FAST AND SLOW COMPONENTS OF UNITARY EPSCs ON STELLATE CELLS ELICITED BY FOCAL STIMULATION IN SLICES OF RAT VISUAL CORTEX

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SUMMARY

1. Voltage and current recordings were made from visually identified non-pyramidal neurones in slices of layer IV of rat primary visual cortex using the whole-cell configuration of the patch clamp technique. These neurones are characterized by high input resistance (0.5–2 GΩ) and a non-adaptive behaviour of action potential frequency following depolarizing current injection, which suggests that they are stellate cells.

2. Excitatory postsynaptic currents (EPSCs) were recorded from these neurones during focal stimulation of neighbouring cells by a second patch pipette, the tip of which was placed on the soma of the stimulated cell. The response amplitude as a function of stimulus strength showed a sharp increase at a critical stimulus strength suggesting that stimulus-evoked currents represent unitary EPSCs.

3. In most cases the latencies of stimulus-evoked EPSCs were unimodally distributed with means in the range of 2.1–3.6 ms. In some experiments two peaks were seen in the distribution of latencies. The EPSC rise times, measured as the time from 20 to 80% peak amplitude, fell into a distribution ranging from 0.1 to 0.8 ms with a peak at 0.2 ms. The EPSC decay time course at −70 mV membrane potential was fitted by a single exponential with a time constant of 2.39 ± 0.99 ms (mean ± s.d.). The rise and decay times were independent of EPSC peak amplitudes.

4. The peak amplitude of successive unitary EPSCs, elicited by a constant stimulus, fluctuated at random. At a holding potential of −70 mV the peak amplitudes varied between 5 and 90 pA. In two out of ten cells the histogram of peak amplitudes could be well fitted by the sum of several equidistant Gaussians with a peak distance of around 10 pA. This suggests that the quantal conductance change underlying the peak current fluctuations is of the order of 100 pS.

5. At membrane potentials more positive than −70 mV the decay of stimulus-evoked EPSCs showed two components with very different time courses. In standard extracellular solution the current–voltage (I–V) relation for the fast component was almost linear whereas the slow component showed a J-shaped I–V relation with a region of negative slope conductance between −30 and −70 mV.

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6. The two components of stimulus-evoked EPSCs had different sensitivities to blockers of \( \alpha \)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate (KA) and \( N \)-methyl-\( d \)-aspartic acid (NMDA) subtypes of the glutamate receptor (GluR) channel, respectively. The fast EPSC component was blocked by 2–3 \( \mu \)M 6-cyano-7-nitroquininaline-2,3-dione (CNQX), the slow component by 20 \( \mu \)M DL-2-amino-5-phosphono-pentanoic acid (APV). This suggests that EPSCs are mediated by both the AMPA/KA and the NMDA subtypes of the GluR channel.

7. The \( I-V \) relationship of the slow component of EPSCs changed when \( \text{Mg}^{2+} \) was omitted from the solution. In \( \text{Mg}^{2+} \)-free solution the slope conductance of the slow component showed a linear voltage dependence suggesting that the region of negative slope conductance arises from the voltage-dependent \( \text{Mg}^{2+} \) block of NMDA receptor channels.

8. The rise time and the decay time of the slower EPSC component, mediated by NMDA receptors, were considerably longer than those of the AMPA/KA receptor-dependent component. The rising phase showed a sigmoidal time course with rise times of 5-4–6.2 ms. The decay could be described by the sum of two exponentials with time constants of 16–70 and 108–307 ms, respectively.

9. Under experimental conditions where both the fast and the slow components of fluctuating unitary EPSCs could be observed (nominally \( \text{Mg}^{2+} \)-free extracellular solution; –70 mV membrane potential) their relative sizes were almost independent of the EPSC amplitude. This suggests that AMPA/KA and NMDA receptor subtypes are co-activated and that the ratio of the currents mediated by the two receptor subtypes is relatively constant for a particular synapse. EPSCs in different cells showed different ratios of AMPA/KA to NMDA receptor-mediated currents.

10. It is concluded that unitary EPSCs in non-pyramidal (stellate) cells of layer IV of rat visual cortex are mediated by both the AMPA/KA and the NMDA subtypes of the GluR channel. The estimated number of AMPA/KA receptor channels activated by a quantum of transmitter is in the order of ten to twenty and the number of co-activated NMDA receptor channels varies between one and ten, depending on the synapse studied.

INTRODUCTION

Glutamate is the putative neurotransmitter at many excitatory synapses in the central nervous systems (for reviews see Streit, 1985; Headley & Grillner, 1990). According to pharmacological criteria glutamate receptors (GluRs) are divided into two major groups (for review see Dinglestone, Boland, Chamberlain, Kawasaki, Kleckner, Traynelis & Verdoorn, 1988), namely the AMPA (\( \alpha \)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid)/KA (kainate) and NMDA (\( N \)-methyl-\( d \)-aspartic acid) subtypes of GluR channels.

Several studies measuring the functional and pharmacological properties of excitatory postsynaptic potentials (EPSPs) or currents (EPSCs) have established the participation of both receptor subtypes in synaptic transmission in many mammalian cortical pathways (for reviews see Nicoll, Malenka & Kauer, 1990; Tsumoto, 1990). However, in these studies stimulation of fibre tracts was used to elicit EPSCs, which recruits many fibres synchronously and probably activates synapses also on remote positions of the dendritic tree. It is thus unclear how the two receptor subtypes
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contribute to EPSCs mediated by a single synapse. This question is of particular importance with respect to mechanisms that involve both AMPA and NMDA receptors, such as long-term potentiation (Collingridge & Bliss, 1987).

In cultured neurones Hirano & Hagisawa (1988) found that EPSCs on single synapses between cerebellar granule cells and Purkinje cells were due exclusively to the activation of AMPA/K+ type receptors. In contrast, Bekkers & Stevens (1989) reported that in cultured hippocampal neurones the majority of spontaneous EPSCs were mediated by both NMDA and AMPA/K+ receptors. However, because of intrinsic problems in studying synapses formed between cultured neurones, e.g. the need to keep cells under artificial circumstances for several days, the lack of cell type specificity, and the possible destruction of receptors during dissociation (Allen, Brady, Swann, Hori & Carpenter, 1988), the degree of relevance of these results to excitatory synaptic transmission in the CNS is unclear. We therefore used the method of whole-cell recording with patch pipettes in brain slices (Edwards, Konnerth, Sakmann & Takahashi, 1989; Sakmann, Edwards, Konnerth & Takahashi, 1989). With this technique it is possible to identify individual neurones visually in the slice and perform high-resolution recordings leaving most of the local neuronal environment intact. By placing the tip of another patch electrode extracellularly on the soma of a neighbouring neurone and stimulating it, unitary EPSCs were elicited. We were thus able to investigate the properties of EPSCs of an excitatory synapse in which a single presynaptic neurone is stimulated.

We investigated neurones in layer IV of rat primary visual cortex (Krieg, 1946; Zilles, Zilles & Schleicher, 1980). This layer receives the majority of synaptic input from previous relay stations of the visual pathway (Ribak & Peters, 1975) and is important for higher visual processing. In addition, in non-pyramidal neurones excitatory synapses are located on or close to the soma which avoids problems arising from inadequate space clamp.

