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Ultrastructural Distribution of the $\alpha 7$ Nicotinic Acetylcholine Receptor Subunit in Rat Hippocampus

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Acetylcholine (ACh) is an important neurotransmitter in the mammalian brain; it is implicated in arousal, learning, and other cognitive functions. Recent studies indicate that nicotinic receptors contribute to these cholinergic effects, in addition to the established role of muscarinic receptors. In the hippocampus, where cholinergic involvement in learning and memory is particularly well documented, $\alpha 7$ nicotinic acetylcholine receptor subunits ($\alpha 7$ nAChRs) are highly expressed, but their precise ultrastructural localization has not been determined. Here, we describe the results of immunogold labeling of serial ultrathin sections through stratum radiatum of area CA1 in the rat. Using both anti-$\alpha 7$ nAChR immunolabeling and $\alpha$-bungarotoxin binding, we find that $\alpha 7$ nAChRs are present at nearly all synapses in CA1 stratum radiatum, with immunolabeling present at both presynaptic and postsynaptic elements. Morphological considerations and double immunolabeling indicate that GABAergic as well as glutamatergic synapses bear $\alpha 7$ nAChRs, at densities approaching those observed for glutamate receptors in CA1 stratum radiatum. Postsynaptically, $\alpha 7$ nAChRs often are distributed at dendritic spines in a perisynaptic annulus. In the postsynaptic cytoplasm, immunolabeling is associated with spine apparatus and other membranous structures, suggesting that $\alpha 7$ nAChRs may undergo dynamic regulation, with insertion into the synapse and subsequent internalization. The widespread and substantial expression of $\alpha 7$ nAChRs at synapses in the hippocampus is consistent with an important role in mediating and/or modulating synaptic transmission, plasticity, and neurodegeneration.

Key words: acetylcholine receptors; nicotine; dendritic spines; postsynaptic density; immuno-electron microscopy; glutamate; GABA; $A_\beta_{1-42}$

Acetylcholine (ACh) is the major excitatory neurotransmitter in the peripheral nervous system. Ionotropic nicotinic receptors mediate postsynaptic excitatory responses at the neuromuscular junction, and there is evidence that nicotinic receptors may also act presynaptically to modulate acetylcholine release in the periphery (Wessler et al., 1992; Liang and Vizi, 1997). In the mammalian CNS, specific receptors for nicotinic ligands have been recognized for many years (Arimatsu et al., 1978; Dudai and Segal, 1978; Hunt and Schmidt, 1978; Segal et al., 1978), but only recently has evidence begun to emerge for their functional roles, including possible mediation of fast postsynaptic responses at certain brain sites (Zhang et al., 1993; Roerig et al., 1997; Chu et al., 2000) and modulation of release of various transmitters, including glutamate (Vidal and Changeux, 1993; McGehee et al., 1995; Gray et al., 1996), GABA (Lena et al., 1993), ACh (McGehee et al., 1995), and dopamine (Rapier et al., 1988). Nicotinic receptors constitute a heterogeneous family of ion channels. In the nervous system, nine different $\alpha$ subunits (\(\alpha 2-\alpha 10\)) and three different $\beta$ subunits (\(\beta 2-\beta 4\)) have been described. Most are assumed to form heteropentameric structures, with various combinations of $\alpha$ and $\beta$ subunits. There is also evidence in heterologous expression systems that some subunits, particularly $\alpha 7$, form homopentamers (Courtjier et al., 1990; Schoepfer et al., 1990; Seguela et al., 1993). Nicotinic acetylcholine receptors containing $\alpha 7$ subunits ($\alpha 7$ nAChRs) are, along with those containing the $\alpha 4/\beta 2$ combination, the most abundant in brain. The distribution of these receptors is specific, with the $\alpha 7$ subunit, which is selectively bound by $\alpha$-bungarotoxin ($\alpha$Bgtx) (Chen and Patrick, 1997; Orr-Urtreger et al., 1997), abundant in particular cortical and subcortical areas (Bina et al., 1995) of the mammalian brain; conspicuous among these is the hippocampus (Dominguez del Toro et al., 1994). The $\alpha 7$ subunit appears to participate in numerous important processes, including modulation of release of several neurotransmitters, mediation of postsynaptic excitatory responses, long-term potentiation (LTP), and cognitive function (Hunter et al., 1994; Fujii et al., 2000; Mansvelder and McGehee, 2000) (for review, see Role and Berg, 1996; Wonnacott, 1997; Radcliffe et al., 1999; Levin and Rezvani, 2000).

