Ultrastructural Correlates of Quantal Synaptic Function at Single CNS Synapses

Paul J. Mackenzie,1 Gail S. Kenner,1 Oliver Prange,1 Hossein Shayan,1 Masashi Umemiya,3 and Timothy H. Murphy1,2

Kinsmen Laboratory of Neurological Research, Departments of 1Psychiatry and 2Physiology, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada, and 3Department of Neurophysiology, Tohoku University School of Medicine, Sendai 980-8575, Japan

We have tested the hypothesis that functional differences between synapses are associated with ultrastructure in cultured cortical neurons. Using Ca\(^{2+}\) imaging, we measured NMDA receptor-mediated miniature synaptic calcium transients attributed to the spontaneous release of single transmitter quanta. After imaging, the identified neurons were processed for serial transmission electron microscopy. At sites of quantal NMDA receptor-dependent Ca\(^{2+}\) transients, we confirmed the presence of excitatory synapses and measured spine size and synapse size correlates positively with the amplitude of the NMDA receptor-mediated postsynaptic response, suggesting that larger synapses express a greater number of NMDA receptors. Therefore, regulation of quantal amplitude may involve processes that alter synapse size.

Key words: NMDA; quantal; spine; dendrite; postsynaptic density; PSD; LTP

Studies using electron microscopy (EM) have indicated that the morphology of cortical excitatory glutamatergic synapses is highly variable (Harris et al., 1992). Bouton size, vesicle number, vesicle diameter, and postsynaptic spine volume vary widely within a relatively homogenous population of neurons (Pierce and Lewin, 1994; Schikorski and Stevens, 1997). It has been hypothesized that structural differences between synapses underlie some of the functional differences that are observed between neurons (Calverley and Jones, 1990; Lisman and Harris, 1993; Edwards, 1995). Accordingly, it is necessary to assess both synaptic structure and function at single CNS synapses. A few reports have combined EM and paired cell electrophysiology to measure the properties of single synapses (Gulyás et al., 1993; Buhl et al., 1994, 1997), but these experiments could not compare multiple synapses within a single neuron. To compare multiple synapses along a region of dendrite, we have measured spontaneous synaptic events using Ca\(^{2+}\) imaging at identified synapses in cultured cortical neurons and subsequently processed the same specimens for serial transmission EM. Ca\(^{2+}\) transients in individual spines can be resolved in an acute brain slice (Petrozzino et al., 1995; Yuste and Denk, 1995; Schiller et al., 1998; Yuste et al., 1999), but subsequent identification of the same synapses with EM would be problematic because of the higher spine density in the slice. Also, the three-dimensional (3-D) nature of neuronal dendrites in situ, as opposed to more planar dendrites found in culture, makes simultaneous imaging of multiple synapses within a slice difficult.

Using Ca\(^{2+}\) imaging, we have measured the NMDA receptor-mediated component of spontaneous miniature EPSCs (mEPSCs), termed the miniature synaptic Ca\(^{2+}\) transient, (MSCT; Murphy et al., 1994, 1995). Sensitivity to the NMDA receptor antagonist DL-APV suggests that under the conditions we have used, Ca\(^{2+}\) transients associated with miniature synaptic activity are primarily attributed to NMDA receptors (Murphy et al., 1994). We have previously reported a positive correlation between MSCT amplitude and mEPSC amplitude (Murphy et al., 1995), indicating that Ca\(^{2+}\) imaging can be used to evaluate the local characteristics of synaptic events. After MSCT imaging, we performed serial reconstruction of transmission EM images to identify synapses at the origins of the Ca\(^{2+}\) transients. This enabled the ultrastructural characterization of the specific synapses where MSCTs were measured. We show that MSCT amplitude is positively correlated with attributes of synapse size, including synaptic contact area and spine volume.