By pharmacological and by functional means the unitary EPSCs could be separated into two components: a rapid component mediated by AMPA/K+ receptors and a slower component mediated by NMDA receptors. The peak amplitude of EPSCs fluctuated in a manner consistent with a quantal mechanism. The size of the slower NMDA receptor-mediated component relative to the AMPA/K+ receptor component was almost constant for different peak amplitudes. This suggests that at a given synapse AMPA/K+ and NMDA receptor subtypes are co-localized, possibly at a fixed ratio.

METHODS

Tissue preparation

Preparation of tissue slices was as previously described (Edwards, Konnerth, Sakmann & Takahashi, 1989). In brief, young rats (postnatal day 5-20) were decapitated and the brains quickly (in less than 1 min) immersed in ice-cold oxygenated extracellular solution. Slices of visual cortex (150-250 μm thickness) were cut using a vibrating microslicer (DTK 1000, Dosaka Co., Kyoto, Japan) and immediately transferred into a holding chamber filled with extracellular solution, continuously bubbled with carbogen (95% O₂, 5% CO₂). Neurones from slices prepared this way could be patch clamped up to 14 h after slicing and stable recordings could still be obtained. For the actual experiment slices were transferred into a recording chamber which could be perfused continuously with oxygenated solution (2 ml/min). Cleaning and recording of neurones was done under a 40× water immersion objective with 1.6 mm working distance (40.0/0.75 W, Carl
Zeiss, Oberkochen, Germany). The cleaning of the surface of tissue slices for patch clamping was essentially as described by Edwards et al. (1989).

**Solutions**

Standard extracellular solution contained (in mM): NaCl, 125; NaHCO₃, 25; NaH₂PO₄, 1.25; glucose, 25; KCl, 25; MgCl₂, 1; CaCl₂, 2 (pH 7.4 when bubbled with carbogen). For some experiments MgCl₂ was omitted from this solution (referred to as 'nominally Mg²⁺-free extracellular solution'). Patch pipettes were filled with standard intracellular solution containing (in mM): KCl, 125; MgCl₂, 2; CaCl₂, 2; EGTA, 10; Na-ATP, 2; HEPES, 10 (pH 7.3 with KOH). Before use, the intracellular solution was filtered with a 0.2 μm pore size filter (Schleicher & Schuell, Dassel, Germany). In some experiments 125 mM-CaCl₂ instead of KCl was used. The pH was then adjusted with CaOH.

**Drugs**

Drugs were stored in frozen stock solutions and dissolved in oxygenated extracellular solution in the concentration indicated and bath-applied by use of a hydrostatic pressure system. When stimulus-evoked EPSCs were recorded, all experiments were done in the presence of 5-6 μM-bicuculline to block GABA<sub>A</sub> receptor-mediated inhibitory currents. CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) was bought from Tocris Neuramin (Buckhurst Hill, UK). All other drugs were purchased from Sigma (St. Louis, MO, USA).

**Current recording**

Recording and stimulation pipettes were pulled from borosilicate glass capillaries (Hillegaert, Malsfeld, Germany) with an outer diameter of 2 mm and a wall thickness of 0.3 mm. Recording pipettes were coated with Sylgard 184 (Dow Corning, Midland, MI, USA). They had resistances of 3-5 MΩ when filled with intracellular solution. Whole-cell current recordings (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) were made using an EPC-7 patch-clamp amplifier (List, Darmstadt, Germany). For stimulation the tip of a second patch pipette (resistance 1-5 MΩ) filled with standard extracellular solution was placed onto the soma of a nearby neuron. Stimulus pulses were generated by a microcomputer (AIM, Rockwell, Newport Beach, CA, USA) which triggered a stimulus isolation unit (model 5333P, Devices Instruments, Welwyn Garden, UK). The voltage of the stimulus was varied between 1 and 50 V with a duration of 50-100 μs and stimuli were applied at a frequency of 0.5-1 Hz. The amplitude of the stimulus was increased until a stimulus-evoked synaptic current was recorded. Usually several neighboring cells had to be tried before an excitatory connection between the stimulated and the recorded neuron was found. Currents were filtered (10 kHz, Band filter of EPC 7), digitized (modulated Sony PCM-701ES), stored on a videotape (Panasonic video recorder, NV-H75 HQ), and later analyzed with a Motorola VM/Bus Computer (Motorola Delta series 1147, Tempe, AZ, USA). All experiments were performed at room temperature (20-24 °C).

**Analysis**

Whole-cell currents were sampled at 5 or at 10 kHz (MPV 958, Burr-Brown/Pentrland Systems Ltd, Livingston, Scotland, UK) and filtered at 2 kHz (3 dB, 8-pole Bessel filter, Frequency Devices, Haverhill, MA, USA). Latencies, amplitudes, and rise and decay times of stimulus-evoked EPSCs were measured offline by a semiautomatic procedure using movable cursors. Latencies were analyzed as the time from the beginning of the stimulus artifact to the beginning of the EPSC. Rise times were calculated as the time from 20 to 80% of the EPSC amplitude. The beginning of the EPSC was determined by the crossing point between the baseline and a back-extrapolated line connecting the curve points at 20 and 80% of the amplitude. Decay time constants of EPSCs were measured by fitting exponentials from peak to baseline. For fitting, a non-linear simplex fit routine based on the least-squares method was used.

For analysis of the NMDA component in nominally Mg²⁺-free extracellular solution thirty to forty responses starting at the stimulus trigger were averaged. Having selected for latency, the amplitudes and the decay time constants of the averaged current were measured as described above. To avoid distortion of the rising phase of averaged current traces due to variability of the latencies, single current sweeps were aligned by moving the traces until the points of the maximal
slope of rise were superimposed. A different approach was used when analysis of individual traces containing an NMDA component was necessary. Because of the large current fluctuations during the activation of the slow EPSC component a time window at a fixed interval after the peak amplitude was set and the mean current in this interval was measured.

Fig. 1. Three-dimensional surface reconstruction of a dye-filled neurone using a laser-scanning microscope. Neurone in the slice of the visual cortex from a 15-day-old animal. The cell was identified and selected according to the criteria in Methods. The cell body was non-pyramidal since no main apical dendrite was visible. After preparation the cell was held in the patch-clamp whole-cell configuration for 15 min. For staining Lucifer Yellow (1 mg/ml) was allowed to diffuse from the electrode into the neurone. After filling with the fluorescent dye the slice was fixed in paraformaldehyde and embedded in clearing solution (Mowiol). Eighty optical sections (63 × oil-immersion objective, 0.8 μm intervals between sections with a resolution of 0.25 μm in the horizontal plane) were scanned using a 'Phoibos' laser-scanning microscope. Calibration bar, 10 μm.