Light microscopic immunostaining has revealed the presence of $\alpha 7$ nAChRs in both somatic and dendritic regions in all hippocampal areas (Dominguez del Toro et al., 1994). Hippocampal cells in culture were found to exhibit patchy $\alpha 7$ nAChR immunolabeling on somata and dendrites, colocalized with presynaptic markers (Barrantes et al., 1995; Zarei et al., 1999), but the identity of the labeled cells was unspecified, nor was it possible at the light microscopic level to establish the presynaptic versus postsynaptic nature of these receptors. The current study examines the distribution of $\alpha 7$ nAChRs in the rat hippocampus at the ultrastructural level to determine their presynaptic versus postsynaptic localization.

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post synaptic nature of the labeling. Electron microscopic (EM) analysis of 125I-Bgtx binding provided evidence for α7 nAChRs at hippocampal synapses (Hunt and Schmidt, 1978), but the large grain radius and relative insensitivity of the method prevented firm conclusions about incidence or distribution of the αBgttx binding sites. Full understanding of the varied and subtle functional roles recently attributed to α7 nAChRs in the hippocampus (Radcliffe et al., 1999) will require high-resolution analysis of the subcellular distribution of this subunit. To this end, we have performed light and electron microscopic immunostaining of CA1 stratum radiatum, and here report that α7 receptors are highly abundant at almost all synapses in this region. The intensity of the signal suggests that the importance of α7-mediated nicotinic cholinergic signaling may be far greater than is currently recognized.

**MATERIALS AND METHODS**

**Light microscopic α7 nAChR immunolabeling.** Intact brain preparations were obtained from two adult male Sprague Dawley rats. Animals were anesthetized with urethane (1.5 mg/kg, i.p.) and perfused over ~20 min with 200 ml of 4% paraformaldehyde (PFA)/0.3% glutaraldehyde (GA) in PBS (0.1 M, pH 7.2). After dissection the brains were immersed in the same fixative for 2 hr and embedded in 7% Agarose. Vibratome sections 30 μm thick were cut transversely through the brain using a Leica VT1000S and rinsed in PBS (4 × 10 min). Sections containing the hippocampal formation were then incubated in 1% glycine in PBS to quench residual reactive aldehyde groups, washed in PBS (2 × 10 min), permeabilized in 0.1% saponin (S2149; Sigma, St. Louis, MO)/PBS for 15 min, and incubated with the primary monoclonal anti-α7 nAChR antibody Mab 306 (M220, Sigma) (Schoepfer et al., 1990) at 4°C overnight. The antibody was diluted 1:3000 in an incubation medium (IM) consisting of PBS with 1% bovine serum albumin (A4503, Sigma), 5% normal goat serum, 0.5% cold water fish skin gelatin (G7765, Sigma), and 0.01% saponin. After incubation, preparations were rinsed thoroughly in PBS. To detect anti-α7 nAChR antibody binding, a secondary goat anti-mouse antibody coupled to 10 nm gold plus mouse anti-β2/3 nAChR antibody (1:100 in IM; SC5544; Santa Cruz Biotechnology, Santa Cruz, CA) was applied at a dilution of 1:100 for 4 hr at 37°C. Preparations were washed subsequently in IM (10 min) and PBS (3 × 10 min) before final rinsing in double-distilled water.

For the double labeling of (1) glutamate and α7 nAChR, (2) GABA and α7 nAChR, or (3) dual epitopes of α7 nAChR, the following antibodies were used: (1) rabbit anti-glutamate (1:20,000 in IM; G66422; Sigma) detected with goat anti-rabbit 10 nm gold plus mouse anti-α7 nAChR (1:4000 in IM) detected with goat anti-mouse 5 nm gold (EM.GAM5; British Biocell, Cardiff, Wales, UK); (2) rabbit anti-GABA (1:4000 in IM; A2052; Sigma) detected with goat anti-rabbit 10 nm gold plus mouse anti-α7 nAChR (1:4000 in IM) detected with goat anti-mouse 5 nm gold, or (3) rabbit anti-α7 nAChR (1:100 in IM; SC5544; Santa Cruz Biotechnology, CA) detected with goat anti-rabbit 10 nm gold plus mouse anti-α7 nAChR (1:4000 in IM) detected with goat anti-mouse 5 nm gold. All secondary gold-conjugated antibodies were used at a dilution of 1:100 in IM. The sections were contrasted with uranyl acetate (5 min) and Reynold’s lead citrate (50 sec) according to standard EM methods. The preparations were examined using JEOL JEM-100 CX and JEOL JEM-1010 electron microscopes.