MATERIALS AND METHODS

Cortical neurons and glia were dissociated from 17–18 d gestation rat fetuses, placed in culture, and allowed to mature for 17–26 d in vitro as previously described (Mackenzie et al., 1996). Whole-cell recording was used to load neurons with fluo-3 and furaptra. The patch pipette solution contained (in mM): 3–5 fluo-3 K\(^+\) salt, 5 furaptra K\(^+\) salt (relatively...
Ca²⁺-insensitive and used to view basal fluorescence). 92 KMeSO₄, 20 NaCl, 5 Mg-ATP, 0.3 GTP, 10 HEPES, and 0.3–0.8% biocytin HCl, pH 7.3 (280 mOsM, adjusted with KMeSO₄). After neurons were loaded, the electrode was removed, and the cells were allowed to recover in the presence of 0.3 μM tetrodotoxin (TTX) containing saline for 1–2 hr. The concentration of fluo-3-loaded into the cell was estimated to be 300–500 μM. The following extracellular solution was used to measure the Ca²⁺-component of mEPSCs (in mM): 137 NaCl, 5 KCl, 5 CaCl₂, 0 MgCl₂, 0.34 Na₃HPO₄(7H₂O), 10 Na-HEPES, 22 glucose, 1 NaHCO₃, 0.02 picrotoxin, and 0.0003 TTX, pH 7.4 (Murphy et al., 1994).

Imaging and analysis. Imaging was performed with a 100× 1.3 NA Zeiss (Thornwood, NY) objective on a Zeiss Axiovert microscope with an Epix (Northbrook, IL) 4M12-64 MB frame grabber. For each experiment, 300 fluo-3 images were collected in 10 sec sweeps (15–17 sweeps per experiment) and were analyzed off-line in Interactive Data Language (Research Systems Inc., Boulder, CO). To view processes under baseline conditions and to correct for process variation processes under baseline conditions and to correct for process variation, we performed analysis of variance (ANOVA) on raw fluorescence difference data collected in consecutive trials. The analysis of variance was used to determine whether there was a significant change in fluorescence over consecutive trials, and this change was not progressive. The first measure, fluorescence scaled to spine volume (ΔFₐₘₚ₅₈), was used to measure a correction for suboptimal focus, and the second measure, the ratio of fluorescence to spine volume (ΔFₐₘₕ₃₈), was used to measure the difference in spine volume (see Eqs. 1, 2). Relative spine size (scaled to mean) was used to calculate correlations between Ca²⁺ response amplitude and spine size, because differences in spine size were normalized by dividing the ΔFₐₘₕ₃₈ by the spine size (Fig. 2a). The use of ΔFₐₘₕ₃₈/Fₐₘₕ₃₈ ratios would cause us to underestimate, rather than account for, observed differences in Ca²⁺ response (Iₐₚ₃ₜ /vol) between spines of different volume (see Eq. 4, Fig. 2a, dashed line). For example, if all spines had identical ΔFₐₘₕ₃₈/Fₐₘₕ₃₈ ratios (and thus Ca²⁺ concentration) during mEPSCs, then the larger spines must have had a NMDA current that was of relatively higher amplitude (Eqs. 3, 4). Similar correlations between synaptic size and response amplitude were observed with both methods of measurement (data not shown). Relative Ca²⁺ transient amplitude (scaled to mean) was used to calculate correlations between Ca²⁺ response amplitude and spine size, because differences in spine size were normalized by dividing the ΔFₐₘₕ₃₈/Fₐₘₕ₃₈ by the spine size (Fig. 2a). The use of ΔFₐₘₕ₃₈/Fₐₘₕ₃₈ ratios would artifactualy increase the variability of correlations. Using other methods of normalizing the MSCT amplitude (scaling to median or maximum response) did not appreciably alter the correlations that were observed. To examine the correlation between means of spine size and responsiveness, we pooled data from multiple neurons to obtain higher statistical power. Although lacking statistical power, analysis of single experiments indicated that all four EM samples showed a positive correlation between the ΔFₐₘₕ₃₈/Fₐₘₕ₃₈ and spine volume (indicating a trend toward significance). None of the samples showed a negative correlation.

$$\Delta F_{380} = I_{NMDA} \times (F_{380\text{max}} - F_{380})$$
$$F_{380} \alpha \text{ vol} = (F_{380\text{max}} - F_{380})$$
$$\Delta F_{380}/F_{380} \alpha \text{ NMDA}/\text{vol}$$
$$I_{NMDA} \alpha (\Delta F_{380}/F_{380})^\alpha \text{vol}$$

(4) Spine size was scaled to the average spine size observed in a particular specimen to control for potential differences in spine size attributable to fixation conditions, development, or phenotype of neuron (Papa et al., 1995; Boyer et al., 1998). We have also used other methods of calculating relative spine size (scaled to median or maximum spine volume) and have found that the correlations do not vary significantly with the method used.