Identification of cells
To assure that the visually identified cells were layer IV non-pyramidal neurones, ten cells were stained intracellularly with Lucifer Yellow, dissolved in the pipette solution (1 mg/ml). Following establishment of the whole-cell recording mode, the dye solution was allowed to diffuse into the cell over a period of 15 min during which dendrites were well filled. The electrode was then removed and
the cells were viewed in the microscope with epifluorescence. For confocal laser-scanning microscopy of dye-filled neurones slices were then taken out of the recording chamber, embedded in clearing solution (Mowiol; Osborn, 1981), and mounted on glass slides. Three-dimensional surface reconstructions of the fluorescent cells were then made using a Ploem 1000 confocal laser-

Fig. 2. Photomicrograph of a slice preparation of the visual cortex of a 12-day-old animal as seen during an experiment. 40 × water-immersion objective, Nomarski optics. Final magnification was 400 ×. Calibration bar, 50 μm. The neurone labelled R was voltage clamped in the patch clamp whole-cell mode. The tip of a stimulating electrode was placed externally onto the membrane of a neighbouring cell (S) and increasing voltage pulses were applied via this pipette until a postsynaptic current in R could be recorded.

scanning microscope (Sarastro AB, Stockholm, Sweden). For scanning and data acquisition a Personal Iris +D20 (Silicon Graphics, Mountain View, CA, USA) was used and for data processing a C 210 computer (Convex Corporation, Richardson, TX, USA) was used.

RESULTS

Identification of neurones in rat visual cortex

Non-pyramidal neurones were identified visually with Nomarski optics by focusing up and down through the brain slice. The main criteria for cell selection were a non-pyramidal shape of the cell body and the absence of a main apical dendrite. Figure 1 shows a three-dimensional reconstruction of the surface of a layer IV neurone selected according to these criteria after filling with Lucifer Yellow and using confocal laser-scanning microscopy.
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Fig. 3. For legend see p. 254.
In slices obtained from animals older than 9 days, following the establishment of whole-cell recording in a non-pyramidal cell the extracellular stimulation of a neighbouring neurone within layer IV of visual cortex often resulted in stimulus-evoked postsynaptic currents. These experiments were done in extracellular solution containing the GABA\(_A\) receptor antagonist bicuculline (5–6 µM) and therefore the most likely explanation is that these currents were stimulus-evoked EPSCs (for further evidence see later). In most experiments several neighbouring cells had to be tested before an excitatory connection was found. Figure 2 shows the recording and stimulation pipettes in a slice preparation as seen through the microscope during an experiment. Whole-cell configuration was established in one neurone with the recording pipette (R). Application of short stimulus pulses via a stimulating pipette (S) with its tip placed on the soma of a nearby neurone resulted in stimulus-evoked EPSCs.

**Voltage recording from non-pyramidal cells and pyramidal cells**

When recordings were made with pipettes filled with standard intracellular solution non-pyramidal neurones had a mean resting membrane potential of \(-69.2 \pm 8.6\) mV (mean ± s.d.; \(n = 43\)) and did not show spontaneous action potentials. The input resistance estimated from voltage changes produced by hyperpolarizing current injections was \(1.1 \pm 0.5 \, \Omega\) (mean ± s.d.; \(n = 9\)). Following depolarization by current injection the neurones responded with a sustained train of action potentials (Fig. 3A). The extrapolated slope of the frequency–input current plot was linear and had a value of 200 Hz/µA (data not shown). Action potentials showed a positive overshoot. After the train of action potentials no slow after-hyperpolarization could be detected. This behaviour is regarded to be characteristic for 19 of 19 pyramidal cells.

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**Fig. 3. Voltage recording from different neuronal cell types in layer IV of rat primary visual cortex.**

A, recording from a fast spiking non-pyramidal neurone. Records were filtered at 2 kHz (−3 dB) and sampled at 5 kHz. The membrane potential was −80 mV. The recording electrode was filled with standard intracellular solution. Depolarizing and hyperpolarizing current pulses of increasing amplitude (40–140 pA) and of 1 s duration were applied. The voltage response in the hyperpolarizing direction could be fitted by two exponentials with time constants of 53 and 35 ms. The calculated DC input resistance of this neurone was 760 MΩ. Action potentials elicited by depolarization showed a positive overshoot and their frequency was not adaptive. At the end of the depolarizing current pulse there was no slow after-hyperpolarization as indicated by the dashed line showing the baseline membrane potential. B, the voltage response of a pyramidal neurone in contrast exhibited a large after-hyperpolarization. C and D, recording of stimulus-evoked EPSPs. The dashed lines indicate the baseline value of the membrane potential. Arrows indicate the stimulus artifacts. The traces in C show the responses of a non-pyramidal neurone in a visual cortical slice from a 15-day-old rat. A neurone in the neighbourhood was stimulated (17 V, 0.5 Hz, 50 µs). A single stimulus was applied at the point marked by an arrow. At a recording potential of −70 mV only an EPSP could be elicited. At a more depolarized potential (−55 mV) a series of action potentials followed the excitatory input. Between these action potentials no after-hyperpolarization could be detected. In D the response of a layer V pyramidal neurone from the slice of a 13-day-old animal is shown for comparison. The stimulus voltage at the nearby neurone was 30 V (0.5 Hz, 50 µs). Already at a recording membrane potential of −65 mV a stimulus-evoked EPSP could elicit an action potential. This action potential showed a marked after-hyperpolarization.
for interneurones in other cortical areas (McCormick, Connors, Lighthall & Prince, 1985; Connors & Gutnick, 1990). In contrast, when similar measurements were made from neurones which had a pyramidal shape of their cell soma, adaptive behaviour of action potential frequency upon current injection was seen. At the end of the

![Image of a graph showing EPSCs](image)

Fig. 4. Unitary EPSCs. Recording from a non-pyramidal neurone of a 14-day-old animal during focal stimulation of another neurone in close proximity (~ 25 μm). The presynaptic neurone was stimulated externally with a patch electrode filled with extracellular solution (resistance of 2 MΩ). Stimulus pulses were of 50 μs duration and were applied at a frequency of 0.5 Hz at different stimulus voltages. Records were filtered at 2 kHz (−3 dB) and sampled at 10 kHz. Holding potential was ~70 mV. A, records of stimulus-evoked EPSCs at different stimulus strengths. For each stimulus strength averages of thirty to forty EPSCs are shown. Initial downward deflections (arrow) represent stimulus artifacts. B, stimulus–response curve from the same neurone as shown in A. Each circle indicates the mean of the peak amplitude of thirty to forty EPSCs. Vertical bars represent standard deviation. The abrupt increase in the amplitude shown in B suggests that the EPSCs were unitary.

depolarizing step pyramidal neurones showed a prolonged after-hyperpolarization (Fig. 3B).

When recording from a non-pyramidal neurone in the current clamp mode the stimulation of a nearby neurone often evoked the appearance of a depolarizing EPSP which sometimes was followed by several action potentials (Fig. 3C). In pyramidal cells on the other hand stimulus-evoked EPSPs elicited a single action potential followed by a long hyperpolarizing after-potential (Fig. 3D). Thus visual criteria, as well as the recorded electrophysiological properties of the neurones, suggest that the recordings were made from an almost homogeneous population of non-pyramidal cells probably corresponding to stellate cells as seen in Golgi preparations.
Basic features of stimulus-evoked EPSCs

Neurones were voltage clamped at a holding potential of \(-70\) mV to study the size and shape of stimulus-evoked currents. These currents remained unaffected in the presence of bicuculline \((5-6 \mu\text{M})\), but disappeared upon addition of \(1 \mu\text{M}\)-tetrodotoxin (TTX) or of the glutamate receptor antagonist CNXQ \((2 \mu\text{M})\). The stimulus-evoked currents were thus identified as EPSCs.