For αBgttx labeling, ultrathin sections were incubated in IM containing biotin-XX-conjugated αBgttx (1:300 for 4 hr at room temperature; B-1196; Molecular Probes). After rinsing in PBS, sections were incubated overnight at 4°C in IM containing mouse anti-biotin antibody (1:250; A-11242; Molecular Probes). For detection of the mouse anti-biotin antibody, a goat anti-mouse antibody coupled to 10 nm gold was used.
The homogenate was then centrifuged at 2000 rpm (400 × g) for 10 min at 4°C in a Beckman JA-20. The supernatant was removed, leaving P1, and centrifuged again at 14,000 rpm (17,500 × g) for 15 min under the same conditions. The resulting supernatant S2 was removed, and the pellet was resuspended in ice-cold homogenization buffer and centrifuged again at 9000 rpm (7000 × g) for 15 min to generate a supernatant S3 and a pellet enriched for mitochondrial fraction. This operation was repeated twice more to wash the mitochondrial fraction. Equivalent amounts of each fraction and the initial homogenate were analyzed by Western blot analysis. Proteins were separated by SDS-PAGE as described previously (Schägger and von Jagow, 1987) using a 10% separating gel, and transferred to Immobilon-P by electroblotting. Membranes were blocked in either 2% (w/v) BSA (Fraction V, 735086, Boehringer Mannheim), 0.05% (v/v) Nonidet P40 (BDH, Poole UK) in PBS, or 3% nonfat milk powder, 0.05% (v/v) NP40 in PBS. Primary antibodies were applied in the same buffer at dilutions of 1:10,000 for anti-α7 nAChR (M220) and 1:30,000 for anti-Hsp60 (SPA-804; StressGen, Victoria British Columbia, Canada). Immunoreactivity was detected using HRP-conjugated donkey anti-rabbit (711-035-152; StressGen, Victoria British Columbia, Canada) or anti-mouse (715-035-150; Stratech, Luton, UK) antibodies at 1:10,000 and ECL (RPN 2106, Amersham Pharmacia Biotech, Little Chalfont, UK).

Controls. The specificity of the mouse anti-α7 nAChR antibodies used here has been described previously (Schepfer et al., 1990; Dominguez del Toro et al., 1994). Specificity of antibody binding was confirmed by immunoblots and by the absence of immunolabeling in preparations from which primary antibodies were omitted. The anti-synaptophysin antibody used here is well characterized and is known to bind selectively to presynaptic sites (Wiedenmann and Franke, 1985). Additional controls were as described below.

Image analysis and processing. Because sections from all three animals processed for electron microscopy showed similar labeling intensity, randomly selected serial sections from all animals were combined for analysis. Gold particles at synaptic sites were counted manually on printed electron micrographs from 14 series (10,000× magnification), each consisting of three serial sections. For the purpose of this study, structures considered to be synapses satisfied the following criteria: (1) presence of a synaptic cleft; (2) accumulation of synaptic vesicles in the presynaptic terminal close to the synaptic cleft; and (3) presence of a postsynaptic density in the postsynaptic profile. According to the appear-
ance of the postsynaptic density, two different synapse types have been distinguished in the vertebrate CNS (Gray, 1959; Colonnier, 1968). The first type (type 1 or "asymmetrical synapse") has an extensive postsynaptic density and a population of large, round, electron-lucent vesicles in the presynaptic profile. Type 2 ("symmetrical synapses") are characterized by a less conspicuous postsynaptic density and a presynaptic population of small, pleomorphic, electron-lucent vesicles. It is generally accepted that glutamatergic synapses have asymmetric morphology, whereas GABAergic synapses are symmetric. Complete counts of all gold particles at each synapse would require full three-dimensional reconstruction of each synapse, a prohibitively time-consuming task. As a useful approximation that largely eliminates false-negative (unlabeled) synapses, we analyzed sets of three serial sections. All synapses in randomly selected fields of view that were present throughout all three serial sections were counted. For presentation, micrographs and slides were digitized at high resolution using a Mustek flatbed scanner or a Nikon LS-1000 slide scanner. Contrast and brightness were optimized in Adobe Photoshop 5.0.