Electron microscopy. After MSCT imaging, preparations were fixed with 4% paraformaldehyde and 0.2–0.5% glutaraldehyde in 0.1 M Sorenson’s Na⁺ phosphate buffer, pH 7.2–7.4 (1.5 hr) at room temperature (RT), rinsed briefly in Dulbecco’s PBS (DPBS), permeabilized in 0.1–0.2% Triton X-100 in DPBS (3–4 min, RT), washed with DPBS (3–5 vol over at least 30 min, RT), incubated with Vector laboratories (Burlingame, CA) A/B reagent (avidin/biotinylated peroxidase complex; 1 hr, RT), washed with DPBS (3–5 vol over 30 min, RT), and incubated in 0.5 mg/ml diaminobenzidine (DAB) and 0.015% H₂O₂ in DPBS for 2–5 min (RT, intensity monitored to prevent over-staining). DPBS washing (5 vol over at least 30 min, RT) was followed by further fixation in 2.5% glutaraldehyde in 0.1 M Sorenson’s buffer, pH 7.2–7.4 (1 hr, on ice) and washing in the same buffer (3 vol over 30 min, on ice). Preparations were then post-fixed in 1% OsO₄ in the same buffer (1 hr, on ice). After a final Sorenson’s buffer wash (3 vol over 30 min, on ice), cultures were dehydrated in a graded ethanol series (50, 70, 85, 95, and 100%) and flat-embedded in Spurr resin on Aclar plastic (Proplastics, Linden, NJ). After polymerization, arsine containing single stained neurons were excised, separated from the Aclar, and mounted on blank blocks. Serial sections of ~70 nm thickness were collected on pioform or Formvar-coated single slot grids, stained with 3% aqueous uranyl acetate (UA) or 5% UA in 20% MeOH, followed by lead citrate, and then examined at 80 keV in a Zeiss EM 10C serial transmission electron microscope. A montage of low-power electron micrographs (8000×) was aligned with fluorescence and bright-field images. From this overlay, it was possible to identify DAB-stained dendritic spines where MSCT events occurred. Staining selectivity arises because both the Ca²⁺ indicators and biocytin are injected into a single neuron, allowing the DAB imaging and subsequent staining of the neuron of interest with an immunoperoxidase reaction (Gulyás et al., 1993). EM images were obtained at higher magnification (31,500×) to perform serial reconstruction at sites where MSCT events were initiated. Spine volume and synaptic contact area were measured at sites with more than one MSCT by tracing the outline of DAB-stained spines through serial sections and digitizing the traces. NIH Image and Adobe Ultrastructure and Quantal Synaptic Function.
(Mountain View, CA) Photoshop were used for three-dimensional reconstructions. Profiles were occasionally unobtainable because of section folding. In such cases, spine sizes were estimated from the digitized serial sections by linear interpolation between adjacent sections; data were used only if the amount estimated accounted for <10% of spine volume. The addition of estimated data did not appreciably change the correlations that were obtained. In some specimens detergents required in the staining process degraded the ultrastructure.

Synapses were identified by the presence of presynaptic and postsynaptic membrane apposition, synaptic cleft thickening, a presynaptic paramembranous density, and clustering of at least three vesicles near the presynaptic membrane. The intensity of the DAB staining prevented accurate measurement of the postsynaptic density size in many cases. The area of synaptic contact was defined as the region of increased (and relatively constant) thickness between the presynaptic and postsynaptic membranes, as illustrated in Figure 1d, arrows. Measurements of synaptic contact area in perforated synapses (synapses with two separate clusters of vesicles from the same presynaptic bouton) included the area of the perforation. Because relative spine size (scaled to the mean spine size for each specimen) was correlated with MSCT amplitude (also scaled to mean MSCT amplitude for each specimen), no correction was applied for differences in shrinkage in the 2-dimensional during ethanol dehydration (Trommald and Hullenberg, 1997). Twenty synapses were reconstructed from four specimens (neurons). Of these, 2 were shaft synapses; spine volume was therefore measured at the remaining 18 spines (12 single macular synapses, 4 perforated synapses with the same presynaptic bouton, and 2 spines each contacting 2 presynaptic boutons). At 7 of the 20 synapses, the synaptic contact area was not measurable throughout its full extent because of a tangential plane of section. Synaptic contact area was therefore measured at 13 of the 20 synapses; this included two of the perforated synapses but none of the synapses with more than one presynaptic bouton.

