearance of individual responses of reduced amplitude (Figs 7 and 8). The amplitude distributions of reduced responses could lack a clear peak ($n = 5$) as exemplified in Fig. 7 or they could have a single peak of similar width, but at lower amplitude values ($n = 3$) (Fig. 8). In the latter cases the standard deviations of the reduced responses were in the same range as those of the non-attenuated all-or-none responses and hence were also similar to the standard deviation of the noise.

The increased failure rate of responses during low-frequency depression, paired-pulse depression and high $[Mg^{2+}]$ could have been due to reduced excitability of the stimulated fibres rather than to changes in synaptic transmission. To control for this possibility stimulus-response functions were determined for both the first and second responses to the paired-pulses and before and during high $[Mg^{2+}]$. In all conditions the applied test stimuli were found to remain well within the plateau region of the input-output function. This is exemplified in Figure 9 for the cell whose response modifications are documented in Figure 8.

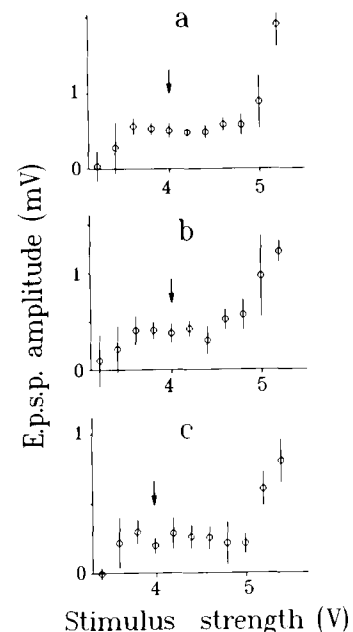


FIG. 9. Stimulus-response curves calculated for the first (a) and second (b) responses in a paired-pulse paradigm during the control period and after increasing extracellular $[Mg^{2+}]$ (c). In the latter case only responses to the first stimulus have been considered. Data are from the same cell as in Fig. 8. The intensity of the stimulus used for the measurements in Fig. 8 was 4.0 V (arrow). Note that for all three curves this intensity falls within the plateau region, suggesting that only one and most likely the same presynaptic fibre was stimulated. Each point with a vertical bar represents an average of 7 (a and b) or 5 (c) responses \pm standard deviation.

Discussion

The synaptic responses selected for this study are with all likelihood monosynaptic and due to activation of a single fibre. In all cases, increasing stimulation intensity led to an abrupt transition of response amplitude from 0 to a plateau level which remained constant over a considerable range of stimulation intensities. At the beginning of this plateau range the only effect of increasing stimulus intensity was a reduction of response failures. This is best explained by assuming that stimuli with intensities in the plateau range activate only a single fibre (Voronin *et al.*, 1977; McNaughton *et al.*, 1981; Stern *et al.*, 1992). The standard deviation of the amplitude fluctuations of the large EPSPs selected for analysis was in all cases similar to that of the noise, indicating that the responses had an all-or-none characteristic. This is further support for the assumption that the responses were due to activation of a single afferent fibre. The occasional occurrence of spontaneous EPSPs, which had a similar amplitude and shape as the evoked responses, points in the same direction. These spontaneous EPSPs could have been due to spontaneous discharges of afferent cells and/or to spontaneous release (for further discussion see below). Large single-fibre EPSPs exceeding 0.5 mV have also been described in experiments in which single fibre activation had been assured by double recordings from coupled pairs of neurons in the visual (Mason *et al.*, 1991) and somatosensory cortex (Thomson *et al.*, 1988; Thomson and West, 1993).

Apart from their large amplitude the EPSPs observed in this study are of interest because of their surprisingly small amplitude variation. In principle such large all-or-none responses can be accounted for in three ways. Firstly, they could be mediated by a single release site, producing an unusually large response with close to zero variance and high, although less than unity, probability of release. Secondly, responses could be mediated by several release sites with smaller, more conventional quantal size, each operating with close to zero quantal variance and a release probability of 1. Thirdly, responses could be mediated by several release sites with close to zero quantal variance but release probabilities of <1 . In this case a mechanism has to be postulated that assures that all sites either release or fail in synchrony. The first possibility can be excluded because of the amplitude changes observed after manipulations known to modify release probabilities such as paired-pulse and low frequency stimulation or by raising extracellular $[Mg^{2+}]$ (McNaughton *et al.*, 1981; Korn *et al.*, 1984; Zucker, 1989; Voronin, 1993a, b). In some cells these reduced responses had the same small amplitude variance as the control responses; in other cells response reduction was associated with an increase of variance. Both results indicate that the large responses are composed of subunits. In the first case it appears as if one or more subunits had dropped out altogether, in the second case there could be some intertrial variability of subunit composition. Our data do not allow us to distinguish unambiguously whether the all-or-none characteristics of the composite EPSPs were due to release sites operating with a probability of 1 or to a synchronizing mechanism assuring that all release sites discharge in unison. Arguments exist for both possibilities.