**Statistical analysis.** Gold particle incidence was compared to determine whether the α7 nAChR immunolabeling over synaptic membranes was significantly higher than background labeling. Gold particles were counted over the synaptic membranes identified in all serial sections. The actual sampled region was the synaptic cleft plus the adjoining 30-nm-wide presynaptic and postsynaptic zones, because the separation between a labeled epitope and a gold particle attached to a secondary antibody can extend up to 28 nm (Matsubara et al., 1996). These counts were compared with the number of gold particles over equivalent areas of regions where no labeling was expected, such as myelin. Because particle counts were not normally distributed, a Welch t test was used for comparisons. The sampled synaptic area for each synapse was estimated by multiplying the total sampled length of the synaptic cleft over the three serial sections by the section thickness. Linear regression analysis was performed to examine possible correlation of the number of gold particles per synapse with the synaptic area.
RESULTS

Light and electron microscopic immunolabeling demonstrates that the α7 nAChR subunit is present in cell bodies and processes of hippocampal neurons

Light microscopic immunolabeling of the hippocampal formation revealed diffuse α7 nAChR-like immunoreactivity (α7 nAChR-LIR) throughout cell bodies and cell processes of neurons in the dentate gyrus and CA3 and CA1 regions (Fig. 1). Immunoreactivity throughout the molecular layer of the dentate gyrus and in the cell body layers of all regions was relatively strong and easily recognizable at low magnification (Fig. 1A). Immunoreactivity in the dendritic fields of CA3 and CA1 was weaker, but clearly visible at higher magnification in Figure 1, B and C. To determine the precise location of the α7nAChRs, we performed postembedding EM immunolabeling. Synaptic contacts were heavily labeled, as were membranous structures within the presynaptic and postsynaptic cytoplasm. Labeling was also present at mitochondria (Fig. 2); to determine whether this labeling represented authentic α7 nAChRs or cross-reactivity of the antibody with other protein(s) present in mitochondria, we performed Western blot analysis on subcellular fractions of brain homogenates. Our results demonstrate that the monoclonal antibody M220 recognizes two bands in crude homogenates, one of ~56 kDa (corresponding to the α7 nAChR) and one of 44 kDa (Fig. 2A). Only the 44 kDa band was present in the purified mitochondrial fraction. That this fraction contained mainly mitochondria was con-
firm ed by its enrichment in the mitochondrial marker, Hsp-60 (Fig. 2A), and by ultrastructural investigation of the fraction (Fig. 2B). We were able to eliminate the 44 kDa band in Western blots using 3% nonfat milk powder in the blocking medium (Fig. 2A) (see Materials and Methods). This blocking procedure, however, was not compatible with immuno-electron microscopy. To confirm the specificity of α7 nAChR immunolabeling, we performed two independent tests: (1) double labeling using the monoclonal antibody (M220) and a polyclonal antiserum (SC5544) raised against a larger epitope of the α7 subunit (polyclonal: amino acids 367–502; monoclonal: amino acids 380–400); and (2) light and electron microscopic labeling for αBgtx. Figure 2C–E demonstrate that both antibodies directed against the α7 subunit labeled synaptic contacts, frequently colocalizing at both presynaptic and postsynaptic sites. As shown in Figure 2, D and E (5 nm particles), the monoclonal antibody yielded stronger labeling, and only the monoclonal antibody labeled mitochondria (Fig. 2C–E).

The presence of α7 nAChR at synaptic sites was further confirmed by αBgtx binding. As demonstrated in Figure 3, light microscopic double labeling for αBgtx and synaptophysin revealed abundant expression of both epitopes in hippocampal synaptic zones, often in close apposition. In keeping with previous findings (Hunt and Schmidt, 1979), αBgtx binding was seen only occasionally throughout neuronal cell bodies; this difference compared with the pattern of anti-α7 nAChR immunolabeling may reflect the post-translational processing required for αBgtx binding (Chen et al., 1998; Aztiria et al., 2000) but not for binding of the monoclonal antibody directed against the peptide epitope. Ultrastructural investigation of hippocampal tissue labeled for αBgtx revealed widespread presynaptic and postsynaptic labeling (Fig. 4) that, although less intense than the labeling observed with the anti-α7 nAChR antibody, was qualitatively similar in distribution, confirming that the synaptic immunolabeling reflects the presence of authentic α7 nAChRs.