**Confocal microscopy.** After fluorescence imaging of MSCTs using wide-field microscopy, cells were fixed with 4% paraformaldehyde in 0.1 M Sörensens's buffer, pH 7.2–7.4 (1.5 hr, RT), rinsed briefly in DPBS, permeabilized in 0.2–0.5% Triton X-100 in DPBS (4–5 min, RT), washed with DPBS (3–5 vol over 5 min, RT), and blocked in 1.2–2.5% normal goat serum in DPBS (4–12 hr, 4°C). Cultures were washed with DPBS (3–5 vol over 30 min, RT), incubated with 20 μg/ml avidin-fluorescein in DPBS (Vector; 1–2 hr, RT), washed with DPBS (3–5 vol over 30 min, RT), and mounted on a coverslip with Antifade in glycerol and DPBS (Molecular Probes, Eugene, OR). Confocal imaging was performed with a Bio-Rad (Hercules, CA) MRC 600 system attached to a Zeiss Axioskop microscope and a 100×/1.3 NA Zeiss objective (laser intensity = 1%; confocal pinhole = 3 Bio-Rad units). Serial images along the vertical axis (z-series) were obtained through the entire dendritic region of interest (step size, 0.5 μm) and a maximal-intensity projection was used to generate a two-dimensional representation of spine size. A maximal-intensity projection flattens 3-D images by creating an image of the maximal pixel value across the sections for each pixel. For a spine to be selected for measurement, it was necessary to clearly resolve at least one MSCT event initiated at the spine. MSCT imaging was performed with wide-field microscopy and a CCD camera that has lower resolution than confocal microscopy. This criterion was the limiting factor in spine determination; therefore, other morphological criteria were not necessary (Trommald et al., 1995). Spines that were selected could be clearly resolved from other structures in the maximal-intensity confocal projection image; at least two rows of pixels of lower intensity were between two adjacent spines or between spine head and dendrite. Cross-sectional spine area was quantified using NIH Image and Adobe Photoshop. Although confocal measurements of absolute spine size are problematic for small spines (Harris, 1994; Trommald and Hullenberg, 1997), a correction was not applied, because relative measurements of spine size versus MSCT response were used. Because of the limiting resolution of confocal microscopy, confocal measurements of spine size are likely to overestimate the size of small spines; this effect would underestimate rather than account for a correlation between spine size and MSCT amplitude.

**RESULTS**

**Calcium imaging and parallel ultrastructural analysis of single synapses**

We have conducted experiments designed to assess both structure and function at the same CNS synapses. Cultured cortical neurones were injected with fluo-3, and MSCT imaging was used to map the NMDA receptor-mediated component of quantal synaptic responses to identified dendritic regions. Co-injection of biocytin allowed the selective staining of the neuron of interest after MSCT imaging. Serial EM reconstruction was performed on 20 synapses (four neurons) from which we had measured the postsynaptic effect of putative single transmitter quanta. Figure 1a shows a basal fluorescence image of a region of dendrite from a cortical neuron. Figure 1b illustrates traces of Ca2+ dynamics at four dendritic sites. A perforated synapse was identified at site 3, where seven synaptic events were initiated. In contrast, most neighboring dendritic synapses were either inactive (for example, site 4) or were the initiation site of only one MSCT event. Sites 1 and 2 represent other sites, subsequently confirmed by serial reconstruction to be single macular synapses, with smaller-amplitude responses. MSCTs were usually localized to dendritic spines, as illustrated by a sequence of images during a single trial of a Ca2+ transient initiated at site 3 (Fig. 1c). After Ca2+ imaging, specimens containing the dendritic region of interest were identified at both the light and EM levels via immunoperoxidase staining (Fig. 1d). EM revealed a single large perforated spine synapse centered at site 3 (Fig. 1d,e). Analysis of serial sections indicated that adjacent synapses were more than 2 μm away. Given the point spread function of the microscope (which describes the attenuation of signal with distance), it is unlikely that fluorescence changes at other synapses contributed significantly to the events measured at this site (our unpublished observations). Figure 1e shows a view of the 3-D reconstruction of site 3 made from serial EM sections, confirming the existence of a large dendritic spine with a single perforated synapse. The combined use of MSCT imaging and serial EM reconstruction thus enables the comparison of NMDA-mediated quantal responses at morphologically identified CNS synapses.