Several lines of evidence suggest that these EPSCs are unitary, i.e. the result of the stimulation of a single presynaptic neurone. First, moving the stimulating pipette about 1–2 \(\mu\text{m}\) away from the soma of the stimulated neighbouring neurone led to the disappearance of the EPSCs. Second, the size of EPSCs showed a characteristic dependence on stimulus strength. Figure 4A shows records of averaged EPSCs evoked by increasing the stimulus strength. Below a certain threshold (12 V stimulus...
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strength, in this example) no response could be detected and EPSCs appeared once a threshold strength was reached. The mean amplitude of the EPSCs then did not change significantly with further increase of stimulus strength (Fig. 4B). This suggests that below the threshold level the stimulated cell does not generate an

![Image](https://via.placeholder.com/150)

Fig. 6. Rise time distributions of stimulus-evoked EPSCs after selecting for latency. Same recording conditions as described in Fig. 3. The stimulus (14 V, 50 μs) was applied at a frequency of 1 Hz. A, latency histogram of all EPSCs. Two peaks with means at 2.73 and 3.71 ms after the beginning of the stimulus can be observed (n = 136). B, rise time histogram of the same EPSCs. C, rise time histogram of selected events with a latency of 2–3 ms i.e., for the EPSCs in the first latency peak (n = 82). D, rise time histogram of selected events with a latency of 3–4 ms (n = 46), i.e., for the EPSCs in the second latency peak.

action potential in response to depolarizing stimuli. Increasing stimulus strength increased the reliability of stimulating the cell which responded in an 'all or nothing' manner. When the stimulus strength was increased to very high values (more than three times the threshold strength) the amplitude of the EPSCs did increase, but the shape of the responses changed also. This could have been due to direct stimulation of the recorded neurone, or due to polysynaptic excitation after stimulation of several other neurones or axons in the vicinity of the stimulating pipette.

Time course and size of stimulus-evoked EPSCs in Mg²⁺-containing solution

The time course and size of EPSCs were studied initially at a holding potential of -70 mV, which was close to the resting potential of these cells. Figure 5A shows
Fig. 7. Fluctuation in amplitudes of unitary EPSCs. A, superimposed records of successive stimulus-evoked EPSCs obtained from a neurone of a 12-day-old animal. The holding potential was \(-70\) mV under standard recording conditions. Stimulus pulses (10 V, 50 \(\mu s\)) were applied at a frequency of 0.5 Hz. Data were recorded at a sampling frequency of 10 kHz and filtered at 2 kHz \((-3\) dB). B, amplitude histogram of all stimulus-evoked EPSCs \((n = 70)\) recorded in this experiment. Background noise is displayed as distribution of 10881 data-points of the baseline of the recording. The distribution of baseline current values was arbitrarily normalized to the peak of the EPSC amplitude at bin No. 15 (original maximal height was 775). The standard deviation of the background noise was 1.5 pA. Due presumably to the large spread of the amplitudes over the range between 11 and 87 pA, and the relatively small number of events, no clear peaks could be detected.

successive records of stimulus-evoked EPSCs recorded from one neurone upon application of suprathreshold stimuli of constant voltage to a neighbouring neurone, to illustrate the fluctuations in latency, rise time and amplitude of the EPSCs. Latencies, measured from the beginning of the stimulus artifact to the beginning of
Fig. 8. Fluctuation in amplitudes of unitary EPSCs. Histogram fit with the sum of Gaussian distributions. A, records of successive stimulus-evoked EPSCs obtained from a neurone of an 11-day-old animal. The holding potential was −70 mV under standard recording conditions. Stimulus pulses (14 V, 50 μs) were applied at a frequency of 1 Hz. Data were recorded at a sampling frequency of 10 kHz and filtered at 2 kHz (−3 dB). B, amplitude histogram of all stimulus-evoked EPSCs (n = 136) recorded in this experiment. Background noise is displayed as distribution of 11840 data points of the baseline of the recording. The distribution of baseline current values was arbitrarily normalized to the peak of the first Gaussian distribution (original maximal height was 845). The standard deviation of the background noise was 1.4 pA. The smooth curve superimposed on the histogram represents the sum of four Gaussian distributions which best fit the data (least-squares fit). The fit was restricted only by the number of Gaussians and the amplitude range to be fitted. The fit range was 5–45 pA as indicated by the arrows at the abscissa.
the EPSC (see Methods), varied between 2 and 6 ms in this neurone (Fig. 5B). The distribution of latencies shows a maximum around 3 ms. The distribution of rise times, measured as the time interval between 20 and 80% of the peak amplitude, showed a single peak at 0·2 ms with a range of values between 0·1 and 0·7 ms (Fig. 5C). Comparable values were measured in nine other cells. Latencies showed minima of 1·5±0·6 ms and maxima of 7·0±2·4 ms with means of 2·9±0·8 ms whereas rise times had minimal values of 0·14±0·03 ms and maximal values of 1·2±0·7 ms with means of 0·4±0·1 ms (all values represent means±standard deviations; n = 10).

The decay of EPSCs at −70 mV could be fitted by single exponentials. The means of the decay time constants obtained from the different neurones were 2·4±1·0 ms (mean±s.d.; n = 9).

The relatively large spread seen in the distribution of latencies observed in some experiments could suggest that focal stimulation activated several excitatory presynaptic neurones or elicited action potentials travelling along axon collaterals. In experiments with a distribution of latencies, which suggested several peaks (Fig. 6D), the rise times of EPSCs were, however, independent of latency (Fig. 6B–D). This indicates that, even if several presynaptic neurones were stimulated, the location of the excitatory synapses on the cell was close to the soma.

The peak amplitudes of stimulus-evoked EPSCs varied between different trials. Figure 7A shows the superposition of successive stimulus-evoked EPSCs recorded from a stellate neurone while stimulating a nearby neurone at constant stimulus strength. Whereas the latencies and rise times of EPSCs recorded in this experiment were homogeneous, their amplitudes were scattered and ranged from 11 to 87 pA (Fig. 7B). Failures, i.e. stimuli not followed by an EPSC, were also seen. The range of EPSC amplitudes was surprisingly small when compared with stimulus-evoked EPSCs recorded from other cell types using fibre stimulation (Brown & Johnston, 1983; Collingridge, Herron & Lester, 1988; Hestrin, Nicoll, Perkel & Sah, 1990). In ten cells measured during focal stimulation of a single presynaptic cell the stimulus-evoked EPSC amplitudes varied between minima of 8·6±1·8 pA and maxima of 52·1±17·8 pA (mean±standard deviation, n = 10). The smallest amplitude spread was seen between 6·2 and 22·4 pA. The largest amplitude spread was seen between 11·4 and 87·6 pA.

In two of these ten experiments, in which the size of peak amplitude varied over a relatively narrow range (<40 pA), the EPSC amplitude histogram could be fitted by the sum of several Gaussian distributions even with small sample sizes. The results from one cell are shown in Fig. 8. In this experiment the peak EPSC amplitudes elicited by successive stimuli seemed to vary in a quantal fashion (Fig. 8A). The histogram of peak amplitudes was fitted by the sum of four Gaussians with their peaks separated by about 10 pA. A comparable distribution of EPSC amplitudes was seen in another experiment.