### Most synapses in CA1 stratum radiatum display α7 nAChR-LIR

The ultrastructural investigation of dentate gyrus and CA1 and CA3 regions revealed α7 nAChR-LIR at synaptic sites in all hippocampal areas (Fig. 5). Gold particles were located mainly (1) at the postsynaptic density, (2) within the synaptic cleft, (3) in the postsynaptic cytoplasm (Figs. 5–7), and (4) at presynaptically located vesicles, often located in close proximity to the synaptic

### Table 1. Quantitative parameters of serial section analysis of immunogold labeling for α7 nAChRs in CA1 stratum radiatum

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of synapses evaluated (3 serial sections per synapse)</td>
<td>158 (100%) in 12 series</td>
</tr>
<tr>
<td>Number of synapses with labeled synaptic membranes in at least one of the three serial sections</td>
<td>151 (96%)</td>
</tr>
<tr>
<td>Number of synapses with unlabeled synaptic membranes in all three serial sections</td>
<td>7 (4%)</td>
</tr>
<tr>
<td>Number of synapses with labeled presynaptic sites (excluding the synaptic membrane) in at least one of the three serial sections</td>
<td>124 (78%)</td>
</tr>
<tr>
<td>Number of synapses with labeled postsynaptic sites (excluding the synaptic membrane) in at least one of the three serial sections</td>
<td>114 (71%)</td>
</tr>
<tr>
<td>Number of background areas evaluated</td>
<td>373 in 12 series</td>
</tr>
</tbody>
</table>
membrane but >30 nm apart from it (indicating that the epitope is located at the vesicles rather than the synaptic membrane). It is not clear whether the latter are synaptic vesicles or specialized transport vesicles. Labeled synapses were present at both den-
glutamatergic and virtually all synapses showed \( \alpha 7 \) nAChR-LIR, most glutamatergic synapses are likely to bear substantial levels of \( \alpha 7 \) nAChRs. Consistent with this interpretation, double labeling for glutamate and \( \alpha 7 \) nAChR shows that most but not all asymmetric \( \alpha 7 \) nAChR-LIR synapses are glutamate-LIR (Fig. 9); glutamate-LIR, \( \alpha 7 \) nAChR-negative synapses were rare. Double labeling for both \( \alpha 7 \) nAChRs/GABA and \( \alpha 7 \) nAChR/glutamate also revealed that most of the postsynaptic neurons with \( \alpha 7 \) nAChRs were glutamatergic, presumably pyramidal cells.

To investigate whether the \( \alpha 7 \) nAChR-immunoreactive contacts include GABAAergic synapses, we performed double labeling for GABA and \( \alpha 7 \) nAChRs. The results show that many but not all GABA-like immunoreactive profiles display \( \alpha 7 \) nAChR-LIR at the synaptic cleft (Fig. 10), suggesting that many GABAAergic synapses in CA1 stratum radiatum are subject to \( \alpha 7 \) nAChR-mediated cholinergic modulation. It is unclear, however, whether the detailed distribution of \( \alpha 7 \) nAChRs at GABAAergic synapses is similar to that at glutamatergic synapses.

**DISCUSSION**

Perhaps the most striking aspects of the current observations are the prevalence and density of \( \alpha 7 \) nAChR immunolabeling at synapses throughout the stratum radiatum. Almost all synaptic profiles in this region appear labeled over the synaptic membranes. Gold particles are also commonly found over presynaptic and postsynaptic elements of the synapse. The mean immunogold particle density at these \( \alpha 7 \) nAChR-labeled synapses (184.01 ± 10.15 particles/\( \mu m^2 \)) is remarkably close to the reported particle densities for NMDA and AMPA glutamate receptor labeling (≈200 particles/\( \mu m^2 \) for each) at synapses in rat CA1 stratum radiatum of similarly prepared tissue (Racca et al., 2000). The synaptic localization of \( \alpha 7 \) receptors reported here is consistent with immunogold labeling in guinea pig medial prefrontal cortex (Lubin et al., 1999); there also \( \alpha 7 \) nAChR-LIR was detected both presynaptically and postsynaptically, at axosomatic (presumably glutamatergic) synapses and at a subset of double-labeled GABAergic synapses. The presence of \( \alpha 7 \) nAChR immunolabeling at presynaptic terminals, although consistent with reported nicotinic stimulation of hippocampal transmitter release (see below), is in contrast to the reported absence of terminal labeling as assessed by light microscopy (Dominguez del Toro et al., 1994).