**The amplitude of the miniature synaptic calcium transient is correlated with synaptic size**

Spontaneous quantal synaptic currents exhibit a heterogeneous amplitude distribution that cannot be fully attributed to spatial distribution and filtering (Manabe et al., 1992; Lisman and Harris, 1993) but may be attributed to difference in response amplitude between synapses. The amplitude distribution may reflect differences in the size of the postsynaptic densities (PSDs), a hypothesis that we sought to test via combined Ca2+ imaging and serial reconstruction EM. Because we were often unable to measure the size of the postsynaptic density because of the intensity of the DAB staining, we measured two other variables that are correlated with PSD size: spine volume and the area of synaptic contact (Harris and Stevens, 1989; Harris and Sultan, 1995; Trommald and Hullenberg, 1997). Both measures of synapse size were significantly positively correlated ($r = 0.75; p < 0.05$) with the NMDA receptor-mediated component of the quantal response amplitude (Fig. 2). Figure 2a indicates a significant positive correlation between relative spine volume and relative MSCT amplitude ($r = 0.51; p < 0.05$). The gray dashed line indicates the predicted relationship between spine volume and MSCT amplitude if no relationship existed between $I_{NMDA}$ and spine volume, that is, for a constant $I_{NMDA}$ (see Eq. 3, Materials and Methods). A significant positive correlation was also observed between relative synaptic contact area and relative MSCT amplitude ($r = 0.75; p = 0.05$). The variables plotted in Figure 2, a and b, may be attributable to chance (given the scatter in the correlations) or may be because synaptic contact...
Figure 1. Quantal synaptic activity and serial reconstruction of identified cortical synapses. 

(a) Basal furaptra fluorescence image ($F_{380}$: Ca$^{2+}$-independent excitation wavelength) of a region of dendrite where Ca$^{2+}$ dynamics were measured. Scale bar, 10 μm. 

(b) Plots of Ca$^{2+}$ response versus time at four dendritic sites (17 trials 10 sec in length are overplotted). MSCTs were initiated and measured at 2 μm$^2$ regions centered over the indicated sites. Units of fluorescence: $F = \Delta F_{480}/F_{380}$. Site 3 exhibited a higher MSCT frequency and larger average MSCT amplitude than other sites, whereas site 4 showed no activity. 

(c) Images of the initiation of an MSCT at 33 msec intervals in the dendritic region encompassing site 3 (in (a, b)). In this trial, an MSCT is first visible at 67 msec. 

(d) Left panel, Bright-field image of the same region of dendrite after fixation and immunoperoxidase staining. The arrow points to site 3 shown above. Scale bar, 5 μm. Right panel, Electron micrograph illustrating a single cross-section through the spine at site 3. The arrowheads demarcate the region of cleft thickening used to measure synaptic contact area. On the left of the image, the DAB-stained spine is visible. A single perforated synapse is visible. Scale bar, 0.4 μm. 

(e) Three-dimensional view of reconstruction of the spine at site 3 confirms the presence of a single perforated synapse onto a large spine head (postsynaptic region only is shown). Examination of >40 serial sections through site 3 indicated only one synapse. The postsynaptic densities, the perforation, and the spine neck are identified by arrows. PSDs were identified from lighter prints of EM images.
area is likely better correlated with postsynaptic density size. To test whether three synapses with high SEM (because of few events) could spuriously account for the significant correlations observed, these data points were removed from the analysis, resulting in a slightly higher correlation. Thus, the behavior of the highly variable synapses did not account for the significant correlations between measures of synaptic size and MSCT amplitude.

Confocal measurement of spine size correlates with miniature Ca^{2+} transient amplitude

To confirm with a larger data set the observed relationship between synapse size as measured by serial EM reconstruction and MSCT amplitude, we performed similar experiments using confocal microscopy to measure spine size. In these experiments neurons were also injected with a combination of biocytin and Ca^{2+} indicators, and MSCT imaging was performed under the same conditions as described above. Figure 3a shows a basal fluorescence image of a region of dendrite captured using wide-field microscopy and a CCD camera. After imaging, specimens were fixed and stained with avidin–fluorescein, and confocal measurements of spine size were performed. Figure 3b shows a confocal image of the region of dendrite. Figure 3c illustrates the average MSCT Ca^{2+} response at three spines, each of which was a site of repeated MSCT initiation. As with the EM data, a significant positive correlation was observed between spine size and Ca^{2+} transient amplitude in 74 spines from five neurons (Fig. 3d; \( r = 0.52; p < 10^{-5} \)).