The results reported so far suggest that the stimulus-evoked EPSCs represent

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Figure 8 legend continued.

The dashed curves represent the individual Gaussian distributions of this fit. The means and standard deviations of the four Gaussians were: 11·22±2·55 pA; 20·28±2·82 pA; 28·26±3·78 pA; 38·1±1·97 pA.
Fig. 9. Rise and decay times of stimulus-evoked EPSCs as a function of EPSC amplitude. 
A–C, same experiment as shown in Fig. 8. A, the rise times (20–80% amplitude) are plotted against EPSC peak amplitudes. The resulting correlation coefficient of the linear regression analysis was −0.19. B, the decay time constants are plotted against EPSC peak amplitudes. The correlation coefficient was 0.06. C, the decay time constants are plotted against rise times. The correlation coefficient was −0.04. D–F, same plots as in A–C for another neuron which showed larger spread of unitary EPSC. The resulting correlation coefficients were −0.01, 0.05 and −0.13 respectively.
unitary EPSCs which fluctuate in a quantal fashion in their peak amplitude. The assumption of unitary EPSCs is further supported by the homogeneity of their rise and decay times. Figure 9 shows the rise times and the decay time constants of EPSCs plotted against the amplitude as well as the decay time constants plotted against the rise times for two experiments. One experiment showed a small spread and a quantal distribution of amplitudes (Fig. 9A–C), whereas the other exhibited a larger spread of amplitudes (Fig. 9D–E). The histograms for both experiments showed no significant dependence on EPSC peak amplitudes. The correlation coefficients were $-0.19$, $0.09$ and $0.04$ for $A$–$C$ and $-0.01$, $0.05$ and $0.13$ for $D$–$E$. In the experiments presented here the means of the rise times were $0.31$ and $0.54$ ms and the means of the decay time constants were $1.87$ and $2.46$ ms respectively. The rise time was about five times and the decay time was about three times shorter than those reported for EPSCs at other excitatory synapses, which were recorded following tract stimulation (Hestrin et al. 1990).

Pharmacological separation of two EPSC components

In many cortical pathways excitatory transmission is mediated by both the AMPA/KA and NMDA subtype of GluR channels (Thomson, West & Lodge, 1986; Jones, 1988). These two components may be separated by either changing the membrane potential or by using pharmacological antagonists specific for the two GluR subtypes.

The time course of unitary EPSCs in stellate cells changed dramatically when the membrane potential was made more positive than $-70$ mV (Fig. 10A). When depolarized an additional slowly decaying EPSC component could be detected (middle and lower record of Fig. 10A). This voltage dependence of EPSC decay was reminiscent of the NMDA receptor-mediated currents previously described in cultured neurones (Nowak, Bregestovsky, Ascher, Herbet & Prochiantz, 1984; Mayer, Westbrook & Guthrie, 1984). To separate the two EPSC components further we used APV (2-amino-5-phosphonovalerate), which is a selective NMDA receptor antagonist (Perkins, Stone, Collins & Curry, 1981; Evans, Francis, Jones, Smith & Watkins, 1982) and CNQX, which selectively blocks AMPA/KA receptors (Honore, Davies, Drejer, Fletcher, Jacobsen, Lodge & Nielsen, 1988; Blake, Brown & Collingridge, 1988). Figure 10B illustrates that CNQX ($2 \mu M$) blocked the fast rising and decaying component present at all potentials but had no effect on the slow EPSC component. On the other hand APV ($20 \mu M$) was without effect on the fast EPSC component while completely abolishing the CNQX-resistant slower component (Fig. 10C). The effects of both antagonists could be fully reversed on wash-out. These results thus suggested that EPSCs at membrane potentials more positive than $-70$ mV are mediated by both the AMPA/KA and the NMDA receptor subtypes of the GluR.

Current–voltage relation of the two EPSC components

To analyse the current–voltage relation of the two EPSC components we measured the peak amplitudes of the EPSCs as well as the mean current in a defined time window after the peak, before and after pharmacological blockade of one or the other GluR channel subtype. Figure 11 illustrates the pharmacological separation of the
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NMDA receptor-mediated EPSC component. Following application of 2 \( \mu \text{M}-\text{CNQX} \), the EPSCs consisted of a slow component with a relatively late peak which was prominent at depolarized membrane potentials (Fig. 11A and B). Figure 11C shows the peak currents measured under these circumstances. The almost linear \( I-V \) relation in control conditions was converted into the J-shaped \( I-V \) relation of the

![Image](image-url)

**Fig. 10.** Pharmacological dissection of two components of stimulus-evoked EPSCs. All traces display the average of thirty to forty stimulus-evoked EPSCs. Neuron in a slice from a 12-day-old rat. The recording pipette was filled with standard intracellular solution. EPSCs were measured at three different holding potential (−70, −50, −30 mV). In column A the appearance of a slowly decaying component of the EPSCs at more positive membrane potentials is illustrated. The current traces in B and C were obtained from the same neuron as shown in A. In column B the fast component was blocked by CNQX (2 \( \mu \text{M} \)). Furthermore the voltage-dependent increase in the slow component between −70 and −30 mV holding potential is illustrated. Column C shows the block of the slow component by APV (20 \( \mu \text{M} \)). Even under depolarized conditions the slow component did not appear. This indicates that the fast component of the EPSCs shown in A and C was mediated by AMPA/KA receptors and the slow component which can be seen in columns A and B was mediated by NMDA receptors.

remaining slow component after addition of CNQX. At −80 mV very little current was measured. The \( I-V \) relation displayed a region of negative slope conductance between −60 to −30 mV and then continued almost linearly at more positive values.

Since the two components have very different rise and decay times it seemed likely that it would be possible to analyse \( I-V \) relations of the slower component even in the absence of CNQX. In Fig. 11D the amplitudes of EPSCs 9–13 ms after the initial
peak in control conditions were compared with the peak EPSC amplitudes in CNQX-containing solution. Both $I-V$s have a very similar shape.

In order to study the current–voltage relation of the component mediated by NMDA receptors EPSCs were recorded in the presence and absence of 20 μM-APV (Fig. 12A and B). The amplitude of the peak current was hardly affected by APV (Fig. 12C) whereas the amplitude of the late current was almost completely blocked (Fig. 12D).

The results presented in Figs 11 and 12 thus suggest that the two EPSC components which are mediated by AMPA/Ka and NMDA receptor subtypes can be separated nearly completely in standard extracellular solution without the use of antagonists by measuring peak amplitudes (AMPA/Ka receptor component) and late mean current amplitudes 9–13 ms after the peak (NMDA receptor component).