The abundance of \( \alpha 7 \) nAChRs at these synapses raises questions about their physiological role. Functional \( \alpha 7 \) nAChR subunits have been demonstrated on hippocampal interneurons, where they constitute 38 pS, inwardly rectifying channels (Shao and Yakel, 2000) mediating strong excitatory effects (Jones and Yakel, 1997; Alkondon et al., 1998; Frazier et al., 1998a,b; McIntosh and Madison, 1999; Sudweeks and Yakel, 2000) including generation of action potentials. The \( \alpha 7 \)-mediated activation of such interneurons can result in either inhibition or disinhibition of pyramidal neurons (Ji and Dani, 2000). The presence of functional \( \alpha 7 \) subunits on hippocampal dentate granule or pyramidal cells has been more controversial. Activation of \( \alpha 7 \) nAChRs on mossy fiber presynaptic terminals has been found to increase intraterminal Ca\(^{2+} \) levels and to increase transmitter release (Gray et al., 1996), but others have failed to observe such Ca\(^{2+} \) elevation (Vogt and Regehr, 2001). Most investigators have found no nAChR-mediated excitation of pyramidal neurons (Frazier et al., 1998b), despite the presence of very low but detectable levels of \( \alpha 7 \) nAChR subunit mRNA in these cells by single-cell RTPCR (Sudweeks and Yakel, 2000). However, in acute and cultured hippocampal slices of 2- to 4-week-old rats, a small component of the EPSC evoked in CA1 pyramidal cells by extracellular...
stimulation in stratum radiatum has been reported to be \( \alpha_7 \) nAChR mediated (Hefft et al., 1999). The reasons for these different observations are not clear, but of some potential relevance may be the recent discovery of lynx1, an endogenous peptide homologous to \( \alpha \)-Bgtx that enhances nicotinic receptor currents (Miwa et al., 1999). The distribution of lynx1 binding is similar to the distribution of \( \alpha_7 \) nAChRs, suggesting that physiological activation of \( \alpha_7 \) nAChRs may be modulated by the simultaneous binding of lynx1.

By producing inward, depolarizing current and particularly by directly mediating Ca\(^{2+}\) influx, activation of \( \alpha_7 \) nAChRs could be expected to influence synaptic transmission and plasticity. Presynaptic \( \alpha_7 \) nAChR activation, induced by repetitive, brief (5 × 200 msec at 8.5 sec intervals) application of 0.5 mM nicotine, can lead to persistent potentiation of glutamatergic synapses between dissociated hippocampal neurons (Radcliffe and Dani, 1998). \( \alpha_7 \) nAChR-mediated nicotinic activation in conjunction with postsynaptic depolarization has also been shown to induce LTP at glutamatergic synapses onto ventral tegmental area dopaminergic neurons. Evidence at those synapses suggested that activation of presynaptic \( \alpha_7 \) nAChRs most likely induced potentiation by acting presynaptically to increase the probability of glutamate release, and it was inferred that the consequent enhanced postsynaptic depolarization produced the NMDA receptor (NMDAR) activation necessary for LTP induction. There is also evidence that arachidonic acid, a putative retrograde messenger in LTP, can facilitate synaptic transmission by increasing \( \alpha_7 \) nAChR-mediated currents and thus enhancing presynaptic transmitter release (Nishizaki et al., 1999).