**DISCUSSION**

It has been widely hypothesized that alterations in synapse structure underlie changes in synapse efficacy (Lisman and Harris, 1993; Edwards, 1995). Many experiments have reported structural changes in synaptic populations after manipulations of synaptic strength or after learning (Greenough et al., 1978; Fifkova et al., 1982; Desmond and Levy, 1988; Hosokawa et al., 1995; Papa and Segal, 1996; Moser et al., 1997; Rusakov et al., 1997; but see Sorra and Harris, 1998). Unlike population studies, we have directly compared structure and function at the same synapses. This is the first report comparing the functional and structural properties of multiple synapses within the same CNS neuron, although previous investigations have obtained functional data from single
synapses that were later investigated at the ultrastructural level (Gulyás et al., 1993; Buhl et al., 1994, 1997). We conclude that the NMDA receptor-mediated component of quantal size (as measured by Ca\(^{2+}\) influx) is correlated with the size-related parameters of spine volume and synaptic contact area.

We have used Ca\(^{2+}\) imaging in cultured cortical neurons to measure the localized Ca\(^{2+}\) component of the miniature synaptic response (the MSCT). Under these conditions, the average MSCT amplitude provides a measure of the average mEPSC amplitude (attributed to NMDA receptors) of an identified synapse for the following reasons. First, synapses are confirmed to be present at each site of MSCT initiation, strongly suggesting that the Ca\(^{2+}\) influx is of synaptic origin. Second, the amplitude of the MSCT is correlated with the amplitude of the underlying mEPSC (Murphy et al., 1995). Third, both MSCTs and the slow component of the mEPSC are blocked in the presence of the NMDA receptor antagonist APV (Murphy et al., 1994, 1995).

We observed that spine size and the area of synaptic contact are significantly correlated with MSCT amplitude. Additional experiments using confocal rather than EM spine measurement confirmed these findings. These results support the hypothesis that larger synapses show larger quantal responses (Harris and Stevens, 1989; Lisman and Harris, 1993), although it is important to stress that the current MSCT imaging method measures the NMDA receptor-mediated component of the quantal response, and thus no conclusions can be made about the AMPA receptor-mediated component. Although we observed significant correlations between size and MSCT amplitude, the considerable scatter in the correlations suggests that other factors may also be contributing to MSCT size, including differences in vesicular transmitter content, and in the stochastic properties of postsynaptic receptors (Frerking et al., 1995; Murphy et al., 1995; Auger and Marty, 1997; Nusser et al., 1997). Additionally, multivesicular release may have contributed to the variability (Auger et al., 1998; Prange and Murphy, 1999). Furthermore, it is conceivable that fixation conditions may have slightly altered the synapse contact area or morphology. A further possibility is that Ca\(^{2+}\) release from intracellular stores may have amplified MSCT amplitudes, as previously results (Murphy et al., 1995) indicated a strong correlation between intracellular stores and MSCT amplitudes, suggesting that Ca\(^{2+}\) release from stores does amplify our optical signals, they are nonetheless proportional to synaptic current amplitude.

Although we were unable to measure directly the PSD area in our study, other measures of synapse size were significantly correlated with response amplitude, suggesting that PSD size is also correlated with NMDA receptor-dependent quantal amplitude. The strongest correlation with MSCT amplitude was observed for the measurement of synaptic contact area (Fig. 2b). Given the scatter in the correlations, this difference may have been attributable to chance; alternatively, this difference may reflect a stronger relationship between synaptic contact area and postsynaptic density size. Larger synaptic contacts and thus PSDs may contain a greater number of functional receptors (Harris and Landis, 1986), suggesting a functional consequence of activity-dependent regulation of PSD composition (Rao and Craig, 1997). In cerebellar stellate cells, postsynaptic GABA\(_A\) receptor density is uniform (Nusser et al., 1997), suggesting that synapse size may be a reliable measure of receptor number. In CA1 of hippocampus, although the density of AMPA receptors may not be constant, AMPA receptor immunoreactivity is greater at larger spines than at smaller spines (Nusser et al., 1998). We are not aware of any reports correlating NMDA receptor density and synapse size. Given the recent identification of relatively complex protein arrays involved in clustering postsynaptic receptors (Sheng, 1997), PSD area may be a limiting factor in controlling receptor expression (Kennedy, 1997). We conclude that mechanisms that control the growth and elaboration of synapses are thus likely to regulate NMDA receptor-mediated quantal amplitude.

REFERENCES


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