**Mg²⁺ dependence of the two EPSC components**

Further support for the activation of the two GluR channel subtypes in stellate cell synapses derives from experiments in which the Mg²⁺ dependence of the EPSC was examined. Changing the extracellular solution from control to nominally Mg²⁺-free solution resulted in the appearance of a slowly decaying component which was clearly visible also at −70 mV membrane potential (Fig. 13A). This component could be blocked completely by 20 μM-APV (Fig. 13B). On the other hand, CNQX
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Fig. 11. Effect of CNQX on current–voltage relation of unitary EPSCs. Neurone from a 17-day-old rat. The membrane potential of the recorded cell was changed between -80 and +40 mV. In this experiment K+ was replaced by Cs+ in the intracellular solution. Stimulus voltage was 7.5 V; stimuli were applied at a frequency of 0.5 Hz and had a duration of 50 µs. A, averages of thirty to forty stimulus-evoked EPSCs at the indicated holding potentials in standard extracellular solution. The peaks of the fast currents are marked by the first dashed vertical line (1). The slow current was calculated by averaging over a fixed interval from 9 to 13 ms after the fast peak indicated by lines (2) and (3). EPSCs are preceded by a stimulus artifact (arrow). B, effect of 2 µM-CNQX. Lines (1)-(3) indicate the same times as in A. C and D, current–voltage relation of the two components. • represent the mean values in control solution while □ show the results after addition of 2 µM-CNQX. Bars represent the standard deviations. In C the currents at lines (1) before and after CNQX application are compared while in D the averaged current values in the intervals between lines (2) and (3) can be seen. The continuous lines were drawn by eye to facilitate the comparison of the curves.
Fig. 12. Effect of APV on current-voltage relation of unitary EPSCs. Neurone from a 13-day-old rat. The membrane potential of the recorded cell was varied between -100 and +20 mV. In this experiment K\(^+\) was replaced by Cs\(^+\) in the intracellular solution. Stimulus voltage was 20 V; stimuli were applied at a frequency of 0.5 Hz and had a duration of 50 μs. A, averages of thirty to forty stimulus-evoked EPSCs at the indicated holding potentials in standard extracellular solution. EPSCs are preceded by a stimulus artifact (arrow). The peaks of the fast currents are marked by the first dashed vertical line (1). The slow current was calculated by averaging over a fixed interval from 9 to 13 ms after the fast peak indicated by lines (2) and (3). B, effect of 20 μM-APV. The numbers of the dashed lines are the same as in A. C and D, current-voltage relation of the two components. • represent the mean values in control solution while □ show the results.
(2 μM) blocked the fast component, but had no effect on the Mg$^{2+}$- and APV-sensitive part of the EPSC (Fig. 13C).

**Time course of the EPSC component mediated by NMDA-type GluR channels**

The high time resolution offered by whole-cell current recordings in stellate cells allowed the dissection of the activation time courses of the synaptic current mediated by AMPA/KA and NMDA receptor subtypes, respectively. Whereas the AMPA/KA receptor-mediated component could be studied at −70 mV in standard extracellular solution, the time course of the NMDA receptor-mediated current was measured in the presence of CNQX. Currents were recorded both in standard extracellular solution under depolarized conditions and at potentials close to the resting potential in nominally Mg$^{2+}$-free solution.

Figure 14A illustrates the difference in the rising phase of the AMPA/KA and the NMDA component of averaged stimulus-evoked EPSC traces at −30 mV holding potential in standard extracellular solution. The NMDA receptor component rose after a longer delay than the AMPA/KA receptor-mediated component and it rose much more slowly to the peak. Figure 14B illustrates essentially the same finding with averaged traces at −70 mV when Mg$^{2+}$ was omitted from the extracellular solution. Again the NMDA receptor-mediated component rose with a longer delay than the AMPA/KA receptor-mediated component (3.4 versus 2.7 ms), and its rise time was much longer. Whereas the AMPA/KA receptor component rose in 0.44 ms the rise time of the NMDA component was 5.6 ms. The AMPA/KA component had decayed to about one-third of its peak value at a time when the NMDA receptor component had risen to one-half of its peak amplitude. The rise time of the averaged NMDA receptor-mediated component of the EPSCs varied between 5.4 and 6.2 ms in different experiments (the EPSCs being aligned on their rising phases before averaging; see Methods).

The decay time course of the NMDA receptor-mediated component was studied in the presence of CNQX (2 μM) in nominally Mg$^{2+}$-free solution and at a holding potential of −70 mV. Figure 15A illustrates the fluctuation of evoked EPSCs between different trials under these conditions. Clearly, both the amplitude and the time course showed a large variability. This is reflected by the fluctuation of charge flow during different single events ranging from 0.3 to 13.8 μC (Fig. 15C).

The decay of the averaged EPSCs mediated by NMDA receptors could be fitted well by the sum of two exponentials (Fig. 15B). In different experiments these time constants varied between 15.7 and 79.4 ms for the fast and between 108.3 and 307.6 ms for the slow component.

**Amplitude fluctuations of fast and slow EPSC components**

The observation that the two EPSC components can be separated by measuring the peak currents (AMPA/KA receptor component) and a mean late current (NMDA after addition of 20 μM-APV. Bars represent the standard deviation. In C the peak currents (indicated by lines (1)) before and after APV are displayed while in D the averaged current values in the intervals between lines (2) and (3) can be seen. The continuous lines were drawn by eye to facilitate the comparison of the curves.
receptor component) offers the possibility to compare their amplitude fluctuations when only a single presynaptic neurone is stimulated. We compared the fluctuations in amplitude of the two EPSC components at $-70$ mV and with nominally Mg$^{2+}$-free extracellular solution. The amplitude of the initial peak of the fast component, and the mean value of the current at an interval of 7–11 ms after the peak, were determined for unitary EPSCs which fluctuated in the peak amplitudes (Fig. 16A).

The two values measured for successive unitary EPSCs were well correlated (Fig. 16B). The correlation coefficients in three experiments were 0.81, 0.9 and 0.92. This

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Fig. 13. Effect of extracellular Mg$^{2+}$ on the two EPSC components. Neurone from a 13-day-old animal. Currents were sampled at 10 kHz and filtered at 2 kHz ($-3$ dB). Holding potential was $-70$ mV. The recording pipette was filled with standard intracellular solution. All traces show averages of ten to thirty single stimulus-evoked EPSCs. Arrows indicate the stimulus artifact. A, in addition to the fast peak under standard extracellular conditions the EPSC in nominally Mg$^{2+}$-free solution was characterized by an additional slow component. B, while APV had no effect on the fast peak it completely abolished the slow component. C, CNQX selectively blocked the fast current but did not affect the slow component in Mg$^{2+}$-containing solution. All records in A, B and C were obtained from the same neurone.
strongly suggests co-activation of AMPA/KA and NMDA receptor channels at an almost constant ratio during unitary EPSCs in a particular synapse.

The ratio of the AMPA/KA and NMDA current components measured in this way varied, however, for different synapses. For the three different synapses studied in this way the ratio between the AMPA/KA and the NMDA peak current was 5 (Fig. 16B), 1.5 (not shown) and 1.25 (Fig. 16C).
Fig. 15. Decay time course of the NMDA component of unitary EPSCs. A, examples of individual current traces at a membrane potential of −70 mV. Records were obtained in nominally Mg²⁺-free solution after addition of CNQX (2 μM) to reveal purely NMDA
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DISCUSSION

Identification of neurones

The classification of neuronal cell types based on morphological criteria in the visual cortex is still somewhat controversial. Werner, Hedlich & Winkelmann (1985) describe a large number of neuronal subtypes, but Winfield, Gatter & Powell (1980) distinguish only between pyramidal neurones and large and small stellate cells. All classifications agree on a major distinction between pyramidal and non-pyramidal cells (Peters & Jones, 1984).