The abundance of postsynaptic \( \alpha_7 \) subunits revealed by our results also suggests, however, a possible postsynaptic locus for \( \alpha_7 \) nAChR-mediated synaptic potentiation. During early postnatal development, when AMPA receptors are absent from many postsynaptic membranes, the \( \alpha_7 \) nAChRs may actually mediate the induction of LTP, either directly or in conjunction with NMDARs. During this time, levels of \( \alpha_7 \) nAChRs in many brain areas, including hippocampus (Hunt and Schmidt, 1979) and somatosensory cortex (Bina et al., 1995), are much higher than in the adult. Activation of postsynaptic, particularly presynaptic, \( \alpha_7 \) subunits, especially during the first postnatal week when their density is presumably highest, could depolarize a dendritic spine sufficiently to relieve the voltage-dependent Mg\(^{2+}\) block of simultaneously stimulated NMDA receptors, thereby inducing NMDA-dependent synaptic plasticity. Indeed, a selective \( \alpha_7 \) nAChR-mediated enhancement of the NMDA component of EPSPs recorded from auditory cortex pyramidal neurons is seen at postnatal day 8–16 but not in older rats (Aramakis and Metherate, 1998). The high Ca\(^{2+}\) permeability of \( \alpha_7 \) nAChRs (Bertrand et al., 1993; Seguela et al., 1993; Castro and Albuquerque, 1995) raises the further possibility that activation of these receptors alone could yield sufficient Ca\(^{2+}\) influx to trigger calcium-dependent processes, including the induction of synaptic plasticity (Ghosh and Greenberg, 1995). The likelihood of \( \alpha_7 \) nAChR-driven synaptic potentiation might be enhanced if the resulting spine depolarization were sufficient to activate voltage-gated calcium channels in the spine membrane (Yuste and Denk, 1995; Reid et al., 2001), or if the resulting Ca\(^{2+}\) influx were amplified by calcium-induced calcium release (CICR) from internal stores in the dendritic spine (Empetage et al., 1999); indeed, there is evidence for nicotinic activation of CICR (Empetage et al., 2001). Such mechanisms may not be essential for gross brain development, because transgenic mice lacking the \( \alpha_7 \) subunit displayed no abnormalities of brain anatomy (Orr-Urtreger et al., 1997) or of behavior (Paylor et al., 1998). However, chronic systemic nicotine administration during the second week of postnatal development has recently been found to lead to persistent electrophysiological abnormalities in auditory neocortex (Aramakis et al., 2000). It is known that \( \alpha_7 \) nAChRs activate and desensitize rapidly in response to brief exposure to high Ach concentrations (Couturier et al., 1990); the half-maximal concentrations for \( \alpha_7 \) activation and inactivation of this subunit under physiological conditions appear to be in the range of 30–90 and 1–2 \( \mu \)M, respectively (Seguela et al., 1993; Fenseter et al., 1997).

Choline, the product of Ach hydrolysis in the extracellular space, is a selective \( \alpha_7 \) nAChR agonist (Alkondon et al., 1997), and ambient levels of choline in the CSF caused by hydrolysis of Ach may be, after strong cholinergic activity, sufficiently high to activate and/or to desensitize \( \alpha_7 \) receptors (Papke et al., 1996). The half-maximal desensitization concentration is sufficiently high, however, for a large fraction of the receptors to remain functional under normal conditions (McGehee et al., 1995; Gray et al., 1996). The physiological consequence of these differential sensitivities is unclear, but it could provide a form of lateral inhibition in time and space: Ach release from an activated cholinergic terminal could result in \( \alpha_7 \)-mediated facilitation of synaptic transmission and plasticity at simultaneously activated glutamergic
synapses close to the activated terminal where the local ACh concentration would transiently be high. As a result of diffusion, synapses farther from the cholinergic terminal, or nearby synapses activated asynchronously, would experience inactivating ACh concentrations.

In addition to labeling of synaptic membranes, we also observed abundant labeling over endoplasmic reticulum and over membranous structures in the postsynaptic cytoplasm, including the spine apparatus (Figs. 5–7). This pattern resembles the reported association of β2 and α4 nAChRs with endoplasmic reticulum and transport vesicles in various neurons, including neocortical pyramidal cells (Hill et al., 1993; Nakayama et al., 1995). These observations of a large pool of intracellular α7 nAChRs suggest that these subunits may be actively internalized or inserted and that the extracellular, functional receptors may thus be dynamically regulated in response to the ongoing activity of the neuron.

Finally, the near-ubiquitous presence of α7 nAChRs at hippocampal synapses described here renders more salient the recent report (Wang et al., 2000b) of high-affinity binding of β-amyloid peptide (Aβ1–42) to α7 nAChRs. Submicromolar concentrations of soluble Aβ1–42, like its fibrillar amyloid precipitate, may be neurotoxic (Roher et al., 1996); toxicity can be partially blocked by nicotine (Wang et al., 2000a). Furthermore, similarly low concentrations of Aβ1–42 can block α7 nAChR-mediated currents in hippocampal interneurons (Petit et al., 2001). Thus the widespread distribution of α7 nAChRs in the hippocampus and their interactions with Aβ1–42 peptide may be important factors in early cognitive impairments and later neuronal loss in Alzheimer’s disease.

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