When stimulation intensity was set high enough to assure consistent activation of the presynaptic fibre, in several cells ($n = 4$) more than 100 consecutive responses could be evoked over 30 min without a failure or a change in amplitude. This indicates the existence of synapses which operate with a release probability close to 1. Indications for a synchronizing mechanism come from the observation that paired-pulse depression ($n = 3$) or raising extracellular $[Mg^{2+}]$ ($n = 2$) produced a significant increase in the number of failures, without

a significant increase in the number of responses of reduced amplitude. If responses occurred they usually had the same amplitude as before depression. We consider it unlikely that the increased failure rate was due to activation failure or conductance block of the afferent fibre because input-output functions were similar for the two responses in paired-pulse experiments and before and during high $[Mg^{2+}]$. Thus the failures are likely to result from reduced release probability but this implies that all release sites had a strong tendency to operate in synchrony. This possibility is further supported by the evidence for spontaneous synchronous multiquantal release observed in other preparations after blockade of impulse conduction with tetrodotoxin (Edwards *et al.*, 1990; Korn *et al.*, 1993; Stevens, 1993). There is yet no established mechanism for the coupling of release probabilities at central synapses (see Kriebel *et al.*, 1990 for discussion of a related problem for peripheral synapses). One possibility is that synaptic boutons with several distinct release sites possess a mechanism for the coupling of release from these sites. Such morphological arrangements are characteristic of perforated synapses (Calverley and Jones, 1990; Geinisman *et al.*, 1993) which represent $\sim 10\%$ of neocortical synapses (see Calverley and Jones, 1990, for a review and references). This percentage corresponds roughly to the incidence of large all-or-none EPSPs in our sample.

These considerations suggest that the large all-or-none EPSPs are composite and consist of several subunits which are always released simultaneously because either release probabilities at different release sites are close to 1 or release is coordinated by a synchronization mechanism. In addition, the low variance of the composite EPSPs indicates that the different release sites operate with a quantal variance close to 0. In nine cases where the standard deviation of amplitude fluctuations was $<10\%$ higher than that of the noise, variability of individual subunits should have been well below 10% of their amplitude.

Our data add to the notion that synaptic connections in the central nervous system are heterogeneous, and that characteristics such as release probability, quantal size and quantal variance can differ markedly at different synapses. Release probability is typically low at central synapses. For example, as estimated for the inputs to hippocampal pyramidal cells, release probability at individual sites varies from <0.1 to 0.54 at the most (Hessler *et al.*, 1993; Rosenmund *et al.*, 1993). Quantal size at hippocampal and neocortical synapses has been reported to be small and to exhibit a high internal variability. Estimations of quantal size yielded values not >0.2 mV for EPSPs, or 15 pA for EPSCs (Larkman *et al.*, 1991, 1992; Kullmann and Nicoll, 1992; Liao *et al.*, 1992; Raastad *et al.*, 1992; Stern *et al.*, 1992; Voronin *et al.*, 1992; Jonas *et al.*, 1993) with the variability of individual quanta ranging from ~ 20 (Jonas *et al.*, 1993) to 50% or more (Bekkers and Stevens, 1989, 1990; Raastad *et al.*, 1992). As a result, the postsynaptic effect of a single fibre is usually weak and fluctuating from trial to trial. Even in rare cases of stronger connections (Thompson *et al.*, 1988, 1993; Mason *et al.*, 1991; Thompson and West, 1993) the amplitude of individual EPSPs was found to be highly variable. It has been inferred from this that central synapses are in general weak, unreliable and imprecise. Our data suggest that there are notable exceptions. At least some synaptic connections in the neocortex are not only strong, but also highly reliable and exhibit very low variance. Unfortunately we are not able to identify the afferents responsible for the large composite all-or-none EPSPs. The fact that these EPSPs could be evoked monosynaptically from layer IV and from sites in supragranular layers adjacent to the recorded cells would be compatible with an intracortical origin of the activated afferents. The supragranular electrode could have activated tangential

connections mediating cross-talk between different columns while the layer IV electrode should have stimulated ascending cortico-cortical association fibres of extrinsic origin and intracolumnar connections which convey activity from layer IV to supragranular cells (Gilbert, 1993).

In our data set manipulations affecting release probabilities had drastic effects on synaptic efficacy, suggesting that modification of release probability is of functional relevance in cortical processing. Several aspects are of interest here. Firstly, as the paired pulse experiments show, release probability can change dramatically and on a short time scale. In the cases of complete paired-pulse depression it seems to switch from ~ 1 to 0 at all release sites following a single response but returns back to 1 rapidly, as another response could be evoked by the next pair of stimuli applied within 10 s. These extreme cases could alternatively be accounted for by activation failures or conduction block of the stimulated fibre but as argued above we consider this possibility as unlikely. Secondly, release probability can switch from ~ 1 to 0 at subsets of the release sites contributing to the large all-or-none responses. This follows from the cases of depression where the amplitude of individual responses was reduced without an increase of variance. If release probability had changed (decreased) at all sites in parallel, it should have resulted in a proportional redistribution of the relative numbers of failures, intermediate and large responses. Thirdly, as discussed above, there are indications that release can be synchronized across several release sites. Synchronization across all release sites of a particular fibre would result in preservation of the all-or-none property of the response despite an increase in failure rates (see above). Finally, when release probability is < 1 , it can be raised to 1. This is suggested by the cases where fluctuating responses to the first pulse were converted into all-or-none responses by paired-pulse facilitation. Therefore it appears that changes in release probability occur under conditions of activation not too far from natural and have a strong effect on synaptic transmission.

However, our data do not exclude a contribution of additional postsynaptic mechanisms. Hestrin (1992) described desensitization of postsynaptic receptors in membrane patches excised from visual cortex neurons in response to external glutamate application. Such a mechanism could also contribute to paired-pulse depression. However, desensitization alone cannot account for the appearance of responses fluctuating between several amplitude levels, the increased number of failures, the cases of complete depression, and the similarity between the effects of high $[Mg^{2+}]$ and paired-pulse depression.

Thus our data indicate that at least some synaptic inputs to supragranular cells in the neocortex are strong, highly reliable and exhibit very low variance. Together with their high susceptibility to undergo modifications of release probability this introduces a powerful mechanism for the activity dependent modulation of synaptic transmission.

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Abbreviations

EPSP excitatory postsynaptic potential
EPSC excitatory postsynaptic current

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