The visual criteria, using Nomarski optics, used in this study to decide which neurones to choose for recording, were the round shape of the cell soma and the lack of a main apical dendrite. A further characterization of the subtypes of non-pyramidal cells could not be made due to the difficulty in following the fine dendritic tree in the cellular periphery. The classification as non-pyramidal cells was supported by the image reconstruction of ten cells filled with Lucifer Yellow. The majority of cells (7/10) stained with this method had the appearance of stellate cells as judged from the shape of the dendritic tree. This is in good agreement with anatomical studies showing that the subgroup of stellate cell is the largest among all non-pyramidal neurones in layer IV (Winfield et al. 1980).

The classification of the recorded neurones as stellate cells is further supported by the electrophysiological characteristics of these cells. In slices of guinea-pig cortex McCormick et al. (1985) found a comparable behaviour of the electrical excitability for non-pyramidal cells, i.e. the fast spiking behaviour without slow afterhyperpolarization which is characteristic for interneurones (Connors & Gutnick, 1990). The observation that the action potential frequency seen with strong depolarization was lower than in sensorimotor cortex interneurones could be due to the fact that cells examined in the visual cortex were less mature (5-20 days), that the intracellular milieu is changed to a larger extent using patch pipettes for whole-cell recording as compared to high-resistance intracellular electrodes, or finally to the lower temperature (20-24 vs. 35-37°C).

A third argument in favour of the characterization of the neurones investigated here as stellate cells concerns the properties of EPSCs. Because of the short rise times of stimulus-evoked EPSCs, the excitatory synapses are probably located on or very close to the cell soma. Electron microscopic studies have shown that only non-pyramidal cells of the visual cortex exhibit asymmetric, putatively excitatory synapses on the cell soma (Parnavelas & Lieberman, 1979; Bähr & Wolff, 1985).

Identification of unitary EPSCs

The action of various blocking substances shows the stimulus-evoked currents to be EPSCs. First, they were not inhibited by the GABA_A receptor antagonist

receptor-mediated EPSCs. B, average of selected current traces of the experiment exemplified in A (n = 49). The current decay was well fitted by the sum of two exponentials with time constants of 70±7 and 307±35 ms. C, change histogram of all stimulus-evoked EPSCs recorded in this experiment (n = 108). Charges showed a large fluctuation ranging from 0.3 to 13.8 pC with a mean of 3.1 pC.
bicuculline in concentrations that were sufficient to block IPSCs. Second, stimulus-evoked currents could be blocked by addition of TTX showing that they do not arise from direct stimulation of nerve terminals but from activation of a presynaptic neurone at a distant site. Third, in standard extracellular solution at holding

Fig. 16. Fluctuation of the two EPSC components. A, examples of EPSCs at high time resolution during stimulation of a neighbouring neurone in nominally Mg²⁺-free extracellular solution. Neurone from a 12-day-old animal. Data were sampled at 10 kHz and filtered at 2 kHz (−3 dB). The stimulus (29 V, 50 ms) was applied at a frequency of 0.5 Hz. The recording electrode was filled with standard intracellular solution. Holding potential was −80 mV. The stimulus artifact was removed by digitally subtracting the average of forty failures from the original records. Arrows indicate the beginning of the stimulus. The peaks of the AMPA/K⁺ currents are marked by the first vertical line (1). The NMDA current was calculated by averaging the current over a fixed time interval after the AMPA/K⁺ peak indicated by lines (2) and (3). B, plot of the correlation between the amplitudes of the NMDA and the AMPA/K⁺ component. The upper graph shows the analysis of all EPSCs recorded in the experiment exemplified in A. The mean current (between (2) and (3)) is plotted against the peak current (1). The regression line had a slope of 0.106. The correlation coefficient was 0.923. The lower graph shows the same analysis of EPSCs in a different experiment with another cell. In this case the regression line had a slope of 0.771. The correlation coefficient was 0.899.

potentials of −70 mV and more negative, the currents were blocked by CNQX, an antagonist of GluR channels of the AMPA/K⁺ subtype as has been demonstrated previously for other excitatory pathways in the central nervous system (Blake et al. 1988; Konnerth, Llano & Armstrong, 1990).

The stellate cell EPSCs are likely to be unitary because evoked EPSCs were not
elicited until a critical threshold level of stimulus strength was reached. Increasing the stimulus voltage above that threshold did not alter the shape and amplitude of the averaged EPSCs and moving the tip of the stimulation pipette a short distance away from its location on the cell soma caused the responses to disappear. Finally, the latencies of the EPSCs fell in a narrow range of 2–4 ms and their distribution was usually characterized by a single peak. The fact that in some experiments a second peak appeared in the latency histogram could have been due to activation of an additional input, perhaps through a polysynaptic connection.

EPSCs could be detected only in slices from animals older than 9 days. Several possibilities could account for this observation. First, the connections might be so rare as to be undetectable or they might not yet exist at all until postnatal day 9. It could thus be a property of these particular synapses to develop at that stage. Second, the synapses could already exist anatomically but they might be still immature and not yet functional. Blue & Parmavelas (1983) described the density of asymmetric synapses in the visual cortex of the rat during postnatal development. In the first few postnatal days the density of these synapses was only about 2% of the value in adult animals. The fastest increase took place during the second postnatal week. Although in the study cited above no distinction between axosomatic and axodendritic excitatory synapses was made, this may indicate that the lack of asymmetric synapses (Gray type I) could be the main reason for the lack of electrophysiologically detectable excitatory connections in very young animals.

Size and time course of unitary EPSCs mediated by AMPA/KA receptors

The EPSCs at membrane potentials close to the measured resting potential (−66 mV), were mediated almost exclusively by the AMPA/KA subtype of GluRs as shown by the action of the different selective GluR antagonists.

The size and time course of the AMPA/KA receptor-mediated EPSC component differed in several aspects from those described for EPSCs in other pathways. The average unitary EPSC did not exceed 100 pA, and the peak EPSC amplitudes fluctuated with peak separations in the range of only tens of picocuries. The EPSC rise times were in the range of 0.2–0.5 ms. Thus stellate cell EPSC amplitudes were much smaller and their rise times much shorter than those of EPSCs recorded in hippocampal pyramidal cells (Hestrin, Nicoll, Perkel & Sah, 1990a; P. Jonas & B. Sakmann, in preparation) or cerebellar neurones (Konnerth et al. 1990). For example, at the excitatory synapses between the Schaffer collateral–commissural tract and CA1 pyramidal cells Hestrin et al. (1990a) reported peak amplitudes of up to 1 nA and rise times of 1–4 ms. This difference in EPSC size and time course probably reflects the fact that EPSCs in these cases were elicited by stimulation of fibre tracts rather than by a single presynaptic neurone and probably EPSCs were generated by summation of currents from several synapses distributed along the dendritic tree.

Two recent publications (Malinow & Tsien, 1990; Bekkers & Stevens, 1990), report relatively small EPSC amplitudes in CA1 pyramidal cells after stimulation of Schaffer collateral fibres (means of 6.7 and 42.3 pA, respectively). In these experiments, however, electrotonic attenuation and inadequate clamp conditions may have interfered with the measurements.

The decay time constants of the AMPA/KA receptor-mediated EPSCs in stellate
cells were also considerably smaller than those reported for other excitatory synapses, where decay time constants between 4 and 14 ms were measured (Konnerth et al. 1990; Hestrin et al. 1990a). This difference could reflect regional differences in gating properties of GluR channel subtypes as suggested by in situ hybridization experiments where a regional specificity in the expression of GluR subunit transcripts was demonstrated (Sommer, Keinänen, Verdoorn, Wisden, Herb, Köhler, Takagi, Burnashev, Sakmann & Seeburg, 1990). With the exception of EPSCs activated by mossy fibre inputs in pyramidal cells of the CA3 region, the longer EPSC decays could also reflect inadequate voltage clamp conditions, since excitatory synapses are located on the dendritic tree in most neurones and voltage clamping the cell soma might not be sufficient to control the membrane potential accurately in the dendritic tree.

Comparably fast rise and decay times have been described previously by Finkel & Redman (1983). They selected for excitatory synapses located on or very close to the soma of cat spinal motoneurones; these experiments were performed at 37 °C rather than at room temperature.

Size and time course of unitary EPSC mediated by NMDA receptors

Several studies have shown that two types of excitatory amino acid receptors, the AMPA/KA and the NMDA receptor subtype, are involved in synaptic transmission in the neocortex (Thomson, 1986; R. S. G. Jones, 1988). In the visual cortex K. A. Jones & Baughman (1988) showed that EPSPs elicited by the stimulation of the layer II/III to layer V circuit were mediated by both receptor subtypes. This was also reported for polysynaptically evoked EPSFs in layer II/III after stimulation of the white matter (Nishigori, Tsumoto & Kimura, 1990). LoTurco, Mody & Kriegstein (1990) showed that evoked synaptic currents in pyramidal cells of layer II/III were composed of NMDA and AMPA/KA receptor-mediated conductances. Spontaneous excitatory currents could be blocked by the AMPA/KA receptor antagonist CNQX while in Mg2+-free solution APV decreased background current fluctuations without affecting discrete synaptic events. In addition in experiments with cultured cells Huettner & Baughman (1988) demonstrated that at excitatory synapses between corticocollateral neurones from the visual cortex in the absence of Mg2+ in the extracellular solution both types of GluR channels could be activated.

The experiments reported here show that EPSCs with slower rise and decay time courses are recorded after block of the AMPA/KA receptors. These EPSCs share many of the characteristics reported for NMDA receptor-mediated currents such as block by Mg2+ and sensitivity towards NMDA receptor antagonists. Thus, unitary EPSFs, generated by transmitter release from a single presynaptic neurone, seem to be mediated by activation of both the AMPA/KA and NMDA subtypes of GluR channels. However, activation of NMDA receptors during EPSCs occurred with a considerable delay; they started at a time when AMPA/KA receptor-dependent current had almost subsided. The rise time of the NMDA component in stellate cell EPSCs was between 5-4 and 6-2 ms which was more than ten times longer than that of the AMPA/KA receptor-mediated component (0-2-0-5 ms). The rise time courses of the NMDA receptor-mediated EPSC component were, however, considerably faster than those reported for EPSCs in CA1 synapses (Collingridge et al. 1988;
Hestrin, Sah & Nicoll, 1990b), but they were comparable to those of EPSCs in cultured hippocampal neurones (Lester, Clements, Westbrook & Jahr, 1990).

A completely different behaviour has been reported at synapses between mossy fibres and cerebellar granule cells (D'Angelo, Rossi & Garthwaite, 1990), where the kinetics of NMDA receptor-mediated EPSCs more closely resembled the fast AMPA/KA receptor-mediated EPSCs seen in other brain regions.

Co-activation of AMPA/KA and NMDA receptors during unitary EPSCs

The co-activation of AMPA/KA and NMDA receptor components raises the question of whether both receptors are co-localized and whether in different synaptic boutons the ratio of the two subtypes is varying. Co-localization of the two GluR channel subtypes was suggested for excitatory synapses between cultured neurones (Bekkers & Stevens, 1989) who investigated the time course of spontaneous miniature EPSCs (MEPSCs) in cultured hippocampal neurones. They assumed that the majority of MEPSCs were mediated by both classes of channels, though with varying ratios. Synapses formed between cultured hippocampal neurones are poorly defined and thus may not reflect the physiological situation in view of the observation that different distributions of functional GluR subtypes are found even within the hippocampal subfields (P. Jonas & B. Sakmann, submitted for publication). Moreover, MEPSCs are generated by different synaptic inputs, and it cannot be excluded that the varying ratios of the two components in MEPSCs in these experiments resulted from the inhomogeneity of the recorded synapses. In the excitatory synapses of stellate cells the ratio of fast to slow EPSC component was almost constant for any particular synapse despite the large fluctuations in peak EPSC amplitudes. This suggests that the ratio of AMPA/KA to NMDA receptors which are activated at different release sites of the same presynaptic neurone is almost constant for a particular synapse. Interestingly, however, in different neurones the ratio of AMPA/KA to NMDA receptors varies for different synaptic inputs over a relatively large range.

Although the sample sizes in this study were too small for a reliable quantal analysis, amplitude distributions such as that shown in Fig. 8B suggest that, similarly to other synapses in the central nervous system (Edwards et al. 1990; Larkman, Stratford & Jack, 1991), the transmission is quantal and that the quantum size is small. The EPSC peak amplitude fluctuations suggest a quantal conduction change in the order of 100 pS for the AMPA/KA receptor-mediated component. Assuming an average single-channel conductance of 4–8 pS for the AMPA/KA receptor channel (P. Jonas & B. Sakmann, submitted for publication) this would indicate that during a quantal event about twenty channels of the AMPA/KA receptor type are activated. The ratio of the peak amplitudes of the NMDA to AMPA/KA receptor-mediated EPSC components, in nominally Mg$^{2+}$-free conditions, varied between 0·2 and 0·8 at different synapses. Assuming a single-channel conductance of 40–50 pS for the NMDA receptor channel (Cull-Candy & Usowicz, 1987; Jahr & Stevens, 1987; Ascher, Bregestovskiy & Nowak, 1988) this could indicate that the number of NMDA receptor channels activated per quantal event varies between one and five channels. Thus the ratio of AMPA/KA to NMDA channels activated at a single synaptic bouton would vary in the range of two to ten.
for different synaptic connections. This ratio might be important for weighting different synaptic inputs to a particular cell.

**Functional implications**

The possibility that a single unitary event can depolarize the cell to the threshold for action potential generation is probably due to the high input resistance of stellate cells and the somal location of excitatory synapses. This, in conjunction with the observation that the spike discharge rate shows little adaptation suggests that stellate cells, which are inhibitory, could be used for exact timing of inhibition of pyramidal cells. This inhibition is important for processing of contrast detection (Perster & Koch, 1987; Bolz, Gilbert & Wiesel, 1989) by larger layer IV pyramidal cells. The electrical characteristics of the stellate cells, high input resistance and the non-adaptive action potential frequency, are such that they would be well suited to integrating incoming excitation accurately according to the number of synaptic boutons that are active, and to generating a specific pattern of inhibition of pyramidal cells.

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